ALPHA AND BETA CONFORMATIONS OF WATER SOLUBLE SEQUENTIAL ISO POLYPEPTIDES

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Dedicated to the memory of Dr Karel Bláha.

Alternating poly(Leu-Lys) and its isopolypeptide poly(Leu-Lys-Lys-Leu) were synthesized via polycondensation of *p*-nitrophenyl esters of the corresponding protected peptides. Addition of one equivalent of 1-hydroxybenzotriazole and varying amounts of a tertiary base allowed to control the molecular weights of the samples. The conformation of the water soluble polypeptides was investigated by circular dichroism. Poly(Leu-Lys) adopts a β-sheet conformation in the presence of salt while poly(Leu-Lys-Lys-Leu) adopts an α-helical conformation. For polypeptides based on a 1 : 1 composition of hydrophobic (A) and hydrophilic (B) residues, the shortest repeat for the formation of a β-sheet is -AB- whereas -AABB- represents the shortest repeat for an α-helix formation.

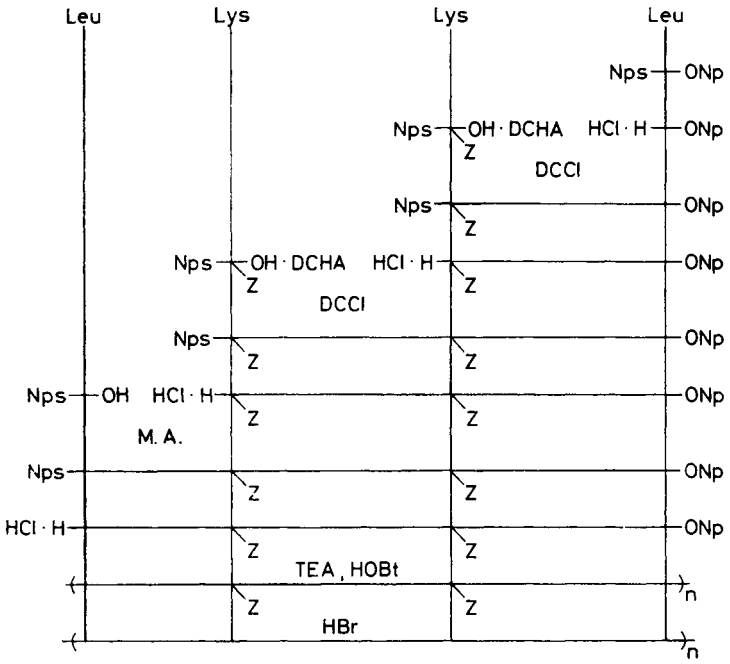
Many periodicities in amino acid sequences have been discovered in animal proteins. In addition to the well-known examples of fibrous proteins of silk fibroin, collagen and keratin[1], tandemly repeats of Ala-Thr-Ala have been found in antifreeze proteins[2]. In the circumsporozite protein of *Plasmodium falciparum*, the main repeating section consists of Asn-Ala-Asn-Pro repeated 37 times[3]. In the organic matrix of mollusk shells, the soluble protein is partly composed of a repeating sequence of aspartic acid separated by either glycine or serine[4]. Tandem repeats were also found in a plant protein, glutelin-2 of maize, in which Val-His-Leu-Pro-Pro-Pro is repeated 6 times[5].

As expected, the conformation of the polypeptides with repeated sequences is related to the nature of the sequence. This is particularly true for the α-helical segments which are thought to be endowed with heptapeptidic periodicities. We have previously shown that polypeptides with binary repeat of hydrophilic and hydrophobic residues adopt a β-sheet structure in water, in the presence of salt[6,7]. Poly(Leu-Lys) represents a typical example of such a behaviour. In the present paper, we show that repeats of Leu-Lys-Lys-Leu induce the formation of an α-helix.

RESULTS AND DISCUSSION

General Procedure for the Synthesis

Poly(Leu-Lys) and poly(Leu-Lys-Lys-Leu) were prepared by condensation of the corresponding *p*-nitrophenyl esters in the presence of 1-hydroxybenzotriazole (HOBt) according to Scheme 1. HOBt was added in order to minimize the extent



SCHEME 1

of racemization[8]. In addition, due to its pK_A of 4·3, the additive may complex to the amino groups of the growing chain, thus acting as a poison. Therefore, varying amounts of a tertiary base were used to polymerize the protected peptide ester hydrochloride in DMF.

Removal of the lysine side-chain protecting groups was achieved by treating the protected polymer with hydrogen bromide in a dichloroacetic acid–chloroform mixture. The cleavage was controlled by UV absorption at 257 nm.

Characteristics of the Samples

Characteristics of the polymers isolated after dialysis are given in Table I. Molecular weights were estimated with a calibration curve which relates the intrinsic viscosity

measured in trifluoroacetic acid to the average number of residues per chain[9]. Lyophilized samples of poly(Leu-Lys) when stabilized in ambient air were found to contain two molecules of water per lysyl residue[7]. The same amount of water was taken for poly(Leu-Lys-Lys-Leu).

Depending on the number of equivalents of tertiary amine used for the polycondensation step, number average molecular weights ranging from 3 700 to 15 600 and from 23 600 to 34 900 were obtained for poly(Leu-Lys) and for poly(Leu-Lys-Lys-Leu), respectively.

No systematic check for racemization was undertaken. However, from previous reports on poly(Arg-Leu) prepared under the same conditions[10], it can be assessed that no more than few percents of the overall residues may have been replaced by D-enantiomers during the polycondensation step. This is confirmed by the fact that the high molecular samples of poly(Leu-Lys) and of poly(Leu-Lys-Lys-Leu) present similar optical rotation, $[\alpha]_{546}^{25}$ $-95{\cdot}5 \pm 0{\cdot}4°$, under identical conditions.

Conformational Study by Circular Dichroism

In pure water, the CD spectrum of the polypeptides is that of a random coil, due to charge repulsion (Figure 1). Addition of salt to the tandemly repeated, but not alternating, poly(Leu-Lys-Lys-Leu) induces the formation of an α-helix (Figure 2). The ellipticities are in good agreement with those published previously[11]. Under the same conditions, alternating poly(Leu-Lys) adopts a β-structure, as demonstrated previously[6,7]. In 0·1M-NaCl, only the highest molecular weight sample exhibits a complete transition (Figure 2). In 0·2M-NaCl, the transition is complete for both samples (Figure 3). Therefore, twelve repeats seem to be the threshold for the formation of a β-structure. However, it must be noted that the samples are polydispersed. Oligopeptides with well-defined length will be required to determine the threshold with more accuracy.

TABLE I
Properties of Free Polymer Samples

Sample	Equivalent of TEA	$[\alpha]_{546}^{25}$ deg. *c* 1·0 TFA	$[\eta]$ cm³/g in TFA	$\overline{M}n$
Poly(Leu-Lys, HCl, 2 H_2O)	1	−87·35	19·0	3 700
Poly(Leu-Lys, HCl, 2 H_2O)	2	−95·1	34·0	15 600
Poly(Leu-Lys-Lys-Leu, 2 HCl, 4 H_2O)	1	−90·9	46·6	23 600
Poly(Leu-Lys-Lys-Leu, 2 HCl, 4 H_2O)	2	−95·8	66·0	34 900

CONCLUSIONS

The syntheses described in this paper indicate that it is possible to control the molecular weight within certain limits by using one equivalent of HOBt as additive and varying amounts of the tertiary base. In the case of poly(Leu-Lys), the molecular weights range between 3 700 and 15 600.

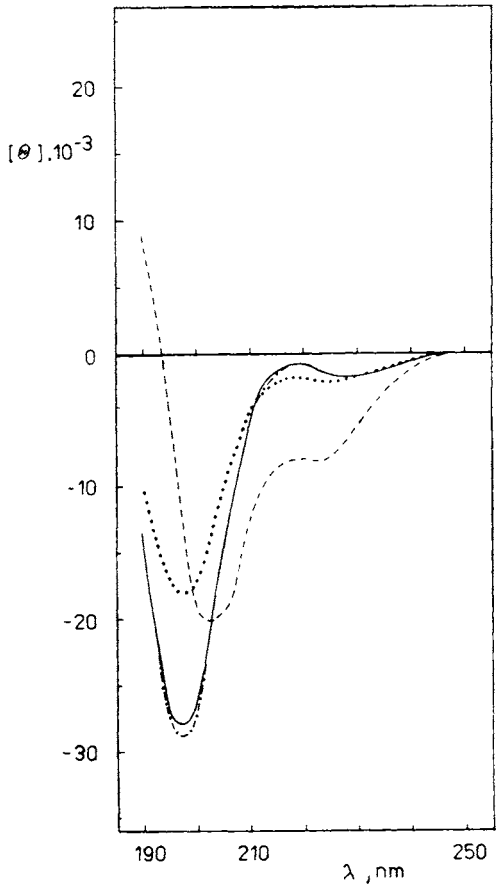


FIG. 1

Circular dichroism spectra of 0·2% solution of poly(Leu-Lys)^{3700} (——), poly(Leu-Lys)^{15600} (·····), poly(Leu-Lys-Lys-Leu)^{23600} (— — —) and of poly(Leu-Lys-Lys-Lys-Leu)^{34900} (-·-·-·) in pure water

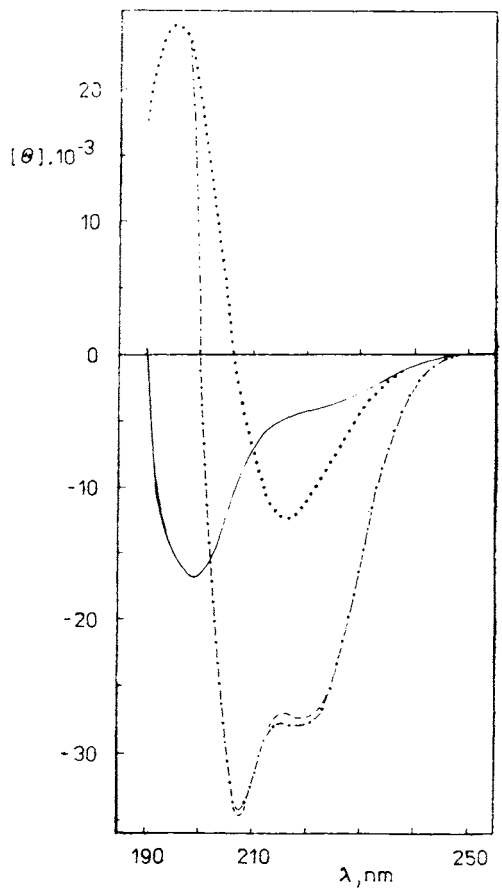


FIG. 2

Circular dichroism spectra of 0·2% solution of the four samples in 0·1M-NaCl: poly(Leu-Lys)^{3700} (——), poly(Leu-Lys)^{15600} (·····), poly(Leu-Lys-Lys-Leu)^{23600} (— — —) and poly (Leu-Lys-Lys-Leu)^{34900} (-·-·-·)

Strict alternation of hydrophilic and hydrophobic residues, i.e. a dipeptidic periodicity, induces the formation of a β-pleated sheet. When analyzing 72 β-sheets in proteins, Van Heijne and Blomberg[12] found a significant over representation of alternating hydrophobic-polar intrastrand pairs of amino acids. The tetrapeptidic periodicity -AABB- induces an α-helix. Such a dramatic change in the conformational tendency depending only on the sequence has already been briefly reported for poly(Arg-Leu) and poly(Leu-Arg-Arg-Leu)[13]. It can therefore be concluded that for a one-to-one composition of hydrophobic residues A and hydrophilic residues B, the shortest repeat for the formation of a β-sheet is -AB-, while -AABB- represents the shortest repeat for an α-helix formation. Syntheses are presently undertaken in our laboratory to determine the minimal peptide length required to obtain these two conformations in aqueous solutions.

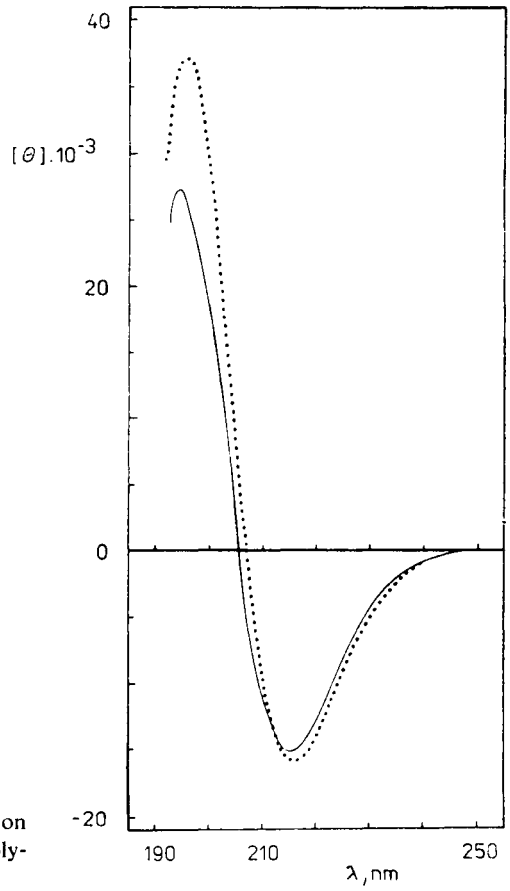


FIG. 3

Circular dichroism spectra of 0·2% solution of poly(Leu-Lys)^{3700} (———) and of poly-(Leu-Lys)^{15600} (·····) in 0·2M-NaCl

EXPERIMENTAL

The purity of the compounds was checked by thin-layer chromatography using Merck precoated plates 60 F 254 (silica gel). Optical rotations were determined using a Perkin–Elmer 141 M polarimeter (1-dm cell). The UV spectra were recorded on a Perkin–Elmer λ 15 spectrometer, while CD spectra were recorded on a Jobin–Yvon Mark IV Auto-dichrograph at 22–24°C. Viscosity was measured with an Ubbelhode viscosimeter Cannon CUSMU size 75. Melting points were determined with a hot-plate Leitz microscope. Solvents and triethylamine used for polycondensation were distilled once from benzyloxycarbonylglycine *p*-nitrophenyl ester and then redistilled.

Nps-Lys(Z)-Lys(Z)-Leu-ONp

Nps-Lys(Z)-OH.DCHA (ref.[14]) (3·68 g, 6 mmol) was dissolved in chloroform (100 ml). The solution was cooled to −10°C, at which point dicyclohexylcarbodiimide (1·24 g, 6 mmol) and HCl.H-Lys(Z)-Leu-ONp (ref.[15]) (3·3 g, 6 mmol) were added. After 2 h at −10°C and 2 h at room temperature, the solution was filtered and extracted twice with 5% aqueous sodium bicarbonate, 0·2M-H_2SO_4 and water. The organic phase was dried over Na_2SO_4 and evaporated to give an oil which crystallized after trituration under petroleum ether. The compound was crystallized from a hot mixture of hexane (100 ml) and 2-propanol (800 ml) (3·4 g, 61%): m.p. 150–155°C, $[\alpha]_{546}^{25}$ −44·3° (*c* 1, hexafluoro-2-propanol), R_F 0·89 (chloroform–methanol 4 : 1).

HCl.H-Lys(Z)-Lys(Z)-Leu-ONp

Nps-Lys(Z)-Lys(Z)-Leu-ONp (3·2 g, 3·5 mmol) was dissolved in acetone (50 ml), and 3·75M hydrogen chloride in diethyl ether (2 ml) was added. After 15 min of stirring, a 1 : 1 mixture of diethyl ether and petroleum ether (200 ml) was added (2·6 g, 91%): m.p. 146–148°C, $[\alpha]_{546}^{25}$ −31·1° (*c* 1, acetic acid), chloride argentometric titration 97%.

Nps-Leu-Lys(Z)-Lys(Z)-Leu-ONp

Nps-Leu-OH.DCHA (ref.[14]) (1·4 g, 3 mmol) suspended in ethyl acetate (15 ml) was vigorously shaken with 0·2M sulphuric acid (25 ml) until dissolved in the organic phase. The organic layer was washed with water, dried over sodium sulphate and evaporated to dryness. The oil was dissolved in tetrahydrofuran. The solution was cooled at −10°C at which time N-methylmorpholine (0·35 ml, 3 mmol) and isobutyl chloroformate (0·4 ml, 3 mmol) were added. After 15 min, HCl.H-Lys(Z)-Lys(Z)-Leu-ONp (2·44 g, 3 mmol) and N-methylmorpholine (0·35 ml, 3 mmol) were added. After 1 h at −10°C and 2 h at room temperature, the solution was filtered and poured in 2-propanol (100 ml) (2·6 g, 83%): m.p. 190–191°C, $[\alpha]_{546}^{25}$ −74·7° (*c* 1, hexafluoro-2-propanol), R_F 0·91 (chloroform–methanol 4 : 1).

HCl.H-Leu-Lys(Z)-Lys(Z)-Leu-ONp

Nps-Leu-Lys(Z)-Lys(Z)-Leu-ONp (2·5 g, 2·4 mmol) was dissolved in hot chloroform. After cooling, 3·75M hydrogen chloride in diethyl ether (1·35 ml) was added. After 15 min of stirring, a 1 : 1 mixture of petroleum ether and diethyl ether was added (200 ml) (2·1 g, 95%): m.p. 265°C (dec.), $[\alpha]_{245}^{25}$ −31·7° (*c* 1, acetic acid), chloride argentometric titration 97%.

Poly(Leu-Lys(Z)-Lys(Z)-Leu)

HCl.H-Leu-Lys(Z)-Lys(Z)-Leu-ONp (0·5 g, 0·54 mmol) was triturated with dimethylformamide (0·5 ml) until a homogeneous paste was obtained. 1-Hydroxybenzotriazole (0·082 g, 0·54 mmol) was added, and the mixture was triturated again. After addition of triethylamine (0·15 ml, 1·1 mmol), the mixture was set aside for 5 days. The paste was diluted with dimethylformamide (5 ml) and isopropylamine was added (0·05 ml, 0·6 mmol) to aminolyse remaining *p*-nitrophenyl ester groups, if any. After 15 min, the polymer was precipitated by addition of 5% aqueous sodium bicarbonate (0·25 g, 87%).

Poly(Leu-Lys(HCl)-Lys(HCl)-Leu)

Chloroform (80 ml) was added to a solution of the protected polypeptide (0·35 g) in dichloroacetic acid (6 ml). Hydrogen chloride was bubbled through for 30 min and then hydrogen bromide for 1 h. The turbid solution was concentrated under vacuum to c. 5 ml; the polymer was precipitated with acetone. The crude polymer was dissolved in water, dialyzed against 0·01M-HCl and water and finally freeze dried (0·2 g, 68%).

Poly(Leu-Lys(HCl))

The procedure used for the synthesis of poly(Leu-Lys-Lys-Leu) was followed starting with HCl.H-Leu-Lys(Z)-ONp (ref.[7]).

Preparation of the Solutions

The polymers are readily soluble in water. However, they cannot be dissolved directly in aqueous solutions of salts. Thus, salt was added to aqueous solutions to bring the final salt concentration to the desired value. 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[16].

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