Volume 224, number 1, 161–171 FEB 05286 November 1987

The geometries of interacting arginine-carboxyls in proteins

J. Singh, J.M. Thornton, M. Snarey* and S.F. Campbell*

Laboratory of Molecular Biology, Department of Crystallography, Birkbeck College, University of London, Malet Street, London WC1E 7HX, and *Pfizer Central Research, Sandwich, CT13 9NJ, England*

Received 25 September 1987

The geometries are reported for interacting arginine-carboxyl pairs obtained from 37 high resolution protein structures solved to a resolution of 2.0 Å or better. The closest interatomic distance between the guanidinium and carboxyl is less than 4.2 Å for 74 arginine and carboxyl groups, with the majority of these lying within hydrogen-bonding distance (2.6–3.0 Å). Interacting pairs have been transformed into a common orientation, and arginine-carboxyl, and carboxyl-arginine geometries have been calculated. This has been defined in terms of the spherical polar angles $T\theta$, $T\varphi$, and the angle P, between the guanidinium and carboxyl planes. Results show a clear preference for the guanidinium and carboxyl groups to be approximately coplanar, and for the carboxyl oxygens to hydrogen bond with the guanidinium nitrogens. Single nitrogen-single oxygen is the most common type of interaction, however twin nitrogen-twin oxygen interactions also occur frequently. The majority of these occur between the carboxyl oxygens and the NH1 and NE atoms of the arginine, and are only rarely observed for NH1 and NH2. The information presented may be of use in the modelling of arginine-carboxyl interactions within proteins.

Arginine-carboxyl interaction; Ion pair; Hydrogen-bonding; Drug design; Protein modelling

1. INTRODUCTION

About 40% of ion pairs within proteins involve arginine-carboxyl interactions [1]. Arginyl residues have been suggested as being an important factor in the thermostability of proteins [2]. For example, the eye-lens protein γ-II crystallin has a functional requirement to be especially stable, since it must exist for the entire life span of the animal. It has been suggested that the above average number of arginine-carboxyl interactions, which form networks of ion pairs on the surface of this protein, make an important contribution to its increased stability [3]. The heat stability of thermophile over mesophile ferredoxins is thought to be a consequence of salt-bridges linking residues at the amino- and carboxy-termini of the protein [4].

Arginine-carboxyl interactions often play an important role in protein-substrate interactions. It has been suggested that as a general rule enzymes acting on anionic substrates will have an arginine residue located at the active site [5]. For example, in carboxypeptidase A, arginines 71, 127 and 145 line the active site groove, and are thought to be involved in the guiding of the carboxyl-termini of the enzyme substrates into their final binding site [6]. The X-ray structure of aspartate carbamoyltransferase complexed with the bisubstrate analogue *N*-(phosphonacetyl)-L-aspartate at 3.0 Å resolution, shows the substrate α-carboxylate in proximity to arginines 105 and 167, and arginine 229 in contact with the β-carboxyl [7].

Due to the importance of arginine-carboxyl pairs in protein structure and function, an analysis of their geometry may prove valuable in understanding the nature of these interactions. We

Correspondence address: J.M. Thornton, Laboratory of Molecular Biology, Department of Crystallography, Birkbeck College, University of London, Malet Street, London WC1E 7HX, England

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

have therefore set out to evaluate whether the arginine-carboxyl pairs from 37 high resolution structures assume preferred geometries. The geometry of an arginine-carboxyl interaction has been calculated by transforming all pairs into a common orientation and then calculating both the approach of a carboxyl towards that of a 'reference' arginine, and that for an arginine towards a 'reference' carboxyl in terms of the spherical polar angles $T\theta$, $T\phi$, and the angle between the guanidinium and carboxyl planes, P. To assess the significance of the observed geometries we have compared them with those we would expect to arise by chance.

2. METHODOLOGY

All arginines, aspartic and glutamic acid

Table 1

37 proteins used in the analysis of arginine-carboxyl geometries

Resolution Å	Protein	Source	Brookhaven code	Number of interactions
2.0	carbonic anhydrase form C	human erythrocyte	1CAC	4
2.0	ferredoxin	*Peptococcus aerogenes*	1FDX	0
2.0	high potential iron protein	*Chromatium vinosum*	1HIP	1
1.38	neurotoxin	snake venom	1NXB	2
2.0	papain	papaya fruit latex	1PPD	6
2.0	Bence-Jones rei protein	human	1REI	1
1.5	cytochrome *c*	tuna	4CYT	1
2.0	leghaemoglobin	yellow lupin	1LH1	1
2.0	lysozyme	hen egg white	2LYZ	1
1.8	penicillopepsin	*Penicillium janthinellium*	2APP	0
1.7	actinindin	Chinese gooseberry	2ACT	4
2.0	actinoxanthin	*Actinomyces globisporus*	1ACX	1
2.0	superoxide dismutase	bovine	2SOD	1
2.0	azurin	*Alcaligenes denitrificans*	1AZA	2
2.0	citrate synthetase	pig	2CTS	8
1.85	parvalbumin	carp	3CPV	1
2.0	calcium binding protein	bovine	1ICB	0
1.54	carboxypeptidase A	bovine	5CPA	5
1.67	α-chymotrypsin	cow	5CHA	0
1.5	ovomucoid third domain	silver pheasant	2OVO	0
1.7	phospholipase a_2	bovine	1BP2	0
1.6	plastocyanin	poplar leaves	1PCY	0
1.6	thermolysin	*B. thermoproteolyticus*	3TLN	6
2.0	cytochrome b_5	bovine	2B5C	3
1.5	crambin	Abyssinian cabbage	1CRN	1
1.8	cytochrome c_3	*Desulfovibrio vulgaris*	2CDV	0
1.7	dihydrofolate reductase	*E. coli* B	4DFR	3
1.5	insulin	porcine	1INS	0
1.4	erythrocruorin	chironomid larva	1ECD	1
2.0	glutathione reductase	human erythrocyte	2GRS	1
2.0	haemerythrin	sipinculid worm	1HMQ	2
1.9	immunoglobulin Fab	human kohl	1FB4	9
1.8	prealbumin	human	2PAB	1
1.45	ribonuclease A	bovine	1RN3	2
1.2	rubredoxin	*Clostridium*	5RXN	0
1.0	trypsin inhibitor	bovine pancreas	5PTI	1
1.5	staphylococcal nuclease	*Staphylococcus aureus*	2SNS	5

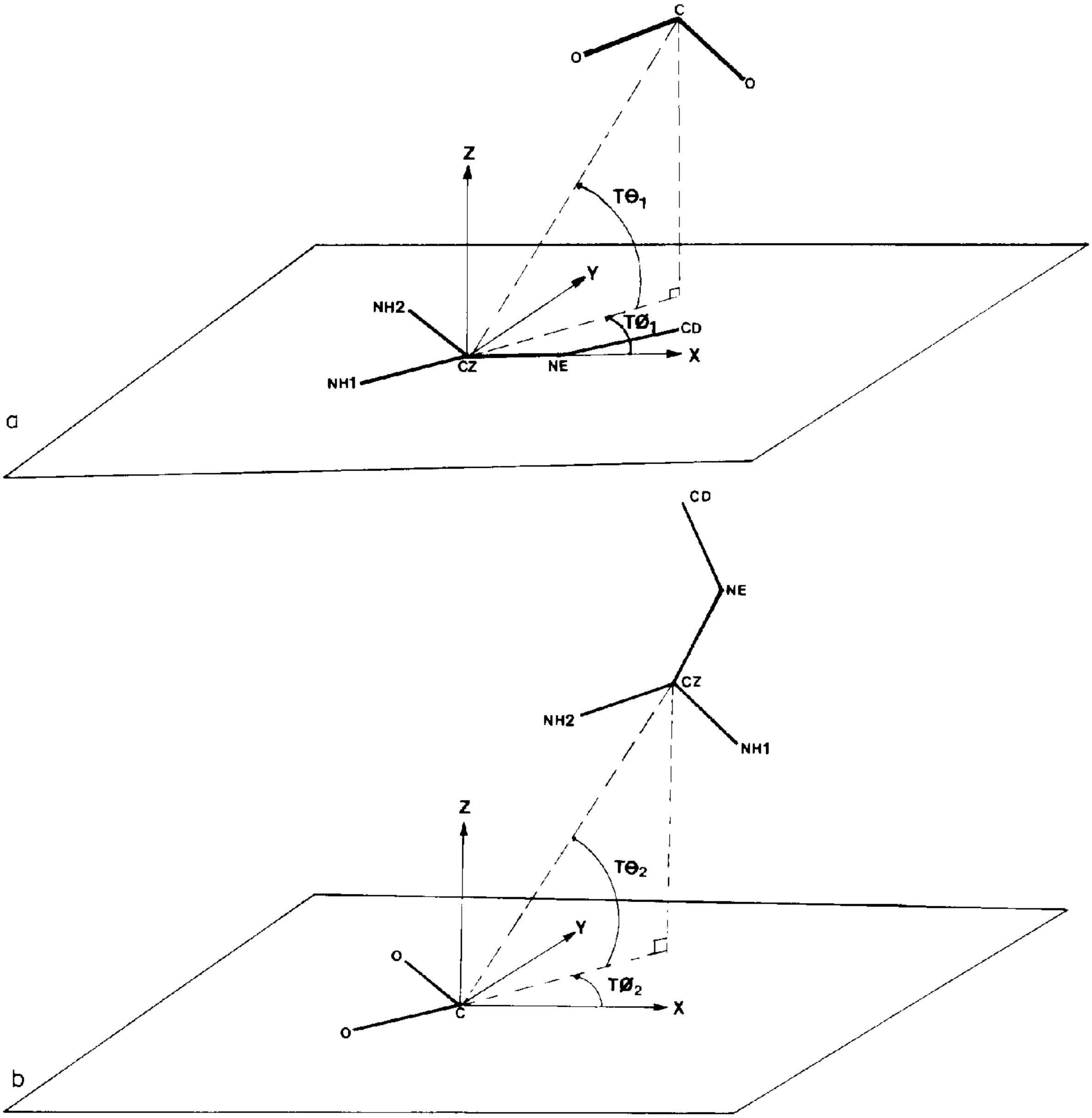


Fig.1. (a) Diagrammatic representation of the spherical polar angles, $T\theta_1$, $T\phi_1$. The 'reference' ARG is lying in the x-y-plane with the interacting carboxyl above it. $T\theta_1$ represents the angle of elevation of the carboxyl carbon above the x-y-plane of ARG. $T\phi_1$ is the equatorial angle of the carbon of the carboxyl measured by taking the projection of the carbon of the carboxyl onto the x-y-plane and calculating the angle between this point and the x-axis. The CZ of the ARG lies at the origin of the coordinate system, whose axes are shown as arrows, denoted X, Y, Z. (b) Diagrammatic representation of the spherical polar angles $T\theta_2$, $T\phi_2$. The 'reference' carboxyl (CARBOXYL) is lying in the x-y-plane with the interacting arginine above it. $T\theta_2$ represents the angle of elevation of the CZ of the arginine above the plane of CARBOXYL. $T\phi_2$ is the equatorial angle of the CZ of the arginine, measured by taking the projection of this atom and calculating the angle between this point and the x-axis. The carbon of the CARBOXYL lies on the origin of the coordinate system whose axes are shown as arrows, denoted X, Y and Z.

residues were extracted from the Brookhaven Protein Databank [8] from 37 high resolution protein structures (see table 1). To ensure reliable data, all residues having low occupancies, or cited in the literature as being poorly defined were excluded. All homologous interactions between the different proteins were removed, and only intramolecular interactions were considered. We were left with 159 arginine, 336 aspartic and 268 glutamic acid residues. The assignment of NH1 and NH2 of the arginine in Brookhaven nomenclature is arbitrary. However, as a consequence of the planarity of the guanidinium cation, the CD atom lies in the plane on the same side as one of the aforementioned nitrogens, and it is important to be able to distinguish between them. Therefore, in order to transform our coordinates into a common orientation all nitrogens connected to the CZ which were found to be on the same side of the arginine as the CD, were defined as NH2, and all the remaining nitrogens as NH1.

Geometrical parameters were calculated using the generalised side chain interaction program SIRIUS [9]. The program possesses the capability of calculating the pairwise geometry between atoms, groups of atoms, and side chains of the 20 amino acids in proteins. To define an interaction between an arginine and carboxyl, the interatomic distance (*d*) was calculated between the NE, CZ, NH1 and NH2 atoms of the guanidinium, with the carboxyl carbon and oxygens in the dataset. Residues were regarded as interacting if the observed distance between any of the above atoms was less than that given by summation of the respective van der Waals radii [10], plus a 1 Å cutoff to allow for coordinate error.

If an arginine and carboxyl were found to be interacting, their geometry was calculated as follows:

(i) Calculate the parameter P which is independent of the coordinate system: P = angle between the planes of the guanidinium and carboxyl (least-squares plane defined for arginine as the plane occurring through atoms NE, CZ, NH1 and NH2, and for the carboxyl through the carbon and carboxyl oxygens).

(ii) Define a 'reference' arginine (ARG) constructed from ideal bond lengths and angles [10] with the CZ located at the origin, with its x-axis lying along the CZ-NE bond; the z-axis perpendicular to the plane of ARG, and y-axis orthogonal to x and z (fig.1a).

(iii) Superpose the arginine residue of an arginine-carboxyl pair onto this reference ARG, apply the resulting matrix to the carboxyl residue and calculate the polar coordinates $T\theta_1$ and $T\phi_1$ of the carbon of the carboxyl where: $T\theta_1$ = angle of elevation of the carboxyl carbon from the plane of the ARG (azimuthal angle); $T\phi_1$ = equatorial angle of the carbon of the carboxyl in the plane of ARG.

(iv) Repeat the above procedure but this time using a 'reference' carboxyl (CARBOXYL) constructed from ideal bond lengths and angles. The carbon of CARBOXYL is located at the origin, the x-axis lies in the opposite direction to that given by bisection of the carboxyl oxygens; the z-axis is perpendicular to the plane of CARBOXYL and the y-axis is in the plane of CARBOXYL, orthogonal to x and z. This time calculate the polar coordinates $T\theta_2$, $T\phi_2$ of the CZ of the interacting arginine where (fig.1b): $T\theta_2$ = angle of elevation of the arginine CZ from the plane of CARBOXYL; $T\phi_2$ = equatorial angle of the CZ of the arginine in the plane of the CARBOXYL.

3. RESULTS

Fig.2a shows the interatomic distance distribution for the closest approach between the guanidinium of the arginine and the carboxyl carbon and oxygens. Since the volume that is sampled varies proportionately with the interatomic distance (*d*), we have normalised our distribution by dividing the number of interacting residues at a given distance interval by d^2. The plot approximates to a normal distribution with the majority of interactions between 2.6 and 3.0 Å, the peak in the distribution at 2.8 Å suggests a preference for the pairs to lie within hydrogen bonding distance of each other. The frequency of the number of residues between interacting arginine-carboxyl pairs (fig.2b) shows that 50% are located within 10 amino acids of each other along the polypeptide chain, while 80% are within 30. Therefore arginine-carboxyl interactions tend to be local in sequence.

The plot of the observed frequency for the angle between the planes gives information about the inclination of the interacting arginine and carboxyl. High values (P ~90°) suggest the two are approximately perpendicular, low values (P ~0°) that they are coplanar. In an analysis of phenylalanine interactions [11,12] in proteins we have shown that to determine the significance of the observed frequencies it is important to take into account the expected frequency distribution. For the angle between the planes (P) the random frequency distribution varies as the sine of this angle (see * in fig.2c). Therefore one would expect purely by chance a larger proportion should be observed at higher angular ranges, and fewer at low. Fig.2c shows the observed frequency for the 74 interacting arginine-carboxyl pairs has a notable excess of low angular values. Therefore despite the bias for high P there exists a statistically significant (a χ^2 analysis, with 7 degrees of freedom shows that the probability of the P distribution arising by chance being less than 1%. This is also the case for the $T\theta_1$, $T\theta_2$, P at $T\theta_1$ = 0–22.5°, and P at $T\theta_2$ = 0–22.5° distributions.) deviation for the observed distribution to favour low P values with all interactions apart from 30° to 40° being more frequent than expected, from 50° to 90° being less. This

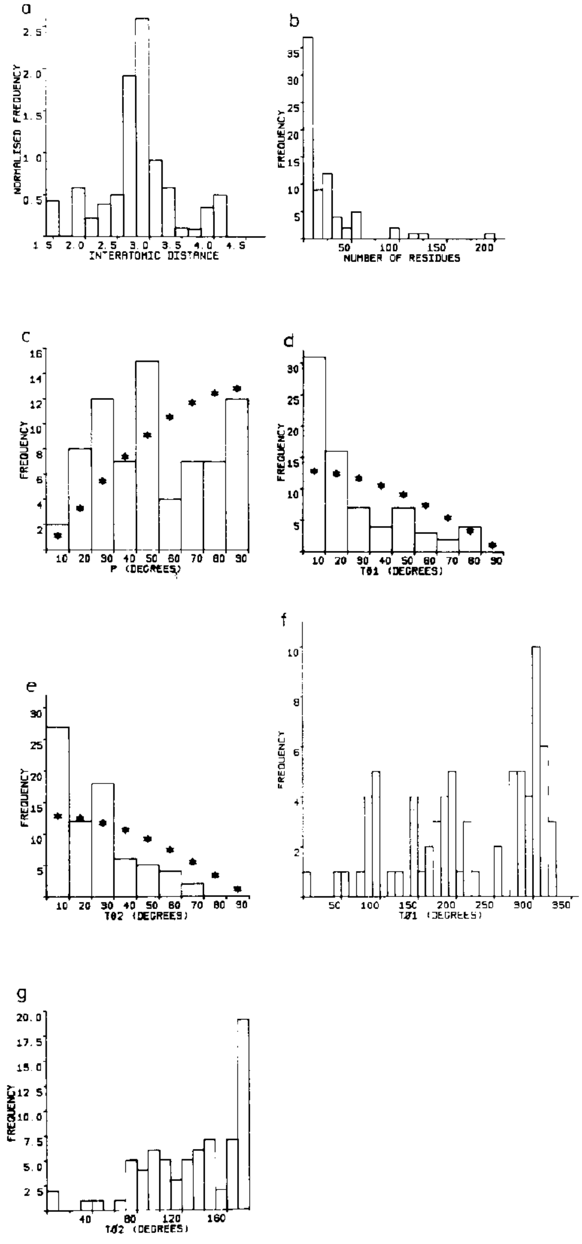


Fig.2. (a) Interatomic distance distribution for the closest approach between the guanidinium and carboxyl groups in 37 high resolution structures. The distribution has been normalised to ensure equal sample sizes are considered by dividing the number of interactions at a given distance interval (d) by d^2. (b) Plot of the frequencies for the number of residues between interacting arginine-carboxyl pairs. (c) Distribution of the angle between the planes (P). The plane for arginine is defined as the least-squares plane between the atoms NE, CZ, NH1 and NH2. Least-squares plane for carboxyl is defined as the plane occurring through carbon and carboxyl oxygens. The expected distribution from a random orientation of two planes [frequency $\propto$ (sin P)] is shown as a *. (d) Distribution of the angle of elevation of the carbon atom of the carboxyl above the plane of ARG ($T\theta_1$). The frequencies obtained from a random distribution of $T\theta_1$ as calculated from volume considerations [frequency $\propto$ (cos $T\theta_1$)] are shown as a *. (e) Distribution of the angle of elevation of the CZ of the arginine above the plane of CARBOXYL ($T\theta_2$). The expected distribution is calculated in the same manner as that for $T\theta_1$, and is shown as a *. (f) Distribution of the equatorial angle $T\phi_1$, obtained by taking the projection of the carboxyl carbon onto the x-y-plane of the reference ARG, and calculating the angle between this point and the x-axis. $T\phi_1$ ranges from 0° to 360°, and has no normalisation factor. (g) Distribution of the equatorial angle $T\phi_2$, obtained by taking the projection of the arginine CZ onto the x-y-plane of the reference CARBOXYL, and calculating the angle between this point and the x-axis. Due to the symmetrical nature of the carboxyl group $T\phi_2$ ranges from 0° to 180°. $T\phi_2$ has no normalisation factor.

suggests an inherent bias for the interacting groups to assume low interplanar angles.

Fig.3 shows stereo pictures of the transformed arginine-carboxyl pair displaying the approach of the carboxyl towards the 'reference' arginine (ARG), and that for the arginine towards the 'reference' carboxyl (CARBOXYL). In both cases the majority of interactions occur in the plane of the 'reference' residue (fig.3b,d), as seen by the peaks in the observed frequency distributions ($T\theta_1$,

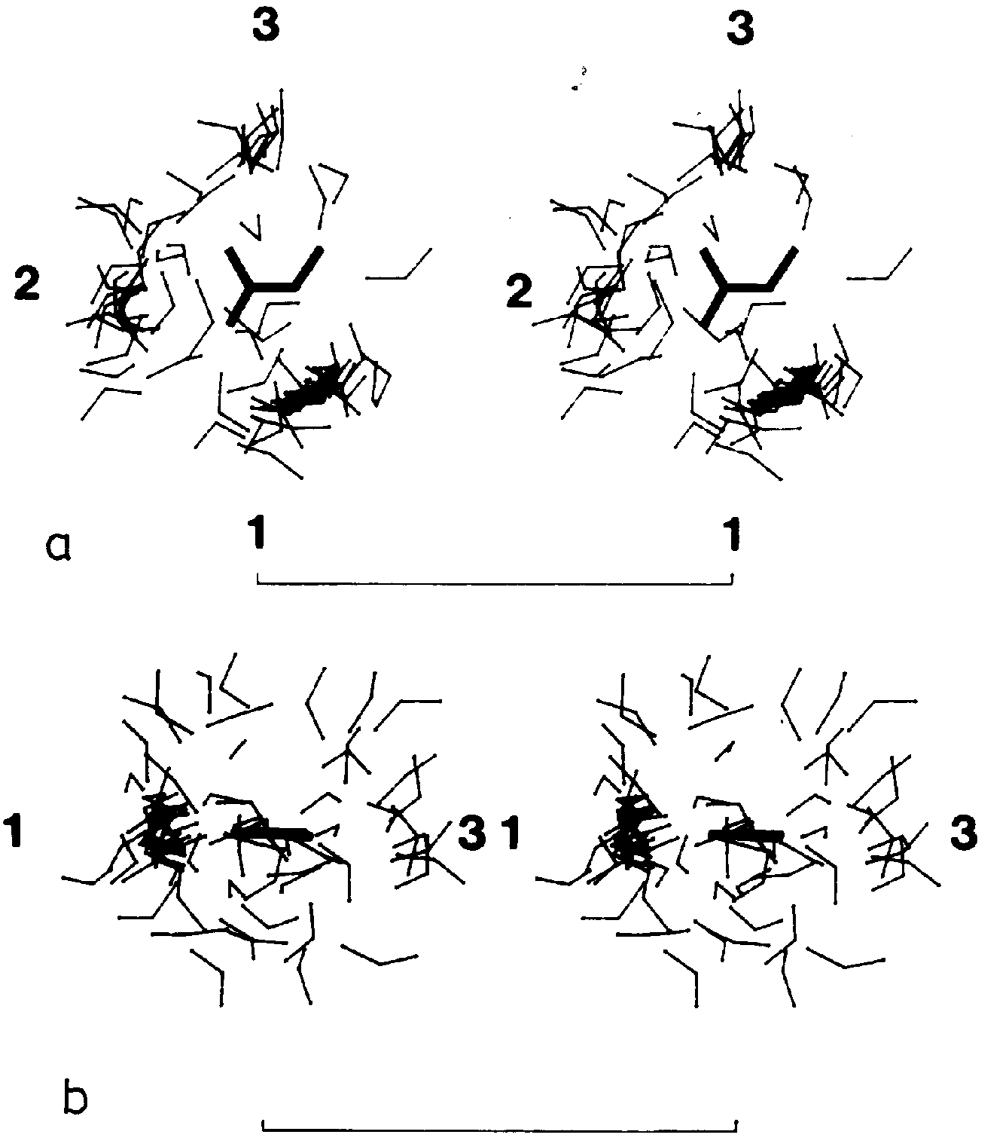
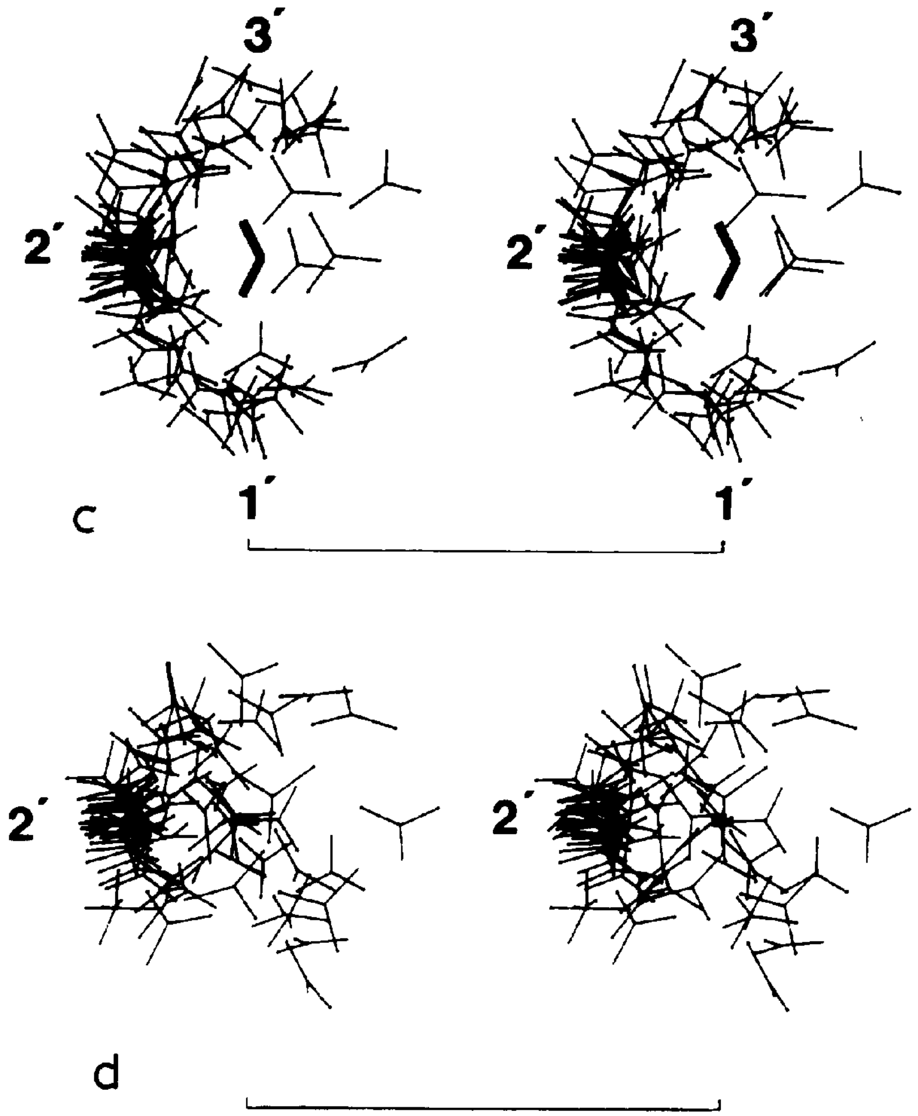


Fig.3. Stereo diagrams of the arginine-carboxyl pairs transformed into a common orientation. (a,b) The approach of carboxyl groups towards a 'reference' arginine (ARG), obtained by fitting the arginine of an arginine-carboxyl pair onto ARG located at the origin, and then applying the resulting matrix to the carboxyl. (c,d) The distributions for the approach of the arginine towards the CARBOXYL, obtained by fitting the carboxyl of an arginine-carboxyl pair onto CARBOXYL located at the origin, and then applying the resulting matrix to the arginine. (a) View down the positive z-axis towards ARG which is lying in the x-y-plane. 1, 2, 3 denote different parts of the distribution; 1 is the cluster of twin nitrogen-twin oxygen contacts between the carboxyl oxygens and the NE and NH1 of the ARG. 2 represents

$T\theta_2$, 0–30°, fig.2d,e). The expected distribution varies as the cosine of the angle (see * in fig.2d,e). Therefore purely on a volume basis in-plane interactions would be expected to be more common. Nevertheless, the excess of in-plane interactions is much higher than would be expected by chance, suggesting a preference for interactions to occur in the plane. In addition to the propensity for in-plane interactions there exists a striking preference for the position in the plane from which the in-



interactions directly in front of NH1 and NH2 atoms. 3 are contacts on the CD site of ARG. (b) View along the x-y-plane, looking directly along the NE-CZ bond such that cluster 1 is on the left hand side of ARG, 3 is on the right, and 2 is below the plane of the paper. (c) View down positive z-axis towards CARBOXYL which is lying in the x-y-plane. 1′, 2′, 3′ represent different parts of the distribution; 2′ is the cluster of twin nitrogen-twin oxygen contacts, 1′ and 3′ are the single oxygen-nitrogen interactions. (d) View along x-y-plane with 2′ seen as a distinct cluster to the left of CARBOXYL, and 3′ below the plane of the paper.

teracting group approaches the 'reference' residue. The distribution of carboxyls around ARG, defined by $T\phi_1$, shows that twin nitrogen-twin oxygen interactions involving NE and NH1 are clearly favourable and this is expressed in the prominent cluster that is observed (fig.2f, $T\phi_1$ = 270–330°, see cluster 1 in fig.3a). However, approach directly in front of the NH1 and NH2 is less common, with almost all being single oxygen-single nitrogen (fig.2f, $T\phi_1$ = 160–200°, see cluster 2 in fig.3a). For approach of the arginines towards the CARBOXYL, a crescent distribution is observed which is heavily populated directly in front of the carboxyl oxygens, due to the presence of twin nitrogen-twin oxygen interactions (fig.2g, $T\phi_2$ = 170–180°, fig.3c cluster 2′). It is seen that very few

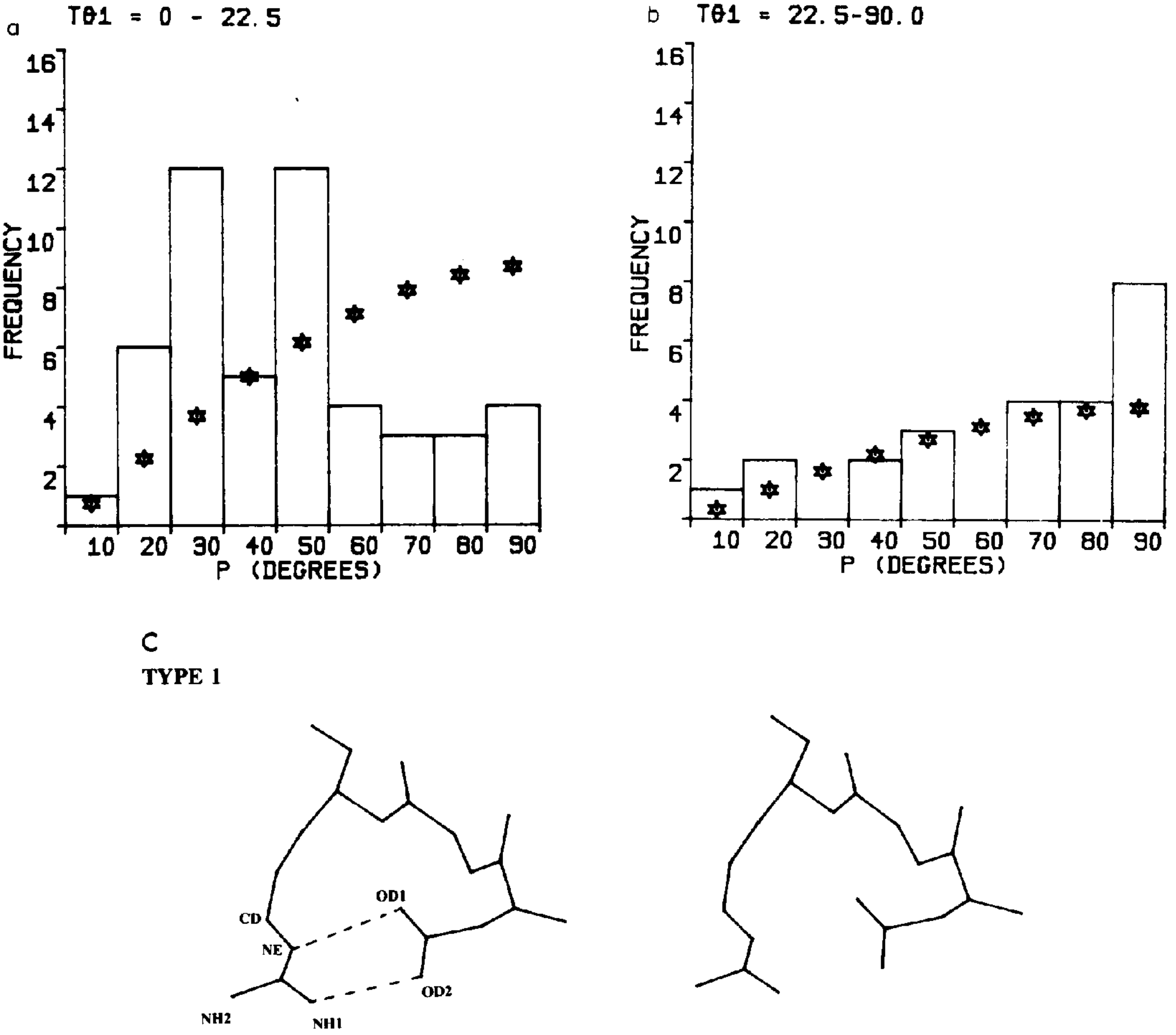


Fig.4. (a,b) Distribution of the angle between the arginine and carboxyl planes (P) for varying angles of $T\theta_1$. (a) $T\theta_1$ = 0–22.5°; (b) $T\theta_1$ = 22.5–90.0°. P distribution for varying $T\theta_2$ follows a similar trend. The expected distribution for a random distribution of the two planes is shown as a *. (c) Stereo diagram of a type1 interaction between Arg 71 and Asp 69 from dihydrofolate reductase. The closest interatomic distance is 3.01 Å and occurs between the NE of the Arg and the OD1 of the Asp. Other geometrical parameters are: P = 15°, $T\theta_1$ = 6.35°, and $T\theta_2$ = 9.3°. The twin nitrogen-twin oxygen interaction has been highlighted by dashed lines.

interactions approach directly behind the carbon of CARBOXYL (fig.2g, $T\phi_2$ = 0–60°), similarly with the distribution of carboxyls around ARG, only a limited number approach around the CD region of the ARG (fig.2f, $T\phi_1$ = 0–80°). In both cases this reflects the occlusion of this volume by side chain carbon and main chain atoms, and the preference of the carboxyl oxygens to approach the hydrogens of the guanidinium nitrogens.

Examination of the geometries of interacting phenylalanines has shown that non-random orientations of the ring planes are only observed for those interactions in the volume directly above the 'reference' residue [12]. The influence of spatial location on the orientation of the arginine-carboxyl pairs can be determined by monitoring variation of P for different $T\theta_1$, $T\theta_2$, respectively (see fig.4a and b for P distribution for different $T\theta_1$ angles. The same trend is observed for the P distribution by varying $T\theta_2$). In both cases, and in contrast to phenylalanine interactions, non-random orientations of P are confined to in-plane interactions only, with a statistically significant preference for them to favour low interplanar angles. The influence of spatial location on P can be seen in fig.3b and d where for those interactions occurring in the plane, the majority assume low P ($P \leq 50°$), suggesting a constraint on these groups to be approximately coplanar.

4. DISCUSSION

Amino acids in proteins are found to pack closely and form a variety of contacts with neighbouring groups. Despite these packing constraints arginine and carboxyl groups are still found to exhibit preferred separation distances and non-random geometries with respect to each other. The results are summarised in table 2 in which the 74 pairs have been categorised into 10 different groups with specified geometries. Approach of a carboxyl resulting in a twin nitrogen-twin oxygen interaction involving the NH1 and NH2 of the arginine is rare (~1% of arginine-carboxyl pairs have type2 geometry). However the twin nitrogen-twin oxygen interactions between the NH1 and NE of the arginine are common (type1, ~22%, fig.4c). Single oxygen-nitrogen are the most frequent type of interaction (type3, 13%; type4, 18%; type5, 28%). This reflects that there are more opportunities to form a single nitrogen-single oxygen interaction than twin nitrogen-twin oxygen interactions. The higher frequency of the type5 interaction is possibly due to favourability of the NE and NH1 to participate in twin nitrogen-twin oxygen interactions. Twin nitrogen-single oxygen and single nitrogen-twin oxygen interactions (type6,7, 8,9) are rarely seen.

The geometrical constraint of the hydrogen bond between the carboxyl oxygens and the hydrogens of the guanidinium nitrogens, is shown in the non-random preferences for the interacting groups to occur in the plane and assume low interplanar angles. This is clearly shown in the case of type1 interactions which assume a narrow range of spherical polar and P angles, and form of a prominent cluster. Single oxygen-single nitrogen interactions also show a preference to lie in the plane (table 2, see mean $T\theta_1$, $T\theta_2$), however they assume a broader range of P and $T\theta$ values than those seen for type1. This probably arises from the packing of the other oxygen.

There are few high resolution crystallographic structures of enzyme-inhibitor/substrate complexes which involve arginine-carboxyl interactions [13–15]. However in both trypsinogen complexed with bovine pancreatic trypsin inhibitor, and dihydrofolate reductase bound with a trimethoprim analogue, a type2 interaction is seen. It appears that geometrical constraints prohibit the formation of a type1 interaction in both of these complexes. At present there are no examples of type1 interactions between enzyme-inhibitor/substrate complexes. This is in contrast to the predominance of type1, and infrequent occurrence of type2 interactions within proteins. More structural data are needed to determine if there is a significant difference in packing between interacting arginine and carboxyls within proteins, and those occurring between enzymes and their substrates.

Packing in small molecule crystal structures of arginine and carboxyl amino acaids reveals no clear preference for type1 interactions over type2 [15–17]. However, quantum mechanical calculations on methylguanidinium-carboxylate suggest an energetic favourability for type1. This arises due to the slightly higher partial positive charge on the hydrogens belonging to the NH1 and NE, together with the electrostatic contribution of the

Table 2

Classification of geometries assumed by interacting arginine-carboxyls and frequencies observed

Type	Structure	Type of contacts	Arginine atoms involved	No. observed	Angular ranges[a]		
					P	$T\theta_1$	$T\theta_2$
1		twin N-twin O	NH1, NE	16	20–50 (32)	0–10 (7)	0–40 (15)
2		twin N-twin O	NH1, NH2	1	10–20 (17)	0–10 (5)	0–10 (2)
3		single N-single O	NE	10	0–90 (50)	0–50 (25)	0–30 (17)
4		single N-single O	NH1	14	20–90 (51)	0–80 (31)	0–60 (30)
5		single N-single O	NH2	21	20–90 (63)	0–50 (20)	0–50 (22)
6		single N-twin O	NH1	2	20–50 (34)	0–20 (10)	0–10 (3)
7		single N-twin O	NH2	2	20–90 (56)	0–10 (4)	0–10 (6)
8		twin N-single O	NH1, NH2	2	20–50 (38)	0–20 (9)	0–30 (16)
9		twin N-single O	NE, NH1	2	40–50 (46)	0–10 (6)	0–10 (6)
10[b]		staggered stacking		4	10–50 (36)	50–70 (57)	20–70 (39)

[a] Values in parentheses represent mean angles

[b] Lines represent planes of arginine and carboxyl edge on

CD of the arginine in proximity to the hydrogen bonding atoms [18].

The observation that arginine-carboxyl pairs adopt preferred orientations in proteins, may be of use in the modelling of ion pairs. While limited structural data exist on the effects of mutations introduced into proteins, a database of side chain interactions may prove useful in determining the conformation of the altered residue, possibly in the design of proteins with increased stability. It could also be used in predicting the packing of substrates to the active sites of proteins, and in modelling drug-receptor interactions.

ACKNOWLEDGEMENTS

J.S. is supported by a CASE studentship, in collaboration with Pfizer, Sandwich, England. We thank Professor T.L. Blundell and Parmageat Kaur for interest and encouragement, J. Baum, V. Horne, M.S. Tute, D. Mahadevan and especially I. Haneef for useful discussion.

REFERENCES

[1] Barlow, D.J. and Thornton, J.M. (1983) J. Mol. Biol. 168, 867–885.

[2] Qaw, F.S. and Brewer, J.M. (1986) Mol. Cell. Biochem. 71, 121–127.

[3] Summers, L., Wistow, G., Narebor, M., Moss, D., Lindley, P., Slingsby, C., Blundell, T.L., Bartunik, H. and Bartels, K. (1984) Pept. Protein Rev. 3, 147–168.

[4] Perutz, M.F. (1975) Nature 255, 256–259.

[5] Riordan, J.F., McElvany, K.D. and Borders, C.L. (1977) Science 195, 884–886.

[6] Nakagawa, S. and Umeyama, H. (1978) J. Am. Chem. Soc. 100, 7716–7725.

[7] Krause, K.L., Volz, K.W. and Lipscomb, W.N. (1985) Proc. Natl. Acad. Sci. USA 82, 1643–1647.

[8] Berstein, F.C., Koetzle, T.F., Williams, G.J.B., Mayer, G.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) J. Mol. Biol. 122, 535–542.

[9] Singh, J., Thornton, J.M. and Blundell, T.L., in preparation.

[10] Momany, F.A., McGuire, R.F., Burgess, A.W. and Scheraga, H.A. (1975) J. Phys. Chem. 79, 2361–2380.

[11] Singh, J. and Thornton, J.M. (1985) FEBS Lett. 191, 1–6.

[12] Blundell, T.L., Singh, J., Thornton, J.M., Burley, S.K. and Petsko, G.A. (1986) Science 1005, 234.

[13] Rees, D.C. (1981) Proc. Natl. Acad. Sci. USA 78, 5455–5459.

[14] Bode, W., Walter, J., Huber, R., Wenzel, H.R. and Tschesche, H. (1984) Eur. J. Biochem. 144, 185.

[15] Kuper, L.F., Roth, B., Baccanari, D.P., Ferone, R., Beddell, C.R., Champness, J.N., Stammers, D.K., Dann, J.G., Norrington, F.E.A., Baker, D.J. and Goodford, P.J. (1985) J. Med. Chem. 25, 1120.

[16] Bray, D., Slattery, N. and Russel, C.S. (1984) Int. J. Pept. Protein Res. 24, 414–418.

[17] Eggleston, D.S. and Hodgson, D.J. (1985) Int. J. Pept. Protein Res. 25, 242–253.

[18] Salunke, D.M. and Vijayan, M. (1981) Int. J. Pept. Protein Res. 18, 348–351.

[19] Sapse, A.M. and Russel, C.S., Int. J. Quant. Chem. 2, 91–99.