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β-Structures of Polypeptides with L- and D-Residues

Part I. Synthesis and Conformational Studies

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Summary. A series of five alternating poly(leucyl-lysyl) samples with varying amounts of L- and D-residues randomly distributed along the chain, but evenly shared out amongst leucyl and lysyl residues were synthesized by condensation of a mixture of the four diastereoisomeric dipeptide *p*-nitro-phenylesters. Their behavior in aqueous solution at various ionic strengths was studied by infrared spectroscopy which allowed measurement of the total amount of β-structures, and by circular dichroism which gives the excess of L-residues over D-residues in the same structures. Comparison with the properties of the all L-poly(Lys-Leu-Lys-Leu) shows that incorporation of a few D-residues in a L-chain seems to reduce the width of the β-sheets obtained in presence of salt. Higher proportions of D-isomers prevent the coil → β transition from occurring when the ionic strength is increased except for segments containing at least 6 to 7 adjacent residues of the same configuration.

Key words: β-structure – Poly DL-peptides – Enantiomer enrichment.

Introduction

Present proteins are built up with amino acid residues of identical handedness, and their backbone itself is folded into regular chiral structures, α-helices and β-sheets, which govern their biological activity. Yet it is generally admitted that the first amino acids available on the primitive earth were most probably racemic mixtures, and that the earlier proteinoids contained both enantiomers. Therefore, it is important to investigate the influence of random incorporation of both enantiomers in a polypeptide chain on the stability of its regular conformations, not only from a pure physicochemical interest, but also from the point of view of evolution.

Ramachandran and Chandrasekaran (1972) have briefly examined this question theoretically. They found that only two large areas were overlapping when the conformational maps of the L- and D-residues are superposed. These are the right and left

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handed α-helical regions. They mentioned also that a few other regions are allowed, such as in the vicinity of the fully extended chain, but these were thought to be energetically much less favorable. No pleated sheet structures with either parallel or antiparallel strands can be built up with chains containing both L- and D-residues randomly distributed. This is also clearly understood when one examines atomic models. Indeed, Cβ atoms of the side chains would be forced into the plane of the sheet, giving rise to conflicting steric contacts.

It is noteworthy that Pauling and Corey previously described sheet structures in which L- and D-residues cohabit, but in a given ordered way. These are the rippled sheet structures (Pauling and Corey, 1953), with either parallel or antiparallel arrangement of chains of an equimolar mixture of poly L- and poly D-peptides, and the so-called β-polar extended structure (Pauling and Corey, 1951) in which alternatingly the torsional angles of the residues correspond to the right and left handed α-helix, so that the conformational repeating unit is a dipeptide. This last structure probably can accommodate randomly distributed enantiomers.

Helices of strictly alternating poly DL-peptides have been described theoretically by several authors (Ramachandran and Chandrasekaran, 1972; Hesselink and Scheraga, 1972; Veatch and Blout, 1974; Colonna-Cesari et al., 1977). These regular structures have a basic DL-dipeptide repeating unit and are either single or double stranded channeled helices. The torsional angles of the enantiomers are located in their respective β-region.

Experimentally, it is well known that random poly DL-peptides can adopt a distorted α-helical conformation, less stable than the α-helix of the optically pure corresponding polypeptide (Spach, 1959; Ballard et al., 1960; Benoit et al., 1967; Spach et al., 1968). Single (π_{DL}) and double ($\pi\pi_{DL}$) stranded helical structures have been characterized for strictly alternating poly-DL-peptides (Heitz et al., 1975; Lotz et al., 1976) as well as the polar β-structure (β_{DL}) (Lotz et al., 1973). The rippled sheet structure has been described for the present time only for a non-chiral polymer, i.e., polyglycine (Lotz, 1974). Akaike et al. (1976) have found, by X-ray crystallography and infrared spectroscopy, a pleated sheet β-structure for a poly DL-valine sample in which D- and L-residues are thought to be randomly distributed along the chain. This result is puzzling in view of the theoretical consideration just discussed.

In this paper, we report experiments run on a polypeptide system giving rise to β-structures in aqueous solutions (Brack and Orgel, 1975; Brack and Caille, 1978). Strict alternation of hydrophilic and hydrophobic residues forces the polypeptide chain to adopt a β-conformation in water by raising the ionic strength. It was previously shown (Brack, 1977; Brack and Caille, 1978) that incorporation of about 5% D-isomer had a sensitive effect on the β-structure formation. It was therefore interesting to increase gradually the proportion of randomly distributed D-residues of both leucine and lysine in poly(Leu-Lys) up to the racemic mixture and to examine what kind of structure, if any, is formed.

Materials and Methods

General Procedures for the Synthesis

As previously described (Brack and Caille, 1978), the optically pure poly(Lys-Leu-Lys-Leu) was prepared by condensation of the corresponding tetrapeptide 2-hydroxyphenyl ester and the partially racemized sample poly(Leu-Lys)^{rac} by condensation of the dipeptide *p*-nitrophenylester.

The random incorporation in the chain of given amounts of D-isomers equally distributed among leucyl and lysyl residues was first attempted by condensation of dipeptide *p*-nitrophenylesters obtained by coupling a mixture of N-protected D- and L-leucine with a mixture, in the same proportion of D- and L-N$^{\epsilon}$-benzyloxycarbonyl-lysine *p*-nitrophylester, according to the general scheme[1]

$$\text{Nps-Lys(Z)-OH, NCHA} \xrightarrow[\text{DCCI}]{p\text{-nitrophenol}} \text{Nps-Lys(Z)-ONp} \xrightarrow{\text{HCl}} \text{HCl,H-Lys(Z)-ODp}$$

$$\xrightarrow[\text{DCCI}]{\text{Nps-Leu-OH,DCHA}} \text{Nps-Leu-Lys(Z)-ONp} \xrightarrow{\text{HCl}} \text{HCl,H-Leu-Lys(Z)-ONp.}$$

This procedure failed since the coupling reaction with a 10% excess of L-isomers led to an optically inactive material in 70% yield. Selective loss of LL- and DD-diastereoisomers probably occurred during the purification of the dipeptides.

Finally, the polymers were prepared by condensation of mixtures of L-Leu-L-Lys (Z), L-Leu-D-Lys(Z), D-Leu-L-Lys(Z) and D-Leu-D-Lys(Z) *p*-nitrophenylesters following the procedure already described for the synthesis of poly(Leu-Lys)^{rac} (Brack and Caille, 1978):

$$\text{HCl,H-Leu-Lys(Z)-ONp} \xrightarrow{\text{TEA}} [\text{Leu-Lys(Z)}]_n \xrightarrow{\text{HBr}} (\text{Leu-Lys})_n$$

The inactive polymer was obtained starting with an equimolar mixture of the four diastereoisomers. Enrichment in L-residues was achieved by co-condensation of equal amounts of LD-, DL- and DD-diastereoisomers with increasing amounts of the LL-isomer.

Peptide Intermediates (Table 1)

General procedures have already been described for HCl,H-L-Leu-L-Lys(Z)-ONp (Brack and Caille, 1978). Compounds were purified by crystallization or precipitation in solvents given in Table 1. The purity of the compounds was checked by t.l.c. using Merck precoated plates of silica gel (60-F-254). Melting points were determined with a hot-plate Leitz microscope.

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[1] *Abbreviations:* The abbreviations employed follow the IUPAC-IUB recommendations (J. Biol. Chem., 1972, 247, 977) with, in addition, DCCI: dicyclohexylcarbodiimide; DCHA: dicyclohexylamine; TFA: trifluoroacetic acid; DCA: dichloroacetic acid; TEA: triethylamine

Table 1. Properties of amino acids and peptides intermediates

Sample	Yield (%)	R_f [a]	M_p (°)	$[\alpha]_{546}^{25}$ (°) c 1.0	Chloride argentometric titration (%)
Nps-D-Leu-OH,DCHA	86[b]	0.63 A	178-180	+ 84.0 DMF	–
Nps-D-Lys(Z)-OH,DCHA	65[b]	0.54 A	180-183	+ 35.5 DMF	–
Nps-D-Lys(Z)-ONp	73[c]	0.80 B	77-78	+118.8 $CHCl_3$	–
HCl,H-D-Lys(Z)-ONp	79[d]	dec.	149-151	- 5.5 AcOH	100
Nps-L-Leu-D-Lys(Z)-ONp	64[e]	0.69 C	138-144	- 73.1 $CHCl_3$	–
Nps-D-Leu-L-Lys(Z)-ONp	65[e]	0.71 C	142-143	+ 73.7 $CHCl_3$	–
Nps-D-Leu-D-Lys(Z)-ONp	62[f]	0.72 C	106-112	+ 63.7 $CHCl_3$	–
HCl,H-L-Leu-D-Lys(Z)-ONp	84[g]	dec.	hyg.	+ 62.4 AcOH	97
HCl,H-D-Leu-L-Lys(Z)-ONp	61[g]	dec.	hyg.	- 62.5 AcOH	97
HCl,H-D-Leu-D-Lys(Z)-ONp	96[h]	dec.	149-153	+ 9.2 AcOH	99

[a]Solvents for chromatography: *A*: chloroform-acetic acid-butanol (85:5:10), *B*: acetone-acetic acid (98:2), *C*: acetone; [b]from ethanol; [c]acetone/di-isopropyl ether; [d]from acetone; [e]acetone/ether; [f]from ethyl acetate and di-isopropyl ether; [g]chloroform/ether-petroleum ether (1:1); [h]tetrahydrofuran/ether

Polymers (Tables 2 and 3)

Triethylamine used for polycondensation reactions was distilled once over benzyloxycarbonyl-glycine *p*-nitrophenylester, and then redistilled.

Cleavage of the side-chain protecting groups with HBr was controlled by UV absorption (Beckman Acta III) on a 1 g l^{-1} aqueous solution in a 1 cm cell at 257 nm. The samples contained less than 0.2% remaining groups.

Concentrations of polymer solutions were determined from the optical density at 205 nm assuming an extinction coefficient of 3,200 per mean residue for the disordered conformation (Rosenheck and Doty, 1961).

Viscosity was measured with an Ubbelhode viscosimeter Cannon CUSMU size 75 for trifluoroacetic acid and size 150 for dichloroacetic acid. Optical rotations were

Table 2. Protected $[Leu\text{-}Lys(Z)]_n$ samples

Polymers	$[\alpha]_{546}$ (°) c 1.0 DCA	$[\eta]$ ml g^{-1} DCA	Yields % Protected	Deprotected
50:50	0	11.9	66	43
40:60	- 2.8	8.8	66	26.4
30:70	-11.6	11.1	80	37
20:80	-18.9	13.6	95	40
10:90	-22.3	9.6	74	30
0:100[a]	-43.4	16.2	48	38

[a]Sample poly(Leu-Lys)^{rac}

Table 3. Properties of polymer samples

D:L ratios in monomers	$[\eta]$ ml g^{-1} in TFA	$[\alpha]^{25}_{546}$ (°) c 1.0 TFA	$[m]_{546}$ (°)	$[m]_{546}$ (°) calculated[e]	α'	D:L residues in polymers
50:50	22.0	0.04	0.0	0.0	0.00	50:50
40:60	24.4	- 18.6	- 29.2	- 28.6	0.18	41:59
30:70	23.5	- 37.4	- 58.6	- 57.2	0.36	32:68
20:80	37.0	- 54.4	- 85.3	- 85.9	0.54	23:77
10:90	19.8	- 68.5	-107.4	-114.4	0.72	14:86
0:100[a]	33.3	- 94.1	-147.5	-143.0	0.90	5:95
0:100[b]	44.0	-103.0	-161.5	-159.2	1.00	0:100
Poly(Lys-HCl, $2H_2O$)		- 83.0[c]	-166.4	–	–	–
Poly(Leu)		-134.5[d]	-152.0	–	–	–

[a]Sample poly(Leu-Lys)[rac]; [b]sample poly(Lys-Leu-Lys-Leu); [c]unpublished data; [d]from Le Guilly et al. (1978); [e]calculated from the relation

$$m = \frac{\alpha}{2}\left\{[m]^{Lys}(1 - f) + [m]^{Leu}\right\}$$

(see text for explanations)

determined using a Perkin-Elmer 141 M polarimeter (1 dm cell). CD spectra were recorded on a Roussel-Jouan 185 model II dichrograph at 22-24°C. Infrared spectra were run on 3% solutions in D_2O in CaF_2 cells. Solid $NaClO_4$ was added to aliquots of polymer solutions.

Results

Characteristics of the Samples

Characteristics of the free polymers isolated after dialysis against water as hydrochloride salt with two molecules of water per lysine residue are given in Table 3.

With regard to the viscosimetric measurements, a molecular weight of 5,200 daltons has been reported for poly(Val-Lys) ($[\eta]$ = 24.8 ml.g^{-1} in TFA). Thus, one can assume that the samples of Table 2 have a molecular weight near that value or higher, since it it known that for a given molecular weight, a poly DL-peptide has a lower reduced viscosity than the corresponding all-L-polypeptide (Spach, 1959; Miller et al., 1967).

The optical purity of the all-L-poly(Lys-Leu-Lys-Leu) was checked using D-amino acid oxydase after acid hydrolysis in conjunction with quantitative analysis of the remaining L-amino acid. This polymer was shown to be optically pure (Brack and Caille, 1978). The same procedure could not be used to determine the percentage of D-residues in the other samples, since D-lysine was shown to be resistant to the enzyme at pH 10, due to an inhibitory effect of L-leucine (Brack and Caille, 1978). Therefore, optical rotations were used to calculate the percentage of D-residues, taking into account the racemization reaction which affects the C-activated lysyl residues with the active

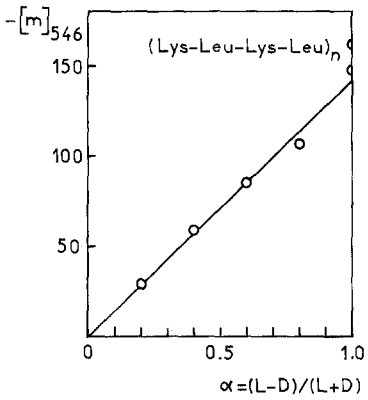

Fig. 1. Plot of $[m]^{25}_{546}$ of the different samples as a function of α, the proportion of L-residues in excess over D-residues in the monomers mixture

ester procedure used to synthesize the samples. We have already reported that polymerization of L-leucyl-N$^{\epsilon}$-benzyloxycarbonyl-L-lysyl *p*-nitrophenyl ester yields a partially racemized polymer (Brack and Caille, 1978).

The molar residue rotations of lysyl and leucyl appear to be additive in the copolymer (compare the rotations of poly(Lys), poly(Leu) and poly(Lys-Leu-Lys-Leu) in Table 3). A plot of the mean molar residue rotations $[m]_{546}$ of the copolymers versus $\alpha = (L - D)/(L + D)$, the fraction of L-residues in excess over D-residues in the monomers mixture, yields a straight line (Fig. 1) from the slope of which an average racemization yield f of 0.20 can be calculated. Indeed, the slope represents

$$1/2 \left\{ [m]^{Lys}_{546} (1 - f) + [m]^{Leu}_{546} \right\} .$$

This value of f means that 20% of the lysyl residues are racemized. Finally the fraction α of L-residues (Lys and Leu) in excess over D-residues in the copolymers is easily shown to be equal to $\alpha(1 - f/2)$. Thus, the D:L residues ratios calculated on that way are reported in Table 3.

Conformational Study

Salt molarities for infrared and circular dichroism measurements were chosen as high as possible, but not so high as to cause precipitation. The allowed salt concentration decreases when the polymer is enriched in L-residues. The concentrations of polymer and salts are given in the captions of Figure 2 for infrared and Figure 3 for circular dichroism. Both spectra, in pure water (D_2O for infrared) and in salt solutions were recorded for each sample.

The infrared spectra obtained in the absence of salt are very similar for all the polymers. The amide I and II absorption bands centered at 1642 cm^{-1} and 1450 cm^{-1} are attributed to a random coil conformation, as confirmed by the CD spectra. In pure water, the CD spectra differ quantitatively since the proportion of L-residues which contribute to the rotational strength varies from one sample to the other. However, they all look like the spectrum previously described for the random coil conformation

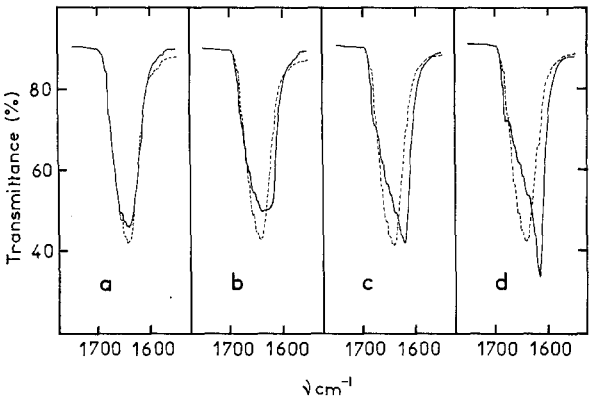

Fig. 2. Infrared spectra of polymers in D_2O (*dashed lines*) and in $NaClO_4$ solutions (*full lines*). (*a*) 50:50 sample (0.40 M $NaClO_4$); (*b*) 30:70 sample (0.38 M $NaClO_4$); (*c*) 20:80 sample (0.21 M $NaClO_4$); (*d*) 10:90 sample (0.11 M $NaClO_4$). Polymer concentration 3%

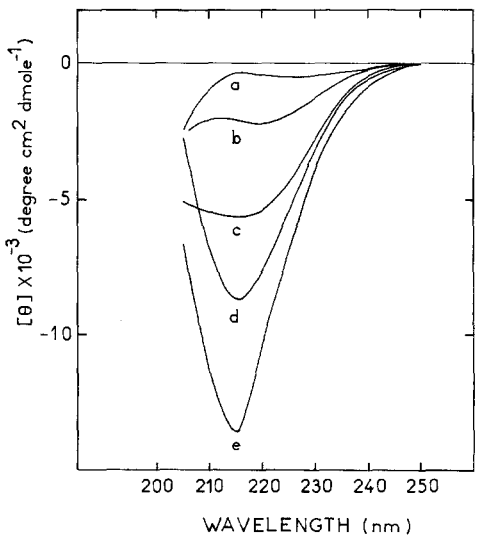

Fig. 3. Circular dichroism spectra of polymers. (*a*) 40:60 sample in H_2O; (*b*) 40:60 sample in 0.5 M $NaClO_4$; (*c*) 30:70 sample in 0.5 M $NaClO_4$; (*d*) 20:80 sample in 0.1 M $NaClO_4$; (*e*) 10:90 sample in 0.1 M $NaClO_4$. Polymer concentration 0.2%

of poly(Leu-Lys)^{rac} (Brack and Caille, 1978). An example is given in Figure 3 for the 40:60 sample.

In the presence of salt, few changes occurred in the infrared spectrum of the 50:50 polymer, suggesting that the random coil conformation is essentially maintained. A small shoulder is visible at 1615 cm^{-1} (Fig. 2a) supporting the idea that a small fraction of β-structure is formed. When the percentage of L-residues increases, evidence is found for the formation of β-structures with antiparallel chains. The infrared spectra show an Amide I band at 1615 cm^{-1} with a shoulder at 1675 cm^{-1}, while CD spectra become typical of mixtures of random coil and β-conformations. The trough at 215 nm, characteristic of the β-form, develops and gradually appears as a single band. A striking feature is that the poly(Leu-Lys)^{rac} sample has a greater molar ellipticity at 215 nm that the optically pure polymer poly(Lys-Leu-Lys-Leu) (Table 4).

Discussion

The overall β fraction in salt solutions was calculated from the infrared spectrum of each sample, taken as a mixture of random coil and β-form. This fraction is expressed as $(l_\beta + d_\beta)/(l + d)$, where l and d represent the total amount of each residue in the polymer, and l_β, d_β, their amount in β-sheets. A standard spectrum for pure random coil was obtained by subtracting from the spectrum of the 50:50 sample a small fraction, arbitrarily taken as 5% of the 10:90 sample spectrum. Owing to the difficulty of dissolving sample poly(Lys-Leu-Lys-Leu), known to be fully in the β-form in salted solution as shown by circular dichroism, at sufficiently high concentration, it was not possible to obtain directly a precise standard spectrum for the β-structure. However, an optical density of 0.520 could be measured at 1615 cm^{-1}. Thus, a β-standard spectrum was calculated by subtracting an increasing fraction of random coil standard from the 10:90 sample spectrum until an optical density of 0.520 was found at 1615 cm^{-1}. Then the experimental spectrum of each sample was compared to mixtures of random coil and β-form standard spectra. Rather good agreement was found between the recombined and the experimental spectra, using the β-form percentages indicated in Table 4.

On the other hand, the molar ellipticity at 215 nm can be used for measuring the overall fraction of L-residues in β conformation in excess over D-residues in the same form, expressed as $(l_\beta - d_\beta)/(l + d)$. First, the contribution of the random coil calculated from the infrared results and from the values of $[\theta]_{215}$ found in pure water (Table 4) must be subtracted from the molar ellipticity measured in salt solution. Although this procedure is not exact, as it supposes that the enantiomer composition of the random coil fraction remains the same as in pure water when the β-conformation is formed, the corrections are small (see Fig. 5, dotted line) and do not alter the conclusions. Then, the corrected $[\theta]_{215}$ values must be standardized by dividing them by the 0:100 sample value which, in fact, is not well defined (see Fig. 5). The unexpected large ellipticity of the negative n-π^* transition of sample poly(Leu-Lys)^{rac}, as compared with the ellipticity of the optically pure polymer, can be explained on theoretical grounds. Indeed, calculation of the magnitude of this spectroscopic transition shows

Table 4. β-form and CD characteristics

Sample	D:L residues in polymer	% β-form	$[\theta]_{215}$ water	$[\theta]_{215}$ salt solution	$[\theta]_{215}^{corr\ a}$ salt solution	$\frac{[\theta]^{corr}}{[\theta]^{extrapc}}$
50:50	50:50	4.0	0.0	0	0	0
40:60	41:59	18.0	- 380	- 2,100	- 1,950	0.09
30:70	32:68	34.0	- 380	- 5,600	- 5,400	0.25
20:80	23:77	54.5	- 650	- 8,700	- 8,400	0.39
10:90	14:86	81.5	- 1,400	-13,700	-13,400	0.62
(Leu-Lys)^{rac}	5:95	90.0	- 2,100	-20,900[b]	-20,700	0.96
(Lys-Leu-Lys-Leu)$_n$	0:100	100	-17,100	-16,500[c]	-16,500	0.77

[a]The contribution of the random coil conformation was subtracted (see text); [b]$NaClO_4$, 0.05 M; [c]$NaClO_4$, 0.04 M

that it should increase on decreasing the number of chains that are associated in the β-sheets (Woody, 1969). If, as we will conclude later, each β-sheet is formed with residues of the same configuration, it may be limited on its fringes by chain containing residues of foreign configuration, thus precluding larger aggregations, and leading to rather narrow β-sheets. When the proportion of D-residues exceeds 10% the ellipticity at 215 nm then decreases because the proportion of L-residues in β-conformation decreases, and also because the coil-to-β transition occurs to a lesser extent. An extrapolated value of $[\theta]_{215}$ for a D:L ratio of 0:100 of about 21.500 appears then reasonable.

The ratios $l_\beta/(l + d)$ and $d_\beta/(l + d)$ expressing the fraction of L- and D-residues inserted in β-structures were calculated as a function of n_β, the minimum number of contiguous residues of same chirality required for the formation of β-sheet structures, and of $x = L/(L + D)$, the relative amount of L-residues in the chains. The calculation is given in the appendix. The chain length was supposed to be infinite with LL, LD,

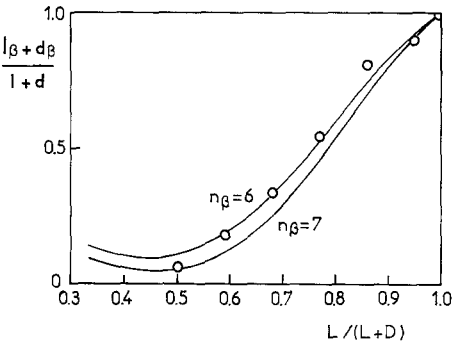

Fig. 4. Variation of the overall $(l_\beta + d_\beta)/(l + d)$ β-fraction with $x = L/(L + D)$. The points are taken from the β-form percentage calculated from infrared spectra (Table 4). The curves were calculated from relations (11) and (12) with $n_\beta = 6$ and $n_\beta = 7$

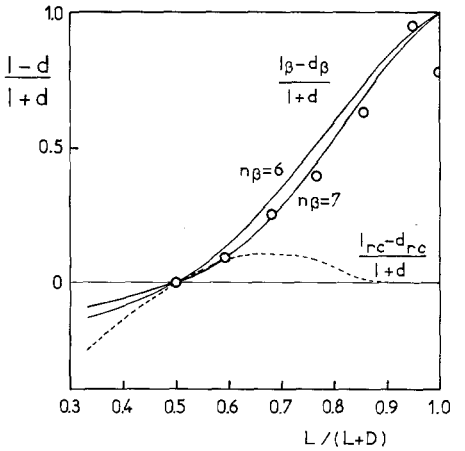

Fig. 5. Variation of the fraction of L-residues in a given conformation in excess over D-residues in the same conformation with x, the fraction of L-residues. *Full lines and points:* β-sheet structure. The curves were calculated from relations (11) and (12) with $n_\beta = 6$ and $n_\beta = 7$. The points are taken from Table 4, last column. *Dotted lines:* random coil conformation. The curve was calculated from relation (18) with $n_\beta = 7$

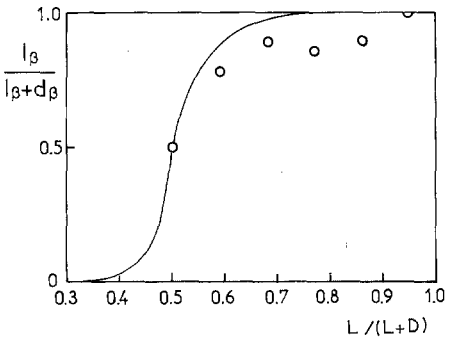

Fig. 6. Fraction of L-residues in the overall β-sheets $l_\beta/(l_\beta + d_\beta)$ as a function of x = L/(L + D). The points are taken from Figures 4 and 5. The curve was calculated from relation (15)

DL and DD monomeric units randomly distributed. From these ratios, one calculates the total fraction of β-structure $(l_\beta + d_\beta)/(l + d)$ and the excess L-residues in these structures $(l_\beta - d_\beta)/(l + d)$ and compares their values with the corresponding experimental data found from infrared and circular dichroism measurements. In Figures 4 and 5, two curves were calculated for varying x and two numerical values of n_β. It can be seen that the experimental points fall in both cases near the theoretical curve obtained with $n_\beta = 7$, an expected value for the formation of β-sheets (Bonora and Toniolo, 1974).

From the above calculations it is also possible to evaluate the fraction of L-residues in the β-sheets $l_\beta/(l_\beta + d_\beta)$ as a function of x. The theoretical curve corresponding to $n_\beta = 7$ is given in Figure 6 together with the experimental values obtained from $(l_\beta + d_\beta)/(l + d)$ and $(l_\beta - d_\beta)/(l + d)$. It can be seen that β-sheets are rapidly enriched in L-residues when x varies from 0.5 to 1.0. Within the limits of experimental errors, the experimental values follow the calculated curve.

Conclusion

If the residues are of uniform chirality, the formation of β-sheet structures in polymers with alternating hydrophilic-hydrophobic residues is readily and completely achieved in aqueous solutions when the ionic strength is increased. The results presented here clearly show that if enantiomer residues of opposite chirality are randomly introduced in the polymer chain, the formation of β-structure is depressed the more there are foreign residues, even at higher salt concentrations. This tends to indicate that, as expected from steric considerations, it is not possible to force D-residues into sheets made of L-isomers, and vice-versa, at least in our case. This is in contradiction with the data of Akaike et al. (1976) who found β-structures in random poly DL-valine, however in the solid state.

The second point, emerging from these calculations is that, as seen from the curve in Figure 6, β-sheet structures are rapidly enriched in L-residues when the ratio x = L/(L + D) increases from 0.5 to 1.0.

From the good agreement between experimental results and theoretical calculations based on the sole formation of β-structure with chain segments containing at least seven adjacent residues of uniform chirality, we think that molecules in the range of

x equal to 0.75 and above can be visualized as particles of almost optically pure β-sheets surrounded by random coil portions containing both L- and D-residues. This image suggests processes of asymmetry amplification which will be discussed in the accompanying paper. Therefore, from the chemical evolution point of view, it seems unlikely that large soluble β-sheets could be formed with racemic polymers, even when a small excess of one enantiomer is present. However, special attention should be devoted to the possible role of small β-surfaces as matrices for stereoregulation and as "seeds" for conformational organization.

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Appendix

Calculation of the fractions of residues engaged in different conformations, for infinite chain length and random distribution of the monomeric dipeptide units.

The relative amount of each LD, DL, and DD dipeptide unit in the polymer chains being represented by y, that of the LL unit is (1 - 3y). Then the amount of L-(and D-) residues present in segments of the chain of n residues of same configuration is calculated equal to, for L-residues:

(1) $l/(l + d) = 2\, n_0 y^2 (1 - 3y)^{(n_0-1)/2}$, for odd n ($n_0$), and

(2) $l/(l + d) = 2\, n_e y^2 (1 - 3y)^{n_e/2-1} (5/4 - 3y)$ for even n (n_e)

and for D-residues:

(3) $d/(l + d) = n_0 y^{(n_0+1)/2} (1 - 2y)$, for odd n, and

(4) $d/(l + d) = (n_e/2)\, y^{n_e/2} (1 - 3y + y^2)$, for even n.

Summing for all $n \geqslant n_\beta$, the minimum number of consecutive residues of same chirality required for the formation of β-sheet structure, one obtains in case of $n_\beta = n_0 = n_e - 1$, the fraction $l_\beta/(l + d)$ or $d_\beta/(l + d)$ of residues in the β conformation:

(5) $l_\beta/(l + d) = (1 - 3y)^{(n_\beta-1)/2} \cdot [1 + (3n_\beta - 7)y/2 - 2(n_\beta - 1)y^2]$, and

(6) $d_\beta/(l + d) = y^{(n_\beta+1)/2} \cdot [(3n_\beta + 1)/2 - 2(n_\beta - 1)y]$,

and in case of $n_\beta = n_e = n_0 - 1$:

(7) $l_\beta/(l + d) = (1 - 3y)^{n_\beta/2-1} [1 + (3n_\beta/2 - 5)y - 2(2n_\beta - 3)y^2]$, and

(8) $d_\beta/(l + d) = y^{n_\beta/2} (n_\beta/2 + 2y)$.

These relations (5)-(8) can be expressed as functions of x = L/(L + D), for $n_\beta = n_0 = n_e - 1$:

(9) $l_\beta(l + d) = [(3x - 1)/2]^{(n_\beta-1)/2} \cdot [(n_\beta - 1)/4 + (n_\beta + 3)x/4 - (n_\beta - 1)x^2/2]$

(10) $d_\beta/(l + d) = [(1 - x)/2]^{n_\beta/2-1} \cdot [(n_\beta + 3)/2 + (n_\beta - 1)x]$,

and for $n_\beta = n_e = n_0 - 1$:

(11) $l_\beta/(l + d) = [(3x - 1)/2]^{n_\beta/2-1} [-n_\beta/4 + (5n_\beta - 2)x/4 - (2n_\beta - 3)x^2/2]$

(12) $d_\beta/(l + d) = [(1 - x)/2]^{n_\beta/2} [(2 + n_\beta)/2 - x]$

Other interesting relations are:

(13) $(l_\beta + d_\beta)/(l + d)$

(14) $(l_\beta - d_\beta)/(l + d)$, readily calculated from relations (9) and (10) or (11) and (12), and

(15) $l_\beta/(l_\beta + d_\beta)$, obtained by dividing (9) or (11) by (13) which represents the fraction of L-residues in the overall β-segments, and

(16) $l_{rc}/(l + d) = x - l_\beta/(l + d)$, expressing the fraction of L-residues engaged in random coil segments, and

(17) $l_{rc}/(l_{rc} + d_{rc}) = [x - l_\beta/(l + d)]/[1 - (l_\beta + d_\beta)/(l + d)]$

(18) $(l_{rc} - d_{rc})/(l + d) = (2x - 1) - (l_\beta - d_\beta)/(l + d)$, related to the optical activity of the random coil segments.

Relations (13) and (14) were calculated numerically for $n_\beta = 6$ and $n_\beta = 7$, and reported in Figures 4 and 5. Relations (15) and (18) are shown in Figure 5 for $n_\beta = 7$. The last relation shows that probably almost no corrections should be done for the $[\theta]_{215}$ values in Table 4.

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