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Distribution of D- and L-Amino Acids along the Chain of Poly-DL-Alanine

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The question concerning the distribution of D- and L-forms along the peptide poly-DL-alanine has been investigated by digestion with carboxypeptidase and aminopolypeptidase which preferably attack bonds between two L-amino acids. The analysis of the digestion curves makes it likely that the distribution of D- and L-forms is very nearly random in the particular preparation under investigation.

In two previous papers [1,2] Arieh Berger and the author have explained the relatively high stability of the helix of poly-DL-alanine (PDLA) in aqueous solution by assuming that a certain number of pairs of D- and L-methyl groups form internal hydrophobic bonds from one turn of the helix to the next. Provided that the distribution of D- and L-forms is entirely random one fourth of all methyl groups would be able to form such bonds. Fig. 1 shows a model of the helix in question. It is built up by 30 alanine residues [1] and is right-handed. The pitch is 1.5 Å and there are 15 residues in four turns (see *e.g.* Refs.[3-5]). The D- and L-forms have been distributed by throwing a coin and it is therefore by chance that 7 out of 30 methyl groups are able to form hydrophobic bonds of the type assumed. Fig. 2 shows some of the characteristic distances in the model. The most important of these are:

1. Distance $C\beta\ L_5 \rightarrow C\beta\ D_8$, the closest approach of two methyl groups, one belonging to an L-alanine residue (8) and one to a D-alanine residue (5) (see Fig. 1). This distance is 3.74 Å.

2. Distance $C\beta\ D_8 \rightarrow O_8$ from a D-methyl group in a given residue to the carbonyl oxygen of the same residue. This distance is 2.70 Å.

The X-ray diffraction pattern of DL-alanine crystals [6,7] indicate that the corresponding minimum distances in the crystals are 3.64 and 2.52 Å, respectively. Hence from this comparison it may be concluded that there is no particular strain in our helical model.

However, other possible helical models have not been investigated and in view of reported strains in right-handed helical structures containing D-residues [8,9] an attempt was made to determine the distribution of D- and L-forms in the particular preparation of PDLA, the stability of which has

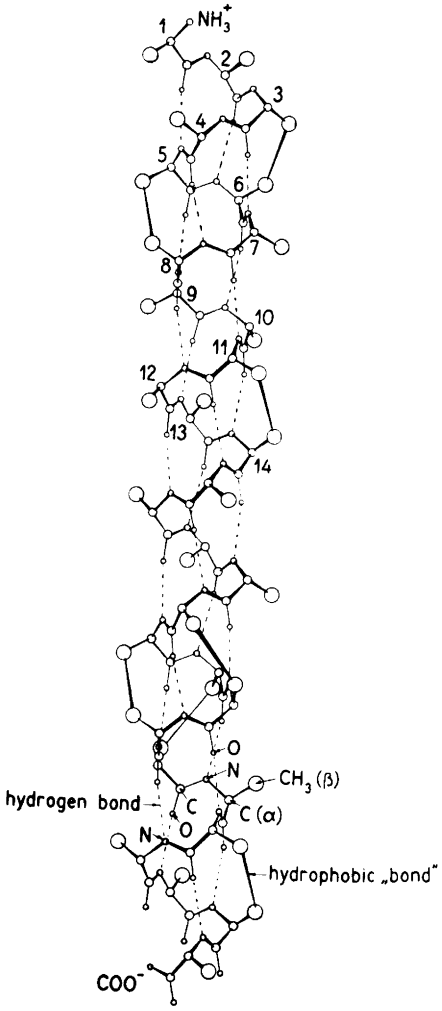

Fig. 1. Right-handed α-helix with 30 residues.

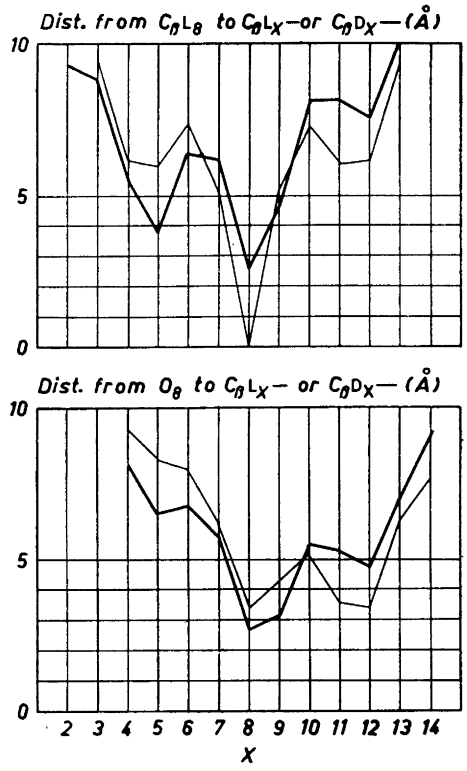

Fig. 2. Distances from atoms in residue 8 (see Fig. 1) to other atoms, numbered X. The distance $C\beta L_8 - C\beta D_8$ is between two β-carbon atoms (two methyl groups) on one α-carbon atom. Many of the other distances are calculated for arbitrary distributions of D- and L-forms and are not found in Fig. 1.

been studied [1,2]. In theory an optically inactive PDLA may consist of equal parts of right-handed helices with L-amino acid residues only and left-handed helices with D-amino acid residues only. Both these forms are generally acknowledged as being without strain. If now our preparation were constituted in this way, the above mentioned explanation of the stability as being due to internal hydrophobic bonds would fail, since the distance between the L-methyl groups in our right-handed model is too large to give effective hydrophobic bonds from one helical turn to another. The same is true for a left-handed helix with D-residues. In order to decide this important ques-

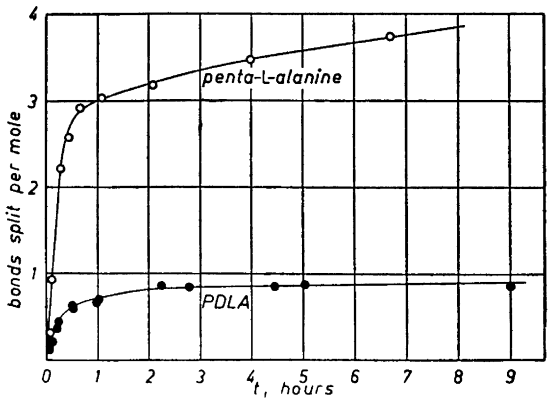

Fig. 3. Splitting of penta-L-alanine and PDLA by carboxypeptidase.

tion our PDLA preparation was digested with carboxypeptidase *, an enzyme which splits polypeptides from the carboxyl end provided the terminal amino acid residues have the L-configuration. Thus in penta-L-alanine ** 3 bonds were broken very fast while the last — in the remaining alanylalanine — was slowly split due to the proximity of the amino end group (Fig. 3). If now half of our PDLA preparation consisted of poly-L-alanine, we should expect a splitting of 14—15 bonds per mole of preparation, the remaining 14—15 bonds being situated in poly-D-alanine which is not attacked by the enzyme. As shown in Fig. 3 only 1/2—1 bond is actually split, indicating that D-amino acid residues are situated close to the carboxyl end in the majority of the molecules. It is easy to show that for random distribution of D- and L-residues the number of bonds, n, split per mole is given by

$$n = \frac{1 - (\frac{1}{2})^{q-1}}{2}$$

where q is the number of residues in the polypeptide, provided the action of carboxypeptidase is restricted to bonds between two L-amino acid residues, and

$$n = 1 - \tfrac{1}{2}^{q-1}$$

if bonds between L- and D-amino acid residues

$$\text{---}\ \text{---}\ \text{---}\ \text{D} \underset{\uparrow}{-} \text{L}$$

are attacked as well. For q equal to 30 it is therefore compatible with a random distribution of D- and L-residues that a fast reaction involving 1/2 bond per mole is observed. The further reaction leading to a splitting of another 1/2 bond may be due to a slow attack of the enzyme on the L—D-bonds, but it may also be the result of the action of a slight proteinase impurity in the

* The enzyme used was kindly placed at our disposal by Professor H. Neurath.
** This preparation was kindly given us by Professor Hans T. Clarke.

carboxypeptidase (see below). Such an impurity might open a bond in the middle of the chains thus exposing new C-terminal L—L-sequences to the carboxypeptidase. Finally the results might indicate a slight deviation from randomness of the distribution of D- and L-amino acid residues.

Similar experiments with aminopolypeptidase * gave essentially the same, though slightly lower results which might be explained by a low content of substituted terminal amino groups in PDLA [1]. We feel therefore justified in assuming random — or nearly random — distribution of D- and L-alanine residues along the chain of PDLA.

In the case of PDLA the carboxypeptidase experiments were carried out as follows: To 500 μl 1.57×10^{-2} M PDLA solution were added 500 μl 0.2 M veronal buffer pH 8.61 and 5 μl of a 1.8×10^{-4} M carboxypeptidase solution which was pre-treated with di*iso*propylfluorophosphate to knock out proteinase contaminations. The digestion mixture was prepared at 39.7°C and samples of 100 μl were taken out at intervals and mixed with 85 μl N/10 alcoholic HCl whereby the enzymatic reaction stopped. For determination of the splitting 2 ml acetone were added and the solution titrated with N/10 HCl in alcohol, using naphthylred as an indicator.

Substrate concentration	7.3×10^{-3} M
Enzyme »	8.6×10^{-7} M
Temperature	39.7°C

In the penta-alanine experiments the procedure was the same.

Substrate concentration	6.7×10^{-3} M
Enzyme »	8.6×10^{-7} M
Temperature	39.7°C

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REFERENCES

1. Berger, Arieh and Linderstrøm-Lang, K. *Arch. Biochem. and Biophys.* **30** (1957) 106.
2. Linderstrøm-Lang, K. *Symposium on Protein Chemistry*, Paris 1957 (*In press*).
3. Pauling, L., Corey, R. B. and Branson, H. R. *Proc. Natl. Acad. Sci. U. S.* **37** (1951) 205.
4. Pauling, L. *Record of Chemical Progress, Fall Issue, 1951*, p. 155.
5. Low, B. W. and Grenville-Wells, H. J. *Proc. Natl. Acad. Sci. U. S.* **39** (1953) 785.
6. Levy, H. A. and Corey, R. B. *J. Am. Chem. Soc.* **63** (1941) 2095.
7. Donohue, J. *J. Am. Chem. Soc.* **72** (1950) 949.
8. Yang, J. T. and Doty, P. *J. Am. Chem. Soc.* **79** (1957) 749, 761.
9. Elliott, A., Hanby, W. E. and Malcolm, B. R. *Nature* **178** (1956) 1170.

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