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The structure of carboxypeptidase A†.

VIII. Atomic interpretation at 0.2 nm resolution, a new study of the complex of glycyl-L-tyrosine with CPA, and mechanistic deductions

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[Plates 58 to 61]

1. Introduction

Bovine pancreatic carboxypeptidase A_α (CPA),‡ the subject of these studies, is a zinc containing enzyme of molecular weight 34600, which catalyses the hydrolysis of polypeptides and esters at the C-terminal peptide or ester bond. Experiments to date have shown that in order to be hydrolysed, the substrate must contain a C-terminal residue in the L configuration, with the carboxyl group free and α to the peptide or ester bond which is to be cleaved. In addition, the reaction is favoured if the C-terminal residue is aromatic.

Our crystallographic studies of CPA have yielded electron density maps of the native enzyme at 0.6 nm (Lipscomb, Coppola, Hartsuck, Ludwig, Muirhead, Searl & Steitz 1966), 0.28 nm (Ludwig, Hartsuck, Steitz, Muirhead, Coppola, Reeke & Lipscomb 1967) and 0.20 nm resolution (Reeke, Hartsuck, Ludwig, Quiocho, Steitz & Lipscomb 1967; Lipscomb, Hartsuck, Reeke, Quiocho, Bethge, Ludwig, Steitz, Muirhead & Coppola 1968). Concurrently, a study of the binding of a number of substrates and inhibitors at 0.6 nm resolution was under way (Steitz, Ludwig, Quiocho & Lipscomb 1967). Subsequently, the most promising of these complexes, that of glycyl-L-tyrosine with CPA, was carried to atomic resolution (Reeke *et al.* 1967; Lipscomb *et al.* 1968). Even though chemical sequence is not available for several of the binding and catalytic groups of the enzyme, we have been able to deduce the identity of the binding residue Arg-145 and the catalytic residue Glu-270 (Reeke *et al.* 1967), to describe the mode of binding of Gly-Tyr, to extrapolate these conclusions to the binding of polypeptides, and to propose a mechanism, with certain ambiguities, for the action of the enzyme (Lipscomb *et al.* 1968).

We have now completed the detailed atomic interpretation of the 0.20 nm electron density map, supplying residue identifications where the primary sequence is not available, and have subjected the resulting atomic coordinates to a model building procedure (Diamond 1966) which forces them to conform to standard bond distances and angles. The improved coordinates have been entered in a structure factor calculation, which gave a standard crystallographic R factor of 0.44.

We have also analysed some characteristics of the various structural features in the molecule—helices, pleated sheet, and random coil—and we present the distribution of residues among them. The structure of the complex between Gly-Tyr and CPA has been further clarified at

† To M. L. Anson, 1901–1968.

‡ Abbreviations used in this paper are: CPA, Carboxypeptidase A in the α form (Sampath-Kumar, Clegg & Walsh 1964); MIR, multiple isomorphous replacement; SF, structure factor; F_o, observed structure factor; F_c, calculated structure factor; HPLA, hippuryl-L-β-phenyllactate; ES, enzyme–substrate complex; CBZ, carbobenzoxy.

0.20 nm resolution by use of the phases from the structure factor calculation and a new function for computing the difference electron density map. The conclusions which we have previously made concerning the mechanism of action of CPA are essentially unchanged but have become less ambiguous in certain aspects.

2. Structure of carboxypeptidase A at 0.20 nm resolution

(a) *Determination of structure*

Carboxypeptidase A_α crystallizes in the monoclinic space group $P2_1$, with unit cell dimensions $a = 5.141$ nm, $b = 5.989$ nm, $c = 4.719$ nm, $\beta = 97.58°$. The preparation of heavy metal derivatives (Lipscomb *et al.* 1966) and the measurement of X-ray diffraction intensities (Lipscomb *et al.* 1968) have been described previously. In summary, complete data (20600 reflexions) to 0.20 nm resolution were measured on the native enzyme, complete data to 0.28 nm resolution were measured on four heavy atom derivatives—Pb (2 sites), Hg (3 sites), Hg (1 site), and Pt (4 sites)— and those 6000 of the 14000 reflexions between 0.30 and 0.20 nm which gave the largest intensities in the native data set were also measured for the Pb(2) and Hg(3) derivatives.

Table 1. Heavy atom binding to carboxypeptidase†

			heavy atom coordinates§			
atom	Z, e/mol	B‡/nm²	x	y	z	residue
Pb_1	58	0.09	−0.094	0.500	−0.089	Glu-270
Pb_2	53	0.26	−0.087	0.540	−0.147	citrate, not protein
Hg, g	50	0.16	−0.071	0.455	−0.115	His-69, Glu-72, Lys-196
Hg, s_1	47	0.13	−0.071	0.452	−0.115	His-69, Glu-72, Lys-196
Hg, s_2	46	0.31	−0.506	0.069	−0.257	His-29
Hg, s_3	48	0.25	−0.475	0.109	−0.136	His-29, Lys-84
Pt_1	74	0.71	0.341	0.430	0.034	Cys-161
Pt_2	45	0.71	−0.438	0.305	−0.568	Met-103
Pt_3	68	1.03	−0.292	0.082	0.141	N-terminus: Ala-1
Pt_4	27	0.69	−0.484	0.485	−0.500	His-303
Ag_1	—	—	0.238	0.498	−0.278	His-166, Ser-158
Ag_2	—	—	−0.082	0.220	0.193	His-120
Ag_3	—	—	−0.457	0.090	−0.143	His-29, (Lys-84)
Ag_4	—	—	−0.483	0.477	−0.516	His-303
Co_1	—	—	−0.500	0.500	−0.500	His-303
Co_2	—	—	−0.500	0.070	−0.130	His-29, (Lys-84)
Zn‖	—	—	−0.087	0.443	−0.155	His-69, Glu-72, Lys-196

† All of these derivatives except Hg, g were prepared by dialysing CPA crystals against solutions of the indicated composition (mol l^{-1}).

Pb: 0.003 $PbCl_2$, 0.01 Na citrate, 0.2 LiCl, 0.02 tris pH 7.5.

Hg, s: data in this paper were taken on crystals soaked against 0.0008 $HgCl_2$, 0.2 LiCl, 0.02 tris, pH 7.5, but the fourth site is not occupied except under prolonged soaking against 0.003 M *p*-acetoxymercurianiline, 0.2 Na acetate and 0.02 tris pH 8. This fourth site is at $x = -0.448$, $y = 0.507$, $z = 0.575$, and is associated with His-303.

Pt: 0.003 K_2PtCl_4, 0.2 LiCl, 0.02 tris, pH 7.5.

Ag: 0.005 $AgNO_3$, 0.2 Na acetate, pH 8.

Co: 0.01 $CoCl_2$, 0.01 tris, pH 7.5.

Hg, g: 5×10^{-4} CPA was dialysed against 0.001 $HgCl_2$, 1 LiCl, 0.02 tris pH 8 and crystallized by dialysis against 0.18 LiCl, 0.02 tris, pH 8.

‡ The effective isotropic temperature factor B for the protein is 0.16 nm².

§ The transformation to the symmetry related position is $x' = -x$, $y' = \frac{1}{2}+y$, $z' = -z$.

‖ Zn coordinates were found by interpolation of the electron density map.

Heavy atom positional, thermal, and occupancy parameters, obtained and refined previously (Lipscomb *et al.* 1968), are given in table 1, which also shows the binding site on the protein of each heavy atom. The 0.20 nm electron density map was computed from multiple isomorphous replacement (MIR) phases and contours were plotted on Mylar sheets. The sheets were supported vertically in a frame designed so that any desired portion of the map could be viewed by a seated observer. The detailed atomic interpretation was accomplished by viewing simultaneously the map and appropriate Kendrew models, which were on the same scale, and by placing coloured markers on the map at proposed atomic positions chosen to place the structure as much as possible in the density. Since the complete sequence was not known, at first only the backbone was constructed. Then an analysis (Lipscomb *et al.* 1968) was made of the known portion of sequence at the N-terminus of the molecule and atomic positions were derived for all side chains in this fragment. Simultaneously, tentative identifications of all residues not in known terminal sequences were made from the map. These tentative identifications enabled us to locate in the complete sequence all other chemically sequenced fragments known to us,† and to assign numbers to the residues in these fragments. The known fragments were then used to revise our initial identifications. A complete (but still tentative) sequence was then drawn up for use in refinements, structure factor calculations, and model building. This sequence, which is listed in table 2, contains 93 residues for which only X-ray identifications have been made. These identifications will be compared to the chemical results when they become available.

TABLE 2. SEQUENCE USED FOR THE STRUCTURE FACTOR CALCULATION‡

A R S T B T F B Y A	T Y H T L B Z I Y B	F M B L L V G Z H P
Z L L S K L Z I G R	T Y Z G R P I Y V L	K F S T G G S B R P
A L W I B L G I H S	R Z W I T Z A T G V	W F A K K F T Z B Y
G B Z P S F T A I L	B S M K L F L Z I V	T B P B G F A F T H
S Z Z R L W Z Z T R	S T G S G G Z C V G	V B A B R T W B A G
W G K K G A S S S P	C S Z T Y Y G K Y A	Z S Z T Z V K S I V
B F V K B H G B F K	A F S S L K G Y S Z	B S L Y P Y G Y T T
Z S L P B K T Z L B	Z V A K S A V A A L	K S L Y G T S Y K R
G S I I S S I Y Z A	S G G I S B H S Y B	Z G I K R S F V F Z
L R B V G S W G F B	H P A K Z I L P V S	H Z L W B G V B T I
M Z H T V B B		

A, Ala; B, Asx; C, Cys; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; P, Pro; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; Z, Glx.

‡ This table lists the X-ray identifications in the case of those residues for which the chemical and X-ray sequences have not yet been reconciled or for which no chemical identification is yet available. Except for those residues whose identification has been published by Professor Neurath's laboratory (see text) and for which the X-ray and chemical identifications are in agreement, this sequence should be treated as provisional. The present authors accept total responsibility for any errors in this sequence of CPA.

The mathematical model building program of R. Diamond (1966) was used to improve the atomic positions. This is a 'guided' model building program; that is, the atomic coordinates from the map are used to guide the positioning of the atoms of a model of idealized geometry. The criterion used is a least squares minimization of the distances between the guide atoms and the corresponding atoms of the model. The idealized coordinates for the peptide group and the

† These fragments are residues 1–22 (Sampath Kumar *et al.* 1964), 23–103 (Neurath *et al.* 1968), 138–144 and 152–165 (Sampath Kumar, Walsh, Bargetzi & Neurath 1963; Neurath 1964), 247–250 (Roholt & Pressman 1967) and 301–307 (Bargetzi *et al.* 1964). Other tentatively sequenced fragments (Neurath 1968 private communication) have been assigned provisionally by us as 104–125, 178–184, 198–201, 202–239, and 258–264. Only the X-ray evidence is available for the total number of residues, the identification of the 93 residues not in the above fragments, and the numbering beyond residue 103, including the numbering of the heptapeptide which is known chemically to be C-terminal.

various side chains were taken from X-ray structure determinations of appropriate model molecules. Initially, only rotations about single bonds in the model were allowed to vary, and all guide atoms differing in position by more than 0.05 nm from the corresponding refined model atoms were examined for errors and readjusted if necessary. During this examination we observed that some regions of the molecule must be distorted from the idealized geometry of the model we were attempting to fit. Inspection of the density contours suggested that in many cases these distortions involved the backbone, principally the backbone angle τ ($C_{carbonyl}$-C_α-N). Accordingly, after all guide atoms were in satisfactory positions the model building program was repeated, this time allowing the bond angles around the alpha carbons to vary.† All distortions were accommodated as variations in these angles because it was not convenient to vary the shape of the peptide group itself. After this procedure, unrealistic bond angles (differing by more than $\pm 10°$ from tetrahedral) were found at 8 % of the alpha carbons in the molecule. Where these occurred at large residues, the program probably placed too much weight on the guide positions of the side chains, and bond angle distortions were probably also present at the β carbons. The average value of τ for the entire molecule is $112 \pm 5°$ rather than the expected 109.2°. This value probably reflects the fact that all peptide distortions were taken up in this one parameter, but might also indicate that packing of side chains in such a large globular molecule may require that, on the average, the peptides be spread apart slightly at the α carbons. The r.m.s. deviation of guide atom positions from final coordinates is 0.023 nm. The final atomic coordinates resulting from the Diamond program are given in table 3.

A structure factor calculation was performed with the atomic positions given by the Diamond program. All atoms were given the Wilson plot isotropic thermal parameter of 0.12 nm^2 except the atoms of residues 133–137, a loop on the outside of the molecule, which were assigned thermal parameters of 0.23 nm^2. A total of 128 atoms, or 5.3 % of the molecule, was omitted either because these atoms had uncertain positions or because they belonged to important side chains, whose identities we wish to confirm by calculating a new electron density map from the structure factor phases and observed intensities. Solvent structure was also omitted from the structure factor calculation. Structure factors were calculated for 16642 reflexions, all those measured to 0.20 nm resolution except those which our data correlation program had indicated were measured very poorly. The results of this structure factor calculation constitute table 4.‡ The overall standard crystallographic R factor is 0.44. The R factor and the difference between the MIR and calculated (SF) phases were examined as functions of structure factor and resolution (table 5). The value of R is 0.36 for the range 0.2 to 0.5 nm resolution, and surprisingly does not increase at the higher resolutions. The reflexions with large interplanar spacings give poor agreement because they are most affected by the solvent structure, which has not been

† In the notation of Diamond (1966), five probes were used with the following parameters:

probe	residues	delay 2	min. ratio (C_1)	min. value (C_2)
1	0	30	1.00×10^{-4}	1.0
2	1	30	1.00×10^{-4}	1.0
3	3	30	1.00×10^{-4}	1.0
4	3	10	0.20×10^{-1}	1.0
5	6	10	0.60×10^{-3}	1.0

These values were adopted after some experimentation and may be helpful to others using this program.

‡ Table 4 is deposited in the Archives of the Royal Society where it may be consulted, and also at the National Lending Library (see note on p. 214) where copies may be ordered (reference SUP 10002).

TABLE 3. THE COORDINATES OF ATOMS OF CARBOXYPEPTIDASE A USED IN THE STRUCTURE FACTOR CALCULATION

1	ALA	CB	-0.293	0.060	0.109	15		CB	-0.076	0.160	0.027	29		CA	-0.442	0.044	-0.241
1		CA	-0.322	0.063	0.100	15		CA	-0.105	0.161	0.033	29		CO	-0.424	0.034	-0.262
1		CO	-0.331	0.042	0.082	15		CO	-0.118	0.138	0.027	29		O	-0.412	0.047	-0.277
1		O	-0.320	0.037	0.061	15		O	-0.132	0.134	0.005	30		N	-0.424	0.012	-0.263
2		N	-0.351	0.032	0.091	16		N	-0.112	0.123	0.049	30	PRO	CG	-0.436	-0.026	-0.257
2	ARG	NH1	-0.349	-0.052	0.185	16	ASP	OD1	-0.075	0.067	0.080	30		CD	-0.440	-0.002	-0.247
2		NH2	-0.371	-0.084	0.169	16		OD2	-0.071	0.101	0.087	30		CB	-0.413	-0.024	-0.273
2		CZ	-0.366	-0.063	0.165	16		CG	-0.085	0.085	0.082	30		CA	-0.409	-0.001	-0.281
2		NE	-0.378	-0.052	0.142	16		CB	-0.114	0.090	0.077	30		CO	-0.414	0.003	-0.314
2		CD	-0.377	-0.028	0.138	16		CA	-0.122	0.100	0.048	30		O	-0.400	-0.006	-0.330
2		CG	-0.365	-0.022	0.112	16		CO	-0.152	0.104	0.043	31		N	-0.435	0.015	-0.322
2		CB	-0.379	-0.001	0.097	16		O	-0.166	0.096	0.022	31	GLU	OE1	-0.483	0.004	-0.309
2		CA	-0.362	0.011	0.077	17		N	-0.161	0.115	0.064	31		OE2	-0.495	-0.031	-0.310
2		CO	-0.380	0.017	0.049	17	GLU	OE1	-0.197	0.167	0.138	31		CD	-0.487	-0.014	-0.319
2		O	-0.391	0.002	0.034	17		OE2	-0.198	0.134	0.157	31		CG	-0.477	-0.014	-0.350
3		N	-0.381	0.038	0.044	17		CD	-0.193	0.147	0.140	31		CB	-0.472	0.010	-0.361
3	SER	OG	-0.443	0.044	0.001	17		CG	-0.177	0.140	0.114	31		CA	-0.444	0.020	-0.352
3		CB	-0.424	0.042	0.025	17		CB	-0.194	0.140	0.085	31		CO	-0.445	0.045	-0.360
3		CA	-0.397	0.047	0.018	17		CA	-0.188	0.120	0.064	31		O	-0.459	0.052	-0.382
3		CO	-0.396	0.072	0.010	17		CO	-0.204	0.122	0.035	32		N	-0.429	0.058	-0.342
3		O	-0.387	0.086	0.028	17		O	-0.223	0.110	0.026	32	LEU	CD1	-0.488	0.101	-0.352
4		N	-0.406	0.077	-0.016	18		N	-0.196	0.139	0.019	32		CD2	-0.449	0.126	-0.349
4	THR	OG1	-0.433	0.089	-0.071	18	ILE	CD1	-0.176	0.190	-0.054	32		CG	-0.463	0.109	-0.333
4		CG2	-0.387	0.095	-0.073	18		CG1	-0.191	0.169	-0.048	32		CB	-0.445	0.090	-0.324
4		CB	-0.411	0.103	-0.061	18		CB	-0.198	0.167	-0.018	32		CA	-0.427	0.082	-0.346
4		CA	-0.408	0.099	-0.028	18		CG2	-0.175	0.170	0.007	32		CO	-0.400	0.088	-0.332
4		CO	-0.428	0.114	-0.017	18		CA	-0.208	0.144	-0.010	32		O	-0.387	0.104	-0.340
4		O	-0.425	0.135	-0.014	18		CO	-0.202	0.129	-0.035	33		N	-0.390	0.075	-0.310
5		N	-0.450	0.104	-0.013	18		O	-0.220	0.125	-0.056	33	LEU	CD1	-0.390	0.116	-0.264
5	ASN	ND1	-0.514	0.089	-0.059	19		N	-0.178	0.121	-0.033	33		CD2	-0.342	0.116	-0.256
5		ND2	-0.515	0.125	-0.049	19	TYR	OH	-0.077	0.158	-0.107	33		CG	-0.366	0.104	-0.250
5		CG	-0.510	0.106	-0.040	19		CD2	-0.097	0.110	-0.067	33		CB	-0.367	0.080	-0.262
5		CB	-0.497	0.101	-0.011	19		CE2	-0.081	0.124	-0.082	33		CA	-0.364	0.077	-0.294
5		CA	-0.472	0.115	-0.002	19		CZ	-0.092	0.145	-0.093	33		CO	-0.342	0.061	-0.298
5		CO	-0.464	0.117	0.030	19		CE1	-0.116	0.151	-0.089	33		O	-0.345	0.040	-0.295
5		O	-0.476	0.130	0.045	19		CD1	-0.133	0.136	-0.074	34		N	-0.320	0.070	-0.304
6		N	-0.444	0.104	0.040	19		CG	-0.122	0.116	-0.063	34	SER	OG	-0.294	0.083	-0.347
6	THR	OG1	-0.453	0.071	0.088	19		CB	-0.139	0.101	-0.048	34		CB	-0.294	0.061	-0.339
6		CG2	-0.406	0.074	0.100	19		CA	-0.169	0.107	-0.054	34		CA	-0.297	0.058	-0.308
6		CB	-0.429	0.078	0.078	19		CO	-0.190	0.089	-0.052	34		CO	-0.274	0.070	-0.291
6		CA	-0.433	0.103	0.070	19		O	-0.203	0.081	-0.074	34		O	-0.273	0.091	-0.290
6		CO	-0.408	0.117	0.076	20		N	-0.193	0.084	-0.025	35		N	-0.255	0.056	-0.278
6		O	-0.394	0.115	0.100	20	ASP	OD1	-0.191	0.031	0.015	35	LYS	NZ	-0.176	0.028	-0.152
7		N	-0.404	0.130	0.055	20		OD2	-0.187	0.051	0.052	35		CE	-0.178	0.050	-0.167
7	PHE	CD2	-0.323	0.136	0.034	20		CG	-0.196	0.048	0.028	35		CD	-0.186	0.047	-0.199
7		CE2	-0.296	0.142	0.038	20		CB	-0.212	0.067	0.015	35		CG	-0.204	0.065	-0.212
7		CZ	-0.288	0.164	0.038	20		CA	-0.212	0.068	-0.018	35		CB	-0.228	0.056	-0.230
7		CE1	-0.307	0.180	0.036	20		CO	-0.241	0.072	-0.032	35		CA	-0.231	0.064	-0.261
7		CD1	-0.334	0.174	0.033	20		O	-0.255	0.057	-0.043	35		CO	-0.209	0.057	-0.277
7		CG	-0.342	0.152	0.031	21		N	-0.248	0.093	-0.031	35		O	-0.205	0.037	-0.282
7		CB	-0.370	0.147	0.028	21	PHE	CD2	-0.301	0.150	-0.078	36		N	-0.194	0.074	-0.284
7		CA	-0.381	0.145	0.056	21		CE2	-0.326	0.160	-0.091	36	LEU	CD1	-0.154	0.047	-0.356
7		CO	-0.386	0.168	0.069	21		CZ	-0.349	0.153	-0.084	36		CD2	-0.195	0.063	-0.380
7		O	-0.408	0.178	0.062	21		CE1	-0.349	0.139	-0.062	36		CG	-0.180	0.056	-0.352
8		N	-0.366	0.175	0.087	21		CD1	-0.325	0.130	-0.048	36		CB	-0.178	0.076	-0.331
8	ASN	ND1	-0.373	0.234	0.131	21		CG	-0.302	0.135	-0.056	36		CA	-0.171	0.071	-0.299
8		ND2	-0.349	0.223	0.172	21		CB	-0.277	0.126	-0.040	36		CO	-0.146	0.082	-0.286
8		CG	-0.359	0.218	0.147	21		CA	-0.274	0.101	-0.043	36		O	-0.147	0.100	-0.273
8		CB	-0.355	0.196	0.133	21		CO	-0.280	0.093	-0.074	37		N	-0.124	0.071	-0.289
8		CA	-0.367	0.197	0.101	21		O	-0.297	0.079	-0.082	37	GLN	NOE1	-0.035	0.041	-0.207
8		CO	-0.352	0.213	0.084	22		N	-0.264	0.103	-0.091	37		NOE2	-0.065	0.022	-0.237
8		O	-0.327	0.214	0.087	22	MET	CE	-0.191	0.155	-0.132	37		CD	-0.054	0.040	-0.228
9		N	-0.367	0.227	0.067	22		SD	-0.196	0.126	-0.138	37		CG	-0.065	0.061	-0.242
9	TYR	OH	-0.405	0.163	-0.033	22		CG	-0.229	0.124	-0.131	37		CB	-0.081	0.059	-0.269
9		CD2	-0.403	0.211	0.015	22		CB	-0.238	0.100	-0.130	37		CA	-0.098	0.079	-0.278
9		CE2	-0.411	0.190	0.003	22		CA	-0.266	0.098	-0.122	37		CO	-0.087	0.092	-0.301
9		CZ	-0.398	0.183	-0.020	22		CO	-0.274	0.074	-0.126	37		O	-0.082	0.083	-0.323
9		CE1	-0.378	0.194	-0.029	22		O	-0.292	0.068	-0.145	38		N	-0.083	0.114	-0.295
9		CD1	-0.370	0.216	-0.015	23		N	-0.260	0.059	-0.109	38	ILE	CD1	-0.079	0.186	-0.275
9		CG	-0.383	0.223	0.007	23	ASP	OD1	-0.206	-0.000	-0.096	38		CG1	-0.081	0.161	-0.280
9		CB	-0.375	0.246	0.021	23		OD2	-0.227	0.015	-0.132	38		CB	-0.081	0.154	-0.311
9		CA	-0.357	0.244	0.049	23		CG	-0.224	0.012	-0.107	38		CG2	-0.107	0.154	-0.331
9		CO	-0.355	0.265	0.066	23		CB	-0.244	0.022	-0.090	38		CA	-0.072	0.129	-0.314
9		O	-0.345	0.282	0.057	23		CA	-0.265	0.035	-0.109	38		CO	-0.042	0.130	-0.308
10		N	-0.363	0.264	0.091	23		CO	-0.292	0.028	-0.106	38		O	-0.028	0.138	-0.325
10	ALA	CB	-0.386	0.281	0.128	23		O	-0.302	0.010	-0.119	39		N	-0.033	0.120	-0.283
10		CA	-0.363	0.283	0.111	24		N	-0.305	0.041	-0.090	39	GLY	CA	-0.004	0.119	-0.273
10		CO	-0.338	0.281	0.133	24	LEU	CD1	-0.333	0.020	-0.019	39		CO	0.003	0.112	-0.241
10		O	-0.340	0.285	0.159	24		CD2	-0.320	0.059	-0.007	39		O	-0.014	0.104	-0.228
11		N	-0.317	0.276	0.123	24		CG	-0.321	0.041	-0.029	40		N	0.027	0.116	-0.231
11	THR	OG1	-0.269	0.261	0.183	24		CB	-0.335	0.049	-0.057	40	ARG	NH1	0.108	0.068	-0.174
11		CG2	-0.289	0.233	0.150	24		CA	-0.332	0.036	-0.084	40		NH2	0.093	0.045	-0.141
11		CB	-0.291	0.256	0.163	24		CO	-0.351	0.047	-0.108	40		CZ	0.092	0.053	-0.167
11		CA	-0.291	0.274	0.140	24		O	-0.368	0.036	-0.122	40		NE	0.073	0.044	-0.187
11		CO	-0.269	0.270	0.122	25		N	-0.346	0.068	-0.113	40		CD	0.067	0.053	-0.216
11		O	-0.274	0.263	0.096	25	LEU	CD1	-0.358	0.140	-0.171	40		CG	0.046	0.071	-0.217
12		N	-0.245	0.274	0.135	25		CD2	-0.397	0.116	-0.170	40		CB	0.056	0.091	-0.198
12	TYR	OH	-0.233	0.377	0.104	25		CG	-0.367	0.116	-0.169	40		CA	0.038	0.111	-0.201
12		CD2	-0.221	0.324	0.143	25		CB	-0.355	0.106	-0.141	40		CO	0.054	0.133	-0.192
12		CE2	-0.230	0.347	0.137	25		CA	-0.361	0.082	-0.135	40		O	0.067	0.147	-0.208
12		CZ	-0.225	0.355	0.111	25		CO	-0.364	0.070	-0.164	41		N	0.052	0.139	-0.165
12		CE1	-0.215	0.343	0.091	25		O	-0.385	0.071	-0.181	41	THR	OG1	0.055	0.188	-0.122
12		CD1	-0.206	0.321	0.098	26		N	-0.344	0.058	-0.168	41		CG2	0.064	0.152	-0.099
12		CG	-0.210	0.312	0.124	26	VAL	CG1	-0.305	0.061	-0.215	41		CB	0.052	0.164	-0.125
12		CB	-0.200	0.288	0.131	26		CG2	-0.313	0.019	-0.220	41		CA	0.065	0.158	-0.152
12		CA	-0.221	0.270	0.122	26		CB	-0.315	0.040	-0.202	41		CO	0.095	0.155	-0.145
12		CO	-0.214	0.246	0.124	26		CA	-0.343	0.044	-0.194	41		O	0.105	0.137	-0.148
12		O	-0.218	0.234	0.145	26		CO	-0.359	0.023	-0.198	42		N	0.108	0.173	-0.136
13		N	-0.203	0.238	0.103	26		O	-0.372	0.019	-0.222	42	TYR	OH	0.257	0.198	-0.085
13	HIS	CD2	-0.264	0.208	0.086	27		N	-0.357	0.010	-0.175	42		CD2	0.186	0.198	-0.080
13		NE2	-0.280	0.197	0.101	27	ALA	CB	-0.361	-0.022	-0.146	42		CE2	0.214	0.198	-0.071
13		CE1	-0.268	0.181	0.116	27		CA	-0.371	-0.011	-0.175	42		CZ	0.230	0.198	-0.093
13		ND1	-0.243	0.181	0.110	27		CO	-0.400	-0.008	-0.172	42		CE1	0.221	0.198	-0.121
13		CG	-0.240	0.198	0.091	27		O	-0.415	-0.024	-0.173	42		CD1	0.192	0.198	-0.129
13		CB	-0.214	0.202	0.080	28		N	-0.406	0.013	-0.169	42		CG	0.176	0.198	-0.108
13		CA	-0.194	0.215	0.101	28	GLU	OE1	-0.434	0.061	-0.092	42		CB	0.146	0.197	-0.117
13		CO	-0.166	0.213	0.094	28		OE2	-0.412	0.037	-0.064	42		CA	0.137	0.174	-0.128
13		O	-0.156	0.228	0.080	28		CD	-0.425	0.043	-0.085	42		CO	0.143	0.156	-0.106
14		N	-0.154	0.194	0.103	28		CG	-0.434	0.026	-0.111	42		O	0.164	0.144	-0.106
14	THR	OG1	-0.139	0.153	0.113	28		CB	-0.435	0.037	-0.141	43		N	0.126	0.153	-0.088
14		CG2	-0.121	0.178	0.150	28		CA	-0.433	0.020	-0.166	43	GLU	OE1	0.147	0.188	0.001
14		CB	-0.120	0.170	0.120	28		CO	-0.448	0.029	-0.194	43		OE2	0.129	0.159	0.019
14		CA	-0.127	0.189	0.098	28		O	-0.472	0.030	-0.198	43		CD	0.133	0.171	0.001
14		CO	-0.129	0.179	0.068	29		N	-0.432	0.035	-0.213	43		CG	0.120	0.168	-0.031
14		O	-0.151	0.179	0.052	29	HIS	CD2	-0.453	0.088	-0.194	43		CB	0.115	0.144	-0.040
15		N	-0.107	0.171	0.061	29		NE2	-0.477	0.096	-0.187	43		CA	0.129	0.136	-0.066
15	LEU	CD1	-0.091	0.162	-0.025	29		CE1	-0.495	0.094	-0.209	43		CO	0.118	0.112	-0.073
15		CD2	-0.044	0.161	-0.007	29		ND1	-0.485	0.084	-0.230	43		O	0.125	0.096	-0.057
15		CG	-0.071	0.169	-0.001	29		CG	-0.458	0.080	-0.221	44		N	0.102	0.111	-0.097
						29		CB	-0.441	0.069	-0.240	44	GLY	CA	0.090	0.090	-0.108

TABLE 3 (*cont.*)

44		CD	0.061	0.088	-0.104	59		O	-0.318	0.192	-0.546	73		O	-0.196	0.326	0.011
44		O	0.049	0.070	-0.107	60		N	-0.350	0.211	-0.574	74		N	-0.196	0.363	0.017
45		N	0.050	0.107	-0.098	60	PRO	CG	-0.382	0.235	-0.599	74	ILE	CD1	-0.274	0.417	-0.004
45	ARG	NH1	0.065	0.170	0.012	60		CD	-0.374	0.211	-0.595	74		CG1	-0.264	0.393	-0.001
45		NH2	0.066	0.145	0.050	60		CB	-0.359	0.248	-0.583	74		CB	-0.233	0.391	0.006
45		CZ	0.057	0.150	0.023	60		CA	-0.342	0.233	-0.564	74		CG2	-0.217	0.403	-0.015
45		NE	0.041	0.136	0.007	60		CO	-0.343	0.235	-0.532	74		CA	-0.223	0.367	0.005
45		CD	0.027	0.142	-0.021	60		C	-0.363	0.231	-0.521	74		CO	-0.228	0.359	-0.026
45		CG	0.024	0.122	-0.041	61		N	-0.320	0.242	-0.517	74		O	-0.251	0.355	-0.038
45		CB	0.019	0.129	-0.072	61	ALA	CB	-0.292	0.232	-0.473	75		N	-0.207	0.356	-0.038
45		CA	0.023	0.110	-0.093	61		CA	-0.317	0.245	-0.486	75	THR	CG1	-0.162	0.344	-0.071
45		CO	0.006	0.113	-0.123	61		CO	-0.309	0.267	-0.472	75		CG2	-0.180	0.380	-0.083
45		O	0.009	0.130	-0.138	61		O	-0.298	0.282	-0.484	75		CB	-0.185	0.355	-0.084
46		N	-0.011	0.097	-0.130	62		N	-0.314	0.269	-0.445	75		CA	-0.207	0.348	-0.067
46	PRO	CG	-0.041	0.067	-0.125	62	LEU	CD1	-0.360	0.324	-0.447	75		CO	-0.214	0.323	-0.073
46		CD	-0.017	0.079	-0.110	62		CD2	-0.381	0.297	-0.417	75		O	-0.234	0.318	-0.089
46		CB	-0.044	0.076	-0.155	62		CG	-0.358	0.301	-0.434	76		N	-0.197	0.310	-0.058
46		CA	-0.029	0.097	-0.156	62		CB	-0.332	0.298	-0.414	76	GLU	OE1	-0.147	0.308	-0.024
46		CO	-0.046	0.118	-0.162	62		CA	-0.309	0.289	-0.427	76		OE2	-0.109	0.296	-0.033
46		O	-0.058	0.126	-0.142	62		CO	-0.287	0.279	-0.404	76		CD	-0.132	0.296	-0.034
47		N	-0.046	0.127	-0.187	62		O	-0.290	0.261	-0.392	76		CG	-0.149	0.277	-0.052
47	ILE	CD1	-0.006	0.149	-0.186	63		N	-0.266	0.292	-0.399	76		CB	-0.176	0.273	-0.042
47		CG1	-0.019	0.164	-0.210	63	TRP	CD1	-0.180	0.267	-0.355	76		CA	-0.200	0.285	-0.060
47		CB	-0.048	0.160	-0.220	63		CE1	-0.185	0.245	-0.361	76		CO	-0.226	0.278	-0.049
47		CG2	-0.066	0.180	-0.224	63		CZ1	-0.167	0.230	-0.346	76		O	-0.238	0.261	-0.058
47		CA	-0.061	0.147	-0.197	63		CH	-0.147	0.236	-0.325	77		N	-0.233	0.292	-0.029
47		CO	-0.086	0.134	-0.209	63		CZ2	-0.141	0.257	-0.318	77	ALA	CB	-0.259	0.309	0.004
47		O	-0.085	0.119	-0.227	63		CE2	-0.158	0.273	-0.333	77		CA	-0.257	0.289	-0.016
48		N	-0.108	0.141	-0.200	63		NE	-0.157	0.294	-0.331	77		CO	-0.281	0.292	-0.038
48	TYR	CH	-0.111	0.035	-0.152	63		CD2	-0.179	0.303	-0.350	77		O	-0.298	0.276	-0.043
48		CD2	-0.129	0.094	-0.147	63		CG	-0.192	0.286	-0.365	78		N	-0.284	0.311	-0.051
48		CE2	-0.119	0.072	-0.139	63		CB	-0.217	0.288	-0.389	78	THR	OG1	-0.299	0.355	-0.059
48		CZ	-0.120	0.056	-0.160	63		CA	-0.244	0.286	-0.378	78		CG2	-0.314	0.348	-0.109
48		CE1	-0.126	0.061	-0.188	63		CO	-0.245	0.301	-0.352	78		CB	-0.297	0.340	-0.083
48		CD1	-0.136	0.083	-0.196	63		C	-0.253	0.321	-0.355	78		CA	-0.305	0.317	-0.073
48		CG	-0.136	0.099	-0.175	64		N	-0.237	0.292	-0.327	78		CO	-0.304	0.300	-0.096
48		CB	-0.146	0.123	-0.184	64	ILE	CD1	-0.314	0.305	-0.298	78		O	-0.325	0.291	-0.109
48		CA	-0.134	0.132	-0.210	64		CG1	-0.287	0.309	-0.309	79		N	-0.280	0.295	-0.102
48		CO	-0.151	0.149	-0.227	64		CB	-0.263	0.302	-0.287	79	GLY	CA	-0.275	0.279	-0.123
48		O	-0.150	0.169	-0.221	64		CG2	-0.257	0.315	-0.260	79		CO	-0.286	0.257	-0.116
49		N	-0.167	0.140	-0.248	64		CA	-0.237	0.303	-0.300	79		O	-0.295	0.243	-0.135
49	VAL	CG1	-0.149	0.171	-0.291	64		CO	-0.214	0.293	-0.279	80		N	-0.285	0.253	-0.088
49		CG2	-0.195	0.169	-0.319	64		O	-0.211	0.273	-0.276	80	VAL	CG1	-0.262	0.216	-0.038
49		CB	-0.175	0.159	-0.295	65		N	-0.198	0.309	-0.266	80		CG2	-0.308	0.224	-0.026
49		CA	-0.186	0.154	-0.268	65	ASP	OD1	-0.144	0.345	-0.231	80		CB	-0.286	0.230	-0.044
49		CO	-0.211	0.140	-0.272	65		OD2	-0.143	0.346	-0.275	80		CA	-0.295	0.233	-0.076
49		O	-0.211	0.120	-0.278	65		CG	-0.146	0.335	-0.254	80		CO	-0.325	0.233	-0.081
50		N	-0.233	0.152	-0.269	65		CB	-0.151	0.311	-0.258	80		O	-0.338	0.215	-0.087
50	LEU	CD1	-0.264	0.115	-0.217	65		CA	-0.175	0.303	-0.245	81		N	-0.336	0.253	-0.080
50		CD2	-0.282	0.149	-0.197	65		CO	-0.178	0.318	-0.218	81	TRP	CD1	-0.419	0.291	-0.090
50		CG	-0.264	0.141	-0.218	65		O	-0.191	0.335	-0.221	81		CE1	-0.415	0.301	-0.116
50		CB	-0.274	0.149	-0.248	66		N	-0.166	0.309	-0.194	81		CZ1	-0.437	0.310	-0.132
50		CA	-0.259	0.142	-0.272	66	LEU	CD1	-0.241	0.324	-0.159	81		CH	-0.462	0.311	-0.124
50		CO	-0.273	0.149	-0.302	66		CD2	-0.222	0.300	-0.193	81		CZ2	-0.467	0.301	-0.099
50		O	-0.275	0.169	-0.308	66		CG	-0.217	0.321	-0.175	81		CE2	-0.445	0.292	-0.082
51		N	-0.282	0.132	-0.318	66		CB	-0.192	0.318	-0.154	81		NE	-0.445	0.282	-0.058
51	LYS	NZ	-0.299	0.112	-0.473	66		CA	-0.166	0.320	-0.166	81		CD2	-0.418	0.276	-0.048
51		CE	-0.298	0.129	-0.451	66		CO	-0.145	0.308	-0.147	81		CG	-0.403	0.281	-0.068
51		CD	-0.300	0.118	-0.422	66		O	-0.142	0.287	-0.149	81		CB	-0.372	0.277	-0.068
51		CG	-0.285	0.132	-0.398	67		N	-0.131	0.320	-0.128	81		CA	-0.364	0.257	-0.084
51		CB	-0.286	0.120	-0.369	67	GLY	CA	-0.110	0.311	-0.107	81		CO	-0.374	0.259	-0.116
51		CA	-0.296	0.136	-0.347	67		CO	-0.083	0.314	-0.116	81		O	-0.398	0.258	-0.125
51		CO	-0.326	0.132	-0.346	67		O	-0.064	0.301	-0.108	82		N	-0.355	0.261	-0.133
51		O	-0.335	0.113	-0.342	68		N	-0.081	0.331	-0.134	82	PHE	CD2	-0.329	0.312	-0.198
52		N	-0.340	0.151	-0.350	68	ILE	CD1	-0.052	0.337	-0.223	82		CE2	-0.334	0.335	-0.201
52	PHE	CD2	-0.387	0.161	-0.286	68		CG1	-0.040	0.346	-0.194	82		CZ	-0.353	0.345	-0.189
52		CE2	-0.379	0.162	-0.256	68		CB	-0.061	0.351	-0.173	82		CE1	-0.367	0.333	-0.172
52		CZ	-0.357	0.170	-0.244	68		CG2	-0.061	0.374	-0.161	82		CD1	-0.362	0.310	-0.168
52		CE1	-0.340	0.180	-0.260	68		CA	-0.057	0.337	-0.146	82		CG	-0.343	0.299	-0.181
52		CD1	-0.347	0.180	-0.290	68		CO	-0.040	0.348	-0.121	82		CB	-0.338	0.275	-0.175
52		CG	-0.370	0.170	-0.303	68		O	-0.016	0.346	-0.116	82		CA	-0.360	0.263	-0.164
52		CB	-0.376	0.171	-0.335	69		N	-0.054	0.361	-0.105	82		CO	-0.368	0.240	-0.178
52		CA	-0.368	0.151	-0.351	69	HIS	CD2	-0.014	0.406	-0.115	82		O	-0.388	0.238	-0.195
52		CO	-0.378	0.152	-0.383	69		NE2	-0.010	0.423	-0.133	83		N	-0.351	0.224	-0.169
52		O	-0.374	0.169	-0.397	69		CE1	-0.029	0.438	-0.133	83	ALA	CB	-0.340	0.185	-0.162
53		N	-0.391	0.134	-0.393	69		ND1	-0.046	0.431	-0.116	83		CA	-0.361	0.201	-0.179
53	SER	OG	-0.387	0.111	-0.462	69		CG	-0.036	0.410	-0.104	83		CO	-0.387	0.192	-0.173
53		CB	-0.381	0.128	-0.441	69		CB	-0.051	0.398	-0.084	83		O	-0.404	0.186	-0.194
53		CA	-0.403	0.131	-0.423	69		CA	-0.043	0.373	-0.080	84		N	-0.390	0.191	-0.146
53		CO	-0.425	0.114	-0.427	69		CO	-0.052	0.362	-0.053	84	LYS	NZ	-0.461	0.116	-0.097
53		O	-0.425	0.097	-0.412	69		O	-0.071	0.370	-0.042	84		CE	-0.470	0.139	-0.102
54		N	-0.444	0.119	-0.448	70		N	-0.039	0.344	-0.044	84		CD	-0.449	0.153	-0.114
54	THR	OG1	-0.478	0.139	-0.480	70	SER	OG	0.003	0.328	-0.014	84		CG	-0.443	0.174	-0.097
54		CG2	-0.499	0.132	-0.439	70		CB	-0.020	0.320	-0.004	84		CB	-0.419	0.186	-0.105
54		CB	-0.489	0.122	-0.465	70		CA	-0.044	0.331	-0.020	84		CA	-0.415	0.183	-0.136
54		CA	-0.467	0.105	-0.457	70		CO	-0.058	0.344	0.002	84		CO	-0.438	0.194	-0.154
54		CO	-0.462	0.089	-0.480	70		O	-0.080	0.338	0.008	84		O	-0.457	0.183	-0.165
54		O	-0.474	0.071	-0.483	71		N	-0.044	0.361	0.015	85		N	-0.436	0.216	-0.156
55		N	-0.445	0.095	-0.497	71	ARG	NH1	-0.001	0.471	0.009	85	LYS	NZ	-0.539	0.265	-0.154
55	GLY	CA	-0.438	0.082	-0.521	71		NH2	0.023	0.467	0.053	85		CE	-0.517	0.276	-0.165
55		CO	-0.423	0.096	-0.540	71		CZ	0.009	0.458	0.031	85		CD	-0.496	0.260	-0.171
55		O	-0.409	0.112	-0.530	71		NE	0.004	0.436	0.031	85		CG	-0.470	0.271	-0.176
56		N	-0.426	0.090	-0.567	71		CD	-0.016	0.425	0.009	85		CB	-0.448	0.255	-0.173
56	GLY	CA	-0.414	0.101	-0.589	71		CG	-0.019	0.400	0.016	85		CA	-0.455	0.230	-0.172
56		CO	-0.385	0.094	-0.592	71		CB	-0.038	0.398	0.038	85		CO	-0.465	0.223	-0.203
56		O	-0.371	0.087	-0.571	71		CA	-0.053	0.375	0.036	85		O	-0.489	0.221	-0.212
57		N	-0.378	0.098	-0.617	71		CO	-0.083	0.380	0.033	86		N	-0.446	0.218	-0.218
57	SER	OG	-0.323	0.100	-0.660	71		O	-0.095	0.377	0.053	86	PHE	CD2	-0.384	0.228	-0.268
57		CB	-0.349	0.101	-0.654	72		N	-0.092	0.387	0.007	86		CE2	-0.372	0.249	-0.274
57		CA	-0.352	0.092	-0.624	72	GLN	NOE1	-0.091	0.435	-0.075	86		CZ	-0.386	0.268	-0.277
57		CO	-0.331	0.103	-0.603	72		NOE2	-0.099	0.445	-0.031	86		CE1	-0.411	0.268	-0.272
57		O	-0.327	0.097	-0.577	72		CD	-0.096	0.430	-0.049	86		CD1	-0.423	0.247	-0.265
58		N	-0.317	0.119	-0.614	72		CG	-0.098	0.406	-0.041	86		CG	-0.410	0.228	-0.264
58	ASP	OD1	-0.235	0.124	-0.567	72		CB	-0.123	0.400	-0.031	86		CB	-0.423	0.207	-0.256
58		OD2	-0.244	0.157	-0.578	72		CA	-0.120	0.392	-0.001	86		CA	-0.450	0.211	-0.247
58		CG	-0.248	0.138	-0.582	72		CO	-0.139	0.372	-0.000	86		CO	-0.466	0.189	-0.251
58		CB	-0.268	0.132	-0.607	72		O	-0.151	0.365	-0.023	86		O	-0.482	0.186	-0.273
58		CA	-0.296	0.132	-0.598	73		N	-0.139	0.363	0.026	87		N	-0.460	0.174	-0.230
58		CO	-0.310	0.154	-0.593	73	TRP	CD1	-0.129	0.323	0.109	87	THR	OG1	-0.458	0.152	-0.180
58		O	-0.299	0.172	-0.597	73		CE1	-0.145	0.326	0.130	87		CG2	-0.435	0.128	-0.210
59		N	-0.333	0.152	-0.584	73		CZ1	-0.136	0.317	0.157	87		CB	-0.461	0.137	-0.205
59	ARG	NH1	-0.455	0.117	-0.556	73		CH	-0.112	0.307	0.164	87		CA	-0.473	0.153	-0.229
59		NH2	-0.472	0.145	-0.530	73		CZ2	-0.095	0.303	0.144	87		CO	-0.500	0.154	-0.220
59		CZ	-0.455	0.139	-0.548	73		CE2	-0.104	0.312	0.117	87		O	-0.515	0.137	-0.220
59		NE	-0.439	0.154	-0.558	73		NE	-0.093	0.311	0.094	88		N	-0.507	0.175	-0.212
59		CD	-0.417	0.148	-0.572	73		CD2	-0.109	0.323	0.071	88	GLU	OE1	-0.514	0.183	-0.099
59		CG	-0.399	0.168	-0.575	73		CG	-0.131	0.330	0.080	88		OE2	-0.520	0.216	-0.120
59		CB	-0.371	0.163	-0.561	73		CB	-0.154	0.343	0.064	88		CD	-0.519	0.196	-0.119
59		CA	-0.350	0.171	-0.579	73		CA	-0.155	0.344	0.031	88		CG	-0.527	0.181	-0.147
59		CO	-0.338	0.192	-0.565	73		CO	-0.184	0.344	0.019	88		CB	-0.526	0.194	-0.175

TABLE 3 (*cont.*)

88		CA	-0.532	0.180	-0.203	104		CD	-0.352	0.143	-0.481	119		CG2	-0.007	0.315	0.066
88		CO	-0.551	0.194	-0.222	104		CG	-0.357	0.168	-0.488	119		CB	-0.018	0.304	0.091
88		O	-0.575	0.192	-0.224	104		CB	-0.347	0.183	-0.463	119		CA	0.004	0.292	0.112
89		N	-0.540	0.210	-0.236	104		CA	-0.366	0.200	-0.456	119		CO	-0.009	0.283	0.136
89	ASP	CD1	-0.564	0.256	-0.213	104		CO	-0.352	0.219	-0.436	119		O	-0.022	0.295	0.151
89		CD2	-0.548	0.282	-0.233	104		O	-0.348	0.237	-0.446	120		N	-0.006	0.261	0.140
89		CG	-0.552	0.263	-0.232	105		N	-0.346	0.213	-0.410	120	HIS	CD2	-0.067	0.246	0.108
89		CB	-0.541	0.249	-0.253	105	LEU	CD1	-0.343	0.257	-0.326	120		NE2	-0.094	0.248	0.105
89		CA	-0.554	0.226	-0.256	105		CD2	-0.387	0.258	-0.356	120		CE1	-0.103	0.239	0.127
89		CO	-0.562	0.219	-0.288	105		CG	-0.358	0.254	-0.356	120		ND1	-0.083	0.232	0.145
89		O	-0.572	0.232	-0.306	105		CB	-0.353	0.230	-0.367	120		CG	-0.060	0.236	0.134
90		N	-0.555	0.198	-0.293	105		CA	-0.333	0.227	-0.388	120		CB	-0.034	0.229	0.149
90	TYR	OH	-0.569	0.132	-0.438	105		CO	-0.310	0.214	-0.371	120		CA	-0.017	0.248	0.162
90		CD2	-0.549	0.139	-0.361	105		O	-0.314	0.195	-0.362	120		CO	0.003	0.240	0.187
90		CE2	-0.556	0.128	-0.388	106		N	-0.288	0.225	-0.367	120		O	-0.002	0.237	0.211
90		CZ	-0.561	0.142	-0.412	106	PHE	CD2	-0.247	0.174	-0.392	121		N	0.027	0.236	0.179
90		CE1	-0.558	0.164	-0.411	106		CE2	-0.253	0.160	-0.416	121	SER	OG	0.069	0.195	0.181
90		CD1	-0.551	0.175	-0.384	106		CZ	-0.262	0.169	-0.442	121		CB	0.069	0.219	0.181
90		CG	-0.546	0.162	-0.359	106		CE1	-0.265	0.191	-0.445	121		CA	0.049	0.228	0.199
90		CB	-0.539	0.173	-0.330	106		CD1	-0.258	0.205	-0.422	121		CO	0.064	0.247	0.216
90		CA	-0.561	0.188	-0.322	106		CG	-0.250	0.197	-0.395	121		O	0.061	0.250	0.242
90		CO	-0.586	0.176	-0.318	106		CB	-0.243	0.213	-0.371	122		N	0.078	0.260	0.202
90		O	-0.587	0.162	-0.299	106		CA	-0.264	0.216	-0.351	122	GLU	OE1	0.143	0.226	0.238
91		N	-0.606	0.181	-0.338	106		CO	-0.257	0.230	-0.325	122		OE2	0.120	0.230	0.195
91	GLY	CA	-0.632	0.171	-0.339	106		C	-0.250	0.250	-0.327	122		CD	0.133	0.236	0.217
91		CO	-0.653	0.185	-0.328	107		N	-0.259	0.220	-0.300	122		CG	0.140	0.262	0.221
91		O	-0.677	0.184	-0.340	107	LEU	CD1	-0.291	0.220	-0.209	122		CB	0.121	0.278	0.205
92		N	-0.645	0.198	-0.306	107		CD2	-0.277	0.258	-0.216	122		CA	0.093	0.280	0.214
92	ASP	OD1	-0.678	0.181	-0.243	107		CG	-0.273	0.234	-0.224	122		CO	0.085	0.304	0.208
92		OD2	-0.692	0.213	-0.238	107		CB	-0.278	0.231	-0.256	122		O	0.088	0.318	0.228
92		CG	-0.677	0.201	-0.247	107		CA	-0.254	0.231	-0.272	123		N	0.076	0.308	0.181
92		CB	-0.655	0.212	-0.260	107		CO	-0.229	0.221	-0.255	123	GLU	OE1	0.072	0.389	0.155
92		CA	-0.662	0.214	-0.293	107		O	-0.228	0.201	-0.249	123		OE2	0.109	0.394	0.138
92		CO	-0.659	0.238	-0.303	108		N	-0.210	0.235	-0.248	123		CD	0.095	0.385	0.151
92		C	-0.678	0.251	-0.306	108	GLN	NOE1	-0.111	0.251	-0.249	123		CG	0.103	0.362	0.168
93		N	-0.635	0.243	-0.309	108		NOE2	-0.093	0.220	-0.231	123		CB	0.089	0.341	0.155
93	GLN	NOE1	-0.599	0.330	-0.330	108		CD	-0.112	0.231	-0.241	123		CA	0.068	0.330	0.171
93		NOE2	-0.566	0.305	-0.321	108		CG	-0.140	0.220	-0.242	123		CO	0.042	0.334	0.151
93		CD	-0.589	0.312	-0.318	108		CB	-0.162	0.236	-0.246	123		O	0.041	0.332	0.125
93		CG	-0.605	0.298	-0.299	108		CA	-0.185	0.229	-0.232	124		N	0.022	0.340	0.165
93		CB	-0.604	0.274	-0.302	108		CO	-0.185	0.239	-0.202	124	ARG	NH1	-0.077	0.375	0.115
93		CA	-0.629	0.265	-0.319	108		O	-0.177	0.258	-0.196	124		NH2	-0.120	0.381	0.119
93		CO	-0.627	0.262	-0.351	109		N	-0.193	0.225	-0.184	124		CZ	-0.097	0.372	0.129
93		O	-0.614	0.247	-0.361	109	ILE	CD1	-0.255	0.193	-0.150	124		NE	-0.096	0.358	0.152
94		N	-0.640	0.278	-0.367	109		CG1	-0.241	0.215	-0.150	124		CD	-0.072	0.346	0.163
94	PRO	CG	-0.676	0.303	-0.382	109		CB	-0.211	0.214	-0.139	124		CG	-0.048	0.360	0.164
94		CD	-0.659	0.293	-0.357	109		CG2	-0.203	0.220	-0.107	124		CB	-0.023	0.346	0.173
94		CB	-0.665	0.293	-0.408	109		CA	-0.194	0.230	-0.154	124		CA	-0.004	0.344	0.150
94		CA	-0.641	0.279	-0.398	109		CO	-0.170	0.240	-0.136	124		CO	-0.007	0.365	0.132
94		CO	-0.617	0.289	-0.408	109		C	-0.170	0.258	-0.123	124		O	-0.027	0.369	0.114
94		O	-0.600	0.278	-0.418	110		N	-0.148	0.228	-0.138	125		N	0.013	0.379	0.137
95		N	-0.616	0.311	-0.405	110	VAL	CG1	-0.138	0.221	-0.078	125	LEU	CD1	-0.016	0.440	0.130
95	SER	OG	-0.567	0.358	-0.406	110		CG2	-0.090	0.231	-0.076	125		CD2	0.021	0.457	0.161
95		CB	-0.587	0.346	-0.396	110		CB	-0.115	0.222	-0.095	125		CG	0.007	0.436	0.153
95		CA	-0.594	0.325	-0.413	110		CA	-0.122	0.234	-0.123	125		CB	0.026	0.420	0.141
95		CO	-0.572	0.308	-0.411	110		CO	-0.102	0.232	-0.144	125		CA	0.015	0.401	0.122
95		O	-0.563	0.301	-0.434	110		O	-0.094	0.214	-0.151	125		CO	0.029	0.397	0.096
96		N	-0.562	0.302	-0.385	111		N	-0.094	0.252	-0.152	125		O	0.037	0.414	0.083
96	PHE	CD2	-0.486	0.278	-0.344	111	THR	OG1	-0.108	0.274	-0.199	126		N	0.033	0.376	0.090
96		CE2	-0.462	0.267	-0.337	111		CG2	-0.065	0.278	-0.214	126	TRP	CD1	0.104	0.346	0.050
96		CZ	-0.461	0.246	-0.325	111		CB	-0.081	0.276	-0.189	126		CE1	0.119	0.358	0.072
96		CE1	-0.484	0.236	-0.321	111		CA	-0.074	0.255	-0.171	126		CZ1	0.145	0.360	0.069
96		CD1	-0.508	0.247	-0.329	111		CO	-0.046	0.255	-0.155	126		CH	0.157	0.353	0.046
96		CG	-0.509	0.268	-0.340	111		O	-0.027	0.247	-0.166	126		CZ2	0.143	0.341	0.024
96		CB	-0.535	0.279	-0.348	112		N	-0.044	0.264	-0.129	126		CE2	0.117	0.338	0.027
96		CA	-0.541	0.285	-0.379	112	ASP	OD1	0.029	0.308	-0.081	126		NE	0.100	0.327	0.010
96		CO	-0.543	0.264	-0.397	112		OD2	0.025	0.274	-0.078	126		CD2	0.075	0.329	0.021
96		O	-0.525	0.258	-0.411	112		CG	0.016	0.291	-0.086	126		CG	0.078	0.339	0.046
97		N	-0.565	0.253	-0.396	112		CB	-0.011	0.290	-0.102	126		CB	0.057	0.345	0.066
97	THR	OG1	-0.617	0.232	-0.418	112		CA	-0.019	0.266	-0.110	126		CA	0.047	0.369	0.066
97		CG2	-0.597	0.215	-0.375	112		CO	-0.022	0.250	-0.085	126		CO	0.031	0.374	0.036
97		CB	-0.596	0.218	-0.406	112		O	-0.024	0.258	-0.061	126		O	0.007	0.373	0.033
97		CA	-0.571	0.232	-0.411	113		N	-0.021	0.229	-0.092	127		N	0.046	0.378	0.016
97		CO	-0.567	0.234	-0.443	113	PRO	CG	-0.026	0.196	-0.120	127	GLN	NOE1	0.064	0.450	-0.031
97		O	-0.556	0.219	-0.455	113		CD	-0.017	0.220	-0.120	127		NOE2	0.027	0.454	-0.062
98		N	-0.576	0.253	-0.454	113		CB	-0.024	0.190	-0.089	127		CD	0.043	0.442	-0.045
98	ALA	CB	-0.582	0.284	-0.486	113		CA	-0.023	0.211	-0.071	127		CG	0.038	0.417	-0.044
98		CA	-0.574	0.259	-0.484	113		CO	-0.002	0.213	-0.046	127		CB	0.040	0.408	-0.015
98		CO	-0.545	0.261	-0.487	113		O	-0.007	0.220	-0.022	127		CA	0.035	0.383	-0.014
98		O	-0.537	0.257	-0.510	114		N	0.022	0.209	-0.052	127		CO	0.046	0.371	-0.038
99		N	-0.530	0.266	-0.463	114	ASP	OD1	0.102	0.194	-0.066	127		O	0.032	0.360	-0.057
99	ILE	CD1	-0.474	0.323	-0.422	114		OD2	0.069	0.178	-0.055	128		N	0.072	0.372	-0.037
99		CG1	-0.487	0.308	-0.446	114		CG	0.081	0.195	-0.057	128	GLU	OE1	0.146	0.423	-0.072
99		CB	-0.490	0.283	-0.436	114		CB	0.069	0.216	-0.047	128		OE2	0.165	0.394	-0.051
99		CG2	-0.465	0.271	-0.423	114		CA	0.046	0.210	-0.031	128		CD	0.147	0.405	-0.061
99		CA	-0.501	0.268	-0.460	114		CO	0.045	0.228	-0.008	128		CG	0.118	0.396	-0.061
99		CO	-0.486	0.246	-0.458	114		O	0.051	0.224	0.018	128		CB	0.115	0.371	-0.056
99		O	-0.470	0.241	-0.475	115		N	0.037	0.248	-0.019	128		CA	0.087	0.362	-0.058
100		N	-0.492	0.233	-0.437	115	GLY	CA	0.034	0.268	-0.001	128		CO	0.087	0.337	-0.054
100	LEU	CD1	-0.444	0.206	-0.379	115		CO	0.012	0.271	0.017	128		O	0.078	0.328	-0.034
100		CD2	-0.459	0.168	-0.389	115		O	0.013	0.286	0.036	129		N	0.098	0.326	-0.074
100		CG	-0.467	0.191	-0.381	116		N	-0.008	0.258	0.011	129	THR	OG1	0.128	0.311	-0.109
100		CB	-0.488	0.200	-0.404	116	PHE	CD2	-0.089	0.269	0.026	129		CG2	0.084	0.301	-0.127
100		CA	-0.480	0.211	-0.431	116		CE2	-0.114	0.269	0.036	129		CB	0.107	0.296	-0.105
100		CO	-0.485	0.196	-0.457	116		CZ	-0.129	0.251	0.036	129		CA	0.101	0.301	-0.074
100		O	-0.469	0.182	-0.463	116		CE1	-0.120	0.231	0.026	129		CO	0.124	0.294	-0.053
101		N	-0.508	0.200	-0.473	116		CD1	-0.095	0.230	0.017	129		O	0.138	0.308	-0.039
101	ASP	OD1	-0.557	0.153	-0.496	116		CG	-0.080	0.249	0.016	130		N	0.128	0.272	-0.050
101		OD2	-0.575	0.180	-0.476	116		CB	-0.053	0.247	0.006	130	ARG	NH1	0.168	0.302	0.068
101		CG	-0.560	0.173	-0.492	116		CA	-0.031	0.258	0.025	130		NH2	0.193	0.271	0.083
101		CB	-0.546	0.191	-0.506	116		CO	-0.024	0.245	0.053	130		CZ	0.174	0.280	0.065
101		CA	-0.516	0.188	-0.500	116		O	-0.029	0.252	0.076	130		NE	0.161	0.267	0.045
101		CO	-0.505	0.193	-0.528	117		N	-0.011	0.226	0.050	130		CD	0.138	0.275	0.025
101		O	-0.507	0.179	-0.548	117	ALA	CB	0.009	0.189	0.063	130		CG	0.122	0.256	0.011
102		N	-0.493	0.212	-0.529	117		CA	-0.002	0.211	0.073	130		CB	0.137	0.245	-0.012
102	SER	OG	-0.510	0.246	-0.579	117		CO	0.020	0.223	0.092	130		CA	0.149	0.262	-0.031
102		CB	-0.489	0.244	-0.557	117		O	0.024	0.219	0.119	130		CO	0.171	0.252	-0.046
102		CA	-0.480	0.220	-0.553	118		N	0.033	0.237	0.079	130		O	0.183	0.235	-0.036
102		CO	-0.450	0.222	-0.548	118	PHE	CD2	0.119	0.252	0.105	131		N	0.175	0.263	-0.069
102		C	-0.437	0.219	-0.567	118		CE2	0.142	0.259	0.123	131	SER	OG	0.188	0.285	-0.124
103		N	-0.441	0.227	-0.521	118		CZ	0.146	0.281	0.128	131		CB	0.187	0.261	-0.118
103	MET	SD	-0.415	0.299	-0.522	118		CE1	0.128	0.296	0.118	131		CA	0.195	0.256	-0.087
103		CG	-0.422	0.270	-0.528	118		CD1	0.105	0.289	0.101	131		CO	0.221	0.268	-0.076
103		CB	-0.404	0.255	-0.508	118		CG	0.101	0.267	0.094	131		O	0.221	0.289	-0.070
103		CA	-0.413	0.230	-0.511	118		CB	0.076	0.260	0.076	132		N	0.242	0.255	-0.073
103		CO	-0.404	0.217	-0.485	118		CA	0.055	0.251	0.093	132	THR	CG1	0.275	0.225	-0.054
103		O	-0.420	0.210	-0.469	118		CO	0.043	0.269	0.110	132		CG2	0.271	0.248	-0.012
104		N	-0.379	0.213	-0.480	118		O	0.055	0.277	0.132	132		CB	0.281	0.246	-0.041
104	LYS	NZ	-0.366	0.124	-0.526	119		N	0.019	0.275	0.099	132		CA	0.268	0.263	-0.062
104		CE	-0.343	0.131	-0.507	119	THR	OG1	-0.036	0.287	0.082	132		CO	0.286	0.265	-0.086

TABLE 3 (*cont.*)

132		O	0.276	0.270	-0.111	150		CO	0.102	0.504	-0.401	166		CB	0.166	0.449	-0.229
133		N	0.311	0.262	-0.078	150		O	0.110	0.518	-0.382	166		CA	0.163	0.430	-0.207
133	GLY	CA	0.331	0.263	-0.098	151		N	0.109	0.482	-0.400	166		CO	0.174	0.409	-0.218
133		CO	0.338	0.287	-0.104	151	TRP	CD1	0.162	0.428	-0.345	166		O	0.160	0.397	-0.236
133		O	0.321	0.302	-0.108	151		CE1	0.146	0.426	-0.325	167		N	0.198	0.404	-0.207
134		N	0.363	0.290	-0.105	151		CZ1	0.157	0.415	-0.299	167	GLY	CA	0.212	0.384	-0.214
134	SER	CG	0.345	0.335	-0.144	151		CH	0.183	0.407	-0.295	167		CO	0.231	0.387	-0.236
134		CB	0.352	0.329	-0.115	151		CZ2	0.199	0.408	-0.315	167		O	0.232	0.405	-0.249
134		CA	0.374	0.312	-0.111	151		CE2	0.188	0.419	-0.340	168		N	0.246	0.369	-0.238
134		CO	0.395	0.322	-0.089	151		NE	0.199	0.422	-0.362	168	LYS	NZ	0.265	0.277	-0.218
134		O	0.407	0.310	-0.069	151		CD2	0.181	0.434	-0.383	168		CE	0.254	0.290	-0.243
135		N	0.399	0.343	-0.092	151		CG	0.158	0.437	-0.373	168		CD	0.274	0.307	-0.250
135	GLY	CA	0.419	0.356	-0.073	151		CB	0.133	0.449	-0.387	168		CG	0.272	0.329	-0.235
135		CO	0.403	0.370	-0.053	151		CA	0.128	0.473	-0.378	168		CB	0.283	0.348	-0.251
135		O	0.400	0.390	-0.055	151		CO	0.155	0.483	-0.368	168		CA	0.265	0.368	-0.258
136		N	0.394	0.357	-0.033	151		O	0.169	0.490	-0.386	168		CO	0.256	0.367	-0.290
136	GLY	CA	0.379	0.367	-0.011	152		N	0.161	0.484	-0.340	168		O	0.271	0.374	-0.308
136		CO	0.367	0.348	0.004	152	GLY	CA	0.186	0.493	-0.326	169		N	0.232	0.360	-0.297
136		O	0.361	0.329	-0.008	152		CO	0.185	0.518	-0.330	169	TYR	OH	0.245	0.254	-0.311
137		N	0.363	0.352	0.031	152		O	0.206	0.530	-0.328	169		CD2	0.258	0.313	-0.321
137	GLU	OE1	0.402	0.369	0.077	153		N	0.161	0.526	-0.335	169		CE2	0.262	0.291	-0.312
137		OE2	0.433	0.343	0.084	153	LYS	NZ	0.114	0.566	-0.469	169		CZ	0.242	0.276	-0.320
137		CD	0.411	0.350	0.084	153		CE	0.135	0.563	-0.445	169		CE1	0.220	0.281	-0.337
137		CG	0.389	0.335	0.094	153		CD	0.124	0.560	-0.417	169		CD1	0.216	0.304	-0.346
137		CB	0.360	0.342	0.081	153		CG	0.145	0.562	-0.392	169		CG	0.236	0.320	-0.338
137		CA	0.352	0.337	0.049	153		CB	0.134	0.556	-0.364	169		CB	0.232	0.344	-0.348
137		CO	0.322	0.339	0.043	153		CA	0.155	0.550	-0.339	169		CA	0.219	0.358	-0.327
137		O	0.307	0.323	0.046	153		CO	0.148	0.560	-0.311	169		CO	0.189	0.357	-0.329
138		N	0.314	0.360	0.035	153		O	0.142	0.548	-0.292	169		O	0.178	0.353	-0.308
138	CYH	SG	0.296	0.411	0.039	154		N	0.150	0.582	-0.310	170		N	0.178	0.361	-0.355
138		CB	0.285	0.388	0.013	154	LYS	NZ	0.207	0.685	-0.256	170	ALA	CB	0.142	0.372	-0.392
138		CA	0.286	0.366	0.027	154		CE	0.206	0.660	-0.260	170		CA	0.149	0.361	-0.363
138		CO	0.273	0.349	0.007	154		CD	0.178	0.653	-0.272	170		CO	0.138	0.337	-0.366
138		O	0.283	0.341	-0.014	154		CG	0.177	0.628	-0.278	170		O	0.141	0.326	-0.388
139		N	0.249	0.343	0.013	154		CB	0.149	0.621	-0.289	171		N	0.127	0.330	-0.344
139	VAL	CG1	0.199	0.300	0.012	154		CA	0.144	0.596	-0.286	171	GLU	OE1	0.072	0.321	-0.422
139		CG2	0.238	0.311	0.048	154		CO	0.116	0.587	-0.284	171		OE2	0.054	0.329	-0.383
139		CB	0.228	0.307	0.016	154		O	0.099	0.590	-0.305	171		CD	0.067	0.318	-0.398
139		CA	0.232	0.326	-0.004	155		N	0.113	0.577	-0.260	171		CG	0.081	0.296	-0.387
139		CO	0.205	0.335	-0.017	155	GLY	CA	0.088	0.568	-0.254	171		CB	0.110	0.298	-0.374
139		O	0.194	0.349	-0.005	155		CO	0.098	0.547	-0.237	171		CA	0.115	0.308	-0.343
140		N	0.197	0.325	-0.042	155		O	0.082	0.531	-0.233	171		CO	0.133	0.291	-0.326
140	GLY	CA	0.171	0.330	-0.058	156		N	0.123	0.548	-0.226	171		O	0.128	0.271	-0.326
140		CO	0.171	0.348	-0.082	156	ALA	CB	0.131	0.510	-0.231	172		N	0.153	0.301	-0.310
140		O	0.187	0.363	-0.080	156		CA	0.136	0.530	-0.208	172	SER	OG	0.182	0.323	-0.265
141		N	0.153	0.345	-0.104	156		CO	0.166	0.530	-0.203	172		CB	0.193	0.303	-0.275
141	VAL	CG1	0.185	0.341	-0.153	156		O	0.179	0.538	-0.222	172		CA	0.173	0.288	-0.291
141		CG2	0.151	0.365	-0.183	157		N	0.176	0.521	-0.178	172		CO	0.157	0.275	-0.271
141		CB	0.157	0.350	-0.156	157	SER	OG	0.237	0.515	-0.127	172		O	0.163	0.255	-0.263
141		CA	0.149	0.360	-0.129	157		CB	0.211	0.523	-0.137	173		N	0.137	0.286	-0.263
141		CO	0.121	0.367	-0.133	157		CA	0.204	0.519	-0.169	173	GLU	OE1	0.145	0.292	-0.174
141		O	0.103	0.354	-0.127	157		CO	0.220	0.499	-0.177	173		OE2	0.165	0.306	-0.208
142		N	0.116	0.387	-0.143	157		O	0.208	0.481	-0.183	173		CD	0.146	0.302	-0.196
142	ASP	OD1	0.040	0.421	-0.155	158		N	0.245	0.502	-0.175	173		CG	0.118	0.310	-0.208
142		OD2	0.058	0.445	-0.127	158	SER	OG	0.274	0.512	-0.215	173		CB	0.103	0.295	-0.231
142		CG	0.060	0.430	-0.143	158		CB	0.284	0.493	-0.199	173		CA	0.119	0.277	-0.244
142		CB	0.088	0.423	-0.145	158		CA	0.263	0.484	-0.181	173		CO	0.102	0.258	-0.257
142		CA	0.090	0.397	-0.148	158		CO	0.279	0.473	-0.155	173		O	0.086	0.261	-0.279
142		CO	0.076	0.387	-0.176	158		O	0.295	0.458	-0.158	174		N	0.107	0.239	-0.243
142		O	0.078	0.396	-0.199	159		N	0.273	0.481	-0.130	174	THR	OG1	0.102	0.180	-0.246
143		N	0.062	0.369	-0.172	159	SER	OG	0.297	0.511	-0.091	174		CG2	0.092	0.199	-0.204
143	ALA	CB	0.026	0.344	-0.184	159		CB	0.289	0.490	-0.080	174		CB	0.105	0.201	-0.230
143		CA	0.047	0.357	-0.196	159		CA	0.285	0.473	-0.103	174		CA	0.092	0.218	-0.251
143		CO	0.031	0.372	-0.219	159		CO	0.268	0.454	-0.094	174		CO	0.063	0.221	-0.249
143		O	0.030	0.367	-0.245	159		O	0.244	0.457	-0.090	174		O	0.047	0.207	-0.260
144		N	0.020	0.389	-0.208	160		N	0.280	0.434	-0.091	175		N	0.057	0.238	-0.233
144	ASN	ND1	-0.047	0.413	-0.256	160	PRO	CG	0.310	0.405	-0.098	175	GLU	OE1	0.077	0.245	-0.181
144		ND2	-0.051	0.443	-0.229	160		CD	0.307	0.430	-0.095	175		OE2	0.071	0.225	-0.144
144		CG	-0.040	0.425	-0.231	160		CB	0.286	0.396	-0.086	175		CD	0.064	0.237	-0.164
144		CB	-0.018	0.416	-0.210	160		CA	0.267	0.414	-0.082	175		CG	0.035	0.244	-0.172
144		CA	0.004	0.405	-0.226	160		CO	0.256	0.417	-0.054	175		CB	0.029	0.257	-0.200
144		CO	0.020	0.423	-0.238	160		O	0.237	0.406	-0.047	175		CA	0.030	0.243	-0.228
144		O	0.011	0.441	-0.246	161		N	0.269	0.433	-0.037	175		CO	0.016	0.256	-0.253
145		N	0.045	0.416	-0.240	161	CYH	SG	0.311	0.430	0.022	175		O	-0.008	0.254	-0.260
145	ARG	NH1	0.043	0.496	-0.167	161		CB	0.285	0.450	0.008	176		N	0.031	0.270	-0.266
145		NH2	0.058	0.531	-0.179	161		CA	0.262	0.439	-0.009	176	VAL	CG1	0.035	0.319	-0.263
145		CZ	0.059	0.509	-0.180	161		CO	0.238	0.455	-0.011	176		CG2	0.030	0.320	-0.317
145		NE	0.078	0.499	-0.195	161		O	0.226	0.458	0.010	176		CB	0.036	0.306	-0.290
145		CD	0.082	0.475	-0.196	162		N	0.232	0.463	-0.037	176		CA	0.021	0.284	-0.290
145		CG	0.079	0.467	-0.227	162	SER	OG	0.201	0.513	-0.072	176		CO	0.020	0.271	-0.318
145		CB	0.082	0.442	-0.228	162		CB	0.212	0.491	-0.072	176		O	-0.000	0.271	-0.336
145		CA	0.064	0.430	-0.252	162		CA	0.210	0.479	-0.044	177		N	0.042	0.260	-0.320
145		CO	0.083	0.418	-0.270	162		CO	0.184	0.467	-0.045	177	LYS	NZ	0.145	0.252	-0.355
145		O	0.097	0.429	-0.284	162		O	0.180	0.448	-0.056	177		CE	0.128	0.238	-0.375
146		N	0.081	0.396	-0.269	163		N	0.166	0.478	-0.034	177		CD	0.099	0.246	-0.377
146	THR	OG1	0.134	0.359	-0.273	163	GLU	OE1	0.086	0.448	0.010	177		CG	0.081	0.228	-0.369
146		CG2	0.092	0.340	-0.274	163		OE2	0.128	0.440	0.016	177		CB	0.071	0.233	-0.341
146		CB	0.108	0.361	-0.266	163		CD	0.110	0.452	0.013	177		CA	0.044	0.246	-0.345
146		CA	0.097	0.381	-0.285	163		CG	0.114	0.478	0.011	177		CO	0.023	0.228	-0.351
146		CO	0.083	0.375	-0.314	163		CB	0.122	0.486	-0.018	177		O	0.010	0.226	-0.375
146		O	0.092	0.361	-0.330	163		CA	0.139	0.470	-0.033	178		N	0.019	0.216	-0.328
147		N	0.060	0.386	-0.321	163		CO	0.128	0.469	-0.065	178	SER	OG	0.013	0.175	-0.288
147	TRP	CD1	-0.017	0.358	-0.335	163		O	0.115	0.453	-0.075	178		CB	-0.005	0.192	-0.299
147		CE1	-0.027	0.350	-0.362	164		N	0.134	0.486	-0.080	178		CA	-0.001	0.199	-0.329
147		CZ1	-0.045	0.332	-0.363	164	THR	OG1	0.156	0.516	-0.116	178		CO	-0.026	0.208	-0.346
147		CH	-0.054	0.324	-0.339	164		CG2	0.120	0.528	-0.093	178		O	-0.042	0.195	-0.360
147		CZ2	-0.045	0.331	-0.312	164		CB	0.129	0.514	-0.116	179		N	-0.029	0.230	-0.344
147		CE2	-0.027	0.348	-0.311	164		CA	0.125	0.489	-0.110	179	ILE	CD1	-0.074	0.243	-0.300
147		NE	-0.015	0.358	-0.288	164		CO	0.142	0.476	-0.129	179		CG1	-0.054	0.260	-0.309
147		CD2	0.001	0.375	-0.297	164		O	0.143	0.481	-0.154	179		CB	-0.056	0.264	-0.342
147		CG	0.001	0.375	-0.326	165		N	0.156	0.459	-0.115	179		CG2	-0.083	0.271	-0.357
147		CB	0.015	0.390	-0.345	165	TYR	OH	0.262	0.384	-0.173	179		CA	-0.051	0.242	-0.358
147		CA	0.043	0.383	-0.349	165		CD2	0.212	0.392	-0.125	179		CO	-0.052	0.251	-0.389
147		CO	0.054	0.399	-0.370	165		CE2	0.230	0.381	-0.141	179		O	-0.071	0.247	-0.408
147		O	0.058	0.419	-0.364	165		CZ	0.244	0.394	-0.158	180		N	-0.030	0.262	-0.393
148		N	0.058	0.390	-0.394	165		CE1	0.242	0.417	-0.160	180	VAL	CG1	0.004	0.304	-0.414
148	ASP	OD1	0.097	0.415	-0.461	165		CD1	0.223	0.428	-0.144	180		CG2	0.007	0.279	-0.456
148		OD2	0.101	0.383	-0.476	165		CG	0.209	0.415	-0.126	180		CB	0.000	0.281	-0.426
148		CG	0.091	0.396	-0.461	165		CB	0.189	0.427	-0.110	180		CA	-0.026	0.271	-0.421
148		CB	0.074	0.385	-0.440	165		CA	0.183	0.445	-0.129	180		CO	-0.034	0.251	-0.440
148		CA	0.069	0.402	-0.417	165		CO	0.159	0.437	-0.157	180		O	-0.053	0.251	-0.459
148		CO	0.052	0.420	-0.433	165		O	0.136	0.430	-0.159	181		N	-0.019	0.233	-0.433
148		O	0.038	0.416	-0.456	166		N	0.173	0.438	-0.178	181	ASP	OD1	0.032	0.207	-0.447
149		N	0.053	0.440	-0.420	166	TYR	OH	0.265	0.449	-0.271	181		OD2	0.035	0.184	-0.415
149	ALA	CB	0.011	0.459	-0.423	166		CD2	0.207	0.430	-0.240	181		CG	0.022	0.195	-0.431
149		CA	0.038	0.459	-0.431	166		CE2	0.232	0.430	-0.250	181		CB	-0.007	0.194	-0.430
149		CO	0.052	0.481	-0.419	166		CZ	0.241	0.450	-0.261	181		CA	-0.022	0.212	-0.449
149		O	0.047	0.488	-0.395	166		CE1	0.227	0.469	-0.260	181		CO	-0.051	0.205	-0.454
150		N	0.068	0.490	-0.435	166		CD1	0.202	0.469	-0.250	181		O	-0.064	0.206	-0.479
150	GLY	CA	0.083	0.510	-0.427	166		CG	0.193	0.449	-0.239	182		N	-0.061	0.197	-0.432

TABLE 3 (*cont.*)

182	PHE	CD2	-0.127	0.152	-0.410	196		O	-0.183	0.450	-0.111	211		CB	-0.047	0.622	-0.532
182		CE2	-0.152	0.141	-0.414	197		N	-0.148	0.432	-0.087	211		CA	-0.061	0.641	-0.518
182		CZ	-0.174	0.152	-0.416	197	GLY	CA	-0.137	0.452	-0.070	211		C	-0.089	0.635	-0.515
182		CE1	-0.173	0.175	-0.415	197		C	-0.158	0.463	-0.055	211		O	-0.094	0.617	-0.502
182		CD1	-0.148	0.186	-0.412	197		O	-0.177	0.453	-0.047	212		N	-0.107	0.649	-0.527
182		CG	-0.126	0.174	-0.408	198		N	-0.155	0.485	-0.053	212	SER	OG	-0.151	0.684	-0.521
182		CB	-0.100	0.187	-0.405	198	TYR	OH	-0.100	0.570	0.039	212		CB	-0.150	0.667	-0.541
182		CA	-0.088	0.190	-0.433	198		CD2	-0.156	0.540	0.001	212		CA	-0.135	0.647	-0.527
182		C	-0.106	0.204	-0.454	198		CE2	-0.140	0.558	0.012	212		C	-0.144	0.625	-0.543
182		O	-0.121	0.194	-0.474	198		CZ	-0.116	0.553	0.028	212		O	-0.137	0.621	-0.567
183		N	-0.104	0.225	-0.450	198		CE1	-0.108	0.531	0.032	213		N	-0.158	0.612	-0.529
183	VAL	CG1	-0.134	0.266	-0.430	198		CD1	-0.125	0.513	0.021	213	LEU	CD1	-0.188	0.543	-0.532
183		CG2	-0.128	0.284	-0.478	198		CG	-0.149	0.518	0.004	213		CD2	-0.211	0.556	-0.493
183		CB	-0.119	0.265	-0.456	198		CB	-0.167	0.499	-0.007	213		CG	-0.188	0.560	-0.509
183		CA	-0.119	0.242	-0.468	198		CA	-0.174	0.500	-0.040	213		CB	-0.190	0.584	-0.521
183		CO	-0.114	0.240	-0.499	198		CO	-0.171	0.524	-0.050	213		CA	-0.169	0.590	-0.540
183		C	-0.133	0.240	-0.519	198		O	-0.150	0.531	-0.057	213		CO	-0.184	0.596	-0.570
184		N	-0.089	0.238	-0.503	199		N	-0.194	0.535	-0.052	213		C	-0.193	0.615	-0.575
184	LYS	NZ	0.034	0.261	-0.549	199	SER	OG	-0.174	0.554	-0.104	214		N	-0.185	0.579	-0.588
184		CE	0.006	0.260	-0.560	199		CB	-0.172	0.564	-0.076	214	PRO	CG	-0.188	0.543	-0.607
184		CD	-0.010	0.253	-0.536	199		CA	-0.196	0.558	-0.062	214		CD	-0.175	0.557	-0.581
184		CG	-0.037	0.244	-0.549	199		CO	-0.219	0.563	-0.084	214		CB	-0.193	0.559	-0.631
184		CB	-0.051	0.234	-0.526	199		O	-0.222	0.582	-0.096	214		CA	-0.198	0.581	-0.618
184		CA	-0.080	0.236	-0.531	200		N	-0.236	0.546	-0.089	214		CO	-0.228	0.584	-0.617
184		CO	-0.091	0.215	-0.549	200	GLU	OE1	-0.306	0.558	-0.026	214		O	-0.241	0.599	-0.631
184		O	-0.101	0.217	-0.574	200		OE2	-0.312	0.584	-0.059	215		N	-0.237	0.569	-0.600
185		N	-0.088	0.196	-0.535	200		CD	-0.303	0.567	-0.048	215	ASP	OD1	-0.239	0.531	-0.613
185	ASP	OD1	-0.047	0.163	-0.519	200		CG	-0.285	0.552	-0.064	215		OD2	-0.275	0.514	-0.625
185		OD2	-0.062	0.139	-0.493	200		CB	-0.283	0.558	-0.095	215		CG	-0.263	0.530	-0.614
185		CG	-0.066	0.153	-0.512	200		CA	-0.260	0.548	-0.109	215		CB	-0.280	0.547	-0.602
185		CB	-0.094	0.157	-0.523	200		CO	-0.255	0.562	-0.135	215		CA	-0.265	0.569	-0.595
185		CA	-0.096	0.175	-0.547	200		O	-0.259	0.582	-0.135	215		CO	-0.268	0.575	-0.565
185		CO	-0.126	0.178	-0.558	201		N	-0.245	0.550	-0.155	215		O	-0.278	0.562	-0.549
185		O	-0.134	0.176	-0.584	201	ASP	OD1	-0.217	0.604	-0.173	216		N	-0.259	0.596	-0.557
186		N	-0.140	0.183	-0.538	201		OD2	-0.179	0.592	-0.176	216	LYS	NZ	-0.213	0.687	-0.475
186	HIS	CD2	-0.226	0.213	-0.525	201		CG	-0.202	0.588	-0.175	216		CE	-0.237	0.675	-0.472
186		NE2	-0.249	0.204	-0.521	201		CB	-0.209	0.564	-0.176	216		CD	-0.247	0.663	-0.499
186		CE1	-0.246	0.183	-0.513	201		CA	-0.239	0.560	-0.182	216		CG	-0.244	0.637	-0.495
186		ND1	-0.221	0.178	-0.510	201		CO	-0.243	0.544	-0.207	216		CB	-0.243	0.626	-0.523
186		CG	-0.207	0.197	-0.518	201		O	-0.235	0.525	-0.205	216		CA	-0.260	0.605	-0.529
186		CB	-0.178	0.197	-0.517	202		N	-0.257	0.553	-0.230	216		CO	-0.288	0.611	-0.525
186		CA	-0.168	0.187	-0.544	202	SER	OG	-0.295	0.518	-0.286	216		O	-0.297	0.609	-0.501
186		CO	-0.175	0.203	-0.569	202		CB	-0.291	0.536	-0.267	217		N	-0.302	0.619	-0.548
186		O	-0.185	0.195	-0.593	202		CA	-0.262	0.540	-0.257	217	THR	OG1	-0.333	0.655	-0.583
187		N	-0.170	0.224	-0.564	202		CO	-0.249	0.553	-0.279	217		CG2	-0.371	0.631	-0.585
187	GLY	CA	-0.175	0.242	-0.585	202		O	-0.256	0.572	-0.287	217		CB	-0.342	0.633	-0.580
187		CO	-0.204	0.246	-0.598	203		N	-0.229	0.542	-0.287	217		CA	-0.330	0.626	-0.550
187		O	-0.210	0.251	-0.623	203	LEU	CD1	-0.183	0.557	-0.241	217		CO	-0.345	0.607	-0.537
188		N	-0.221	0.243	-0.579	203		CD2	-0.200	0.589	-0.271	217		O	-0.356	0.611	-0.515
188	LEU	CD1	-0.291	0.247	-0.631	203		CG	-0.180	0.570	-0.268	218		N	-0.345	0.588	-0.550
188		CD2	-0.306	0.210	-0.620	203		CB	-0.185	0.555	-0.294	218	GLU	OE1	-0.365	0.524	-0.605
188		CG	-0.282	0.224	-0.623	203		CA	-0.213	0.551	-0.308	218		OE2	-0.373	0.492	-0.585
188		CB	-0.264	0.224	-0.595	203		CO	-0.211	0.536	-0.334	218		CD	-0.368	0.512	-0.585
188		CA	-0.249	0.246	-0.586	203		O	-0.210	0.516	-0.332	218		CG	-0.364	0.526	-0.556
188		CO	-0.259	0.260	-0.562	204		N	-0.212	0.547	-0.359	218		CB	-0.349	0.548	-0.558
188		O	-0.282	0.257	-0.557	204	TYR	OH	-0.255	0.636	-0.412	218		CA	-0.359	0.568	-0.541
189		N	-0.242	0.274	-0.550	204		CD2	-0.223	0.584	-0.429	218		CO	-0.352	0.560	-0.510
189	PHE	CD2	-0.233	0.263	-0.467	204		CE2	-0.229	0.608	-0.429	218		O	-0.369	0.559	-0.494
189		CE2	-0.226	0.244	-0.450	204		CZ	-0.249	0.614	-0.412	219		N	-0.327	0.556	-0.503
189		CZ	-0.204	0.232	-0.454	204		CE1	-0.260	0.600	-0.396	219	LEU	CD1	-0.249	0.522	-0.485
189		CE1	-0.188	0.239	-0.473	204		CD1	-0.254	0.576	-0.396	219		CD2	-0.294	0.506	-0.486
189		CD1	-0.195	0.259	-0.489	204		CG	-0.234	0.569	-0.413	219		CG	-0.274	0.523	-0.471
189		CG	-0.217	0.270	-0.487	204		CB	-0.228	0.544	-0.413	219		CB	-0.286	0.546	-0.472
189		CB	-0.223	0.291	-0.504	204		CA	-0.210	0.536	-0.386	219		CA	-0.316	0.548	-0.474
189		CA	-0.247	0.289	-0.527	204		CO	-0.180	0.535	-0.383	219		CO	-0.323	0.565	-0.452
189		CO	-0.254	0.312	-0.540	204		O	-0.167	0.535	-0.359	219		O	-0.331	0.558	-0.429
189		O	-0.242	0.320	-0.559	205		N	-0.171	0.534	-0.408	220		N	-0.320	0.586	-0.459
190		N	-0.274	0.322	-0.530	205	PRO	CG	-0.167	0.505	-0.441	220	ASN	ND1	-0.341	0.663	-0.442
190	LYS	NZ	-0.305	0.295	-0.633	205		CD	-0.186	0.519	-0.427	220		ND2	-0.306	0.648	-0.415
190		CE	-0.313	0.293	-0.604	205		CB	-0.140	0.513	-0.429	220		CG	-0.323	0.646	-0.436
190		CD	-0.325	0.315	-0.596	205		CA	-0.142	0.533	-0.410	220		CB	-0.324	0.627	-0.456
190		CG	-0.321	0.320	-0.564	205		CO	-0.125	0.552	-0.418	220		CA	-0.327	0.605	-0.441
190		CB	-0.310	0.343	-0.558	205		O	-0.135	0.571	-0.425	220		CO	-0.355	0.600	-0.435
190		CA	-0.283	0.344	-0.539	206		N	-0.100	0.547	-0.416	220		O	-0.360	0.600	-0.410
190		CO	-0.286	0.360	-0.515	206	TYR	OH	-0.074	0.517	-0.547	221		N	-0.371	0.596	-0.459
190		O	-0.296	0.379	-0.519	206		CD2	-0.079	0.536	-0.474	221	GLU	OE1	-0.465	0.618	-0.464
191		N	-0.278	0.352	-0.489	206		CE2	-0.075	0.521	-0.497	221		OE2	-0.466	0.582	-0.464
191	ALA	CB	-0.304	0.360	-0.451	206		CZ	-0.077	0.531	-0.524	221		CD	-0.459	0.600	-0.471
191		CA	-0.278	0.364	-0.462	206		CE1	-0.082	0.552	-0.529	221		CG	-0.440	0.602	-0.495
191		CO	-0.257	0.354	-0.440	206		CD1	-0.086	0.567	-0.506	221		CB	-0.414	0.589	-0.489
191		O	-0.257	0.334	-0.433	206		CG	-0.084	0.558	-0.478	221		CA	-0.399	0.590	-0.458
192		N	-0.240	0.370	-0.429	206		CB	-0.088	0.574	-0.453	221		CO	-0.405	0.570	-0.441
192	PHE	CD2	-0.162	0.336	-0.403	206		CA	-0.080	0.563	-0.424	221		O	-0.421	0.571	-0.423
192		CE2	-0.140	0.327	-0.384	206		CO	-0.072	0.575	-0.396	222		N	-0.392	0.552	-0.447
192		CZ	-0.126	0.340	-0.365	206		O	-0.078	0.595	-0.392	222	VAL	CG1	-0.394	0.502	-0.472
192		CE1	-0.132	0.361	-0.361	207		N	-0.057	0.563	-0.376	222		CG2	-0.378	0.490	-0.422
192		CD1	-0.155	0.370	-0.379	207	GLY	CA	-0.047	0.572	-0.348	222		CB	-0.381	0.510	-0.443
192		CG	-0.169	0.358	-0.400	207		CO	-0.017	0.572	-0.349	222		CA	-0.395	0.530	-0.433
192		CB	-0.192	0.369	-0.417	207		O	-0.001	0.577	-0.327	222		CO	-0.389	0.533	-0.400
192		CA	-0.218	0.364	-0.406	208		N	-0.010	0.568	-0.374	222		O	-0.404	0.525	-0.384
192		CO	-0.222	0.380	-0.382	208	TYR	OH	-0.002	0.521	-0.505	223		N	-0.367	0.544	-0.392
192		O	-0.218	0.401	-0.383	208		CD2	0.033	0.547	-0.441	223	ALA	CB	-0.333	0.563	-0.359
193		N	-0.229	0.370	-0.359	208		CE2	0.026	0.542	-0.470	223		CA	-0.358	0.549	-0.362
193	SER	OG	-0.280	0.380	-0.351	208		CZ	0.005	0.527	-0.477	223		CO	-0.378	0.562	-0.347
193		CB	-0.261	0.378	-0.327	208		CE1	-0.009	0.519	-0.457	223		O	-0.384	0.556	-0.324
193		CA	-0.233	0.382	-0.333	208		CD1	-0.002	0.524	-0.427	224		N	-0.387	0.580	-0.362
193		CO	-0.214	0.374	-0.307	208		CG	0.019	0.539	-0.420	224	LYS	NZ	-0.467	0.672	-0.381
193		O	-0.212	0.354	-0.300	208		CB	0.027	0.545	-0.388	224		CE	-0.457	0.651	-0.391
194		N	-0.199	0.391	-0.295	208		CA	0.017	0.569	-0.380	224		CD	-0.428	0.651	-0.391
194	SER	OG	-0.132	0.386	-0.260	208		CO	0.022	0.586	-0.403	224		CG	-0.414	0.636	-0.367
194		CB	-0.154	0.396	-0.277	208		O	0.044	0.588	-0.410	224		CB	-0.414	0.612	-0.377
194		CA	-0.180	0.387	-0.270	209		N	0.001	0.597	-0.414	224		CA	-0.406	0.595	-0.353
194		CO	-0.188	0.403	-0.247	209	THR	OG1	0.002	0.628	-0.483	224		CO	-0.430	0.582	-0.347
194		O	-0.190	0.424	-0.251	209		CG2	-0.015	0.591	-0.480	224		O	-0.443	0.587	-0.327
195		N	-0.194	0.392	-0.224	209		CB	0.005	0.607	-0.467	225		N	-0.436	0.565	-0.365
195	LEU	CD1	-0.255	0.369	-0.225	209		CA	0.001	0.614	-0.436	225	SER	OG	-0.476	0.551	-0.414
195		CD2	-0.255	0.370	-0.172	209		CO	-0.023	0.629	-0.437	225		CB	-0.465	0.537	-0.392
195		CG	-0.239	0.374	-0.196	209		O	-0.045	0.621	-0.433	225		CA	-0.458	0.550	-0.364
195		CB	-0.231	0.398	-0.196	210		N	-0.018	0.650	-0.443	225		CO	-0.452	0.533	-0.340
195		CA	-0.202	0.404	-0.199	210	THR	OG1	-0.011	0.690	-0.468	225		O	-0.467	0.529	-0.322
195		CO	-0.185	0.399	-0.171	210		CG2	-0.008	0.695	-0.417	226		N	-0.429	0.523	-0.340
195		O	-0.167	0.385	-0.168	210		CB	-0.026	0.691	-0.445	226	ALA	CB	-0.389	0.503	-0.321
196		N	-0.191	0.412	-0.149	210		CA	-0.039	0.667	-0.445	226		CA	-0.418	0.506	-0.319
196	LYS	NZ	-0.093	0.443	-0.159	210		CO	-0.056	0.664	-0.474	226		CO	-0.420	0.517	-0.290
196		CE	-0.103	0.423	-0.174	210		O	-0.076	0.675	-0.481	226		O	-0.430	0.507	-0.270
196		CD	-0.130	0.417	-0.167	211		N	-0.047	0.648	-0.490	227		N	-0.410	0.538	-0.288
196		CG	-0.132	0.420	-0.135	211	GLU	OE1	-0.031	0.593	-0.587	227	VAL	CG1	-0.366	0.571	-0.262
196		CB	-0.149	0.402	-0.124	211		OE2	-0.073	0.588	-0.581	227		CG2	-0.405	0.594	-0.252
196		CA	-0.177	0.411	-0.120	211		CD	-0.053	0.598	-0.579	227		CB	-0.395	0.573	-0.267
196		CO	-0.170	0.433	-0.105	211		CG	-0.049	0.622	-0.565	227		CA	-0.409	0.551	-0.262

TABLE 3 (*cont.*)

227		CO	-0.435	0.556	-0.251	243		CO	-0.100	0.615	-0.297	260		N	-0.102	0.434	-0.522
227		C	-0.437	0.555	-0.225	243		C	-0.101	0.626	-0.274	260	ASN	ND1	-0.073	0.477	-0.555
228		N	-0.454	0.562	-0.271	244		N	-0.087	0.620	-0.318	260		ND2	-0.038	0.477	-0.520
228	ALA	CB	-0.499	0.573	-0.293	244	ILE	CD1	-0.009	0.654	-0.350	260		CG	-0.055	0.466	-0.534
228		CA	-0.481	0.567	-0.266	244		CG1	-0.036	0.661	-0.342	260		CB	-0.057	0.441	-0.533
228		CO	-0.493	0.547	-0.252	244		CB	-0.057	0.643	-0.345	260		CA	-0.085	0.433	-0.545
228		O	-0.502	0.549	-0.229	244		CG2	-0.081	0.647	-0.367	260		CO	-0.087	0.410	-0.557
229		N	-0.492	0.528	-0.266	244		CA	-0.070	0.640	-0.317	260		O	-0.078	0.405	-0.580
229	ALA	CB	-0.495	0.487	-0.274	244		CO	-0.087	0.661	-0.315	261		N	-0.099	0.395	-0.543
229		CA	-0.502	0.507	-0.257	244		O	-0.077	0.680	-0.315	261	GLU	OE1	-0.053	0.324	-0.549
229		CO	-0.493	0.504	-0.225	245		N	-0.112	0.657	-0.312	261		OE2	-0.048	0.359	-0.535
229		O	-0.508	0.503	-0.207	245	SER	OG	-0.170	0.682	-0.343	261		CD	-0.059	0.341	-0.537
230		N	-0.467	0.503	-0.219	245		CB	-0.157	0.667	-0.322	261		CG	-0.084	0.337	-0.522
230	LEU	CD1	-0.397	0.512	-0.148	245		CA	-0.131	0.675	-0.309	261		CB	-0.106	0.354	-0.528
230		CD2	-0.381	0.481	-0.176	245		CO	-0.135	0.683	-0.279	261		CA	-0.103	0.372	-0.551
230		CG	-0.405	0.492	-0.167	245		O	-0.130	0.702	-0.270	261		CO	-0.126	0.371	-0.575
230		CB	-0.424	0.499	-0.193	246		N	-0.144	0.667	-0.263	261		C	-0.130	0.355	-0.592
230		CA	-0.453	0.500	-0.190	246	SER	OG	-0.190	0.675	-0.211	262		N	-0.141	0.390	-0.577
230		CO	-0.455	0.518	-0.166	246		CB	-0.175	0.660	-0.227	262	GLY	CA	-0.164	0.393	-0.598
230		O	-0.446	0.514	-0.141	246		CA	-0.150	0.670	-0.234	262		CO	-0.189	0.390	-0.584
231		N	-0.468	0.536	-0.176	246		CO	-0.125	0.664	-0.213	262		O	-0.209	0.382	-0.598
231	LYS	NZ	-0.456	0.657	-0.155	246		O	-0.120	0.674	-0.190	263		N	-0.187	0.397	-0.558
231		CE	-0.475	0.639	-0.148	247		N	-0.111	0.648	-0.223	263	ILE	CD1	-0.171	0.355	-0.539
231		CD	-0.474	0.619	-0.167	247	ILE	CD1	-0.044	0.626	-0.167	263		CG1	-0.197	0.356	-0.524
231		CG	-0.471	0.597	-0.150	247		CG1	-0.063	0.607	-0.178	263		CB	-0.203	0.380	-0.515
231		CB	-0.468	0.578	-0.170	247		CB	-0.083	0.614	-0.204	263		CG2	-0.228	0.382	-0.501
231		CA	-0.473	0.555	-0.157	247		CG2	-0.075	0.608	-0.233	263		CA	-0.209	0.396	-0.540
231		CO	-0.501	0.555	-0.150	247		CA	-0.087	0.639	-0.207	263		CO	-0.216	0.420	-0.530
231		C	-0.508	0.570	-0.134	247		CO	-0.062	0.650	-0.216	263		O	-0.201	0.430	-0.512
232		N	-0.516	0.539	-0.162	247		C	-0.061	0.671	-0.219	264		N	-0.239	0.427	-0.542
232	SER	OG	-0.552	0.505	-0.191	248		N	-0.044	0.636	-0.220	264	LYS	NZ	-0.328	0.477	-0.633
232		CB	-0.559	0.527	-0.185	248	TYR	CH	0.092	0.696	-0.215	264		CE	-0.303	0.470	-0.616
232		CA	-0.543	0.536	-0.158	248		CD2	0.026	0.681	-0.202	264		CD	-0.299	0.483	-0.588
232		CO	-0.551	0.519	-0.136	248		CE2	0.048	0.694	-0.205	264		CG	-0.273	0.477	-0.570
232		O	-0.574	0.516	-0.132	248		CZ	0.070	0.683	-0.212	264		CB	-0.273	0.454	-0.558
233		N	-0.531	0.508	-0.122	248		CE1	0.071	0.661	-0.217	264		CA	-0.250	0.448	-0.536
233	LEU	CD1	-0.552	0.442	-0.095	248		CD1	0.048	0.647	-0.213	264		CO	-0.260	0.448	-0.507
233		CD2	-0.512	0.430	-0.116	248		CG	0.026	0.658	-0.206	264		O	-0.258	0.465	-0.491
233		CG	-0.532	0.449	-0.114	248		CB	0.002	0.644	-0.202	265		N	-0.272	0.429	-0.501
233		CB	-0.517	0.470	-0.103	248		CA	-0.019	0.642	-0.229	265	ARG	NH1	-0.370	0.422	-0.572
233		CA	-0.534	0.491	-0.100	248		CO	-0.010	0.626	-0.251	265		NH2	-0.367	0.460	-0.564
233		CO	-0.529	0.503	-0.071	248		O	-0.022	0.607	-0.255	265		CZ	-0.359	0.440	-0.558
233		O	-0.546	0.504	-0.055	249		N	0.009	0.633	-0.264	265		NE	-0.340	0.437	-0.536
234		N	-0.505	0.511	-0.065	249	GLN	NOE1	0.085	0.648	-0.334	265		CD	-0.331	0.415	-0.525
234	TYR	OH	-0.502	0.416	-0.027	249		NOE2	0.083	0.611	-0.339	265		CG	-0.330	0.414	-0.493
234		CD2	-0.503	0.475	-0.008	249		CD	0.073	0.630	-0.334	265		CB	-0.302	0.406	-0.479
234		CE2	-0.510	0.452	-0.009	249		CG	0.046	0.630	-0.325	265		CA	-0.283	0.426	-0.474
234		CZ	-0.495	0.438	-0.025	249		CB	0.045	0.630	-0.294	265		CO	-0.261	0.421	-0.449
234		CE1	-0.475	0.446	-0.038	249		CA	0.020	0.619	-0.286	265		O	-0.260	0.402	-0.437
234		CD1	-0.468	0.469	-0.037	249		CO	0.025	0.596	-0.271	266		N	-0.245	0.438	-0.442
234		CG	-0.483	0.484	-0.021	249		O	0.038	0.595	-0.246	266	SER	CG	-0.193	0.419	-0.447
234		CB	-0.475	0.509	-0.020	250		N	0.014	0.579	-0.286	266		CB	-0.198	0.439	-0.431
234		CA	-0.496	0.523	-0.038	250	ALA	CB	-0.011	0.549	-0.268	266		CA	-0.224	0.437	-0.418
234		CO	-0.487	0.547	-0.042	250		CA	0.016	0.556	-0.276	266		CO	-0.224	0.457	-0.399
234		O	-0.482	0.560	-0.021	250		CO	0.022	0.543	-0.301	266		O	-0.221	0.477	-0.408
235		N	-0.485	0.553	-0.068	250		C	0.005	0.542	-0.324	267		N	-0.227	0.453	-0.372
235	GLY	CA	-0.476	0.575	-0.077	251		N	0.045	0.533	-0.298	267	PHE	CD2	-0.280	0.459	-0.390
235		CO	-0.447	0.579	-0.067	251	SER	OG	0.101	0.521	-0.301	267		CE2	-0.297	0.461	-0.416
235		O	-0.440	0.595	-0.050	251		CB	0.081	0.526	-0.325	267		CZ	-0.306	0.481	-0.427
236		N	-0.431	0.564	-0.076	251		CA	0.054	0.519	-0.320	267		CE1	-0.299	0.500	-0.413
236	THR	OG1	-0.397	0.530	-0.047	251		CO	0.056	0.494	-0.312	267		CD1	-0.282	0.499	-0.387
236		CG2	-0.361	0.543	-0.070	251		O	0.059	0.488	-0.286	267		CG	-0.273	0.479	-0.375
236		CB	-0.390	0.542	-0.071	252		N	0.052	0.480	-0.334	267		CB	-0.255	0.479	-0.347
236		CA	-0.403	0.565	-0.069	252	GLY	CA	0.053	0.456	-0.331	267		CA	-0.227	0.470	-0.350
236		CO	-0.394	0.580	-0.092	252		CO	0.030	0.447	-0.317	267		CO	-0.217	0.458	-0.322
236		O	-0.397	0.575	-0.118	252		C	0.032	0.431	-0.301	267		O	-0.219	0.438	-0.319
237		N	-0.383	0.599	-0.082	253		N	0.008	0.459	-0.325	268		N	-0.207	0.472	-0.301
237	SER	OG	-0.352	0.653	-0.104	253	GLY	CA	-0.017	0.453	-0.314	268	VAL	CG1	-0.153	0.471	-0.291
237		CB	-0.363	0.637	-0.085	253		CO	-0.033	0.438	-0.335	268		CG2	-0.153	0.451	-0.243
237		CA	-0.372	0.616	-0.101	253		C	-0.028	0.437	-0.361	268		CB	-0.167	0.468	-0.265
237		CO	-0.349	0.607	-0.114	254		N	-0.051	0.426	-0.325	268		CA	-0.196	0.465	-0.273
237		O	-0.327	0.603	-0.099	254	ILE	CD1	-0.082	0.437	-0.296	268		CO	-0.212	0.475	-0.251
238		N	-0.354	0.604	-0.142	254		CG1	-0.090	0.412	-0.295	268		C	-0.218	0.495	-0.251
238	TYR	CH	-0.301	0.495	-0.123	254		CB	-0.087	0.399	-0.323	269		N	-0.220	0.461	-0.232
238		CD2	-0.321	0.539	-0.177	254		CG2	-0.076	0.375	-0.320	269	PHE	CD2	-0.277	0.445	-0.257
238		CE2	-0.309	0.519	-0.164	254		CA	-0.068	0.410	-0.342	269		CE2	-0.300	0.446	-0.277
238		CZ	-0.312	0.514	-0.136	254		CO	-0.085	0.422	-0.366	269		CZ	-0.324	0.450	-0.269
238		CE1	-0.326	0.528	-0.120	254		C	-0.081	0.419	-0.391	269		CE1	-0.326	0.454	-0.241
238		CD1	-0.338	0.548	-0.133	255		N	-0.104	0.435	-0.357	269		CD1	-0.302	0.454	-0.221
238		CG	-0.335	0.553	-0.162	255	SER	CG	-0.154	0.472	-0.359	269		CG	-0.279	0.448	-0.228
238		CB	-0.346	0.575	-0.175	255		CB	-0.127	0.470	-0.363	269		CB	-0.255	0.449	-0.206
238		CA	-0.334	0.595	-0.160	255		CA	-0.122	0.448	-0.377	269		CA	-0.235	0.467	-0.210
238		CO	-0.328	0.612	-0.182	255		CO	-0.109	0.452	-0.404	269		CO	-0.217	0.471	-0.182
238		O	-0.345	0.624	-0.196	255		C	-0.120	0.446	-0.428	269		C	-0.199	0.458	-0.174
239		N	-0.302	0.612	-0.186	256		N	-0.086	0.462	-0.399	270		N	-0.221	0.490	-0.169
239	LYS	NZ	-0.217	0.692	-0.169	256	ASN	ND1	-0.079	0.505	-0.393	270	GLU	OE1	-0.147	0.501	-0.157
239		CE	-0.243	0.693	-0.159	256		ND2	-0.037	0.516	-0.384	270		OE2	-0.142	0.507	-0.111
239		CD	-0.254	0.670	-0.158	256		CG	-0.052	0.501	-0.393	270		CD	-0.151	0.511	-0.135
239		CG	-0.271	0.663	-0.186	256		CB	-0.045	0.480	-0.408	270		CG	-0.172	0.530	-0.144
239		CB	-0.267	0.639	-0.193	256		CA	-0.070	0.467	-0.421	270		CB	-0.201	0.523	-0.146
239		CA	-0.292	0.627	-0.206	256		CO	-0.064	0.448	-0.441	270		CA	-0.206	0.497	-0.142
239		CO	-0.285	0.612	-0.231	256		C	-0.073	0.449	-0.467	270		CO	-0.229	0.496	-0.125
239		O	-0.281	0.591	-0.227	257		N	-0.051	0.431	-0.428	270		C	-0.247	0.510	-0.129
240		N	-0.284	0.623	-0.255	257	HIS	CD2	-0.041	0.351	-0.444	271		N	-0.228	0.479	-0.107
240	ARG	NH1	-0.350	0.638	-0.356	257		NE2	-0.029	0.338	-0.462	271	LEU	CD1	-0.248	0.413	-0.096
240		NH2	-0.382	0.663	-0.349	257		CE1	-0.010	0.349	-0.472	271		CD2	-0.272	0.443	-0.124
240		CZ	-0.364	0.648	-0.339	257		ND1	-0.008	0.369	-0.460	271		CG	-0.264	0.434	-0.094
240		NE	-0.362	0.644	-0.311	257		CG	-0.027	0.371	-0.442	271		CB	-0.247	0.451	-0.077
240		CD	-0.345	0.625	-0.298	257		CB	-0.031	0.392	-0.426	271		CA	-0.248	0.476	-0.088
240		CG	-0.321	0.622	-0.314	257		CA	-0.044	0.411	-0.443	271		CO	-0.254	0.492	-0.065
240		CB	-0.304	0.603	-0.299	257		CO	-0.069	0.402	-0.461	271		C	-0.245	0.512	-0.064
240		CA	-0.278	0.612	-0.281	257		C	-0.071	0.401	-0.488	272		N	-0.268	0.484	-0.046
240		CO	-0.265	0.627	-0.301	258		N	-0.088	0.396	-0.446	272	ARG	NH1	-0.382	0.480	0.040
240		O	-0.268	0.648	-0.300	258	SER	OG	-0.139	0.411	-0.431	272		NH2	-0.412	0.490	0.001
241		N	-0.250	0.617	-0.318	258		CB	-0.133	0.388	-0.437	272		CZ	-0.387	0.487	0.013
241	GLY	CA	-0.235	0.628	-0.338	258		CA	-0.113	0.387	-0.459	272		NE	-0.368	0.490	-0.003
241		CO	-0.212	0.614	-0.342	258		CO	-0.125	0.400	-0.485	272		CD	-0.340	0.489	0.008
241		O	-0.210	0.594	-0.334	258		O	-0.130	0.391	-0.509	272		CG	-0.323	0.486	-0.016
242		N	-0.194	0.624	-0.355	259		N	-0.131	0.421	-0.479	272		CB	-0.294	0.485	-0.003
242	SER	CG	-0.137	0.641	-0.373	259	TYR	CH	-0.194	0.520	-0.575	272		CA	-0.275	0.497	-0.022
242		CB	-0.158	0.626	-0.384	259		CD2	-0.143	0.492	-0.526	272		CO	-0.253	0.507	-0.001
242		CA	-0.169	0.614	-0.361	259		CE2	-0.155	0.507	-0.547	272		C	-0.230	0.500	-0.000
242		CO	-0.150	0.615	-0.333	259		CZ	-0.183	0.505	-0.555	273		N	-0.260	0.524	0.014
242		C	-0.147	0.632	-0.318	259		CE1	-0.197	0.491	-0.542	273	ASP	OD1	-0.239	0.524	0.110
243		N	-0.136	0.596	-0.327	259		CD1	-0.185	0.476	-0.520	273		OD2	-0.233	0.554	0.090
243	ILE	CD1	-0.096	0.532	-0.322	259		CG	-0.157	0.477	-0.512	273		CG	-0.234	0.534	0.089
243		CG1	-0.114	0.552	-0.318	259		CB	-0.144	0.461	-0.490	273		CB	-0.228	0.524	0.062
243		CB	-0.098	0.573	-0.306	259		CA	-0.142	0.437	-0.502	273		CA	-0.242	0.536	0.035
243		CG2	-0.083	0.571	-0.276	259		CO	-0.128	0.437	-0.528	273		CO	-0.227	0.553	0.019
243		CA	-0.117	0.593	-0.302	259		C	-0.140	0.440	-0.552	273		C	-0.216	0.547	-0.002

TABLE 3 (*cont.*)

274		N	-0.228	0.573	0.028	285		CA	-0.350	0.413	0.087	296	GLY	CA	-0.411	0.448	-0.252
274	THR	OG1	-0.249	0.610	0.035	285		CO	-0.346	0.399	0.061	296		CO	-0.403	0.435	-0.277
274		CG2	-0.253	0.610	-0.016	285		C	-0.348	0.407	0.037	296		O	-0.409	0.442	-0.302
274		CB	-0.235	0.612	0.012	286		N	-0.342	0.378	0.067	297		N	-0.391	0.416	-0.270
274		CA	-0.215	0.592	0.016	286	ILE	CD1	-0.284	0.347	0.067	297	VAL	CG1	-0.341	0.388	-0.261
274		CO	-0.189	0.596	0.035	286		CG1	-0.308	0.335	0.079	297		CG2	-0.363	0.361	-0.299
274		C	-0.173	0.610	0.028	286		CB	-0.334	0.337	0.059	297		CB	-0.367	0.380	-0.278
275		N	-0.185	0.583	0.057	286		CG2	-0.336	0.323	0.032	297		CA	-0.383	0.400	-0.291
275	GLY	CA	-0.162	0.584	0.078	286		CA	-0.339	0.361	0.046	297		CO	-0.405	0.394	-0.314
275		CO	-0.168	0.582	0.109	286		CO	-0.362	0.362	0.021	297		O	-0.402	0.394	-0.340
275		O	-0.150	0.583	0.130	286		C	-0.359	0.368	-0.003	298		N	-0.428	0.390	-0.305
276		N	-0.193	0.579	0.111	287		N	-0.385	0.356	0.029	298	ASP	OD1	-0.495	0.360	-0.351
276	SER	OG	-0.241	0.578	0.164	287	LEU	CD1	-0.470	0.323	0.021	298		OD2	-0.520	0.370	-0.321
276		CB	-0.232	0.576	0.137	287		CD2	-0.428	0.310	0.046	298		CG	-0.498	0.370	-0.329
276		CA	-0.203	0.577	0.139	287		CG	-0.441	0.325	0.022	298		CB	-0.477	0.381	-0.309
276		CO	-0.191	0.556	0.154	287		CB	-0.432	0.349	0.026	298		CA	-0.452	0.384	-0.323
276		O	-0.181	0.556	0.179	287		CA	-0.409	0.356	0.010	298		CO	-0.457	0.400	-0.349
277		N	-0.193	0.538	0.137	287		CO	-0.420	0.377	-0.007	298		C	-0.460	0.393	-0.374
277	TYR	OH	-0.260	0.519	0.244	287		O	-0.424	0.377	-0.033	299		N	-0.457	0.422	-0.342
277		CD2	-0.247	0.510	0.170	288		N	-0.424	0.394	0.010	299	THR	OG1	-0.468	0.461	-0.323
277		CE2	-0.261	0.515	0.194	288	PRO	CG	-0.429	0.415	0.049	299		CG2	-0.462	0.482	-0.365
277		CZ	-0.247	0.514	0.221	288		CD	-0.413	0.396	0.040	299		CB	-0.454	0.462	-0.347
277		CE1	-0.221	0.510	0.226	288		CB	-0.432	0.431	0.024	299		CA	-0.461	0.440	-0.363
277		CD1	-0.207	0.505	0.202	288		CA	-0.433	0.416	-0.002	299		CO	-0.444	0.435	-0.387
277		CG	-0.221	0.506	0.174	288		CO	-0.415	0.420	-0.024	299		O	-0.453	0.435	-0.412
277		CB	-0.206	0.500	0.149	288		C	-0.424	0.423	-0.050	300		N	-0.419	0.431	-0.377
277		CA	-0.183	0.516	0.147	289		N	-0.390	0.419	-0.014	300	ILE	CD1	-0.348	0.453	-0.348
277		CO	-0.163	0.507	0.128	289	VAL	CG1	-0.337	0.441	0.005	300		CG1	-0.367	0.433	-0.350
277		O	-0.148	0.491	0.136	289		CG2	-0.319	0.419	-0.034	300		CB	-0.371	0.422	-0.380
278		N	-0.165	0.517	0.103	289		CB	-0.341	0.422	-0.016	300		CG2	-0.354	0.430	-0.402
278	GLY	CA	-0.148	0.512	0.081	289		CA	-0.369	0.422	-0.032	300		CA	-0.399	0.426	-0.396
278		CO	-0.149	0.486	0.076	289		CO	-0.372	0.407	-0.059	300		CO	-0.407	0.406	-0.417
278		O	-0.170	0.477	0.068	289		C	-0.373	0.415	-0.083	300		O	-0.409	0.409	-0.443
279		N	-0.126	0.476	0.081	290		N	-0.375	0.386	-0.054	301		N	-0.411	0.387	-0.404
279	PHE	CD2	-0.061	0.476	0.070	290	SER	CG	-0.374	0.328	-0.073	301	MET	SD	-0.408	0.319	-0.358
279		CE2	-0.049	0.490	0.051	290		CB	-0.385	0.347	-0.060	301		CG	-0.407	0.346	-0.373
279		CZ	-0.055	0.488	0.023	290		CA	-0.379	0.368	-0.076	301		CB	-0.428	0.349	-0.398
279		CE1	-0.073	0.474	0.012	290		CO	-0.403	0.374	-0.098	301		CA	-0.419	0.367	-0.419
279		CD1	-0.086	0.460	0.030	290		C	-0.402	0.372	-0.124	301		CO	-0.441	0.372	-0.443
279		CG	-0.079	0.461	0.059	291		N	-0.424	0.380	-0.086	301		O	-0.440	0.366	-0.468
279		CB	-0.094	0.446	0.078	291	HIS	CD2	-0.495	0.376	-0.045	302		N	-0.461	0.383	-0.435
279		CA	-0.122	0.452	0.078	291		NE2	-0.498	0.355	-0.036	302	GLU	OE1	-0.537	0.422	-0.400
279		CO	-0.133	0.439	0.101	291		CE1	-0.486	0.341	-0.052	302		OE2	-0.544	0.386	-0.396
279		O	-0.141	0.419	0.097	291		ND1	-0.473	0.353	-0.069	302		CD	-0.538	0.402	-0.408
280		N	-0.134	0.450	0.125	291		CG	-0.479	0.375	-0.065	302		CG	-0.530	0.401	-0.439
280	ASP	OD1	-0.095	0.439	0.215	291		CB	-0.468	0.394	-0.082	302		CB	-0.501	0.407	-0.441
280		OD2	-0.107	0.418	0.180	291		CA	-0.450	0.385	-0.103	302		CA	-0.484	0.389	-0.455
280		CG	-0.109	0.435	0.192	291		CO	-0.447	0.404	-0.124	302		CO	-0.478	0.399	-0.483
280		CB	-0.130	0.451	0.178	291		O	-0.449	0.401	-0.151	302		O	-0.487	0.391	-0.506
280		CA	-0.144	0.441	0.150	292		N	-0.442	0.424	-0.112	303		N	-0.462	0.416	-0.480
280		CO	-0.174	0.444	0.147	292	GLU	OE1	-0.387	0.481	-0.067	303	HIS	CD2	-0.420	0.469	-0.538
280		C	-0.184	0.458	0.162	292		OE2	-0.414	0.484	-0.034	303		NE2	-0.427	0.490	-0.549
281		N	-0.187	0.432	0.127	292		CD	-0.409	0.479	-0.057	303		CE1	-0.444	0.498	-0.534
281	HIS	CD2	-0.264	0.459	0.062	292		CG	-0.430	0.470	-0.082	303		ND1	-0.448	0.485	-0.513
281		NE2	-0.270	0.481	0.063	292		CB	-0.418	0.457	-0.106	303		CG	-0.433	0.466	-0.515
281		CE1	-0.253	0.491	0.082	292		CA	-0.438	0.445	-0.128	303		CB	-0.433	0.446	-0.495
281		ND1	-0.235	0.476	0.093	292		CC	-0.421	0.441	-0.152	303		CA	-0.453	0.428	-0.505
281		CG	-0.242	0.455	0.081	292		O	-0.432	0.442	-0.178	303		CO	-0.442	0.410	-0.523
281		CB	-0.226	0.435	0.089	[illegible]		N	-0.396	0.437	-0.144	303		C	-0.452	0.407	-0.548
281		CA	-0.216	0.432	0.120	293	LEU	CD1	-0.325	0.424	-0.176	304		N	-0.422	0.399	-0.509
281		CO	-0.228	0.415	0.138	293		CD2	-0.340	0.387	-0.165	304	THR	OG1	-0.373	0.386	-0.487
281		O	-0.224	0.394	0.134	293		CG	-0.334	0.410	-0.153	304		CG2	-0.376	0.350	-0.509
282		N	-0.243	0.423	0.156	293		CB	-0.358	0.419	-0.143	304		CB	-0.391	0.369	-0.498
282	PRO	CG	-0.262	0.449	0.184	293		CA	-0.377	0.433	-0.163	304		CA	-0.409	0.380	-0.522
282		CD	-0.251	0.446	0.156	293		CO	-0.392	0.418	-0.186	304		CO	-0.429	0.367	-0.543
282		CB	-0.272	0.425	0.190	293		C	-0.394	0.423	-0.212	304		O	-0.431	0.370	-0.569
282		CA	-0.257	0.409	0.175	294		N	-0.404	0.401	-0.175	305		N	-0.443	0.352	-0.530
282		CO	-0.274	0.393	0.154	294	TRP	CD1	-0.467	0.342	-0.223	305	VAL	CG1	-0.486	0.304	-0.530
282		O	-0.284	0.399	0.130	294		CE1	-0.490	0.353	-0.222	305		CG2	-0.509	0.342	-0.531
283		N	-0.276	0.373	0.165	294		CZ1	-0.512	0.345	-0.241	305		CB	-0.483	0.329	-0.527
283	ALA	CB	-0.294	0.335	0.170	294		CH	-0.510	0.326	-0.258	305		CA	-0.462	0.338	-0.546
283		CA	-0.291	0.355	0.150	294		CZ2	-0.487	0.316	-0.260	305		CO	-0.477	0.350	-0.572
283		CO	-0.319	0.362	0.139	294		CE2	-0.466	0.324	-0.242	305		O	-0.483	0.340	-0.595
283		O	-0.329	0.359	0.114	294		NE	-0.442	0.317	-0.239	306		N	-0.481	0.371	-0.568
284		N	-0.331	0.372	0.159	294		CD2	-0.427	0.330	-0.217	306	ASN	ND01	-0.534	0.368	-0.568
284	LYS	NZ	-0.332	0.468	0.180	294		CG	-0.442	0.346	-0.208	306		ND02	-0.549	0.400	-0.551
284		CE	-0.356	0.455	0.180	294		CB	-0.434	0.364	-0.184	306		CG	-0.533	0.391	-0.563
284		CD	-0.350	0.430	0.177	294		CA	-0.420	0.385	-0.193	306		CB	-0.511	0.403	-0.576
284		CG	-0.375	0.416	0.175	294		CO	-0.437	0.395	-0.219	306		CA	-0.494	0.386	-0.590
284		CB	-0.369	0.392	0.179	294		C	-0.434	0.389	-0.244	306		CO	-0.475	0.400	-0.605
284		CA	-0.358	0.380	0.154	295		N	-0.455	0.409	-0.212	306		O	-0.479	0.420	-0.612
284		CO	-0.365	0.398	0.130	295	ASP	CD1	-0.534	0.440	-0.211	307		N	-0.453	0.389	-0.610
284		O	-0.385	0.409	0.129	295		OD2	-0.515	0.409	-0.207	307	ASN	ND01	-0.455	0.448	-0.595
285		N	-0.347	0.398	0.112	295		CG	-0.514	0.429	-0.211	307		ND02	-0.424	0.428	-0.569
285	GLN	NOE1	-0.322	0.487	0.122	295		CB	-0.488	0.438	-0.216	307		CG	-0.435	0.433	-0.593
285		NOE2	-0.329	0.476	0.076	295		CA	-0.473	0.421	-0.233	307		CB	-0.427	0.424	-0.620
285		CD	-0.329	0.473	0.102	295		CO	-0.455	0.433	-0.252	307		CA	-0.432	0.399	-0.624
285		CG	-0.335	0.449	0.112	295		O	-0.463	0.439	-0.277	307		CO	-0.407	0.386	-0.616
285		CB	-0.329	0.431	0.093	296		N	-0.431	0.437	-0.238	307		O	-0.405	0.366	-0.623
												308		N	-0.388	0.397	-0.602

taken into account in the structure factor calculation. As expected, the R factor also increases for the smaller structure factors, reflecting greater measurement errors. The average difference between the MIR and structure factor phase angles is 50.2° and the average of the cosine of the difference is 0.5294. Differences are greatest for the smallest reflexions ($\langle|\Delta\phi|\rangle = 73°$ for

$F_o < 100$) and for the highest resolution reflexions ($\langle|\Delta\phi|\rangle = 57°$ for $d < 0.28$ nm). Whether one set of phases is more reliable at high resolution than the other may become evident when a new electron density map is made using the structure factor phase angles.

TABLE 5. SUMMARY OF STRUCTURE FACTOR CALCULATION†

resolution range/nm	R factor	$\langle\Delta\phi\rangle$	$\langle\cos\|\Delta\phi\|\rangle$
∞–1.00	1.052	26.8	0.796
1.00–0.60	0.866	36.6	0.689
0.60–0.50	0.635	38.6	0.670
0.50–0.42	0.368	39.0	0.669
0.42–0.35	0.329	42.9	0.623
0.35–0.28	0.362	50.9	0.522
0.28–0.20	0.366	57.1	0.444
all data	0.437	50.2	0.529
structure factor range	**R factor**	$\langle\Delta\phi\rangle$	$\langle\cos\|\Delta\phi\|\rangle$
∞–400	0.294	29.8	0.783
400–300	0.358	34.8	0.724
300–200	0.399	44.1	0.610
200–150	0.420	56.3	0.453
150–100	0.538	64.5	0.348
100–0	1.261	73.4	0.209

† $R = \frac{\Sigma||F_o|-|F_c||}{\Sigma|F_o|}$, where F_o is a scaled, observed structure factor, F_c is a calculated structure factor, and the summation is over all reflexions.

The phase angle differences $\Delta\phi$ are reduced to the range $0 \leqslant \Delta\phi \leqslant 180°$. $\Delta\phi = |\phi_{MIR} - \phi_{SF}|$, where ϕ_{MIR} is an MIR phase, and ϕ_{SF} is a structure factor phase.

FIGURE 2. Drawing of the polypeptide chain. The Zn atom is the shaded ball near the centre, the disulphide bond is at the right, the C-terminus is at the left at 307, and the N-terminus is at the bottom at 1.

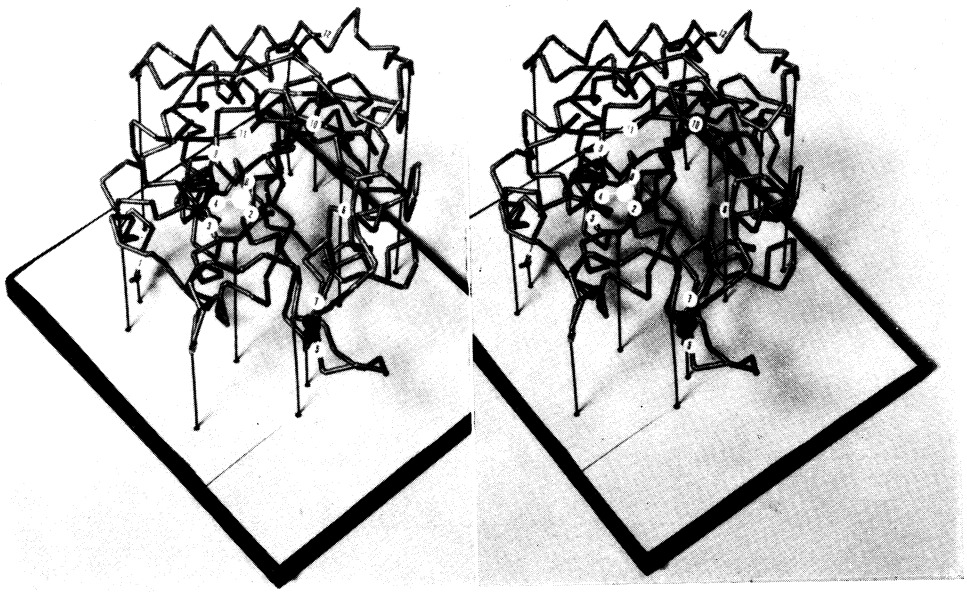


FIGURE 1. For legend see p. 189.

(a)

(b)

(c)

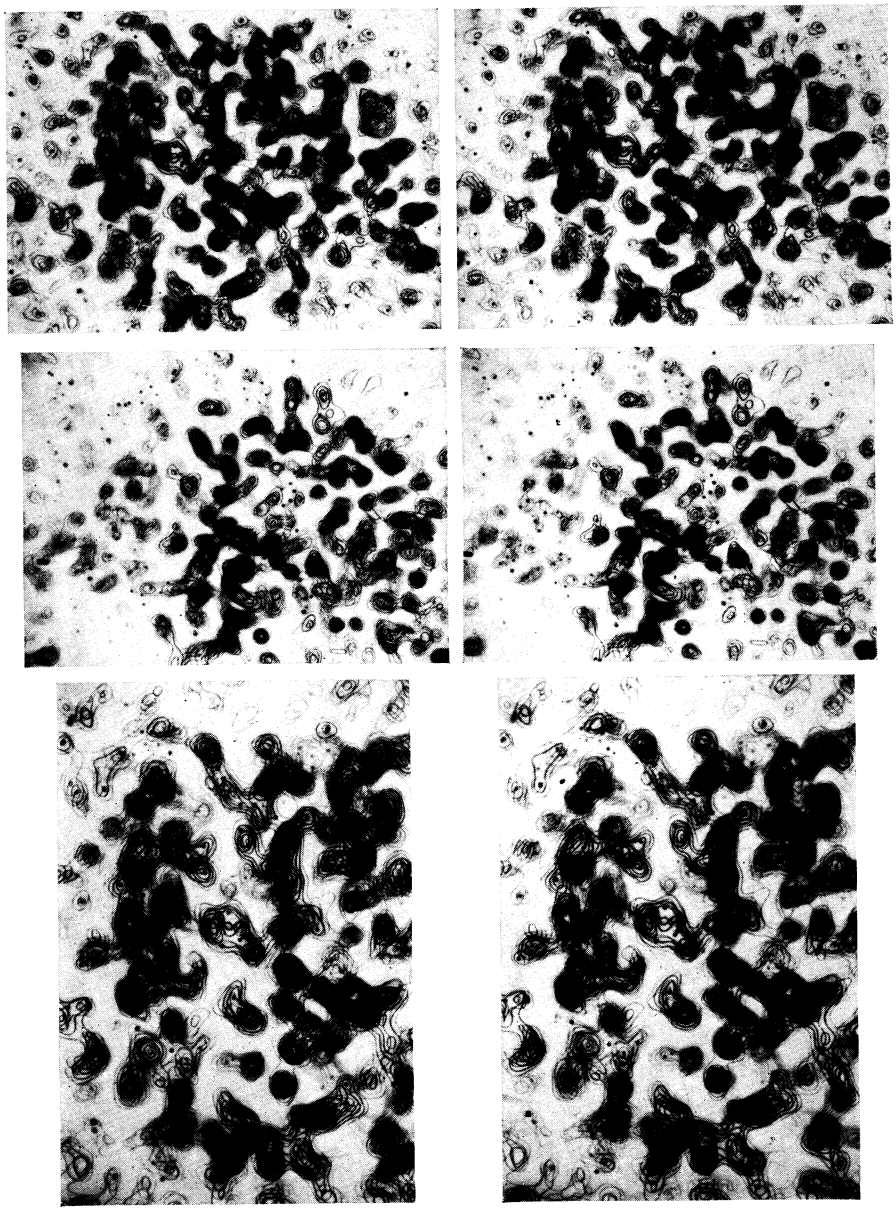


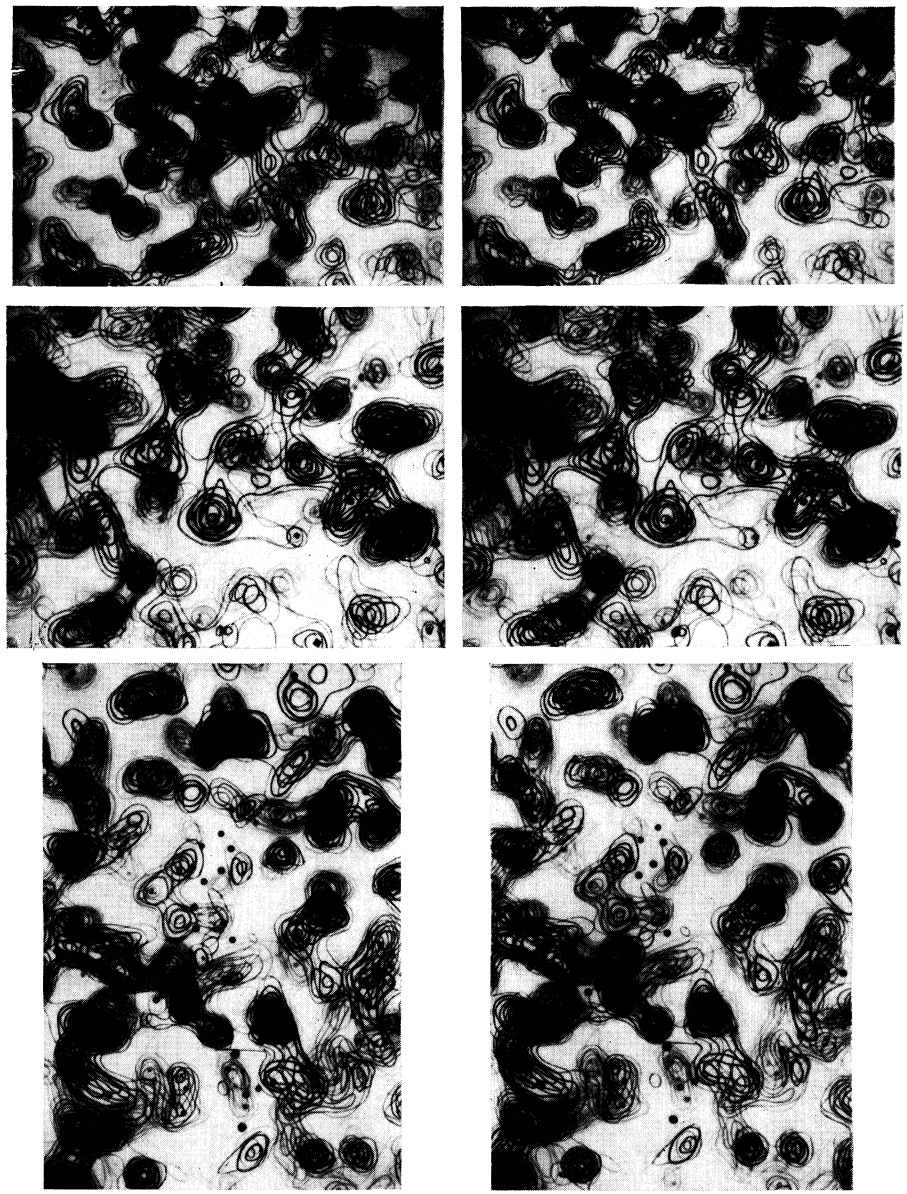
FIGURE 3 *a*, *b*, *c*. For legend see p. 189.

(*Facing p.* 188)

(d)

(e)

(f)



(g)

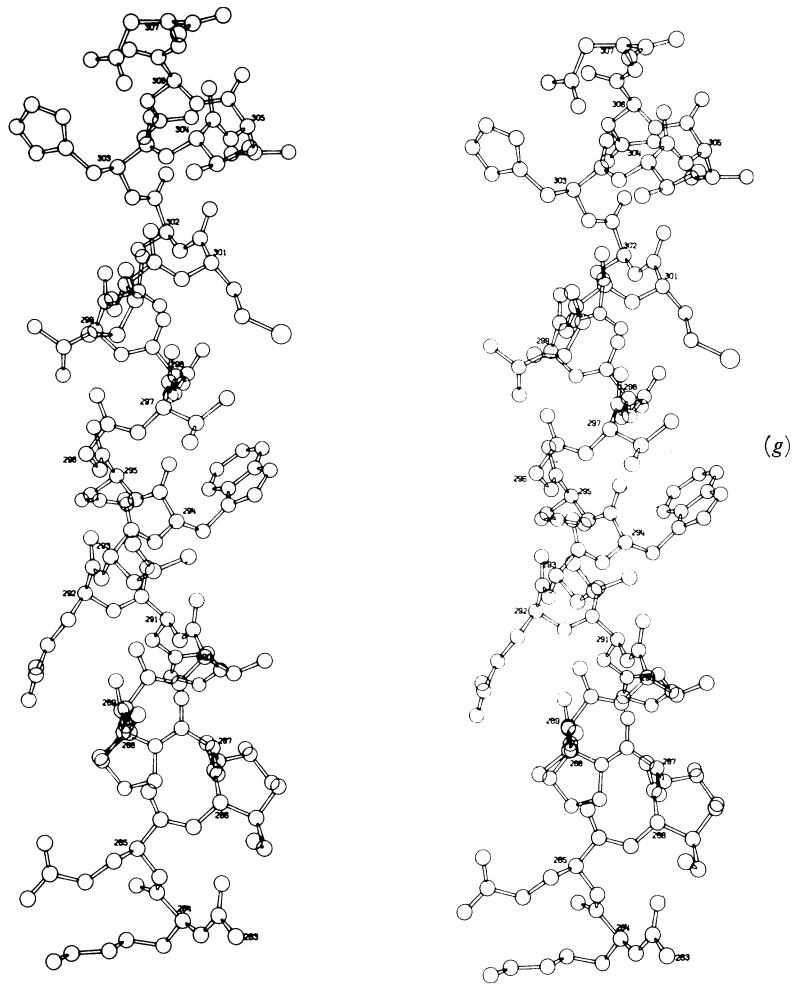


FIGURE 3 *d, e, f, g.* For legend see facing page.

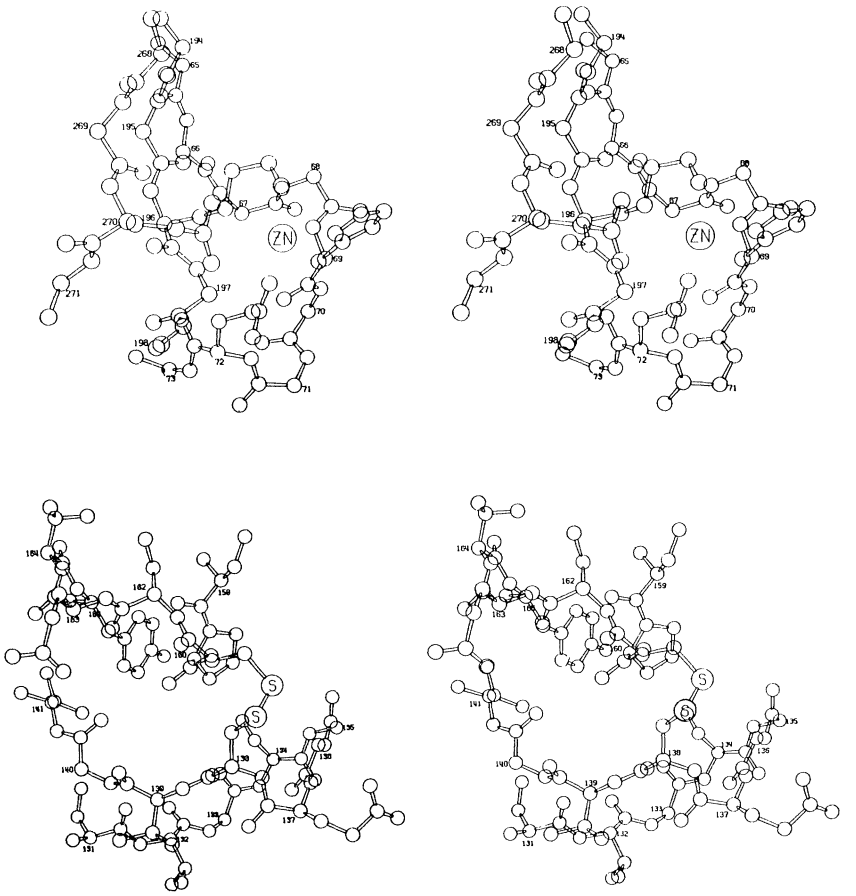


FIGURE 3 *h, i*

DESCRIPTION OF PLATES 58 AND 59

FIGURE 1. Stereophotograph† of the polypeptide chain of CPA. Each short rod is a peptide unit, and each intersection is one of the 307 C_α atoms. The Zn atom is the white sphere and the disulphide bridge is indicated by two black spheres. The numbers are 1, Ala-1; 2, His-69; 3, Arg-71; 4, Glu-72; 5, Cys-138; 6, Arg-145; 7, Cys-161; 8, Glx- or Lys-196; 9, Tyr-198; 10, Tyr-248; 11, Glu-270; 12, Asn-307 (C-terminus).

FIGURE 3 (*a*). Stereocomposite of the electron density sections $y = 0.38$ to $y = 0.46$. From left to right the C-terminal helix, a hydrophobic core, an extended chain, the Zn atom, and the disulphide bond can be seen. (*b*) Stereocomposite of the electron density sections $y = 0.45$ to $y = 0.57$. To the right of the extended chain the pocket region where the substrate binds can be seen. Dots have been added to depict a bound substrate molecule. (*c*) Close-up view of (*a*) which shows the C-terminal helix, the pleated sheet, and the Zn atom. (*d*) The Zn atom and its protein ligands in the electron density map. The water molecule, which completes the near-tetrahedral configuration about Zn, is in sections above those shown. (*e*) The disulphide bond in the electron density map at the right. The bond occurs between S atoms of Cys-138 and Cys-161. The Zn is at the left. (*f*) Close-up view of (*b*) which shows the electron density of the protein in the region where the substrate binds. The substrate molecule is shown as dots which do not match the contours of the protein or solution. (*g*) OR-TEP drawing of (*c*): the C-terminal helix. (*h*) OR-TEP drawing of (*d*): Zn, its protein ligands, and Glu-270. (*i*) OR-TEP drawing of (*e*): the region of the disulphide bond.

† Stereoviewers may be obtained, for example, from Ward's Natural Science Establishment, Inc., Rochester, New York, model 25 W2951. Computer drawn stereo figures were made using the program OR-TEP of Dr Carroll Johnson.

(b) *Description of the structure*

(i) *General features*

The gross shape of the CPA molecule is an ellipsoid of approximate dimensions $5 \times 4.2 \times 3.8$ nm. Views of the entire backbone of CPA are presented in figure 1, plate 58, and figure 2, and smaller portions which contain side chains of particular interest are shown in figure 3, plates 58 and 59. Six of the nine regions of helix are on the left-hand surface of the molecule as viewed in figure 1. A twisted pleated sheet which contains both parallel and antiparallel β structure runs through the centre of the molecule. Between the bank of helices and the pleated sheet there is a core of hydrophobic side chains. A similar hydrophobic trough exists to the right (in figure 1) of the pleated sheet below the level of Zn atom. The Zn is adjacent to the pleated sheet; in fact residue 196 which is one of three Zn ligands is a part of the β structure (figures 3*a*, *d* and *h*). The three ligands from the protein to Zn are His-69, Glu-72 and Lys- or Glx-196. Associated with the Zn and lined on one side by side chains of the pleated sheet are the previously described (Lipscomb *et al.* 1966, 1968) groove and pocket which are essential for substrate binding (figures 3*b* and *f*). With the exception of three short helical regions the half of the molecule to the right of the pleated sheet is random coil. This coil possesses a few hydrogen bonds (approximately 10) and the disulphide bond (Reeke *et al.* 1967) (figures 3*a*, *e* and *i*), but otherwise ought to be quite flexible. Indeed most of the conformational changes observed when a substrate molecule is bound to the enzyme are in this tortuous segment of the molecule.

(ii) *Helical segments*

Table 6 lists the helical segments of the molecule along with their average unit rises, unit rotations and numbers of units per turn. The expected values for an α-helix are also tabulated (Pauling, Corey & Branson 1951; Pauling 1960). Included in the helix classification are those

TABLE 6. PARAMETERS OF HELICES

residues	unit rise nm	unit rotation deg	units per turn
14–28	0.143	95	3.8
72–80	0.162	105	3.4
82–88	0.146	107	3.4
94–103	0.153	96	3.8
112–122	0.156	92	3.9
173–187	0.150	94	3.8
215–231	0.159	90	3.9
254–262	0.160	95	3.8
285–306	0.154	97	3.7
α helix	0.149	100	3.6

residues whose conformation is manifestly helical and which participate in at least one hydrogen bond approximately parallel to the helical axis. This criterion necessitates the breaking of the segment 72-88 into two helices because neither hydrogen bond is formed by Trp-81. The region 112–122 is an exception to this definition of a helix since it is so imperfect that only one or two of the potential hydrogen bonds exist. The averages in the table are made over the entire helical segments even though there are imperfect regions near the ends of several segments. Some of the imperfections resemble α_{II} helix (Nemethy, Phillips, Leach & Scheraga 1967) and others do not fit any of the previously described helical conformations (Bragg, Kendrew & Perutz 1950). The C-terminal helix, residues 285–306 (figures 3*a*, *c* and *g*), is the most nearly perfect helix in the molecule, but even here the helix axis is bent.

(iii) *β-structure*

The pleated sheet, which is twisted by about 120° from the top to the bottom of the molecule (figure 1), contains in its four parallel and three antiparallel pairs of extended chains about 20 % of the backbone atoms. However, only 45 residues, or 17 % of the molecule, form at least one of the hydrogen bonds of β-structure (Pauling & Corey 1951). The β-structure is portrayed schematically in figure 4. The dashed lines representing hydrogen bonds have been drawn if the oxygen–nitrogen distance is within 0.06 nm of the expected value of 0.276 nm (Pauling 1960). Under these conditions there are 33 hydrogen bonds among the 45 residues of the pleated sheet.

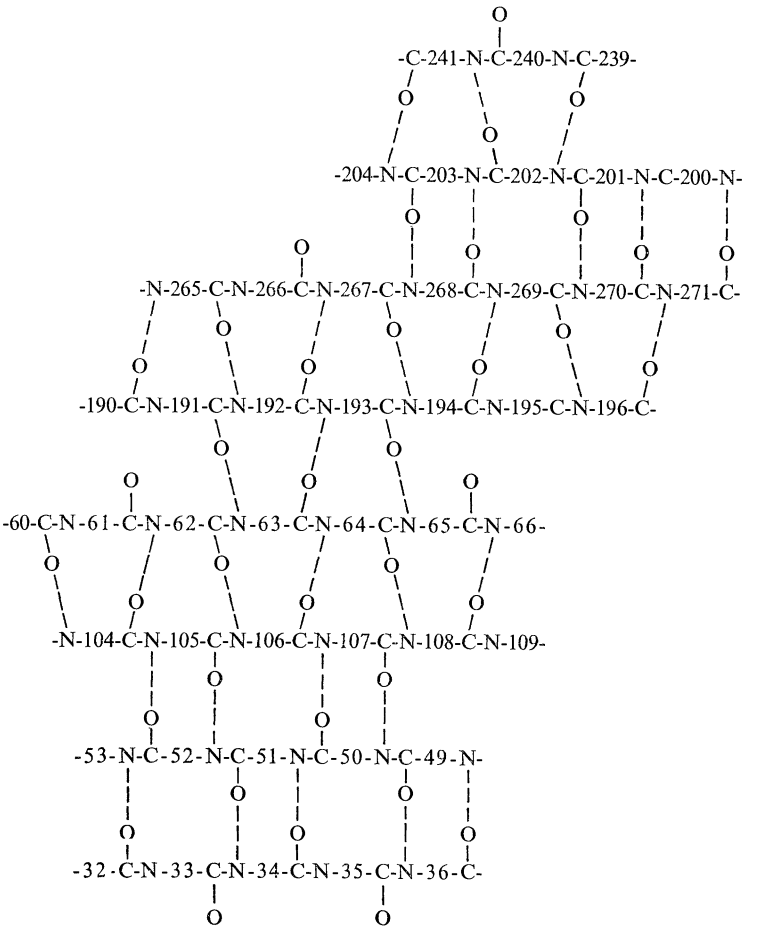


FIGURE 4. Schematic drawing of the pleated sheet (β) structure of CPA. Hydrogen bonds are shown as dashed lines.

(iv) *Folding of the polypeptide chain*

Carboxypeptidase A is biologically synthesized as the zymogen, proCPA (Anson 1937*a*; Brown, Greenshields, Yamasaki & Neurath 1963). The folding of the CPA chain into its native conformation can be considered independently of the conformation of proCPA, however, because CPA constitutes the C-terminal portion of a proCPA chain, and because proCPA exhibits some substrate binding characteristics of active CPA (Piras & Vallee 1967), suggesting that the CPA portion of proCPA is already nearly in its active conformation in the zymogen.

Examining, then, the folding of CPA from its N-terminus, we find that the various chains of the pleated sheet are not in sequential order (figures 1 and 4). Therefore, the final hydrogen bonds of extended chains 60–66 and 200–204 cannot be formed until chains 104–109 and 265–271 respectively have been folded into place. Furthermore, to insert chain 104–109 into the pleated sheet, residues 1–103 cannot be in their final positions for, if they were, chain 104–109 would have to pass, for example, between helices 14–28 and 72–88, whose axes are 1.2 nm apart, and between Phe-52 and Phe-86, which are 0.35 nm apart in the final structure. We have observed, in addition, three other places in the molecule where the final structure must differ from the conformation during the folding process. First, chain 249–254 must pass between two loops of random coil, which at closest approach in the final structure are only 0.55 nm apart between C_α 150 and C_α 208. Secondly, residues Leu-233 and Tyr-234 would interfere with placement of the C-terminal helix. Finally, we observe that the disulphide bond must be formed after residues 163–170 pass through the disulphide loop 138–161.

(v) *Ramachandran plot*

A plot (Ramachandran, Ramakrishnan & Sasisekharan 1963) of the dihedral angles of rotation ϕ (about the C_α–N bond) and ψ (about the $C_{carbonyl}$–C_α bond) (Edsall, Flory, Kendrew, Liquori, Nemethy, Ramachandran & Scheraga 1966) for the peptides of CPA is given in figure 5. We have superposed on the usual (ϕ, ψ) plot the boundaries of Ramachandran's 'outer

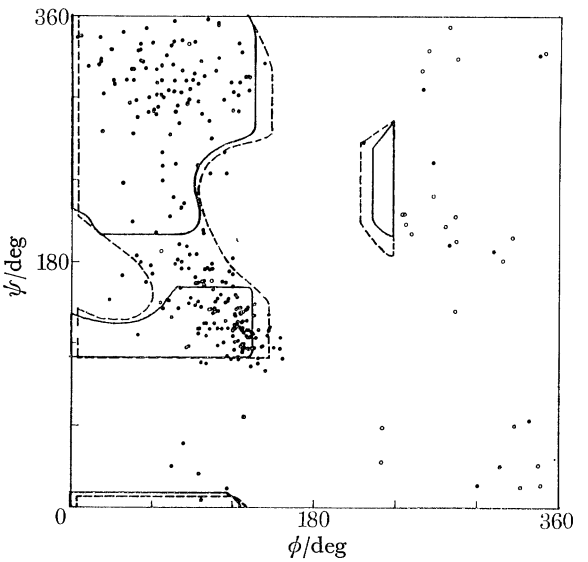


FIGURE 5. Plot of dihedral angles of CPA. Solid lines demarcate allowed regions for $\tau = 110°$ and dashed lines the regions for $\tau = 115°$.

limit' regions allowed for $\tau = 110°$ (solid lines) and $\tau = 115°$ (dashed lines) (Ramachandran & Ramakrishnan 1965). Of course, distortions of the peptide unit could change the shapes of the allowed regions. The distribution of points is similar to that found for lysozyme (Blake, Mair, North, Phillips & Sarma 1967*b*), where there are also violations of the $\tau = 110°$ limits in the regions $\psi \simeq 180°$, $\phi < 180°$ and $\psi \simeq 120°$, $\phi \simeq 180°$.

(vi) *Correlation of sequence and structure*

Table 7, which is similar to the table prepared by Cook (1967) for haemoglobin, myoglobin and lysozyme, is intended to reveal the existence of correlations between structural type and

TABLE 7. CORRELATION OF SEQUENCE AND STRUCTURE

	chemical amino acid analysis	X-ray amino acid analysis	random coil	helix	N helix	C helix	β
Ala	6.50	5.86	5.67	9.84	3.70	3.70	3.92
Arg	3.58	3.58	6.38	—	—	—	3.92
Asx	9.11	9.45	8.51	9.84	18.52	14.81	3.92
Cys	0.65	0.65	1.42	—	—	—	—
Glx	8.13	9.77	7.80	9.84	14.81	14.81	9.80
Gly	7.32	8.79	14.89	3.28	—	14.81	—
His	2.60	2.93	2.84	4.92	—	7.41	—
Ile	6.50	5.21	3.55	6.56	11.11	—	7.84
Leu	7.48	7.17	2.13	8.20	7.41	3.70	21.57
Lys	4.88	5.86	4.26	6.56	4.71	3.70	9.80
Met	0.98	0.98	—	3.28	—	3.70	—
Phe	5.20	4.89	2.13	6.56	7.41	3.70	9.80
Pro	3.25	3.26	4.26	1.64	7.41	—	1.96
Ser	10.73	10.75	13.48	6.56	7.41	7.41	11.76
Thr	9.11	7.49	7.80	6.56	11.11	11.11	3.92
Trp	2.60	2.61	3.55	1.64	3.70	—	1.96
Tyr	6.18	5.54	9.21	3.28	—	—	3.92
Val	5.20	5.21	2.13	11.48	—	11.11	5.88

The values in columns 1 and 2 are percentages of the total molecule. The values in columns 3 to 7 are percentages of the structural types named. For a given residue, the entries in columns 3 to 7 would equal the entry in column 2 if that residue were distributed randomly among the structural types.

sequence. This tabulation is based on the chemical sequence where available and on the X-ray identifications for the other 93 residues. The chemical amino acid analysis (Bargetzi, Sampath Kumar, Cox, Walsh & Neurath 1963), column 1, and the amino acid totals obtained from the X-ray identifications, column 2, have the largest discrepancies for Glx, Gly, Ile and Thr. In the tabulation of N- and C-ends of helices, columns 5 and 6, three residues are included from the respective ends of each helix. If the distribution of a residue were totally unrelated to secondary structure, a given horizontal row of the table (excepting the first column) would have equal entries. We do not consider the results for Cys, Met, His or Trp to be significant because their percentages of the amino acid composition are small.

The residues which appear to display a preference for one type of secondary structure are: Arg, Gly and Tyr for random coil; Ala and Val for helix; Asx, Glx, Gly and Ile for the ends of helices; and Leu and Phe for β structure. These conclusions are in agreement with those drawn from the haemoglobin–myoglobin–lysozyme tabulation with the exception of the distribution of Asx which Cook found more often in random coil segments. Our result for the β

TABLE 8. CONFORMATION OF TYROSINE RESIDUES*

residue number	χ_1	residue number	χ_1
42	180	166†	85
90	180	169†	49
165	186	208	84
248	200	19	267
259	183	198	98
9	314	204	27
12	295	238	236
48	276		
206	271		
234	315		
277†	285		

* See also *Note added in proof*, p. 212. † These residue identifications are by X-ray means only.

structure reflects more the position of the pleated sheet in the core of the molecule than it does the nature of β structure and is not necessarily typical.

It is interesting to note the locations of the 10 proline residues in the CPA structure. Four proline residues, namely 94, 113, 214 and 288, terminate helices at their amino ends. Three other proline residues, 46, 60 and 205, are at the ends of extended chains of the pleated sheet, and the remaining three proline residues, 30, 160 and 282, are situated in random coil.

The conformations of the side chains of the residues are quite varied. For example, table 8 shows the various values of χ_1 (the angle of the free rotation about the C_α–C_β bond) of tyrosine. The staggered conformations have χ_1 equal to 60, 180 or 300°. $\chi_1 = 190$ is found in the tyrosine crystal structure (Smits & Wiebenga 1953). Fourteen of the 18 tyrosine residues identified so far are approximately equally distributed among the three staggered conformations. Four other residues are closer to an eclipsed than a staggered conformation.

A discussion of the locations of charged residues in the CPA molecule must be deferred until the complete chemical sequence is available. For example, the state of amidation of the acidic residues cannot reliably be determined from the crystal structure.

3. The glycyl–tyrosine complex with CPA_α

(*a*) *Preparation of the difference electron density map*

When Gly–Tyr is diffused into crystals of CPA which have been previously crosslinked with glutaraldehyde (Quiocho & Richards 1964) there results an unusually stable enzyme–substrate complex whose crystals are isomorphous with those of the native enzyme (Ludwig *et al.* 1967). Data were collected on this complex to 0.28 nm resolution (Reeke *et al.* 1967) and then to 0.20 nm resolution (Lipscomb *et al.* 1968). The overall agreement factor obtained in the scaling of the various data sets is

$$R = [\Sigma|F_i^2 - F_j^2|]/[\Sigma|F_i^2 + F_j^2|] = 0.1037$$

for the 0.20 nm resolution merged Gly-Tyr data set. For further details see table 7 of Lipscomb *et al.* (1968).

Once the structure factor calculation described above was complete, the new phase information could be used to make an improved Gly-Tyr difference map. Two earlier observations had led us to conclude that the Gly-Tyr occupancy in the crystals was low. First, an occupancy of 1/3 was calculated from peak heights in the difference map computed with Fourier coefficients $(|F_{ES}| - |F_N|) \exp(i\phi_{MIR})$ where $|F_{ES}|$ and $|F_N|$ are scaled observed structure factors for the Gly-Tyr enzyme complex and for the native enzyme respectively. Secondly, an attempt to make a map of the enzyme substrate complex using $|F_{ES}| \exp(i\phi_{MIR})$ as coefficients was unsuccessful because the resulting function had the appearance of the native enzyme electron density. Therefore we endeavoured to correct for the low occupancy by calculating a difference map with coefficients

$$(|F_{ES}| - x|f_c| - (1-x)|F_N|) \exp(i\phi_{SF}),$$

where $|F_{ES}|$ and $|F_N|$ are as defined above, x is the fractional occupancy of the substrate, and f_c is a calculated structure factor for the native enzyme. This function removes the contribution of the unmodified enzyme and enhances substrate features which replace solvent molecules in the complex. The rationale for the function derives from the fact that water is displaced from the active site of CPA when Gly-Tyr is bound. Therefore, for the fraction x of enzyme molecules

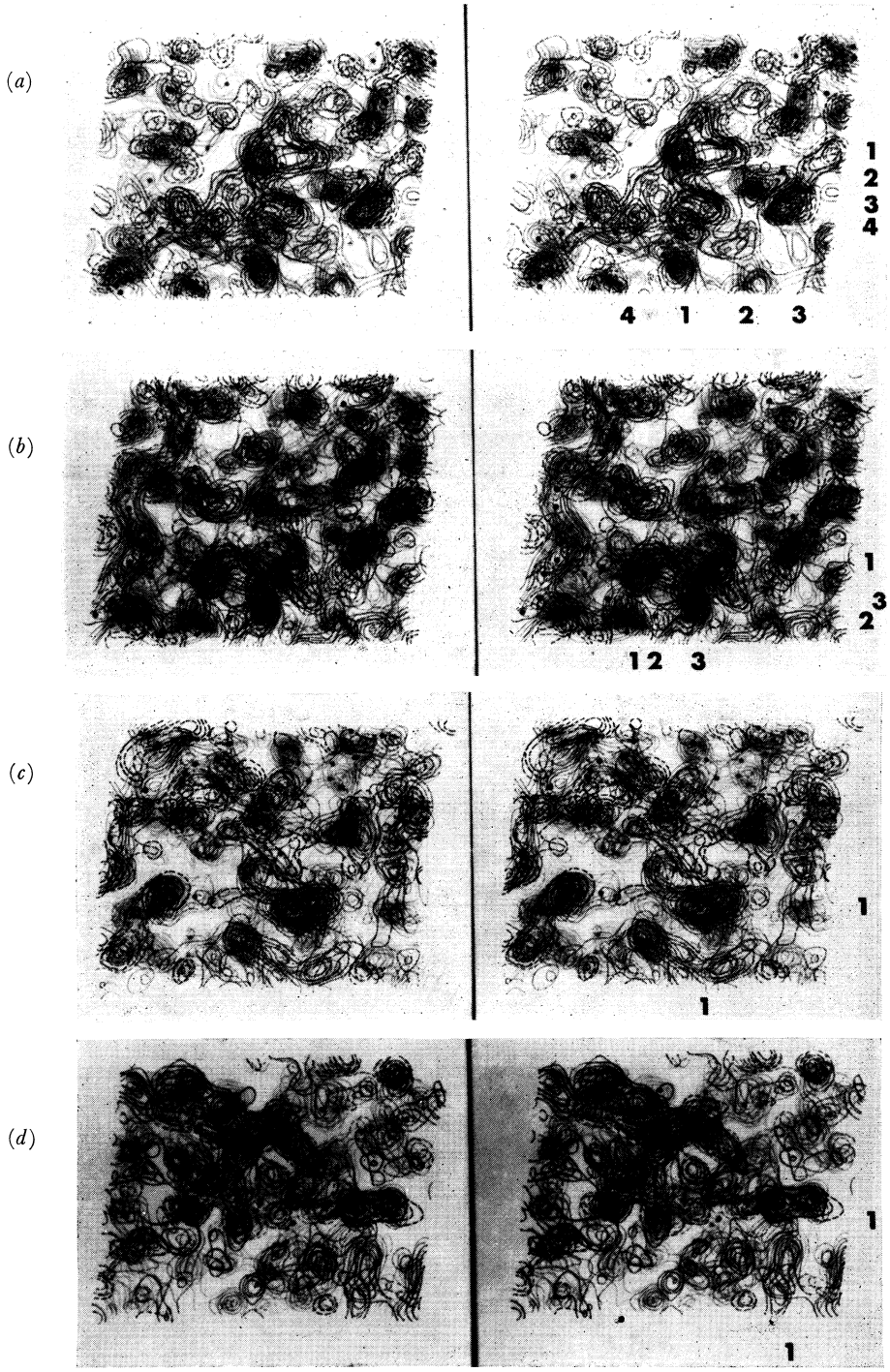


FIGURE 6. The difference electron density function for the complex of Gly-Tyr with CPA is shown as dotted (positive) and dashed (negative) contours. Solid contours show the electron density of CPA at 0.2 nm resolution. Dots are placed at proposed atomic positions. (*a*) Composite of the difference map sections $y = 0.47$ to $y = 0.52$. Near the top centre the positive contours of the tyrosyl side chain of the substrate are visible (1). To the right of the substrate are the positive (2) and negative (3) contours of the Arg-145 guanidinium group and to the left (4) are the native contours of Glu-270. (*b*) Composite of the difference map sections $y = 0.49$ to $y = 0.56$. Near the bottom of the picture are the positive contours of the moved Glu-270 (1), the substrate's terminal amino group (3), and the connecting water molecule (square dot, 2). (*c*) Composite of the difference map sections $y = 0.56$ to $y = 0.62$ shows Tyr-248 after its conformational change in the bottom right portion of the picture (1). (*d*) Composite of the difference map sections $y = 0.63$ to $y = 0.68$ shows Tyr-248 before its conformational change (1).

(*Facing p.* 195)

to which Gly-Tyr is bound, $|f_c|$, which does not include any contribution from water, should be subtracted, and for the fraction $(1-x)$ of enzyme molecules to which Gly-Tyr is not bound, $|F_N|$, which does include water, should be subtracted. The parameter x was decreased in successive maps until at a value of 0.30 water peaks which should be displaced by Van der Waals repulsions with the nearby Gly-Tyr molecule disappeared. The displaced water peaks returned to a small extent when MIR phases were substituted for the calculated structure factor phases in this map. Accordingly, the final difference map of figure 6, plate 60, was made with calculated structure factor phases.

(*b*) *Description and interpretation of the Gly-Tyr difference electron density map*

The revised map of the enzyme–substrate complex does not lead to any conclusions different from those previously stated (Lipscomb *et al.* 1968). However, in several instances, for example the conformational change of Tyr-248, features are more distinct than they were previously. The binding of Gly-Tyr to CPA can be summarized by describing four interactions. We believe that the first three of these are characteristic of a productive enzyme–substrate complex. First, the C-terminal side chain of the substrate is inserted into a pocket in the enzyme with concomitant displacement of several water molecules (figure 6*a*). In agreement with the moderate but not high specificity for the C-terminal side chain of the substrate, this pocket contains no specific binding group, probably no charged group, and is large enough to accommodate a tryptophan side chain. Secondly, the terminal carboxylate group of the substrate (which is essential for susceptibility to cleavage) interacts with the positively charged guanidinium group of Arg-145 (figure 6*a*). Thirdly, the carbonyl oxygen of the scissile peptide bond replaces water as a ligand to Zn (figure 6*a*). This feature, although still not certain, is clearer than it was in the previous 0.2 nm difference electron density map. Fourthly, in an interaction possible only with dipeptide substrates, Glu-270† binds through water to the α-amino group of Gly-Tyr‡ (figure 6*b*). It is probably this interaction which accounts for the unusual stability of the Gly-Tyr-CPA complex. All of these binding interactions are summarized in figure 7.

The native enzyme undergoes several conformational changes when Gly-Tyr is bound. These changes are seen in the difference map as negative density at the native conformation and positive density at the modified conformation. First, the guanidinium group of Arg-145 moves about 0.2 nm by means of a rotation about the C_β–C_γ bond of the side chain (figure 6*a*). Secondly, the carboxylate of Glu-270 moves toward the viewer in figure 6*b* by about 0.2 nm. This motion results from rotations about both the C_α—C_β and C_β—C_γ bonds of Glu-270. In exception to the above statement, there is no negative density at the native Glu-270 position because this side chain was not included in the structure factor calculation. Thirdly, the phenolic hydroxyl of Tyr-248, identified as being involved in the activity of the enzyme (Roholt & Pressman 1967; Reeke *et al.* 1967), moves about 1.2 nm so that the OH group comes from the surface of the molecule to the vicinity of the peptide bond of the substrate (figures 6*c*, *d*). This

† Residue 270 has been identified as glutamic acid from the X-ray map since chemical sequence data are not available for this part of the molecule. Asx and His were also considered, but did not fit the density so well as Glx, nor could they undergo the conformational change observed at 270 when Gly-Tyr binds. The choice of Glu rather than Gln is corroborated by the binding of a Pb ion near this residue (see table 1).

‡ Yanari & Mitz (1957*a*) deduced from the increased efficiency of Gly-Tyr and Leu–Tyr as competitive inhibitors at higher pH that only the anionic form of a dipeptide, i.e. the species with an uncharged amino group, is bound to CPA. Although we cannot distinguish crystallographically which species of Gly-Tyr is bound, it is reasonable that the NH_3^+ of the zwitterion would be repelled by the positive charge on Zn.

motion involves a rotation of the side chain about its C_α—C_β bond by about 120° as well as a limited motion of the peptide backbone. In the new Gly-Tyr difference map the density at the moved position of Tyr-248 is more distinct than previously. However, we are still not able to locate the tyrosyl side chain accurately. In our present interpretation, the tyrosyl oxygen is located 0.27 nm from the nitrogen of the scissile peptide bond, and 0.35 nm from the α-amino group of the substrate. Finally, in the tortuous portion of the native structure, there is a system of four hydrogen bonds which form a link between Arg-145 and Tyr-248. This link goes from Arg-145 to backbone carbonyl 155, from backbone carbonyl 154 to Glx-249, and finally from Glx-249 via H_2O to the hydroxyl of Tyr-248. When Gly-Tyr is bound all these groups move, and we observe that Arg-145 then no longer interacts with backbone carbonyl 155 and that Tyr-248, having undergone an enormous conformational change, no longer interacts with Glx-249.

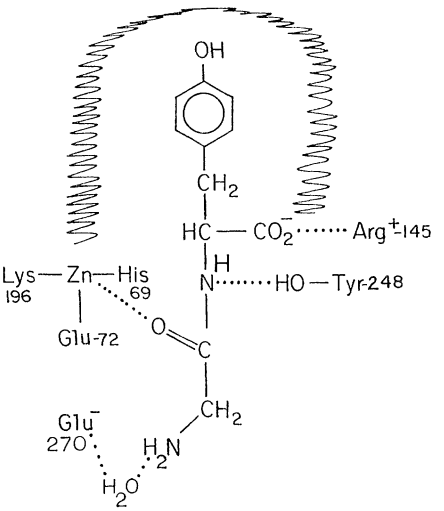


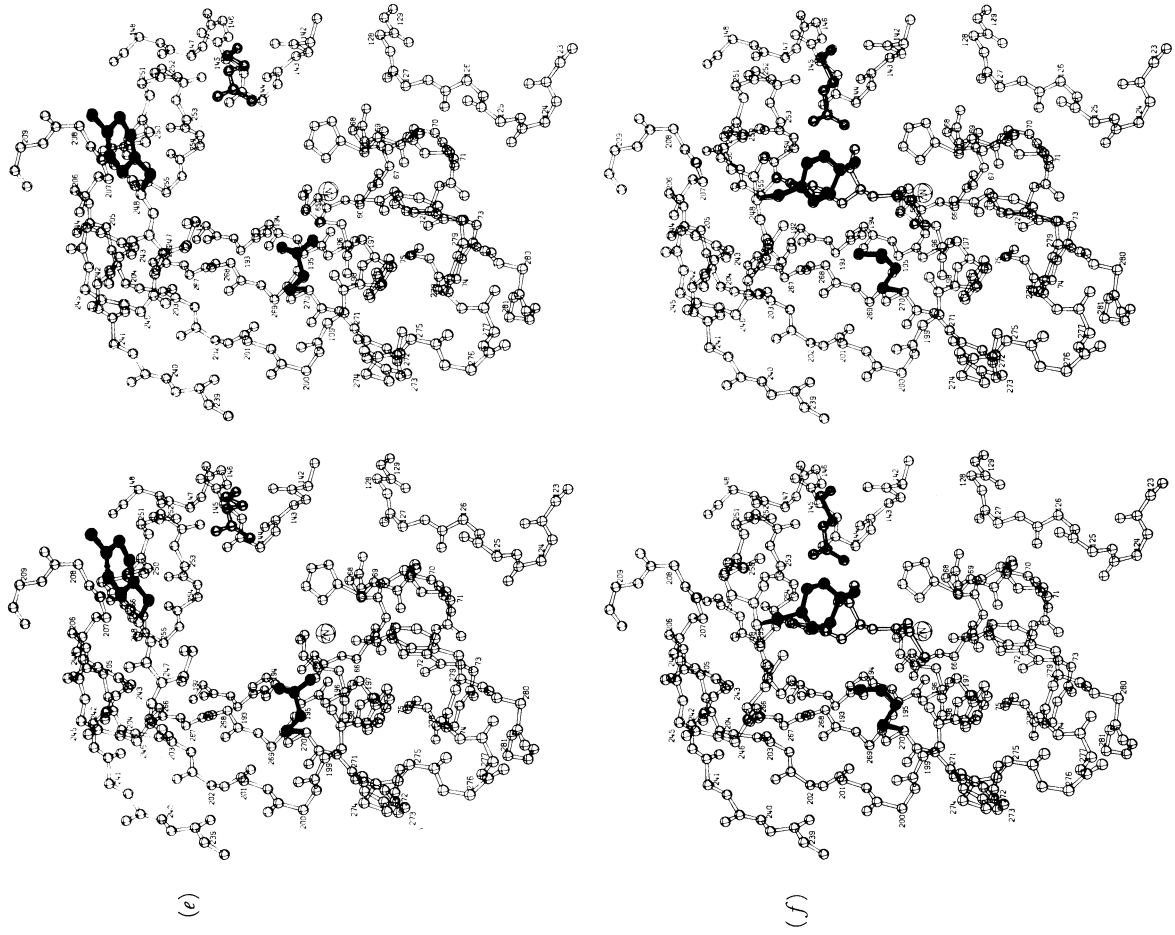
FIGURE 7. Schematic drawing of the binding of Gly-Tyr to CPA.

The binding of glycyl-L-tyrosine, then, results in several complementary rearrangements in the enzyme structure. The most striking of these is the coordinated motion, linked together by movements of several intervening amino acids, of Arg-145 and Tyr-248, which brings a group thought to be required for catalysis into contact with the substrate. Adaptation of an enzyme to its substrate has been suggested by several workers (Karush 1950; Lumry & Eyring 1954; Vaslow 1958), but the conformation changes in CPA are a clear example of the 'induced fit' theory of Koshland (1958, 1963).

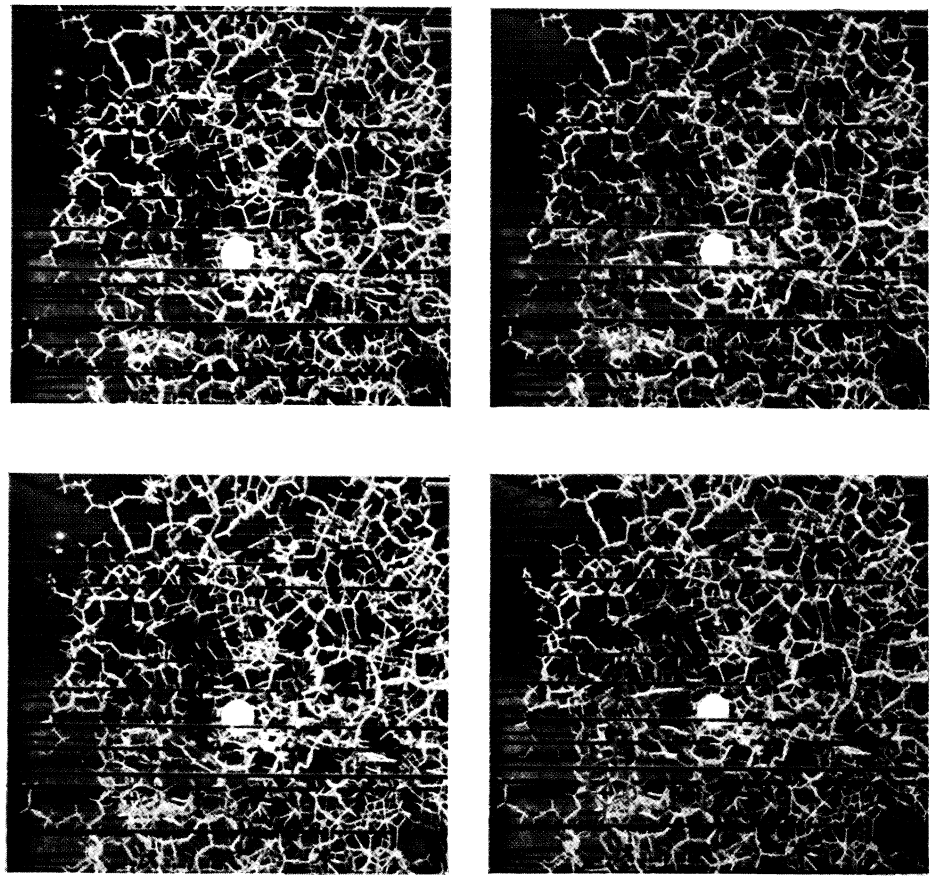
4. ACTIVITY OF CARBOXYPEPTIDASE A

(*a*) *Introductory remarks*

Following the discovery of CPA (Waldschmidt-Leitz & Purr 1929) and the isolation of crystalline CPA (Anson 1935, 1937*b*) a number of important conclusions regarding its specificity were established by about 1950 and were reviewed by Waldschmidt-Leitz (1931), Neurath & Schwert (1950), and Smith (1951). Those features of the substrate which are of particular relevance to our structural study are listed briefly here. First, the peptide bond which is hydrolysed (figure 8) must be adjacent to a terminal free carboxyl group (Hoffman & Bergmann 1940; Waldschmidt-Leitz 1931). Secondly, the rate of hydrolysis is usually enhanced if the terminal residue is aromatic or branched aliphatic (Stahmann, Fruton & Bergmann 1946).



(*e*) (*f*)



(*a*) (*b*)

FIGURE 9 *a*, *b*, *e*,*f*. For legend see facing page.

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Thirdly, dipeptides having a free amino group are hydrolysed slowly, but if this group is blocked by *N*-acylation, the hydrolysis is rapid (Hoffman & Bergmann 1940). Fourthly, the C-terminal residue must be in the L configuration (Bergmann & Fruton 1937; Hanson & Smith 1949; Dekker, Taylor & Fruton 1949). Fifthly, the substitution of a methyl group (in sarcosine) or a methylene group (in proline) for the H atom of the NH group of the peptide bond to be split either prohibits or very greatly reduces hydrolysis (Stahmann *et al.* 1946; Snoke & Neurath

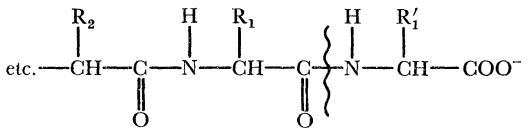

FIGURE 8. Substrate for carboxypeptidase, to be split at the wavy line.

1949). Sixthly, in *N*-acyl dipeptides the rate of hydrolysis is greatly decreased by substitution of sarcosine (Snoke & Neurath 1949) or β-alanine (Hanson & Smith 1948) for the second amino acid. Thus, the presence and integrity of the second peptide bond are important for rapid hydrolysis. Seventh, studies of longer polypeptides (Glantz & Smith 1952), most recently and thoroughly by Abramowitz, Schechter & Berger (1967), indicate that at least the five C-terminal residues of the substrate influence the K_m constant and, to a lesser extent, k_{cat}.

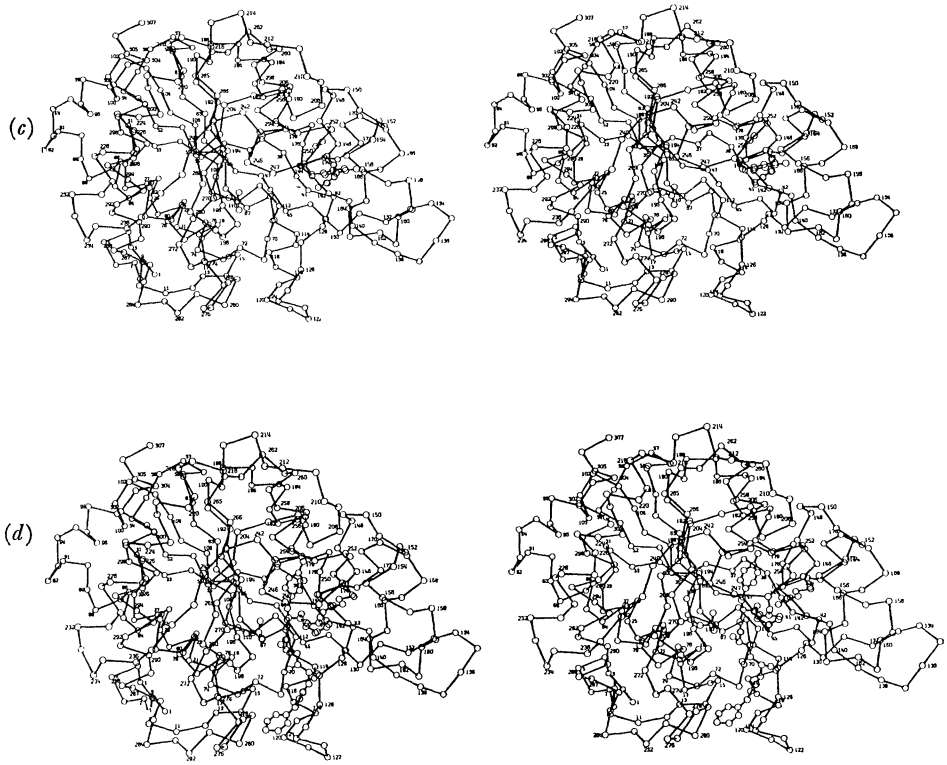

FIGURE 9. CPA with and without substrate. (*a*) Kendrew model of the active site region of the native enzyme. (*b*) Kendrew model after the substrate has been added and the conformational changes of Glu-270, Arg-145, and Tyr-248 have been made. (*c*) OR-TEP drawing of the α carbon model of CPA, with Arg-145, Tyr-248, and Glu-270 before the conformational change. (*d*) OR-TEP drawing of CPA, showing Arg-145, Tyr-248, and Glu-270 after their conformational changes observed when Gly-Tyr is bound. The substrate, CBZ-Ala-Ala-Tyr, has been positioned by model building, starting with the conclusions drawn from the Gly Tyr-CPA map. (*e*) OR-TEP drawing of the active site of native CPA with Arg-145 coloured green and Tyr-248 and Glu-270 coloured red. (*f*) OR-TEP drawing of CPA after the substrate, which is orange, has been added, and the conformational changes have been made.

(b) Binding of active peptide substrates

The above experimental findings, relating primarily to the substrate structure, provide data with which the X-ray diffraction models of the enzymic binding and catalytic steps must be consistent. We assume that in the productive mode substrates are bound with the C-terminal aromatic residue in the pocket, with the terminal carboxyl group salt linked to Arg-145, with the carbonyl group of the susceptible peptide bond bound to Zn, and with the OH of Tyr-248 near enough to donate a proton to the NH of the susceptible peptide bond (figure 9, plate 61). These points of binding are sufficient to establish the stereospecificity of CPA for C-terminal L amino acids. All of these features are unambiguous in the Gly-Tyr difference map. We further assume that the coordinated motion of Arg-145 and Tyr-248 which occurs when Gly-Tyr is bound also takes place when larger substrates are bound (Steitz *et al.* 1967).

We now extrapolate the results obtained with Gly-Tyr to explain the binding and cleavage of other substrates, based on model building and the chemical properties cited above. If all other groups of the protein are assumed to be in essentially the same position in the active substrate complex as they are in the Gly-Tyr complex, we cannot form a link (through water) between the N-terminus, if it exists, and the carboxyl group of Glu-270 when substrates longer than dipeptides are bound. Nevertheless, Glu-270 will be very close to the carbonyl carbon of the hydrolysable bond. The placement in our structure of a longer chain substrate is determined from the importance of the NH of the second peptide bond, from the general shape of the groove in the enzyme structure itself, and from the detailed results of Abramowitz *et al.* (1967). Maximum interaction of the aromatic R groups in positions 3 and 4 (figure 10) with the aromatic enzyme residues Tyr-198 and His(Phe)-279 results if the second substrate NH is allowed to hydrogen bond to the OH of Tyr-248. In addition, the carbonyl group of the third peptide bond, and possibly also that of the fourth, can be placed near Arg-71 in a stabilized situation.

While we would not claim that all experiments relating to binding are uniquely interpretable on the basis of this model, we know of no results which are incompatible with it. Several examples of readily interpretable observations now follow: (*a*) The presence of a dead-end pocket, in addition to a groove, provides an explanation of the early observation that CPA is an exopeptidase, not an endopeptidase (Hoffman & Bergmann 1940; Waldschmidt-Leitz 1931). (*b*) Peptide substrates with a D amino acid at the C-terminus, such as CBZ-Gly-D-Phe, are neither hydrolysed nor bound (Neurath, Elkins & Kaufman 1947). Here we find that the peptide bond, carboxyl group, and R group of a substrate C-terminal D residue cannot easily be accommodated at their correct positions for hydrolysis, and steric interactions which prevent binding may occur. (*c*) The small K_I and K_m (Izumiya & Uchio 1959) of Gly-Tyr as compared to the K_I of *N*-acetyl-Tyr (Yanari & Mitz 1957*a*) indicates that the former is bound more strongly than the latter. Earlier work comparing *N*-acyl dipeptides with analogous dipeptides also indicated that when a free amino group is present, the dipeptide is bound tightly but is hydrolysed very slowly (Hoffman & Bergmann 1940): we find from models that blocking of this amino group destroys the non-productive binding mode, described above as an interaction of the amino group with the carboxyl group of Glu-270, thus freeing Glu-270 to serve in its probable catalytic role. (*d*) Model building indicates that steric hindrances occur if the NH group of the sensitive peptide bond is substituted. The most severe example is CBZ-Gly-thiazolidine-4-carboxylic acid, which is neither bound nor cleaved (Smith 1948), probably because of steric interference by Ile-247. In the case of CBZ-Gly-sarcosine and CBZ-Gly-Pro, neither of which is cleaved (Stahmann *et al.* 1946), model building suggests interference with the final stages of

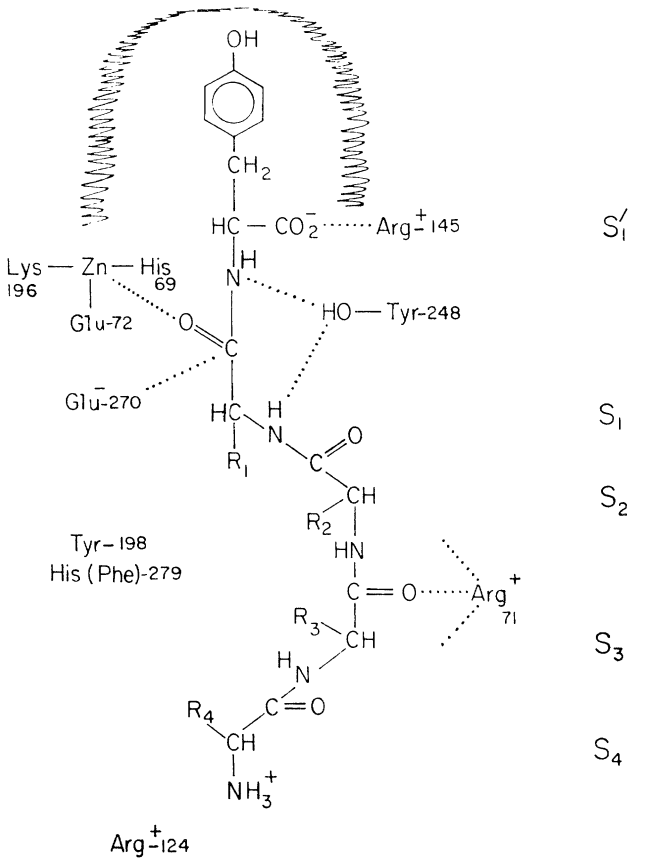

FIGURE 10. Drawing of the binding of a longer substrate to CPA.

the conformation change of Tyr-248. (*e*) The recent experiments of Abramowitz *et al.* (1967) have mapped carefully several effects, of which those relating primarily to the kinetic constant K_m are discussed here. Comparison of K_m for Phe-Ala-Ala with K_m for Ala-Ala-Ala yields a ratio of 72:1 in favour of this N-terminal aromatic at site S_2 (figure 10), but substitution of an aromatic group is also felt at S_1, S_3 or S_4. The effect is strongest when the relatively flexible CBZ group is at S_3, where it can most favourably be placed near Tyr-198 and His(Phe)-279. Also, a consistently lower K_m is noted if the terminal NH_3^+ group in a tetrapeptide is blocked by acetylation or by the introduction of a urethane. Our model indicates that this substitution on NH_3^+ provides additional interactions of lone pairs of added oxygen atoms with the guanidinium group of Arg-71 and removes the possible repulsion of the NH_3^+ from Arg-71. (*f*) Placement of the bulky D-Leu residue as the S_1 peptide in several *N*-acyl-D-Leu-L-Tyr substrates is known to lower the rate of hydrolysis by a factor of about 5000 relative to that of the corresonding *N*-acyl-L-Leu-L-Tyr (Yanari & Mitz 1957*b*): if the amide of the second peptide binds to Tyr-248, the D side chain interferes sterically with the protein, but such repulsion does not occur for the L compounds.

(*c*) *Cleavage*

(i) Zn–*carbonyl mechanisms*

The probable mechanisms of cleavage of a peptide substrate proposed below, while not necessarily exhaustive, are consistent with the importance of the amide group of the substrate's penultimate peptide bond, the effect of pH on cleavage, the kinetic experiments on polypeptide

substrates, and the effects of modification of the enzyme on its activity. One important contribution of X-ray diffraction methods is the identification of the various groups of the protein which are close enough to the substrate to function in the catalytic steps. Mechanistic deductions are based on knowledge of these groups, which are Arg-145, Zn, Glu-270, Tyr-248, and H_2O, and we now discuss the roles of each in turn (figure 11).

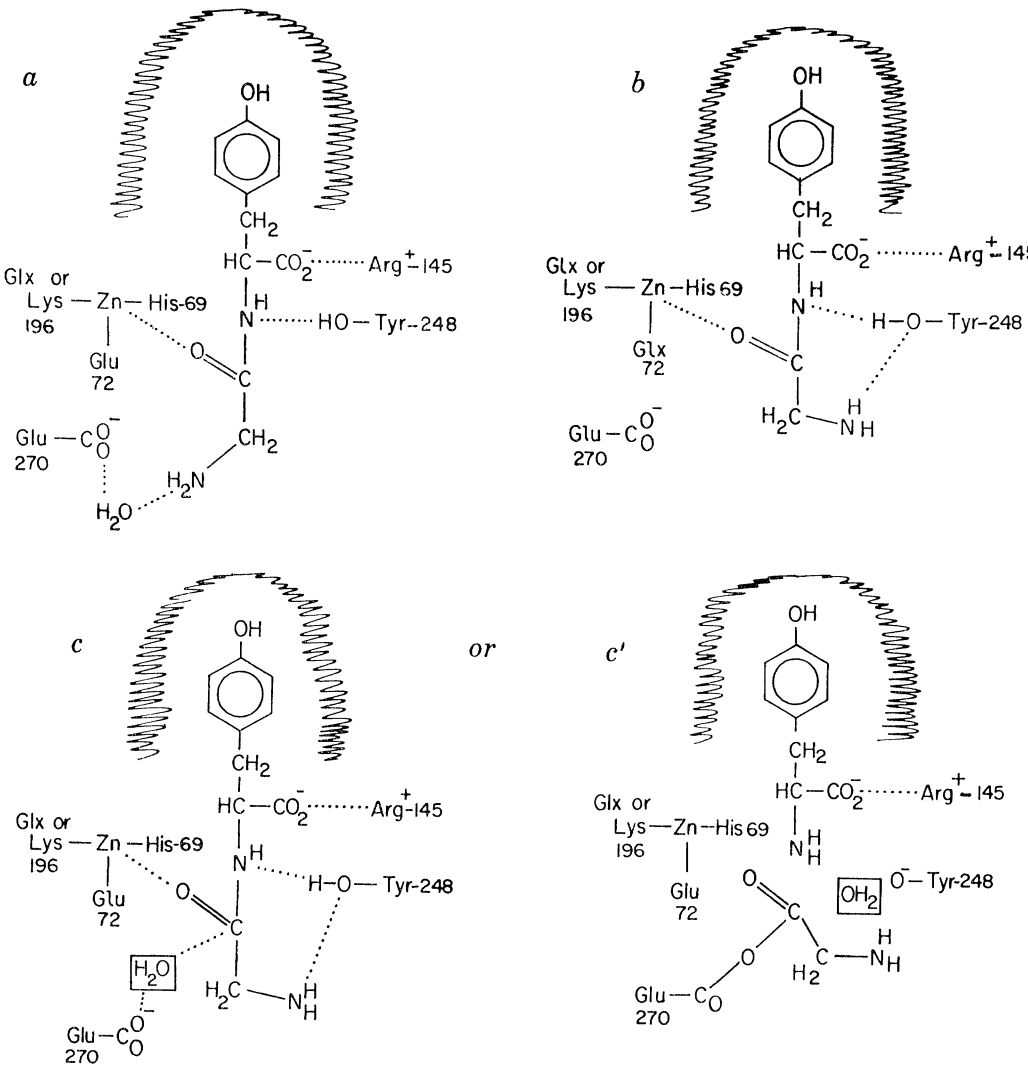

FIGURE 11. Possible stages in the hydrolysis of a peptide by CPA. It is probable that the carbonyl carbon of the substrate becomes tetrahedrally bonded as the reaction proceeds, but it is uncertain at what stage of the reaction the proton is added to the NH group of the susceptible peptide bond. (*a*) Abortive binding of the dipeptide Gly-Tyr. (*b*) Productive binding mode. (*c*) General base attack by water upon the carbonyl carbon of the substrate. (*c'*) Nucleophilic attack by Glu-270 upon the carbonyl carbon of the substrate.

The function of Arg-145 has been shown to be the binding of the terminal carboxyl group of Gly-Tyr. On this basis and from the results at 0.6 nm resolution of binding of longer substrates to acetyl-CPA (Steitz *et al.* 1967), we assign this same function to Arg-145 in the proposed productive mode of binding of longer substrates.

Smith & Hanson (1948) showed that a metal is essential for the activity of CPA, and this metal was later shown by Vallee & Neurath (1954) to be Zn. The Zn atom serves to polarize

the carbonyl group of the substrate, $Zn^{+}...O^{\delta-}—C^{\delta+}$, in order to render the carbon atom of this carbonyl group more susceptible to nucleophilic attack. Thus the Zn atom functions as a general acid, which attacks the basic oxygen atom and serves to reduce the double bond character of this carbonyl group of the substrate. In addition, bonding about this carbonyl carbon can thereby more easily become non-planar, rendering this atom more susceptible to nucleophilic attack.

Glu-270 probably functions either in nucleophilic attack on the carbon of the susceptible carbonyl group ('Nucleophilic pathway' figure 11*c'*) or in promoting general base catalysis of the oxygen atom of a water molecule at this carbon atom of the susceptible peptide bond ('General base path' figure 11*c*). Chemical evidence relating to these possibilities is discussed below. Another possible role for Glu-270 may be to enhance the polarization of the susceptible carbonyl group in the sense $Zn^{+}...O^{\delta-}—C^{\delta+}...Glu^{-}$-270.

After its conformational change Tyr-248 is in a position to donate a hydrogen bond to the NH of the susceptible peptide bond of the substrate, and, within the limits of error of the Gly-Tyr difference map, to receive a hydrogen bond from the NH group of the penultimate peptide bond. The hydrogen bond to the N of the susceptible peptide bond tends to make the bonding around this N non-planar. At some point in the reaction the H of this hydrogen bond may be incorporated in the NH_2 group of the product, the OH of tyrosine being regenerated perhaps simultaneously by water. A second possibility is that the second proton of NH_2 comes directly from water, but the geometry of the Gly-Tyr complex does not favour this mechanism for peptides. The system of two hydrogen bonds between Tyr-248 and the two NH groups of the substrate would further induce strain in the substrate. Finally, in the case of the 'nucleophilic pathway' mentioned above, if the proton transfer to the susceptible NH occurs before the anhydride is cleaved, then either the Tyr O^{-} could promote cleavage by water, or incipient OH^{-} formed in the regeneration of Tyr-248 could attack the anhydride directly in a nearly concerted mechanism. Of course, this incipient OH^{-} would not be in equilibrium with the aqueous medium.

One of the most striking effects of the binding of the substrate to CPA is the conversion of the enzyme cavity from a water-filled to a hydrophobic region. At least four water molecules must be expelled when a substrate C-terminal side chain such as Tyr is inserted into the pocket, and one water molecule is displaced from the Zn atom when the carbonyl group of the substrate is bound. Also, the charge of Arg-154 is somewhat neutralized by its interaction with the terminal carboxylate ion of the substrate. Finally, Tyr-248, in making its conformational change, closes off the enzyme cavity so that it is not in equilibrium with the solvent. The Zn atom probably has one positive charge after the substrate is bound so that the conversion of the surroundings of Zn to a hydrophobic region enhances the ability of Zn to polarize the susceptible carbonyl group. Thus the positive charge is brought more to the surface of the enzyme–substrate complex. It is hard to escape the conclusion that the displacement of water upon binding the substrate and the resultant conversion of the active centre of the enzyme to a hydrophobic area provide a driving force for the reaction.

In summary, we regard the attack on the susceptible carbonyl group as either nucleophilic catalysis or general base catalysis, and we remain unsure of the mechanism or timing of proton transfer to the susceptible NH group. However, the roles of Zn in polarizing the susceptible carbonyl group and of Arg-145 in binding the terminal carboxyl group of the substrate are clear. Nevertheless, Glu-270 and Tyr-248 definitely appear to be involved in the catalysis even though their exact functions remain to be clarified. We now turn to some of the chemistry

relating to this proposed mechanism and later consider briefly some of the less probable alternative mechanisms.

The pH–rate profile for peptidase activity is usually bell shaped, with inflexions at pH 6.7 and pH 8.5 (Utsunomiya 1942; Neurath & Schwert 1950; Lumry, Smith & Glantz 1951; Riordan & Vallee 1963). Assuming that the pH–activity relation arises from two groups on the enzyme, Carson & Kaiser (1966) derived pK values of 6.9 and 7.9 for these two residues from a plot of k_{cat}/K_m against pH for the hydrolysis of *O*-acetyl-mandelate. If these values of pK are assigned to Glu-270 and Tyr-248 respectively, both groups titrate very abnormally. However, factors such as a hydrophobic environment (Blake, Johnson, Mair, North, Phillips & Sarma 1967*a*; Rupley 1967), changes in conformation with pH (Oppenheimer, Labouesse & Hess 1960), the proximity of Tyr-248 to Arg-145, the nature of the rate determining step (Bruice & Schmir 1959) and the nature of the substrate (Izumiya & Uchio 1959) may contribute to the effect of pH on activity.

Unfortunately, we cannot at present choose between the nucleophilic and the general base mechanism of attack on the carbonyl carbon. Steric aspects seem to favour the anhydride pathway. The relative orientation and separation (about 0.25 nm) of the carboxyl group of Glu-270 in its position in native CPA and the hydrolysable bond of the substrate are ideal for nucleophilic attack. On the other hand, the placement of water for general base reaction necessitates a re-orientation of the side chain of Glu-270 from its native conformation (the backbone being fixed by the β-structure) in order to facilitate attack by water and in order not to violate closest acceptable distances of approach between various atoms in the enzyme, the water, and the substrate. This re-orientation and positioning of water is not only possible, but does occur when Gly-Tyr is bound to CPA. The failure to observe transpeptidation (Ginodman, Mal'tsev & Orekhovich 1966; but see also Wood & Roberts 1954) or transesterification (Hall & Kaiser 1967) is not a strong argument against an acyl intermediate, because the anhydride intermediate would be readily susceptible to hydrolysis by water. Moreover, the approach of another C-terminal residue for the transfer reaction would be hindered by Tyr-248. Furthermore, it is known that L-phenylalanine and L-β-phenyllactate, products of the respective peptide and ester substrates, are competitive inhibitors (Whitaker, Menger & Bender 1966; Hall & Kaiser 1967). Thus, the binding of the original C-terminal residue would also hinder its ready replacement by a new C-terminal residue.

Although some implications can be drawn, neither deuterium isotope effects nor ^{18}O exchange in a virtual substrate distinguishes unequivocally the nucleophilic attack by Glu-270 from the attack by H_2O. Deuterium substitution effects seen in the hydrolysis by CPA of CBZ-Gly-L-Phe ($k_{H_2O}/k_{D_2O} = 1.22$) (Lumry & Smith 1955; Lumry *et al.* 1951) or of hippuryl-L-Phe (1.1) (F. A. Quiocho, unpublished results 1968) are small when compared to the effects expected in general base catalysis, which often demonstrates two- to fivefold higher rates in H_2O than in D_2O, as seen in the deacylation step with chymotrypsin (Bender, Clement, Kézdy & Heck 1964) and in similar non-enzymic general base catalysed reactions (Bender, Pollack & Neveu 1962). However, the results observed for CPA, which are fully compatible with nucleophilic attack, may be equally well explained in the case of any general mechanism† where the rate determining step does not involve a proton transfer. Furthermore, although catalysis by CPA of the

† Contrary to common usage (Bruice & Benkovic 1966, pp. 27–29), the phrase general base mechanism is meant here to include all pathways where water attacks the susceptible carbonyl carbon even though proton transfer is not the rate limiting step, and the mechanism is actually concerted.

incorporation of ^{18}O from $H_2{}^{18}O$ into the carboxyl group of a virtual substrate such as acetyl-L-phenylalanine displays a bell-shaped pH–rate profile which is not consistent with a simple ortho-acid mechanism (general base) for this exchange (Ginodman *et al.* 1966), this result is not sufficient to distinguish the peptidase mechanisms in view of the presence of several potential catalytic groups for virtual reaction. However, in cases where a similar virtual reaction occurs in other proteolytic enzymes, the kinetics for these reactions have been shown to be in accord with a double displacement reaction mechanism (Vaslow 1958; Inagami & Sturtevant 1964; Sharon, Grissaro & Neumann 1962; Neumann, Levin, Berger & Katchalski 1959; Grissaro & Sharon 1964; Sun & Tsou 1963). Finally, in studies of model compounds involving the participation of neighbouring carboxyl groups in ester and amide hydrolysis, several examples of nucleophilic catalysis have been observed (Bender, Choupek & Neveu 1958; Bruice & Benkovic 1966, pp. 173–186; Fersht & Kirby 1968*a–c*). However, even after a proton is added to the NH group, the resulting amide is a poor leaving group, and a carboxyl group, such as that of Glu-270, is a poor nucleophile. Ordinarily, such a situation would not be a good case for nucleophilic attack (St Pierre & Jencks 1968; Jencks 1969), but we cannot be sure that these arguments are valid in the case of an enzyme where the geometrical disposition of the catalytic groups and the polarization in the enzyme substrate complex are so important.†

The proposed mechanism is also consistent with the kinetic data on longer peptide substrates and suggests why certain of these substrates show maximal hydrolytic rates. Binding of a model polypeptide substrate to the enzyme is most reasonably accomplished by placing it so that its C-terminal side chain is in the pocket, so that it extends along the groove near aromatics Tyr-198 and His(Phe)-279 and so that the carbonyl of the third peptide bond is directed toward Arg-71. With the substrate in this position, a hydrogen bond can be formed between the NH of the penultimate peptide bond and the OH of Tyr-248 (figure 10). There is no other residue of the protein which can form a hydrogen bond to this second NH group. When the amide of the second peptide is substituted or displaced, e.g. when R_1 is sarcosine or β-alanine respectively, the rates of cleavage are 800 to 2400 times slower than the rates for the analogous glycine compounds (Snoke & Neurath 1949; Hanson & Smith 1948). On the basis of these observations, it was proposed that the penultimate NH is either a hydrogen bond acceptor or donor (Snoke & Neurath 1949). Similarly, the rates of cleavage of acetylated amino acids and acetylated dipeptides differ by a factor of about 1000 (Bergmann & Fruton 1942). This effect is not due primarily to variations of K_m, because nearly analogous acetylated amino acids and dipeptides have comparable K_m values within a factor of about 5 (Snoke & Neurath 1949). These data suggest that in the usual substrate an H bond from Tyr-248 to the penultimate amide might produce distortion at the carbonyl carbon of the susceptible peptide. Three types of distortion are possible: (*a*) the N atom of the peptide bond can tend toward a tetrahedral configuration; (*b*) the peptide bond can rotate to become non-planar; and (*c*) the carbon of the peptide bond can become somewhat tetrahedral as the intermediate is formed. When β-alanine is the penultimate amino acid, our model building experiments indicate that its NH group can still be made to form a hydrogen bond to Tyr-248, but the strain in the β-alanyl residue can be taken up by

† An experiment to detect introduction of an ^{18}O terminal atom into Glu-270 of the protein or into the newly formed carboxyl group of the product under single turnover conditions upon hydrolysis of substrates by CPA in $H_2{}^{18}O$ might distinguish a nucleophilic reaction from a general base mechanism, unless cleavage of the anhydride were to occur preferentially at the Zn polarized carbonyl carbon. The ^{18}O-labelling experiments by Kowalsky & Boyer (1960) and by Ginodman *et al.* (1966) do not bear upon this distinction, because at the small enzyme to substrate ratios employed, ^{18}O would necessarily be incorporated in most product molecules.

the methylene carbon. The importance of adjacent peptides has also been demonstrated for chymotrypsin and papain (Kaufman & Neurath 1949; Bender & Turnquest 1955; Schechter & Berger 1967), and the role of strain has been discussed often (Jencks 1966; Lumry 1959).

Another structural alteration in the substrate which produces a large decrease in k_{cat} with little change in K_m is the substitution of a D residue in the third position of a peptide having at least four residues. For example Ala-L-Ala-Ala-Ala and Ala-D-Ala-Ala-Ala have a K_m ratio of 1.1:1 but a k_{cat} ratio of 50:1 (Abramowitz *et al.* 1967), and we find from our model that if the carbonyl group of the third peptide is directed toward Arg-71, then the methyl group of D-Ala (R_2 in figure 10) interferes sterically with Tyr-248 after the conformational change has taken place. The same type of interference can also occur with the substrate CBZ-sarcosyl-L-Phe, which is hydrolysed very slowly (Snoke & Neurath 1949).

(ii) *Other mechanisms*

A 'Zn–hydroxyl' mechanism (Lipscomb *et al.* 1968) now seems substantially less probable than it did earlier, in view of the absence of density in the region that would be occupied by a substrate carbonyl oriented away from Zn, and the greater density in the region of the Zn–(carbonyl) oxygen bond in the new difference electron density map of the complex of Gly-Tyr with CPA. In the 'Zn–hydroxyl' mechanism, the initial binding step has the susceptible CO group oriented with its O atom away from the Zn, which has OH^- or H_2O, depending on the pH, as a fourth ligand. Possible further steps of the reaction, and the steric problems associated with this mechanism have been discussed (Lipscomb *et al.* 1968), but the characteristics of this proposal are as follows: (*a*) The mechanism is consistent with the lack of a strong effect of Zn removal on the binding of peptides (Coleman & Vallee 1962). In the case of ester substrates, metal substitutions have less effect on catalysis than in the case of peptides (Vallee, Riordan & Coleman 1963); this 'Zn–hydroxyl' mechanism may therefore apply to the hydrolysis of certain esters (Lipscomb *et al.* 1968). (*b*) The pK values of 6.9 and 7.2, characteristic of the free enzyme and of the enzyme–substrate complex, and obtained respectively from the ascending limbs of the K_{cat}/K_m against pH and k_{cat} against pH curves for the hydrolysis of acetyl mandelate (Carson & Kaiser 1966), could be due (as one possibility) to the ionization $Zn(OH_2)^+ = ZnOH + H^+$ (Verpoorte, Mehta & Edsall 1967). (*c*) The low activity or lack of activity of CPA toward substrates in which the H atom of the susceptible peptide NH is replaced by a larger group (Stahmann *et al.* 1946; Smith 1948) is explained in the case of this mechanism by a resulting steric repulsion of this group by the Zn or OH^- on the Zn. However, CBZ-Trp-Pro is cleaved by CPA (Smith 1948) in a reaction which could not proceed by this mechanism. (*d*) The Zn would be neutral, being bonded to Glu-72 and an OH^-, as well as to His and Lys (or Glx). (*e*) This mechanism is similar to a class of mechanisms now under active consideration for carbonic anhydrase (Riepe & Wang 1968; Smith 1949*a*; Davis 1958; Coleman 1967; Whitney, Nyman & Malström 1967). A determination of whether or not water or hydroxide is displaced from the metal upon substrate binding would be helpful in testing this proposed mechanism. Already nuclear magnetic resonance techniques have demonstrated displacement of water or hydroxide from the metal when the inhibitor β-phenylpropionate is bound to MnCPA (Shulman, Navon, Wyluda, Douglass & Yamane 1966).

Proposals have been made earlier for the mechanism of action of CPA. After the necessity of the presence of a metal for CPA activity was demonstrated (Hanson & Smith 1948), Smith (1949*b*) and Lumry & Smith (1955) suggested initial binding of both the free carboxyl group

and the carbonyl group of the scissile bond to the metal. However, we find that the binding site of the substrate's carboxyl group is Arg-145 in the Gly-Tyr complex, and hence we do not believe that the suggested binding of carboxyl to Zn is present in peptide hydrolysis.

Use of one of the Zn ligands, then thought to be Cys, as a nucleophile (Williams 1964) requires enormous motion of the protein backbone in the case of His-69 or Glu-72, or gross distortion of the peptide or anhydride intermediate in the case of Lys- or Glx-196. While we cannot rigorously rule out the involvement of Lys- or Glx-196 in later steps, there is no evidence from our map of the Gly-Tyr complex that such motion occurs in the binding stage. Other possible mechanisms which might involve groups some distance away from the Gly-Tyr location have been considered, but are unlikely (Lipscomb *et al.* 1968).

The extremely general proposal (Vallee, Riordan & Coleman 1963; Vallee 1964) that a proton donor and a nucleophile are required for peptide hydrolysis are features which are to be expected in enzymatic hydrolysis of peptides. In this proposal, the Zn had four bonds, two to the protein (Cys and N-terminus) and two to the substrate (the N atom of the NH and the O atom of the CO, both of the susceptible peptide bond). The suggestion that an NH can be bound to the enzyme seems first to have been made by Balls & Köhler (1930–31). Since modification of CPA by acetylation, iodination, or photo-oxidation decreased the peptidase activity, the suggestion was made that in these reactions the nucleophile was modified (Vallee *et al.* 1963; Vallee 1964). The nature of these modification reactions appeared to implicate either tyrosine or histidine. However, there is only one tyrosine near the susceptible peptide bond of bound Gly-Tyr, and we identify that critical tyrosine as residue 248. We have discussed above various roles for Tyr-248, but in no case do we believe it to be the nucleophile. Concerning a possible role for histidine in the catalysis, Sokolovsky & Vallee (1967) have suggested that His plays a role in either catalysis or binding of peptide substrates, or both. However, the X-ray sequence of the active site region shows that apart from the zinc ligand, His-69, the only other nearby histidine residue is His(Phe)-279, which is far removed (about 0.7 nm) from the glycyl-tyrosine binding site (figure 10). We consider it unlikely that either of these residues is directly involved in the activity, but of course modification of the Zn ligand, His-69, is likely to produce alterations of the enzymatic activity.

(iii) *Active group modifications*

Chemical modifications of the two tyrosine residues which produce changes in the activity of CPA have formed an elegant series of studies from Vallee's group. Study of acetylated CPA indicates the modification of two tyrosine residues which are protected when acetylation is carried out in the presence of the inhibitor β-phenylpropionate (Simpson, Riordan & Vallee 1963). One of these tyrosines can be selectively nitrated by tetranitromethane (Riordan, Sokolovsky & Vallee 1967) and the other can be modified by a small excess of 5-diazonium-1*H*-tetrazole (Sokolovsky & Vallee 1967). In agreement with these data, we find only two tyrosine residues, 248 and 198, in the general area of the active site. Crystallographic studies at 0.6 nm resolution have shown that β-(*p*-iodo)-phenylpropionate binds to CPA at three major sites (Steitz *et al.* 1967). Now that the structures of the native enzyme and of the Gly-Tyr complex are known it seems likely that two phenylpropionate molecules are required to protect both tyrosines.† Binding of a phenylpropionate molecule in the pocket induces the large

† We therefore now believe, contrary to our previous suggestion (Steitz *et al.* 1967), that β-iodo-phenylpropionate can simultaneously occupy both sites.

conformational change of Tyr-248 and accounts for its protection. The second phenylpropionate binding site is near enough to Tyr-198 to account directly for its protection. Nitration destroys peptidase activity while mild diazotization does not, and we therefore suggest in accord with the roles assigned to Tyr-248 in our mechanism that it is Tyr-248 which is nitrated and Tyr-198 which is diazotized. Retention of peptidase activity and an increase in esterase activity occur when CPA is acetylated in the presence of Gly-L-Phe (F. A. Quiocho 1968, unpublished results). Since there is only one dipeptide binding site, only Tyr-248 can be protected. These results confirm our conclusion that of the two tyrosines only Tyr-248 is functional in the catalytic step for peptides. In these experiments, however, substrate inhibition of esterase activity is alleviated, suggesting that Tyr-198, although too far from the bound substrate to be directly involved in the reaction, is involved in the mechanism of substrate inhibition. These results are also consistent with the effects of mild diazotization with 5-diazonium-1*H*-tetrazole.

Subsequent to our suggestion that Arg-145 and Glu-270 are involved in the activity of CPA, Vallee & Riordan (1968) succeeded in producing modification of enzymic activity by reacting arginine groups in CPA with diacetyl and carboxyl groups with cyclohexylmorpholinoethyl carbodiimide.

(iv) *Inhibition and activation*

Carboxypeptidase A is known to exhibit a variety of kinetic anomalies, such as inhibition and activation by certain substrates. Substrate inhibition has been demonstrated both for ester substrates, e.g. hippuryl-L-β-phenyllactate (HPLA) (Snoke & Neurath 1949; McClure, Neurath & Walsh 1964) and hippuryl-L-mandelate (Awazu, Carson, Hall & Kaiser 1967) and for peptide substrates, e.g. CBZ-Gly-Phe (Lumry *et al.* 1951). However, neither hippurylphenylalanine (Whitaker *et al.* 1966) nor acetylated aromatic α-amino or α-hydroxy acids (Snoke & Neurath 1949) exhibit substrate inhibition. Substrate activation is exhibited by CBZ-Gly-Phe (Whitaker *et al.* 1966) and by hippuryl-glycolate (Kaiser, Awazu & Carson 1964). In addition, amino acid products inhibit (Utsunomiya 1942) and acylamino acid products, e.g. CBZ-Gly (Whitaker *et al.* 1966), activate the enzyme. It is noteworthy that these kinetic anomalies have been associated almost exclusively with dipeptide or analogous ester substrates or products which have aromatic *N*-acyl groups.

Model building experiments have so far elicited two abortive modes of binding for substrates which may result in substrate inhibition. In both of these cases the productive binding site which we have deduced from the Gly-L-Tyr crystallographic experiment is occupied and substrate-protein interaction is maximized. Furthermore, in generating these models we have assumed that Tyr-198 is associated with substrate inhibition (see above), and we have been aware that only *N*-acyl dipeptides or their ester analogues with both the *N*-acyl and C-terminal R groups aromatic have so far been shown to exhibit substrate inhibition. The first abortive binding mode has the substrate (CBZ-Gly-L-Phe in figure 12*a*) shifted with respect to the productive mode, so that the C-terminal carboxyl group is bound to the Zn atom, the C-terminal side chain partly extends into the hydrophobic pocket, the aromatic acyl group has a favourable π–π interaction with Tyr-198, and the second peptide carbonyl is bound to Arg-71. The second mode of binding (figure 12*b*) has the substrate reversed so that the aromatic acyl group is in the pocket which accommodates the C-terminal side chain in productive binding. The terminal carboxyl group of the substrate can then bind to Arg-71, and the C-terminal side chain is near Tyr-198. So far we have been unsuccessful in devising a crystallographic experiment to

demonstrate either of these abortive binding modes. The shifted substrate position does not allow as much protein–substrate interaction as does the reversed one, and should be less specific since it admits binding of inhibitors of any length. In both abortive binding modes it is important that the *N*-acyl group or the C-terminal residue respectively be aromatic or large aliphatic in

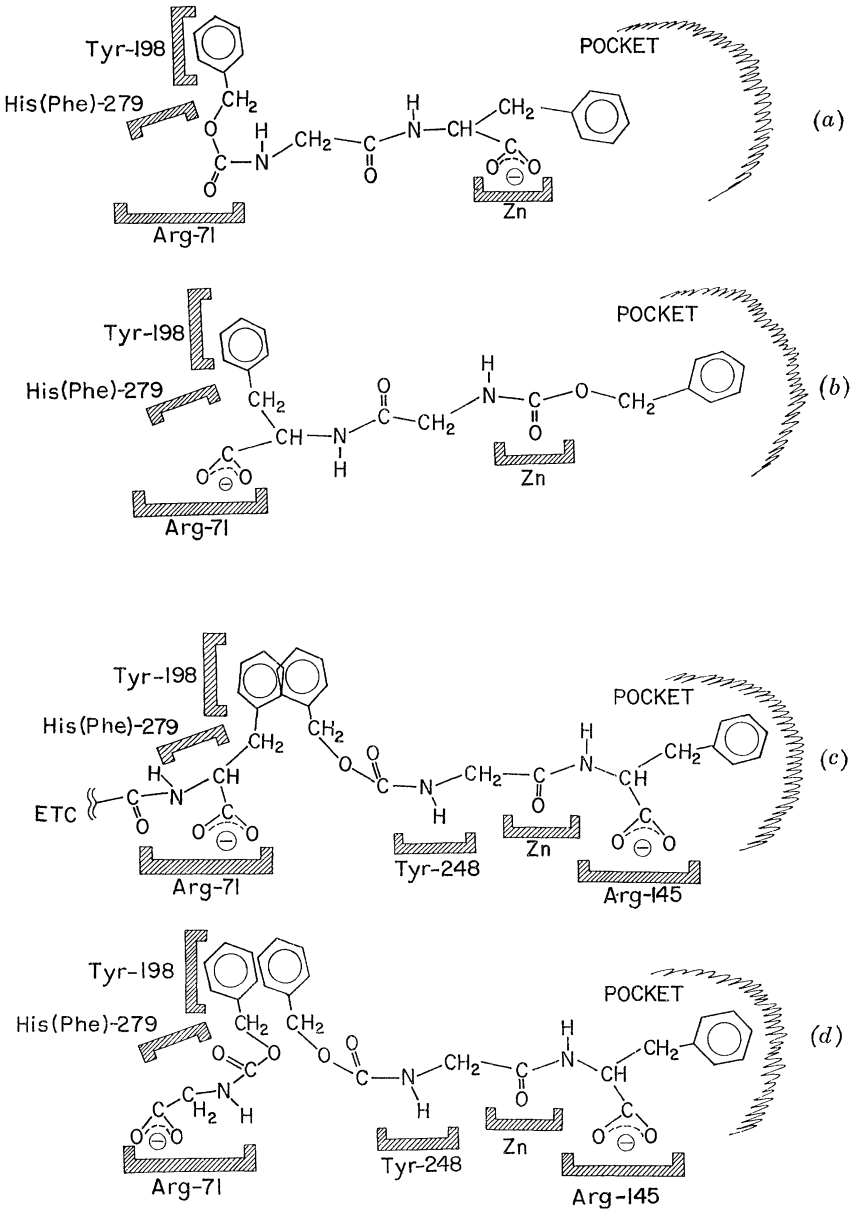

FIGURE 12. Proposed modes of binding of substrate molecules showing: (*a*) displaced binding, (*b*) reversed binding for CBZ-glycylphenylalanine, (*c*) binding of two substrate molecules giving rise to inhibition which would not be competitive, and (*d*) binding of a substrate and of a product molecule in an activation process.

order to have a favourable hydrophobic interaction with Tyr-198. In the case of short substrates, such as acyl amino acids and similar esters, which do not show substrate inhibition, there would be less favourable binding in either of these two abortive modes. For example, in shifted binding the *N*-acyl group would not be near Tyr-198, and in reversed binding the *N*-acyl group would no longer extend into the pocket. Of course the two binding modes discussed here do not

preclude other possibilities, and indeed yet another mode must be involved in order to satisfy the kinetic data (Snoke & Neurath 1949; Lumry *et al.* 1951; Whitaker *et al.* 1966; McClure *et al.* 1964; Quiocho & Richards 1966), which imply binding of more than one substrate molecule in order to inhibit one active centre (Thoma & Koshland 1960). One way to bind two substrate molecules is suggested in figure 12*c*, which shows a second molecule bound farther down the groove in a way that could interfere with precise placement of the first molecule in the active site for hydrolysis. In disagreement with kinetic analyses which suggest that as many as 3 to 5 molecules of CBZ-Gly-Phe (Lumry *et al.* 1951) or HPLA (Dennard & Williams 1960) are bound, we find that the binding region is probably limited to two substrate molecules of this size.

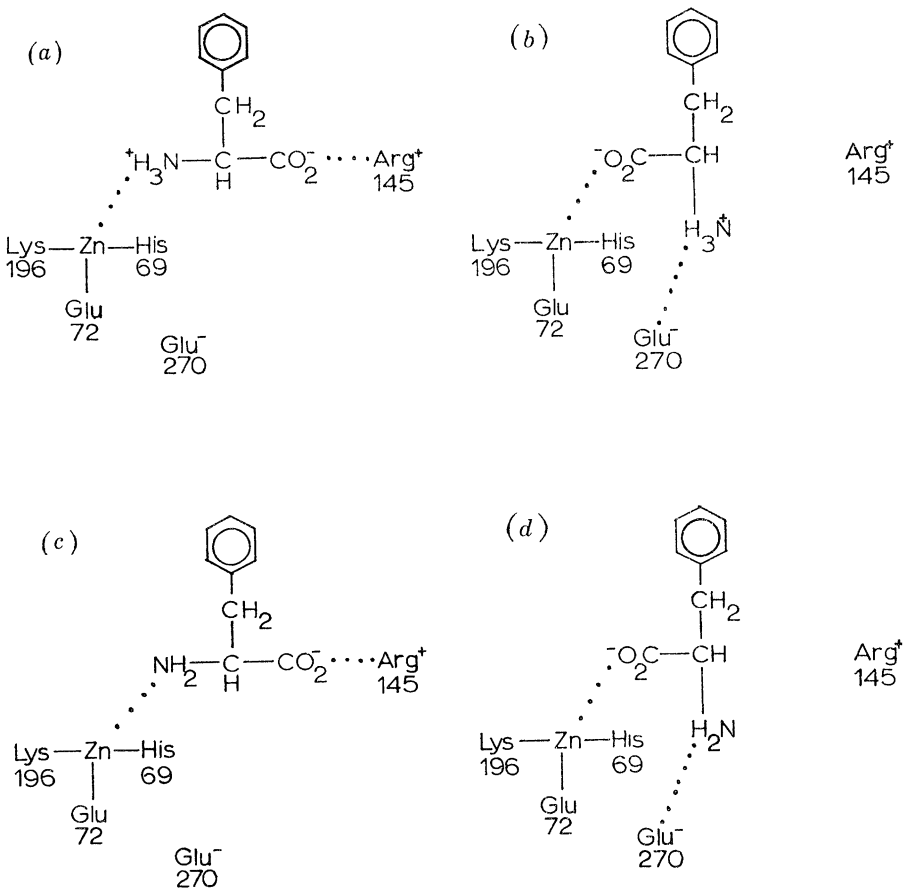

FIGURE 13. Probable binding of (*a*) L-Phe at pH 7.5, (*b*) D-Phe at pH 7.5, (*c*) L-Phe at pH 9.0 and (*d*) D-Phe at pH 9.0. Inhibition (*a*) is one-eighth that of (*b*), and dependent upon phosphate buffer concentration, but at pH 9 L-Phe is about as effective as D-Phe. The binding at (*a*) with the CO_2^- on Arg-145 and the H_3N^+, toward $(ZnL_3)^+$, where L is a Zn ligand, is suggested by the occurrence of the Tyr-248 conformational change in an X-ray study at 0.6 nm resolution of the binding of L-Phe at pH 7.8.

The cluster of side chains composed of Arg-71, Tyr-198, and His(Phe)-279 which is located farther down the cleft may also be used to explain substrate and product activation. If CBZ-Gly, which does activate the enzyme (Whitaker *et al.* 1966) is placed so that the free carboxyl group is bound to Arg-71, then the phenyl ring can interact favourably with Tyr-198 and with the phenyl ring of a productively bound substrate molecule (see figure 12*d*). Similar interactions occur when a second molecule of hippurylglycolate or CBZ-Gly-Phe is bound. Substrates long enough to cover the Arg-71–Tyr-198 region are then not expected to exhibit any of the above

anomalous binding modes. In fact, the data which are presently available indicate that substrates longer than acyl dipeptides are not subject to substantial substrate inhibition or to product activation (Abramowitz *et al.* 1967; Auld 1968).

Competitive inhibition by products such as L-phenylalanine has been found in the CPA-catalysed hydrolysis of CBZ-Gly-L-Phe at pH 9.0 (Neurath & DeMaria 1950). This inhibition is primarily due to the anionic form of L-phenylalanine. Recently, however, it has been shown that L-Phe is a competitive inhibitor of CPA even at pH 7.5 (Whitaker *et al.* 1966). Moreover, L-Phe has been shown crystallographically to bind only in the pocket, and this binding is accompanied by a structural change presumably involving Tyr-248 (Steitz *et al.* 1967). The detailed structural interpretation from model building of the binding of L-Phe places the α-COO^- near Arg-145. D-phenylalanine, on the other hand, inhibits competitively and more effectively at pH 7.5 than at pH 9.0 (Neurath & DeMaria 1950; Elkins-Kaufman & Neurath 1949). This finding has been interpreted by assuming that the charged α-amino group of D-Phe, unlike that of L-Phe, is oriented near a negative group of the protein. Accordingly, our structural interpretation of the binding of D-Phe, based upon model building only, places the α-COO^- near Zn (Dennard & Williams 1960), and the charged α-amino group near Glu-270 (see figure 13). The loss of a proton from the α-NH_3^+ at pH 9.0 would then result in less effective binding.

The strictly competitive inhibition by β-phenylpropionate in the region of equimolecular inhibitor:CPA binding would be analogous to that depicted for L-Phe. However, mixed inhibition, competitive and non-competitive (or uncompetitive), by β-phenylpropionate has also been found (Lumry & Smith 1955) and is probably related to the ability of this inhibitor to bind in two meaningful sites, as demonstrated by X-ray crystallography.

(v) *Esterase activity and metals*

It has long been known that CPA possesses esterase as well as peptidase activity (Snoke & Neurath 1949). The study of the hydrolysis of esters, in particular with the substrate HPLA, has shown differences from the hydrolysis of corresponding peptide substrate analogues. These differences are:† (1) the values of k_{cat} for the esters and peptides are within a factor of five of one another, but the K_m of the esters are 20- to 110-fold less than those of the peptides (Snoke & Neurath 1949; Whitaker *et al.* 1966); (2) tyrosine modifications (Simpson & Vallee 1966; Riordan *et al.* 1967; Vallee 1967) which result in inactivation of peptide hydrolysis do not abolish esterase activity but rather diminish substrate inhibition (for example Whitaker *et al.* 1966); and (3) the pH–rate profile which has apparent points of inflection at pH 6.7 and 8.5 in peptidase activity is replaced in esterase activity by a pH–activity profile which shows an initial rise between pH 5.5 and 7.0, a plateau between 7.0 and 9.0, and a rapid second rise reaching a maximum at pH 10.5 (Riordan & Vallee 1963). The last two observations have led Vallee and co-workers to propose that peptides and esters are hydrolysed by different mechanisms (Vallee *et al.* 1963; Vallee 1964). Specifically, it was proposed that the nucleophilic group B which is required for peptide hydrolysis is not necessary for ester hydrolysis and that a hydroxide ion is the sole attacking group. In order to account for the differences given above for ester and peptide hydrolysis, it has been further proposed that esters have a locus of binding different from that of peptides (Vallee 1967). On the other hand, Bruice & Benkovic (1966, p. 5) have objected to the proposed role of OH^- as nucleophile, on the grounds that the observed increase in rate of

† Although further studies are necessary, it also appears that the isotope effect k_{H_2O}/k_{D_2O} is in the range of 2.0 to 2.5 for HPLA (F. A. Quiocho 1968, unpublished results; B. L. Kaiser & E. T. Kaiser 1968, private communication), substantially greater than the effect for certain peptide substrates.

esterase activity with a thousandfold increase in hydroxide ion concentration is only minimal or non-existent in the pH range of interest.

Unfortunately we have not as yet succeeded in preparing crystals of CPA complexes with ester substrates. However, we present the following observations, which are derived mostly from model building experiments. The only obvious difference between analogous peptides and esters is the steric and electronic configuration about the ester oxygen. The fact that peptide nitrogen can be replaced by oxygen in corresponding active substrates suggests that the essential point of substrate–enzyme interaction at the sensitive bond is the carbonyl group of the peptide or ester. The very nature of the active site especially with respect to its shape, the location of the zinc, and the probable binding and catalytic groups, combined with the characteristics of ester substrates which are most efficiently hydrolysed, severely limits the possible productive modes of binding. Since most of the requisites of substrate and enzyme, i.e. L configuration, C-terminal aromatic group, penultimate peptide (Snoke & Neurath 1949), and presence of a metal (Coleman & Vallee 1962) for peptide substrates are also found in the case of analogous ester substrates, we expect that the most favourable productive binding mode for ester substrates is in its essential interactions that depicted for peptides. However, the tyrosine modification experiments demonstrate that Tyr-248 need not necessarily participate catalytically in hydrolysis of some ester substrates, e.g. HPLA.

There is a class of short ester substrates, including *O*-acetyl-L-mandelate, whose pH–activity characteristics differ from those of HPLA. The pH dependences of K_{cat}, k_{cat}/K_m, and the activity of CPA toward the hydrolysis of *O*-acetyl-L-mandelate are all qualitatively similar to the pH–activity curves for CBZ-Gly-L-Phe and suggest a common mechanism for both this ester and peptides (Carson & Kaiser 1966). This analogy is further substantiated by the finding that acetylated CPA is inactive toward the hydrolysis of *O*-acetyl-L-mandelate (F. A. Quiocho 1968, unpublished results). It would seem essential for *O*-acetyl-L-mandelate and similar short ester substrates, such as *O*-acetyl-DL-β-phenyllactate (Ogilvie, Riordan & Vallee 1963) which behave like peptides to bind like peptides.

Our comments on metal substitution are given previously (Lipscomb *et al.* 1968), and we therefore here refer only to the experimental result that when Hg replaces Zn the Hg atom is at a position shifted about 0.1 nm along the *a* axis (away from the pleated sheet) relative to the original Zn position. Detailed studies of the bonding geometries in the various metal substituted CPA (Vallee *et al.* 1963; Coleman & Vallee 1960) are required in order to provide additional physical data relating to the interesting chemical properties of these metal substituted forms of the enzyme.

5. Summary

The positions of the atoms in the CPA crystal structure have been deduced, based on a tentative amino acid sequence drawn up by us from the known N-terminal sequence, our location on the structure of several chemically sequenced fragments, and X-ray identifications of the remaining 93 amino acids. The resulting atomic coordinates have been used in a structure factor calculation. Even though 5.3 % of the protein atoms and all of the solvent molecules were omitted from the structure factor calculation, the standard crystallographic R factor is 0.44. The degree of perfection of the helices (about 35 % of the structure) and of the β structure (about 20 % of the structure) has been examined. The relation between primary and secondary structure has been considered.

A new function, with Fourier coefficients $(|F_{ES}| - x|f_c| - (1\text{-}x)|F_N|) \exp(i\phi_{SF})$, has been used to prepare a difference electron density map of the complex of Gly-Tyr with CPA. This map shows that (1) the C-terminal side chain of the substrate is inserted into a pocket of the enzyme; (2) the substrate's C-terminal carboxylate ion interacts with Arg-145; (3) the carbonyl group of the susceptible peptide bond is bound to the Zn atom; and (4) in an interaction restricted to dipeptides the terminal amino group of the substrate interacts with the carboxylate of Glu-270 via water. As a result of substrate binding Arg-145 moves 0.2 nm in order to bind the substrate's carboxylate ion. This motion of Arg-145 is coordinated with the 1.2 nm motion of the phenolic oxygen of Tyr-248 which brings Tyr-248 from the exterior of the molecule to a position where it can participate in catalysis. In addition, Glu-270 moves 0.2 nm away from the scissile bond toward the terminal amino group of the substrate. Arg-145 and Glu-270 have been identified only by inspection of the X-ray electron density map but Tyr-248 is contained in a tetrapeptide which was isolated by Roholt & Pressmann (1967) and located in the sequence by us.

Based on the information gained from the Gly-Tyr complex, the placement of substrates in the three-dimensional model of CPA and the wealth of chemical data in the literature we have made several deductions concerning the mechanism of action of CPA. In the active complex we believe that Arg-145 binds the terminal carboxylate ion of the substrate while the C-terminal side chain is inserted into the enzyme's pocket. The carbonyl group of the scissile peptide bond binds to Zn and is consequently polarized thereby rendering its carbon more susceptible to nucleophilic attack. Longer substrates have the carbonyl groups of the third and fourth peptide bonds directed toward Arg-71 and side chains of R_2, R_3 and R_4, especially if they are aromatic, located in the hydrophobic region of Tyr-198 and His(Phe)-279.

The displacement of most of the water molecules from the pocket of the enzyme and from the region near the zinc atom as well as the movement of Tyr-248 into the region of the substrate make the environment of the substrate hydrophobic in nature. Tyr-248, after its conformational change, is in an appropriate position to form two hydrogen bonds with the substrate. The first is donation to the N of the susceptible peptide bond, and the second is reception of H from the second peptide bond of the substrate. At some point in the reaction Tyr-248 may actually donate H to the substrate and be regenerated by H_2O. Glu-270 either acts as a nucleophile to attack the carbonyl carbon of the susceptible bond directly or induces the attack by water in a general base mechanism. Structurally, it is necessary that the conformational change of Tyr-248 be reversed before the product amino acid can leave the enzyme's pocket and another substrate molecule can be bound.

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Note added in proof. (18 August 1969): The sequence of CPA has now been determined chemically and reported in part (H. Neurath, R. A. Bradshaw & R. Arnon, International Union of Biology, Symposium on Structure–function relationships of proteolytic enzymes. Abstracts, Copenhagen, 1969). The X-ray identifications of the critical residues Arg-145, Tyr-248, and Glu-270 have been confirmed, and no changes are required in the mechanistic conclusions of this paper. The most important change in our identifications occurs at Zn ligand residue 196, which is found to be histidine. Also, in table 8 residue 166 should be deleted, and 240 and 265 should be added.

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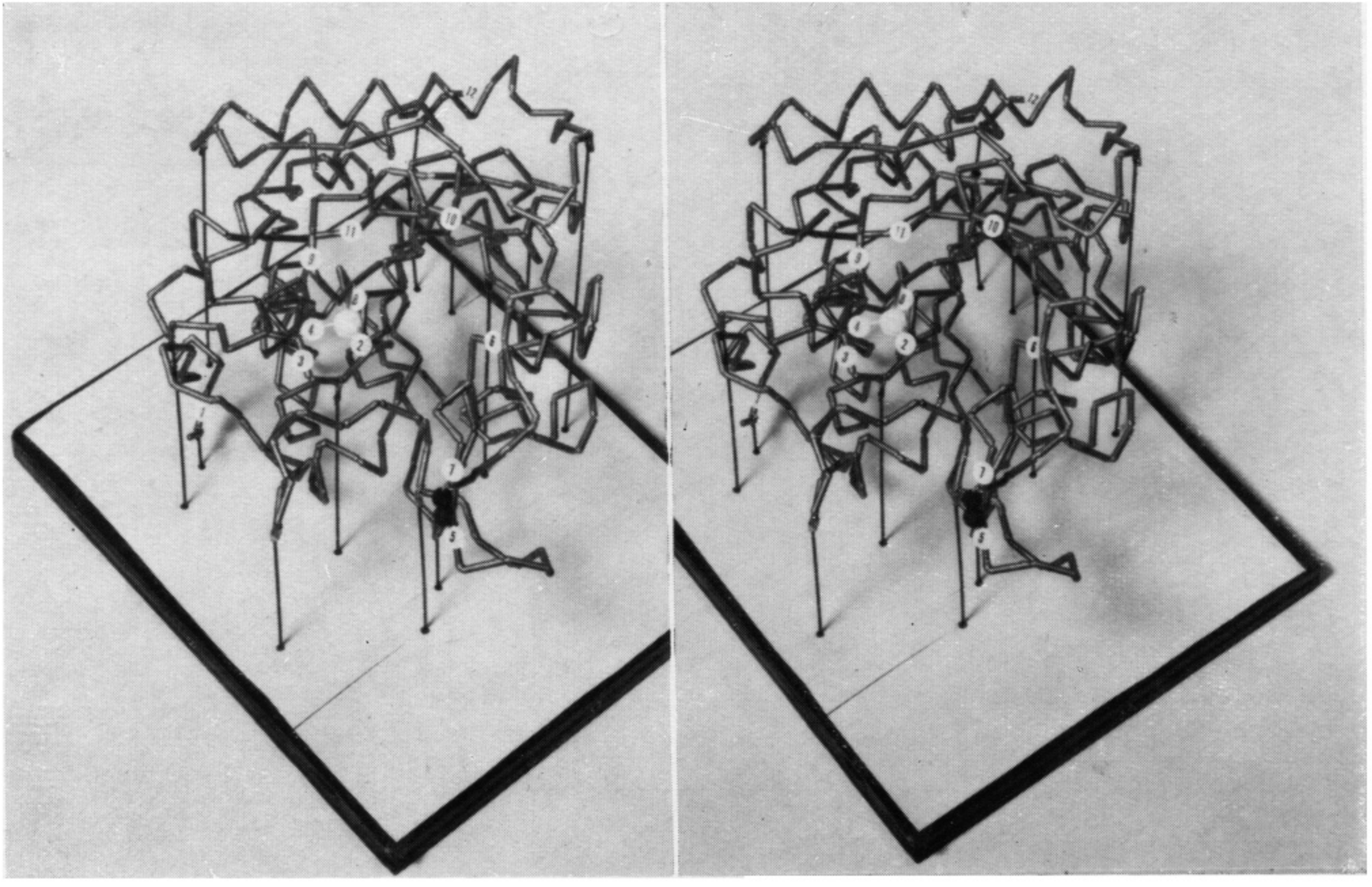
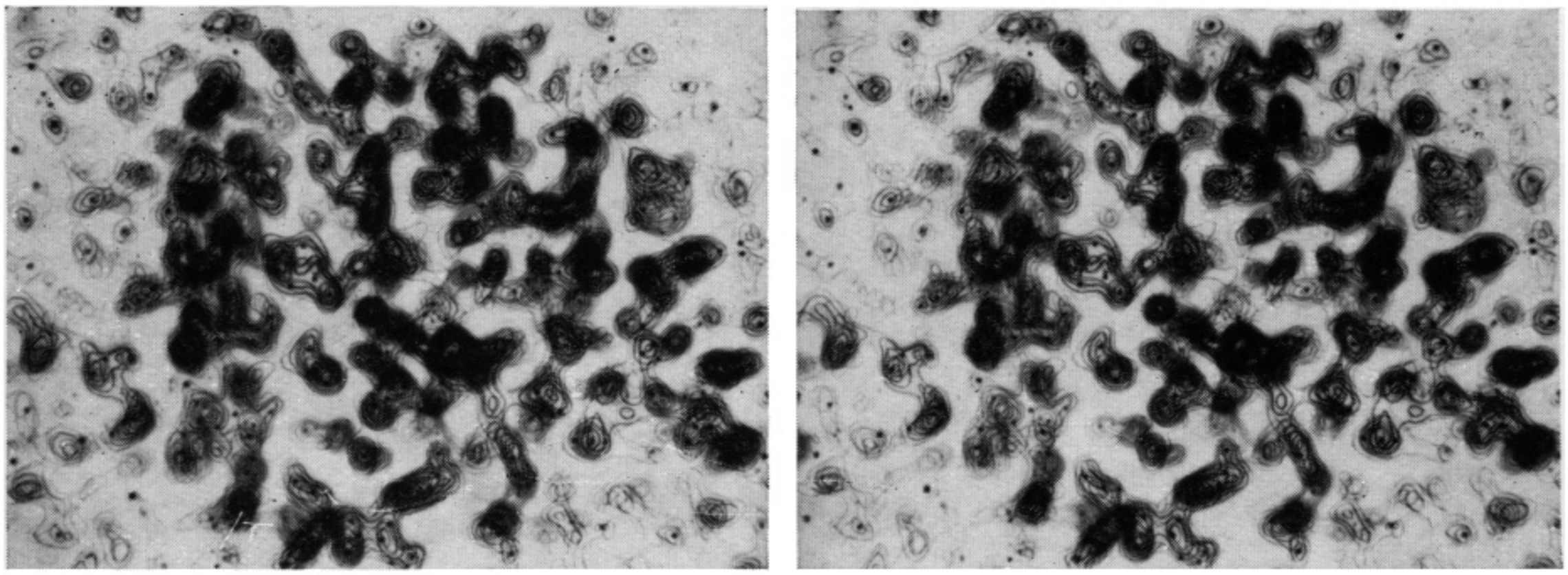


FIGURE 1. For legend see p. 189.

(*a*)



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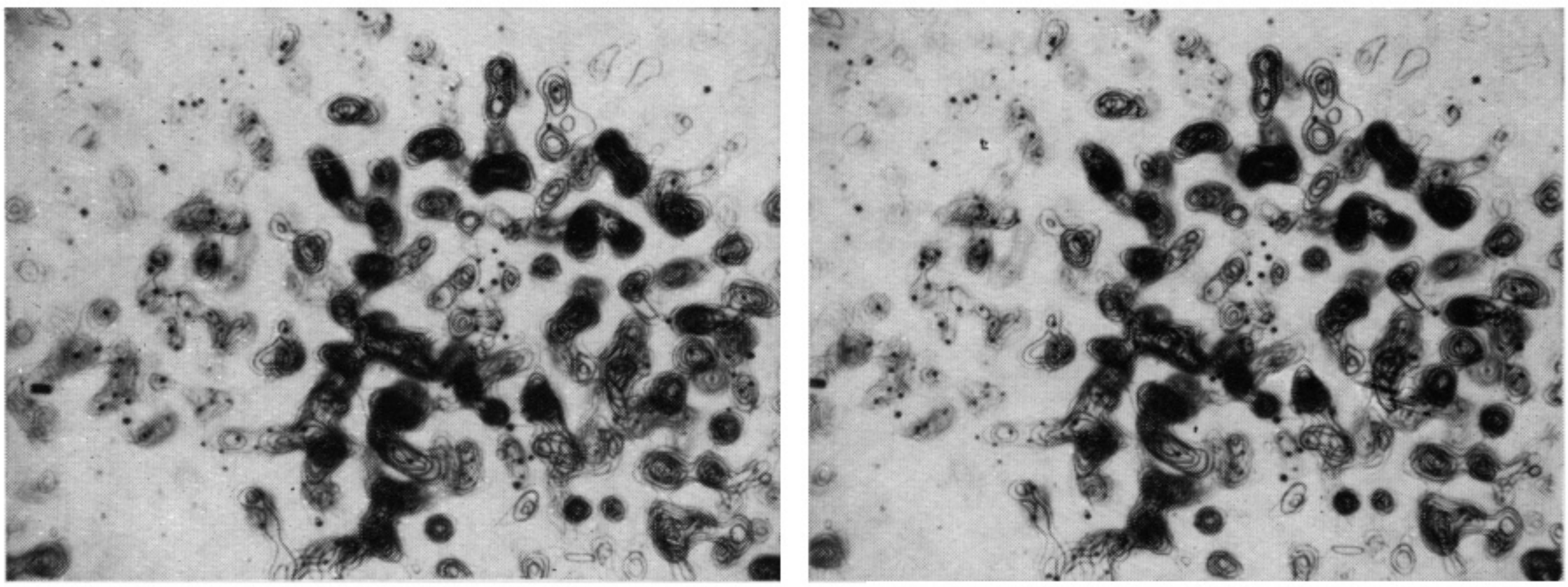

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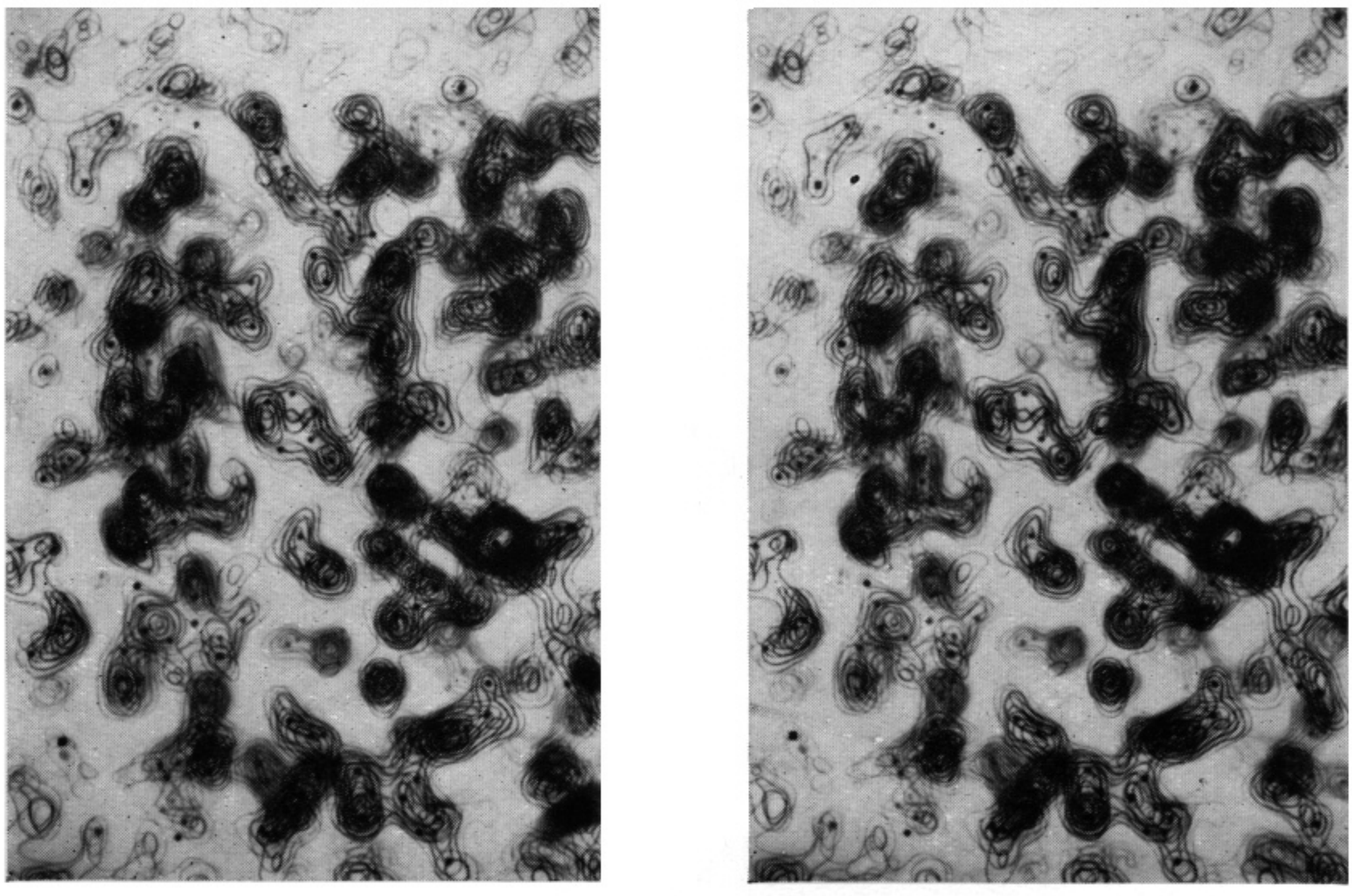
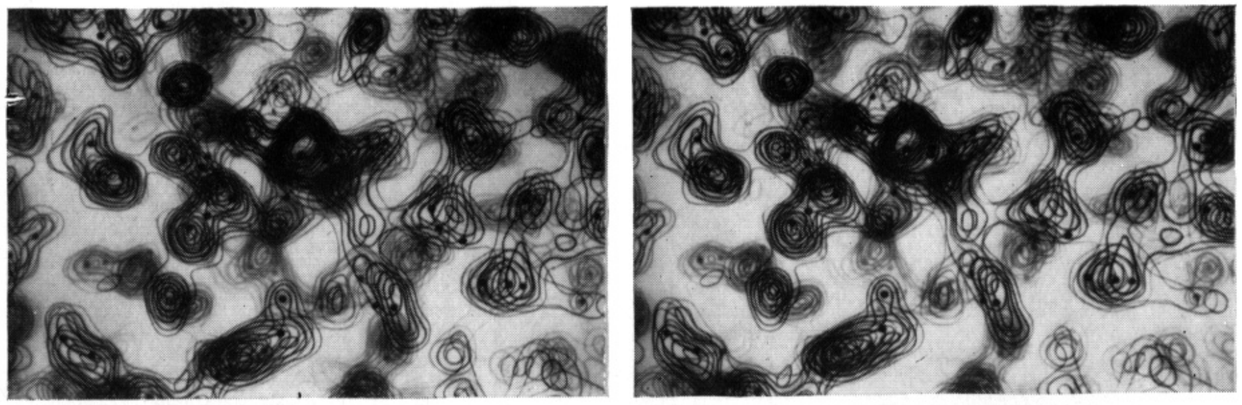
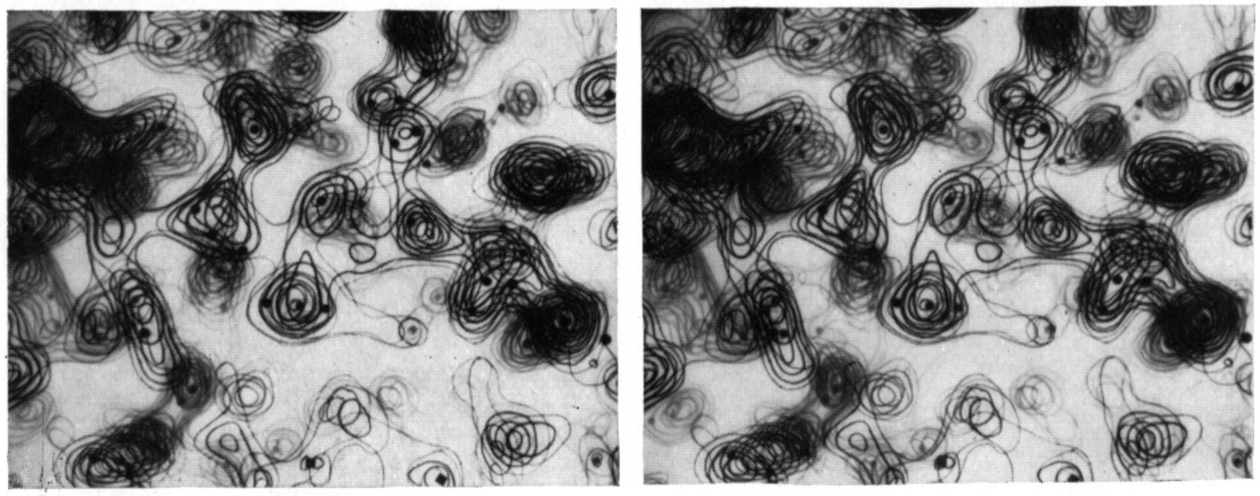


FIGURE 3 *a*, *b*, *c*. For legend see p. 189.

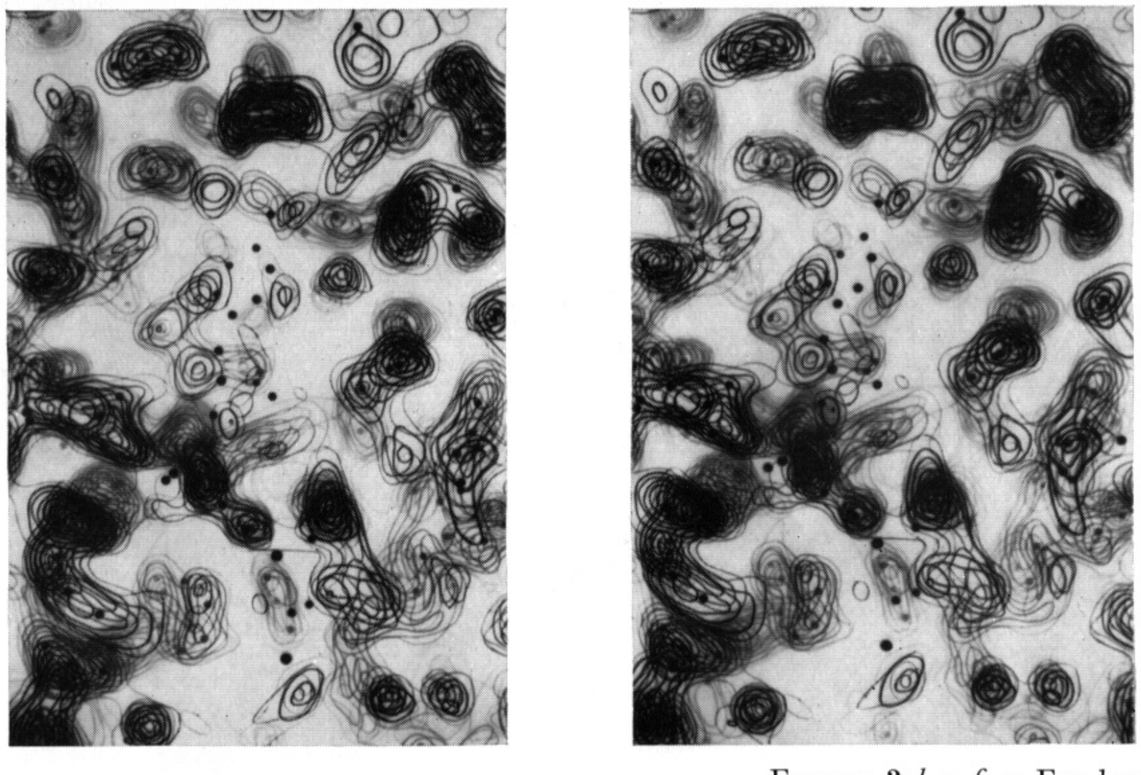
(*d*)

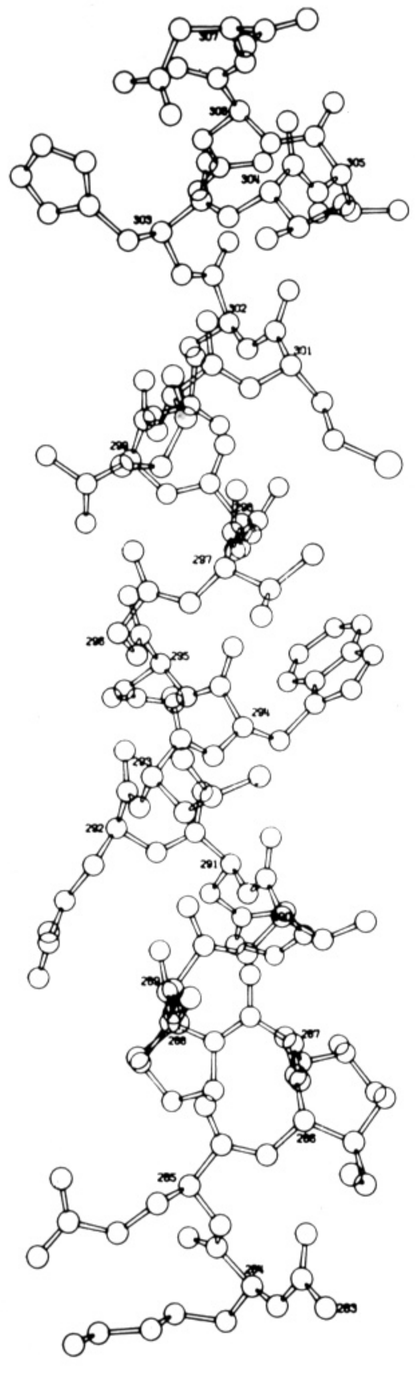


(*e*)



(*f*)





(*g*)

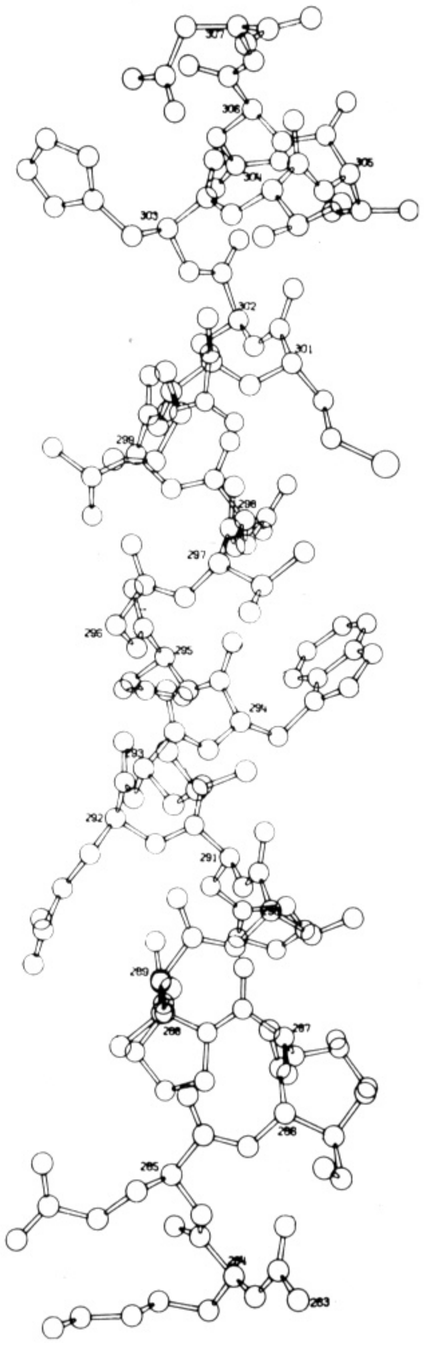
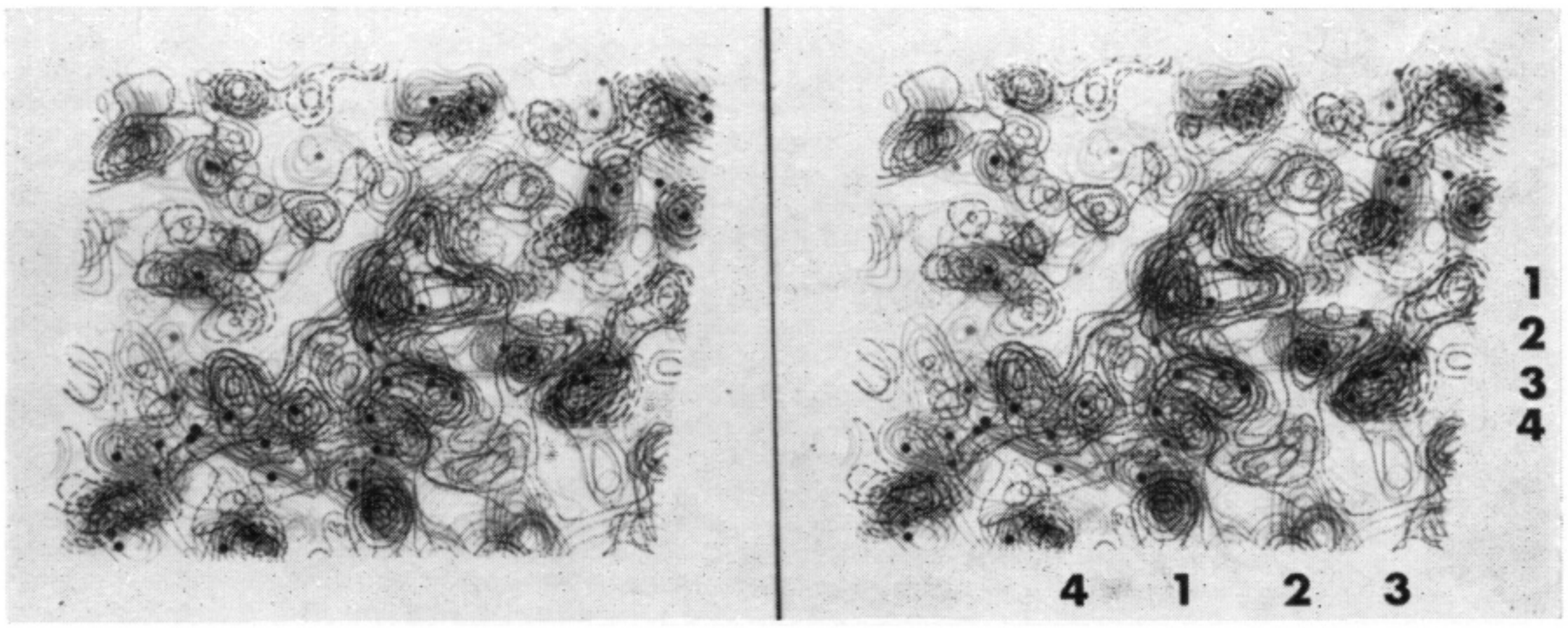


FIGURE 3 *d*, *e*, *f*, *g*. For legend see facing page.

(*a*)



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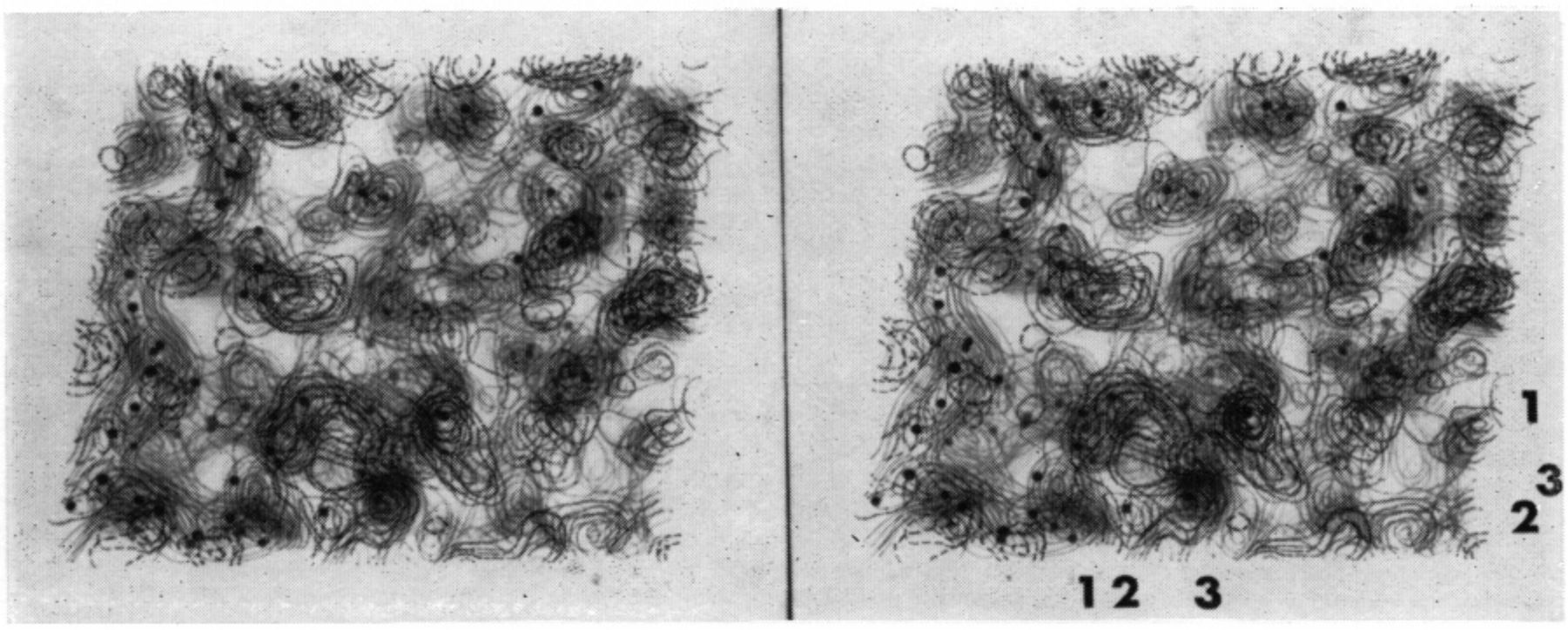

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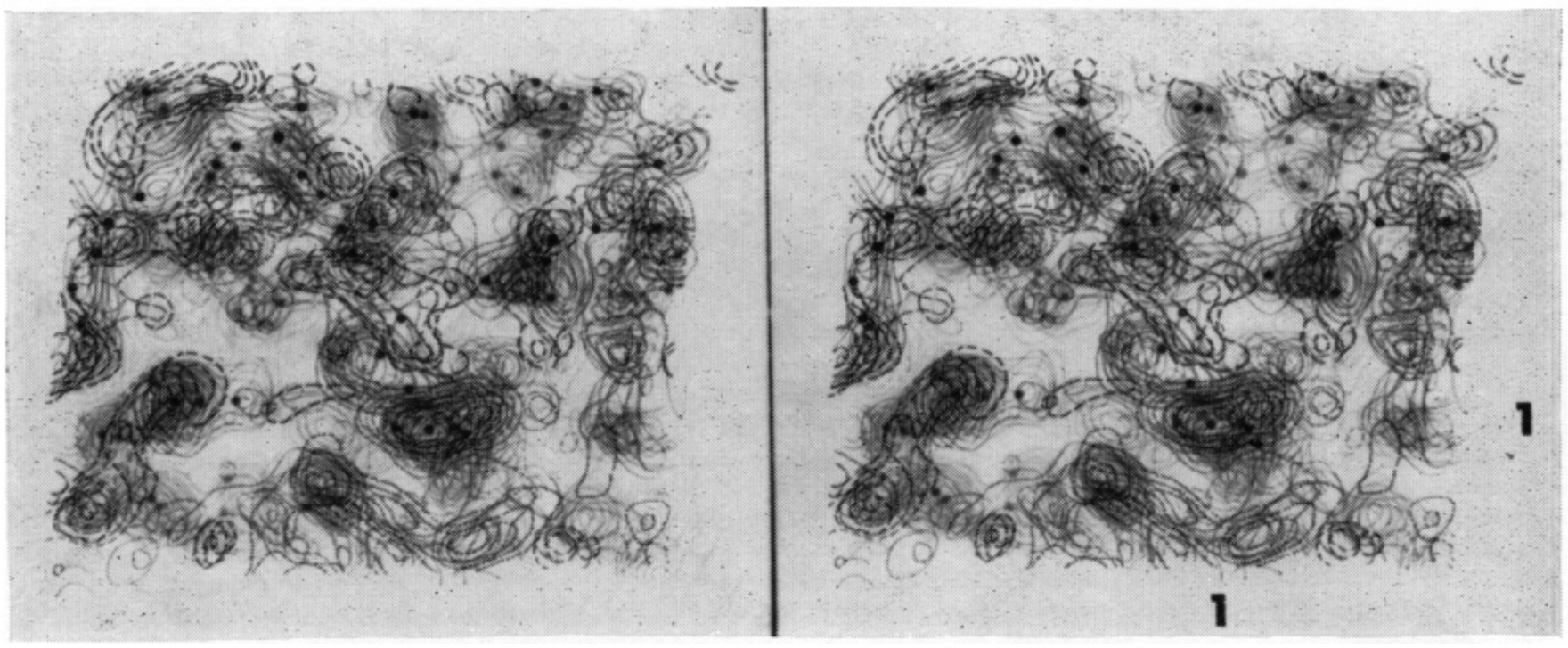


(*d*)

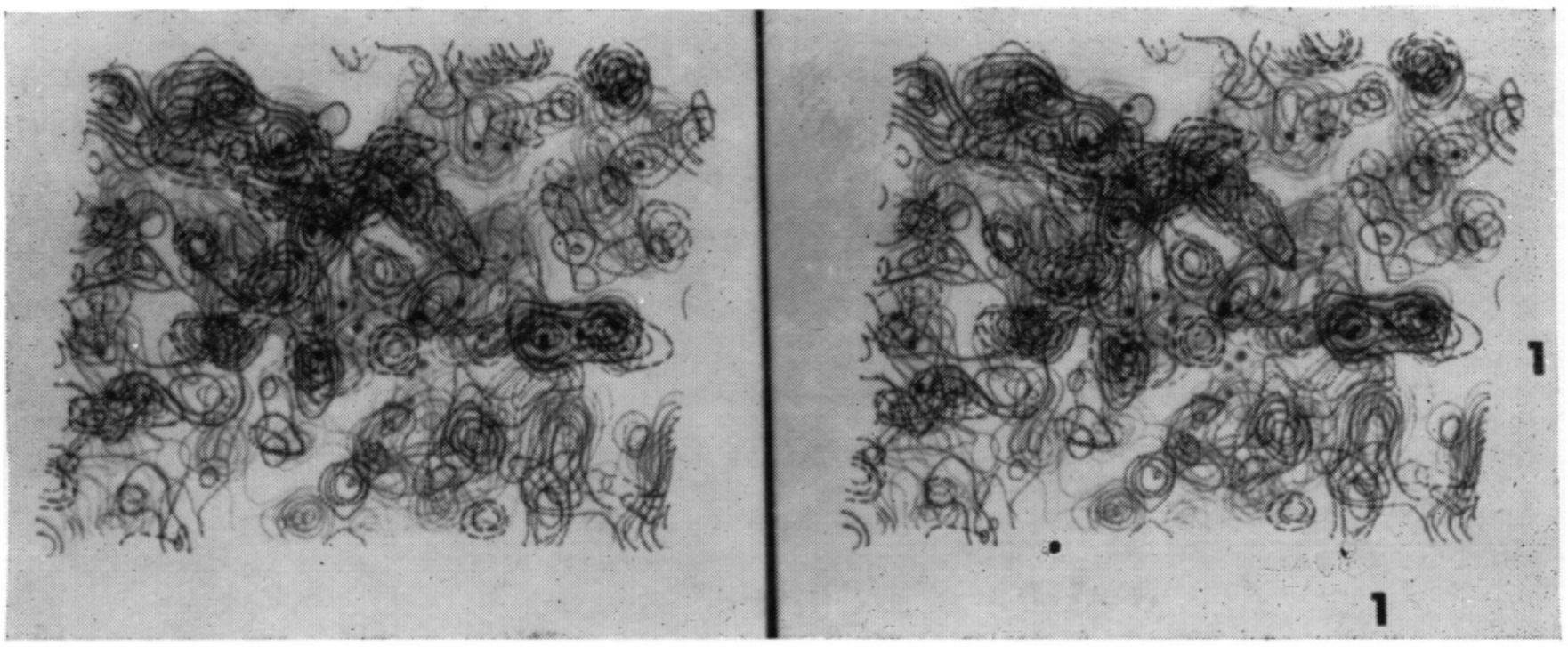
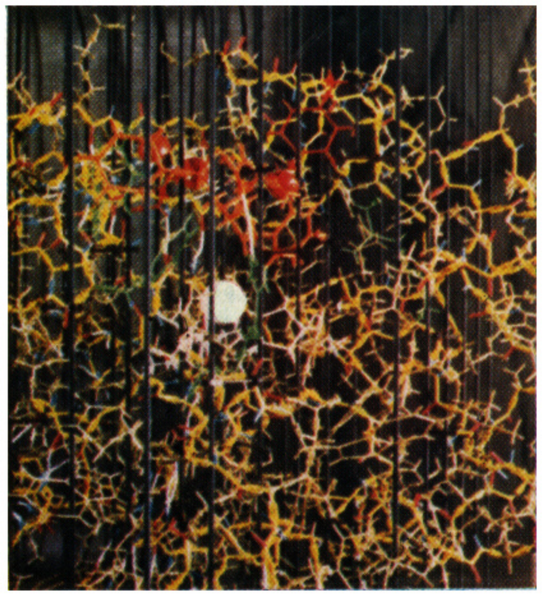


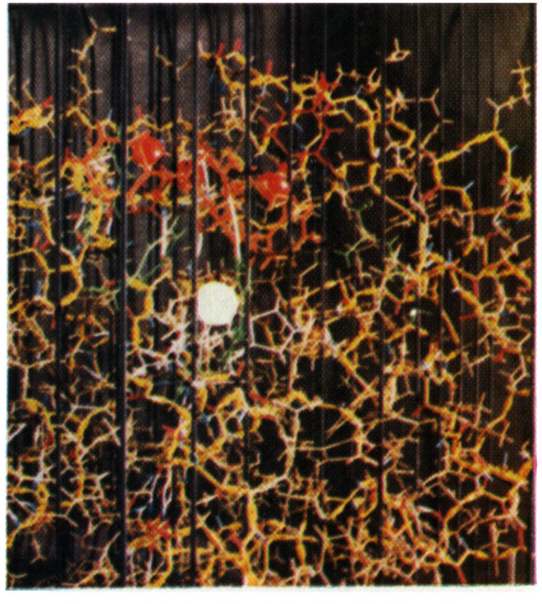
FIGURE 6. The difference electron density function for the complex of Gly-Tyr with CPA is shown as dotted (positive) and dashed (negative) contours. Solid contours show the electron density of CPA at 0.2 nm resolution. Dots are placed at proposed atomic positions. (*a*) Composite of the difference map sections $y = 0.47$ to $y = 0.52$. Near the top centre the positive contours of the tyrosyl side chain of the substrate are visible (1). To the right of the substrate are the positive (2) and negative (3) contours of the Arg-145 guanidinium group and to the left (4) are the native contours of Glu-270. (*b*) Composite of the difference map sections $y = 0.49$ to $y = 0.56$. Near the bottom of the picture are the positive contours of the moved Glu-270 (1), the substrate's terminal amino group (3), and the connecting water molecule (square dot, 2). (*c*) Composite of the difference map sections $y = 0.56$ to $y = 0.62$ shows Tyr-248 after its conformational change in the bottom right portion of the picture (1). (*d*) Composite of the difference map sections $y = 0.63$ to $y = 0.68$ shows Tyr-248 before its conformational change (1).

(*Facing p.* 195)

(*a*)

(*b*)





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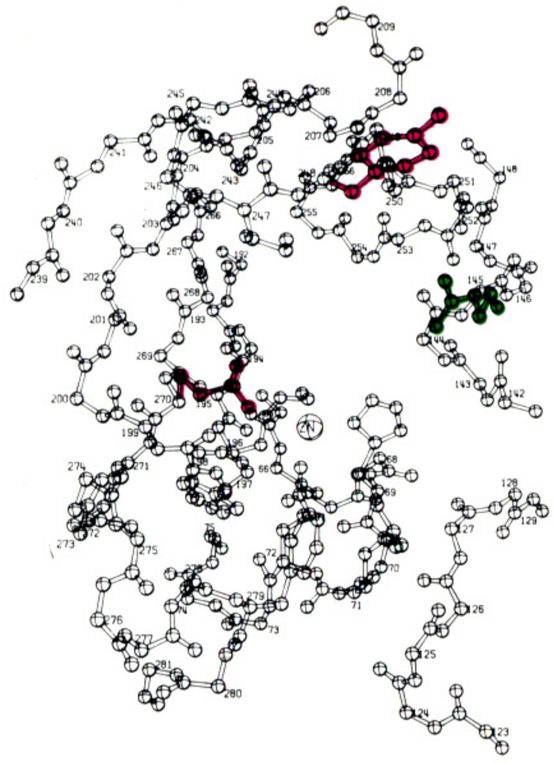

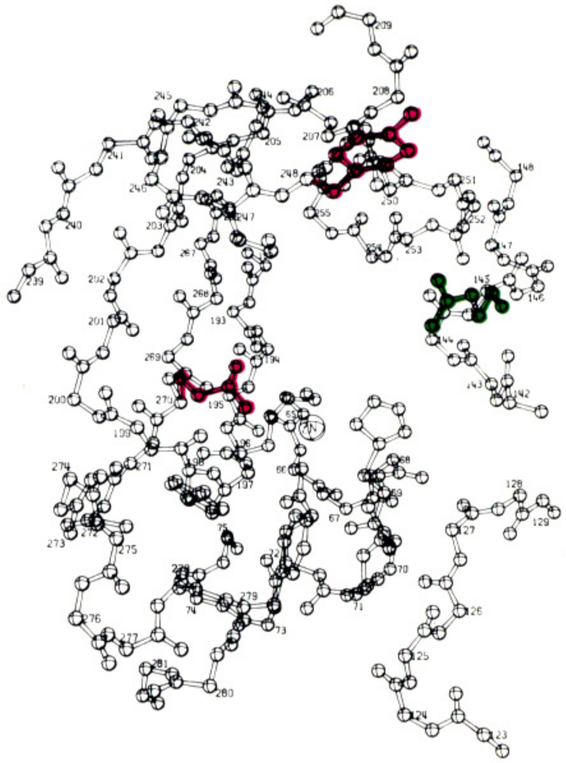

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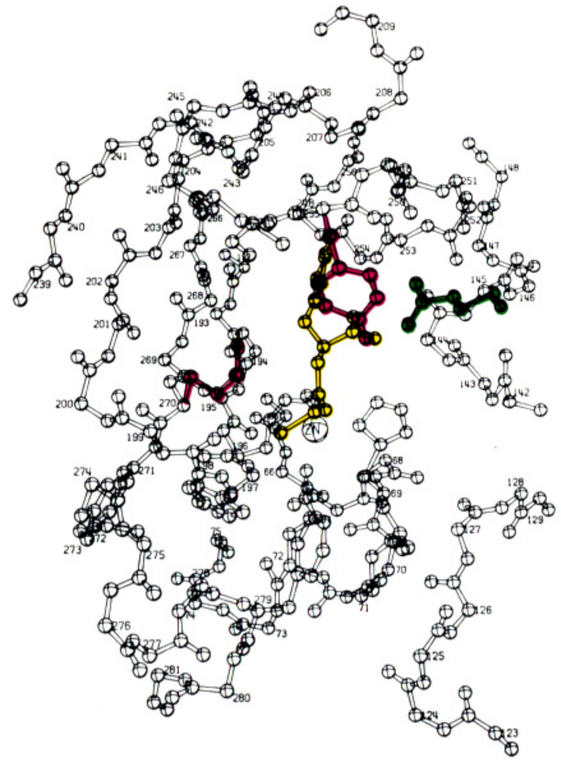

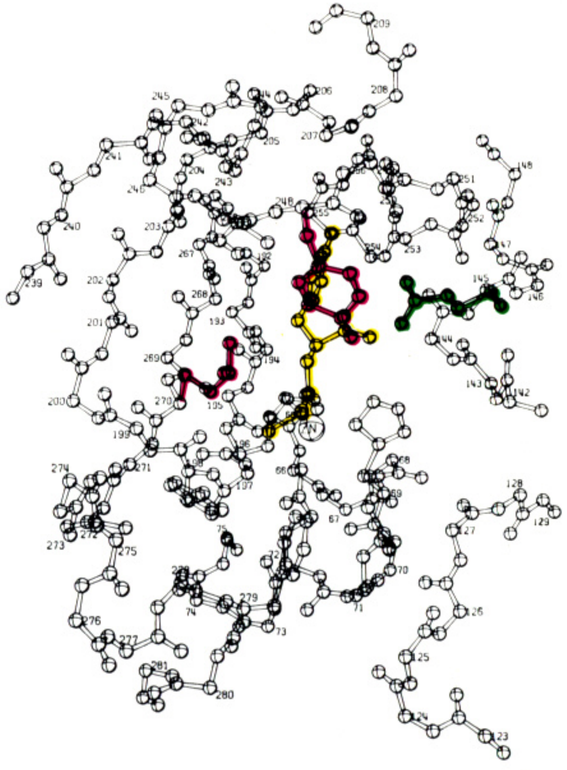

FIGURE 9 *a*, *b*, *e*, *f*. For legend see facing page.