TABLE I

Compound	Sample no.	$(\eta_{sp}/c)^a$	MW_w[b]	CHCl₃ soln. b_0[c]	CHCl₃ soln. $[\alpha]^{22}_{546}$	DCA soln. b_0[c]	DCA soln. $[\alpha]^{22}_{546}$
L-PBA	1354	0.09	4,000	+124	− 84		
	1145	.26	30,000	+411	−168		−18
D-PBA	1335	.16	19,000	−363	165		
	1355	.30	35,000	−425	174		+19
L-PBG	2–10	.21	24,000	−363	+ 14	0	−15
D-PBG	1322	.89	140,000	+482	− 18	0	+17

[a] c = 0.2 in DCA solution. [b] Estimated from the viscosity using the molecular weight (MW_w) calibration furnished by J. C. Mitchell, A. E. Woodward and P. Doty, THIS JOURNAL, **79**, 3955 (1957). [c] The optical rotations were measured between 365 and 578 mμ using a Rudolph high precision photoelectric polarimeter. From these data, b_0 was calculated using λ_0 = 212 mμ as in ref. 3b.

the equation is now known to be incorrect,[5] the equation may still be used empirically. In particular the quantity b_0 has been used as a measure of helix content of proteins[6,7] and polypeptides,[3b] and the *negative values of b_0 for* L-*isomers* of a few polypeptides have been taken to indicate the presence of a definite screw sense of the helical configurations of these molecules. It is the purpose of this communication to describe synthetic polypeptides having *positive b_0 values for* L-*isomers* and negative b_0 values for D-isomers.

We have synthesized poly-β-benzyl-L-aspartate[8] (L-PBA) and poly-β-benzyl-D-aspartate[9] (D-PBA) by the polymerization of the corresponding amino acid-N-carboxyanhydrides in chloroform solution using sodium methoxide initiation.[10] That no inversion of configuration occurred during the synthesis was shown by hydrolysis of the polymers with 6 *N* HCl to the amino acids. L-PBA upon hydrolysis gave $[\alpha]^{22}_{578}$ +23.9 in 6 *N* HCl, reported for L-aspartic acid $[\alpha]^{25}D$ +24.6; D-PBA upon hydrolysis gave $[\alpha]^{22}_{578}$ −23.0. The optical rotatory dispersions of the polybenzyl aspartates were measured in chloroform and dichloroacetic acid (DCA) solutions. Pertinent data are summarized in the table along with data for poly-γ-benzyl-D-glutamate (D-PBG) for comparison.

In DCA solutions both L-PBA and L-PBG show nearly identical negative values for $[\alpha]_{546}$; the D isomer values are positive and the same for both polypeptides. In this solvent b_0 = 0, for each glutamate polymer indicating the normal dispersion behavior characteristic of random configurations.

In chloroform solutions anomalous rotatory dispersion characteristic of helical configurations is observed, and b_0 is positive for L-PBA and negative for D-PBA.[11] This is the converse of the situation with the corresponding optical isomers of polybenzyl glutamate. Two possible explanations are offered for the observed b_0 values in the polybenzyl aspartates. First b_0 values may be sensitive to the nature of the side chain, and in particular a chromophoric group such as the ester group attached to the β carbon atom may influence the absorption and dispersion of the helix (as suggested for the phenol group in poly-L-tyrosine).[12] If this is so, the sense of twist of the helical peptide core could be the same in both the poly-L-aspartates and poly-L-glutamates. The second and alternative explanation is that the helix in L-PBA has a different sense of twist from that in L-PBG.[13] A change in sense of the helix may be caused by steric effects of the large benzyl ester groups which lie closer to the peptide core in L-PBA than in L-PBG. The unexpected results reported here indicate the necessity for both investigations of the optical rotatory properties of other polypeptides and studies of the relevance of their optical properties to similar data from proteins.[14]

(5) W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Nat. Acad. Sci.*, **43**, 723 (1957).
(6) C. Cohen and A. Szent-Gyorgyi, THIS JOURNAL, **79**, 248 (1957).
(7) C. B. Kretschmer, *J. Phys. Chem.*, **61**, 1627 (1957).
(8) A. Berger and E. Katchalski, THIS JOURNAL, **73**, 4084 (1951).
(9) E. R. Blout and R. H. Karlson, to be published.
(10) E. R. Blout and R. H. Karlson, THIS JOURNAL, **78**, 941 (1956).
(11) It should be noted that the value of b_0 seems to depend on the molecular weight—increasing in either a negative or positive sense as the molecular weight increases.
(12) A. Elliott, W. Hanby and B. Malcolm, *Nature*, **180**, 1340 (1957).
(13) A third explanation of the data would be that b_0 is not a satisfactory measure of helical content of all polypeptides and proteins.
(14) We are pleased to acknowledge the support of this work by the Office of the Surgeon General, Department of the Army, Washington 25, D. C.

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AMINO ACID SEQUENCE IN THE REGION OF DIISOPROPYL PHOSPHORYL BINDING IN DIP-TRYPSIN

Sir:

Trypsin has been treated with P^{32}-diisopropyl phosphorofluoridate and the enzymatically inactive, labeled protein degraded by means of performic acid oxidation followed by tryptic hydrolysis. As has been reported elsewhere,[1] several large radioactive peptides were obtained by fractionation of the complex tryptic hydrolyzate by a combination of high voltage ionophoresis[2] and paper chromatography. The smallest peptide contained 15 residues and the largest, from which it appears to be derived, 55 residues. We now wish to report the sequence of amino acids in the smallest labeled peptide which has the composition: $(CySO_3H)_2$, Asp_2, Glu, Ser_3, Gly_4, Val, Pro, Lys, DIP.

Sequential degradation by aminopeptidase[3] indicated that asparagine was the N-terminal residue, whereas application of Sanger's method to the peptide yielded DNP-aspartic acid. The kinetics

(1) H. Neurath and G. H. Dixon, *Fed. Proc.*, **16**, 791 (1957).
(2) H. Michl, *Naturwissenschaften*, **40**, 390 (1953).
(3) D. H. Spackman, E. L. Smith and D. M. Brown, *J. Biol. Chem.*, **212**, 255 (1955).

of release of free amino acids indicated that the N-terminal sequence was Asp(-NH_2)-Ser-Cys-Glu-Gly and this was confirmed by the isolation of a series of labeled peptides from the aminopeptidase digest lacking progressively Asp(-NH_2), Asp(-NH_2) Ser, Asp(-NH_2)Ser CySO_3H and Asp(-NH_2)Ser CySO_3H Glu, as indicated in Fig. 1 (top). In accordance with the specificity of trypsin, the C-terminal residue of the peptide was found to be lysine, using carboxypeptidase B.[4]

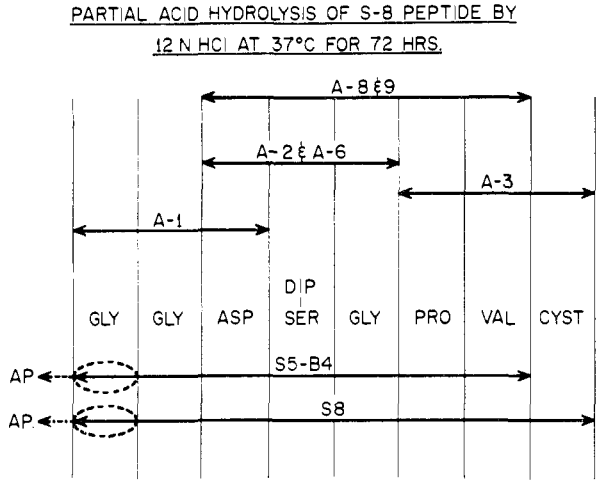

Fig. 1.—Enzymatic degradation of the peptide O-Tr-1: the horizontal arrows delineate the peptides obtained by degradation with subtilisin (S) or aminopeptidase (AP). The vertical arrows denote the major points of cleavage by subtilisin. The sequence denoted by "Army" has been established previously by the authors of ref. 5. Peptides S6 and S7 were present in trace quantitities only and their radioactivities were below the limits of detection.

The remainder of the sequence was determined by subtilisin digestion which yielded 14 peptides. Of the subtilisin peptides, only S5-B4, S8 and S9-B4 were radioactive, and their composition showed in each case the presence of a single serine. Glycine was liberated from both S5-B4 and S8 by aminopeptidase, while neither peptide gave any free amino acid with carboxypeptidase, probably due to the C-terminal cysteic acid (S8) and the penultimate proline (S5-B4). S8 was subjected to partial acid hydrolysis and the resulting peptides could be fitted into a unique sequence (Fig. 2). In Fig. 1, these subtilisin peptides are combined with the sequences found by aminopeptidase and acidic hydrolysis, and provide a unique sequence. The sequence Asp-Ser-Gly around the DIP-seryl confirms that previously determined by Schaffer, *et al.*[5]

Much interest has been focused recently upon the similarity in composition around the phosphorylated serine in those enzymes inhibited by DFP.[6]

(4) J. E. Folk, THIS JOURNAL, **78**, 3541 (1956).

(5) N. K. Schaffer, R. E. Engle, L. Simet, R. W. Drisko and S. Harshman, *Fed. Proc.*, **15**, 347 (1956).

(6) N. K. Schaffer, S. C. May and W. H. Summerson, *J. Biol. Chem.*, **202**, 69 (1953); R. A. Oosterbaan, H. S. Jansz and J. A. Cohen, *Biochim. et Biophys. Acta*, **20**, 402 (1956); G. H. Dixon, S. Go and H. Neurath, *ibid.*, **19**, 193 (1956); D. E. Koshland, Jr., and M. J. Erwin, THIS JOURNAL, **79**, 2657 (1957); F. Turba and G. Gundlach, *Biochem. Z.*, **327**, 186 (1955).

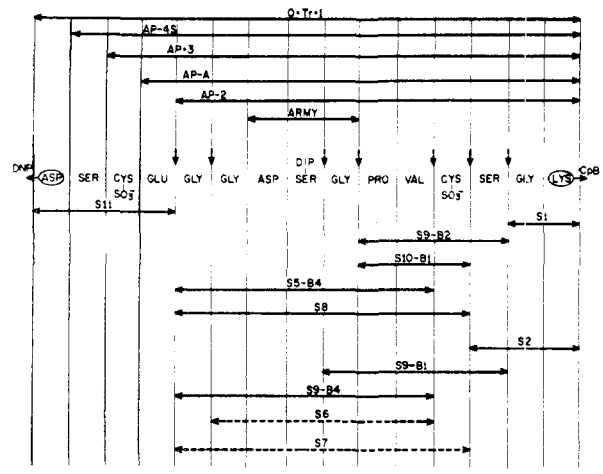

Fig. 2.

Detailed sequences are, however, available only for chymotrypsin[7] and trypsin (above) and these show complete identity over the sequence Gly-Asp-DIP
|
Ser-Gly. Recently, Westheimer[8] has attempted to explain in detail the orientation of the active site of chymotrypsin by assuming the folding of this hypothetical sequence

DIP
|
—X—X—X—His—X—Gly—Asp—Ser—Gly—Glu—
Ala—Val—

in an α-helix, thus bringing the histidine and serine into the favorable orientation previously suggested by Cunningham.[1,9] On the basis of our evidence for the sequence in trypsin which possesses an essentially identical bond-breaking mechanism, the assumption as to the position of the histidine is unjustified. In addition, the presence of two cysteic residues (and proline) close to the serine would probably preclude the formation of an α-helix in this region. The absence of histidine from the trypsin peptide described above (and in fact even from the largest peptide containing 55 residues) would suggest that maintenance of the histidine and serine in a favorable orientation is a question of the tertiary structure of the protein.[10]

(7) N. K. Schaffer, L. Simet, S. Harshman, R. R. Engle and R. W. Drisko, *J. Biol. Chem.*, **225**, 197 (1957).

(8) F. H. Westheimer, *Proc. Nat. Acad. Sci.*, **43**, 969 (1957).

(9) L. W. Cunningham, *Science*, **125**, 1145 (1957).

(10) G. H. Dixon and H. Neurath, *Biochim. et Biophys. Acta*, **20**, 572 (1956).

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ON THE ABSOLUTE CONFIGURATION OF THE ANTIBIOTIC ACTIDIONE

Sir:

The striking results of optical rotary dispersion measurements among polycyclic ketones[1] have

(1) For leading references see C. Djerassi, *Bull. Soc. Chim. France*, 741 (1957), and C. Djerassi, O. Halpern, V. Halpern, O. Schindler and C. Tamm, *Helv. Chim. Acta*, **41**, No. 1 (1958), in press.