portant stabilizing factor for a Si=C bond. Analysis of monoelectronic energy shifts and charge migrations indicates this conjugation to be comparable with that occurring in butadiene, and suggests a somewhat larger interaction in **2** than in **1**; however, the use of bond separation isodesmic reactions does not confirm the π-conjugation to be responsible for the great stability of **2**. Conjugation strongly stabilizes 1-silabutadiene with respect to its silylene isomer, but in the case of 2-silabutadiene the π C=Si—C=C conjugation is balanced by the $\pi_{C=C} \rightarrow 3p_{zSi}$ π delocalization occurring in the silylene isomer.

Acknowledgment. The authors thank Drs. G. Bertrand and P. Mazerolles for stimulating discussions and Dr. J. C. Barthelat for part of the pseudopotential calculations.

Multiconformational Synthetic Polypeptides

André Brack* and Gérard Spach

Contribution from the Centre de Biophysique Moléculaire, C.N.R.S., 45045 Orléans Cédex, France. *Received February 26, 1981*

Abstract: Polypeptides with two amino acid residues, one hydrophobic, L-leucine, the other hydrophilic, L-lysine, were synthesized by condensation of the dipeptide units Lys-Lys, Leu-Leu, Lys-Leu, and, in varying amounts, Leu-Lys. Their chains contained simultaneously sequences of alternatingly and randomly distributed Leu and Lys residues. The former sequences, if long enough, give rise in aqueous salted solutions to the formation of β structures and the latter to α helices, in such a way that for a given amount of each sequence, β, α, and eventually random coil, structures coexist in the same chain. These polypeptides undergo transconformations mimicking the self-organization of protein chains. Heating the samples increases the amount of β structure with loss of α helix. Increasing volumes of alcohols in aqueous solutions of alternating and random poly(Leu-Lys) induce an α helix. A β to α transition is also observed when the alcohol is added to an aqueous solution of alternating poly(Leu-Lys) previously transconformed in the β structure by addition of salt. Thus these simple multiconformational synthetic polypeptides may be useful as protein models for the study of chain folding in different environment.

It has been previously shown that polypeptides with alternating hydrophilic and hydrophobic residues take up systematically a β sheet structure in aqueous solution in the presence of salt. Poly(Glu-Ala) exhibits a circular dichroism spectrum typical of a β sheet structure after standing for several weeks in neutral aqueous solution[1]. Poly(Tyr-Glu) forms a soluble aggregate of antiparallel β chains below pH 10.5.[2] Seipke et al.[3] showed that alternating poly(Lys-Phe) adopts a β sheet structure in the presence of sodium perchlorate; so does poly(Tyr-Lys)[4] in the presence of NaCl. The β structure formation has been generalized to alternating polypeptides built up with leucyl or valyl residues combined to glutamyl or to lysyl residues.[5-7] For poly(Val-Lys), the existence of a specific bilayer with a hydrophobic interior and a hydrophilic exterior has been shown.[5] The corresponding random copolypeptides poly(Lys^{50},Phe^{50})[3] and poly(Leu^{50},Lys^{50})[8] exhibit an α helix under the same conditions. It was therefore thought that polypeptides containing both alternating and random sequences of hydrophilic and hydrophobic residues, i.e., lysyl and leucyl residues, may exhibit simultaneously β sheets and α-helical structures, modeling by the way the ordering of protein chains. Seven samples covering the range from random distribution to strict alternation of leucyl and lysyl residues were synthesized. The conformations of the polymers were studied by circular dichroism and infrared spectroscopy in aqueous solution of varying ionic strength, temperature, and hydrophobicity.

Statistical Analysis of the Sequences

The β fraction determined experimentally was compared with the fraction R_β of alternating leucyl and lysyl residues calculated by statistical analysis in a similar way as for alternating L and D residues,[9] supposing that no selection took place during the polymerization of the dipeptide monomers. For all $n \geq n_\beta$, where n is the number of consecutive alternating hydrophobic hydrophilic residues, and n_β is the minimum number of these residues required for a sequence to be included in a β-sheet structure,

$$R_\beta = (1 - 3\alpha)^{(n_\beta - 1)/2}[1 + \alpha(3n_\beta - 7)/2 + 2\alpha^2(1 - n_\beta)] + \alpha^{(n_\beta+1)/2}[1 + 3n_\beta + 4\alpha(1 - n_\beta)]/2 \quad \text{(for odd } n_\beta\text{)}$$

and

$$R_\beta = (1 - 3\alpha)^{(n_\beta/2)-1}[1 + \alpha(3n_\beta - 10)/2 + 2\alpha^2(3 - 2n_\beta)] + \alpha^{n_\beta/2}(n_\beta + 4\alpha)/2 \quad \text{(for even } n_\beta\text{)}$$

(α is defined below)

These relations take into account the sequences formed by (Leu-Lys) and (Lys-Leu), eventually elongated by one amino acid residue at either extremity by adjunction of a (Lys-Lys) or (Leu-Leu) dipeptide monomer unit. For reasons explained elsewhere,[10] n_β was taken equal to 7 to draw the curve in Figure 2.

General Procedure for the Synthesis[11]

The samples were prepared by condensing *p*-nitrophenyl dipeptide esters according to the following scheme:

$$\left.\begin{array}{l}(\alpha)\ \text{HCl, H-Lys(Z)-Leu-ONp}\\(\alpha)\ \text{HCl, H-Leu-Leu-ONp}\\(\alpha)\ \text{HCl, H-Lys(Z)-Lys(Z)-ONp}\end{array}\right\} +$$

$$(1 - 3\alpha)\text{HCl, H-Leu-Lys(Z)-ONp} \xrightarrow[\text{DMF}]{\text{TEA}}$$

$$[\text{Leu-Lys(Z)}]_n \xrightarrow[\text{CHCl}_3]{\text{HBr}} (\text{Leu-Lys})_n$$

(1) Rippon, W. B.; Chen, H. H.; Walton, A. G. *J. Mol. Biol.* **1973**, *75*, 369–375.
(2) Trudelle, Y. *Polymer* **1975**, *16*, 9–15.
(3) Seipke, G.; Arfmann, H. A.; Wagner, K. G. *Biopolymers* **1974**, *13*, 1621–1633.
(4) Saint-Pierre, S.; Ingwall, R. T.; Verlander, M. S.; Goodman, M. *Biopolymers* **1978**, *17*, 1837–1848.
(5) Brack, A.; Orgel, L. E. *Nature (London)* **1975**, *256*, 383–387.
(6) Brack, A.; Caille, A. *Int. J. Peptide Protein Res.* **1978**, *11*, 128–139.
(7) Brack, A. *BioSystems* **1977**, *9*, 99–103.
(8) Brack, A. unpublished.
(9) Spach, G.; Brack, A. *J. Mol. Evol.* **1979**, *13*, 47–56.
(10) Brack, A.; Spach, G. *J. Mol. Evol.* **1979**, *13*, 35–46.
(11) 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; DMF, dimethylformamide.

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Table I. Properties of Dipeptide Intermediates

	procedure	yield, %	R_f[a]	mp, °C	$[\alpha]^{25}_{546}$, deg (c 1.0)	chloride argent. titration, %
Nps-Leu-Leu-ONp	1a	72[b]	0.56 A	59–60	−79.9 $CHCl_3$	
HCl,H-Leu-Leu-ONp	2a	85[c]	dec	163–165	−34.7 AcOH	98
Nps-Lys(Z)-Leu-ONp	1b	82[d]	0.86 B	126–127	−62.6 $CHCl_3$	
HCl,H-Lys(Z)-Leu-ONp	2b	89[e]	dec	129–130	−18.3 $CHCl_3$	98
Nps-Lys(Z)-Lys(Z)-ONp	1c	83[f]	0.43 A	152–154	−50.2 $CHCl_3$	
HCl,H-Lys(Z)-Lys(Z)-ONp	2c	70[g]	dec	118–120	−0.5 $CHCl_3$	99

[a] Solvents for chromatography: (A) chloroform–acetic acid (95:5), (B) acetone–acetic acid (98:2). [b] Dissolved in acetone and precipitated with diisopropyl ether. [c] Dissolved in tetrahydrofuran and precipitated with ether. [d] Dissolved in acetone–2-propanol and precipitated by evaporation of acetone. [e] Dissolved in chloroform and precipitated with ether–petroleum ether (1:1). [f] From ethyl acetate. [g] From acetone.

Table II. Properties of Protected Polymer Samples

polymer	$[\eta]$, mL g^{-1} (DCA)	$[\alpha]^{25}_{546}$, deg (c 1.0, DCA)	yield (%) of polymerization
0:100	40.0	−44.0	88
20:80	39.0	−41.4	88
40:60	40.5	−44.9	82
60:40	41.7	−42.8	82
70:30	50.0	−49.1	78
80:20	36.0	−46.9	90
100:0	39.6	−42.1	79

Table III. Properties of Free Polymer Samples

polymers	$[\eta]$, mL g^{-1} (TFA)	$[\alpha]^{25}_{546}$, deg (c 1.0, TFA)	yield (%) after dialysis	Leu:Lys after hydrolysis
0:100	60.7	−90.1	68	0.89
20:80	58.2	−88.6	66	0.95
40:60	63.7	−89.1	63	0.97
60:40	54.0	−90.9	65	0.88
70:30	52.6	−92.2	49	1.13
80:20	55.2	−88.8	58	1.10
100:0	33.3	−87.2	24	1.00

where α represents the fraction of activated dipeptides being taken in equal amounts to generate the random sequences, and $(1 - 3\alpha)$ the fraction of Leu-Lys(Z) dipeptide chosen to generate mainly the alternating sequences. Hereafter, the samples will be denoted as $(1 - 3\alpha) \times 100:(3\alpha) \times 100$.

The activated dipeptides (Table I) were obtained as examplified below for Nps-Leu-Lys(Z)-ONp:

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

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

$$\text{Nps-Leu-Lys(Z)-ONp} \xrightarrow{\text{HCl}} \text{HCl, H-Leu-Lys(Z)-ONp}$$

No systematic check for racemization was undertaken. However, from previous studies on alternating poly(Leu-Lys)[7,10] prepared under the same conditions, it can be assessed that no more than 5% of the overall residues may be replaced by D enantiomers during the course of the polycondensation.

Results

Characteristics of the Samples. Characteristics of the protected polymers are given in Table II. They are homogeneous with respect to their intrinsic viscosity ($[\eta] = 39.5 \pm 4.0$ mL g^{-1}) and to their optical rotation ($[\alpha]^{25}_{546} - 44.6 \pm 4.4°$). The corresponding free polymers (Table III) show the same homogeneity ($[\eta] = 57.4 \pm 6.0$ mL g^{-1}) and optical rotation ($[\alpha]^{25}_{546}$ −89.6 ± 3.0°). Such homogeneities together with an average ratio of Leu/Lys of 0.99 after acidic hydrolysis of the samples indicate that the composition of the polymers reflects that of the monomeric mixtures. Moreover, the sample 40:60 was fractionated by gel filtration into four fractions, each of which was subjected to acidic hydrolysis. A very good agreement was observed between the four fractions and the starting unfractionated material (see Experimental Section). In addition, the sample 70:30 was fractionated in three fractions and each fraction was submitted to conformational analysis under the same conditions. A good similarity was found between the 70:30 sample and the most abundant middle fraction representing about 60%. The other fractions also have a very similar conformation composition. The low molecular weight one (10%) is as expected enriched in the random coil, and the high molecular weight fraction (30%) in the β form.

With regards to the viscosimetric measurements, a molecular weight of 5200 has been reported for poly(Val-Lys)[5] ($[\eta] = 24.8$ mL g^{-1} in TFA). One can thus assume that the samples have a molecular weight higher than this value. This is in agreement with the elution profile of sample 40:60 in 0.2 M NaCl (Figure 9) obtained on Sephadex G 200, the molecular weight fractionation of which ranges from 20,000 to 400,000 when calibrated with globular proteins, insofar as such a calibration is valid for the synthetic polypeptide which is mostly α-helical.

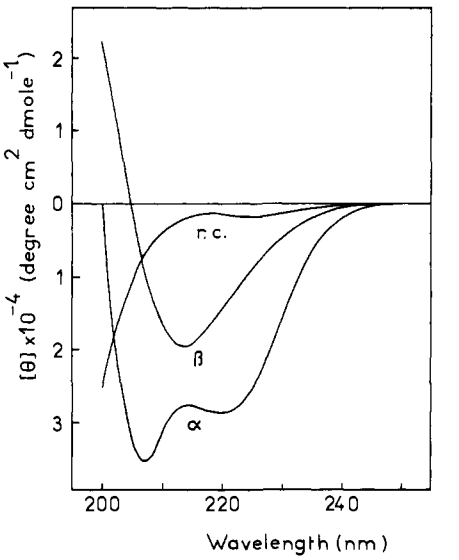

Figure 1. Circular dichroism standards: random coil (rc), poly(Leu-Lys) in pure water (0.2 mg mL^{-1}, cell path 1 mm); β structure (β), poly-(Leu-Lys) in 0.2 M NaCl (0.2 mg mL^{-1}, cell path 1 mm); α helix (α), sample 40:60 in 0.05 M $NaClO_4$ (2 mg mL^{-1}, cell path 0.1 mm).

Conformational Study by Circular Dichroism. In pure water, the CD spectrum of the samples is that of a random coil, due to charge repulsion. In presence of salt, the samples poor in alternating sequences take the α-helical conformation, whereas the alternating polymer adopts the β sheet structure (Figure 1). These spectra were taken as standards for the corresponding conformations. Their $[\theta]$ values are in good agreement with those published in the literature for the random coil and β structure,[10] and for the α helix.[12] Samples containing both alternating and random sequences exhibit a mixed spectrum which was fitted on a computer using the three standards of Figure 1. A very good

(12) Holzwarth, G.; Doty, P. *J. Am. Chem. Soc.* **1965**, *87*, 218–228.

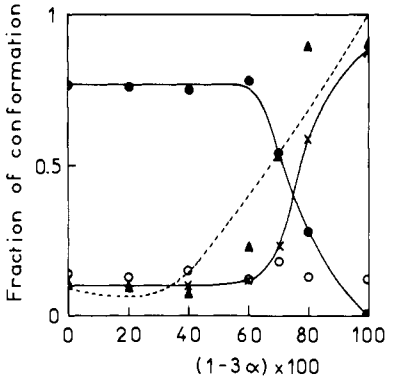

Figure 2. Fraction of different conformations [random coil(○), α helix (●), and β sheet (×)] as a function of (1 − 3α) × 100, the percent of (Leu-Lys) dipeptide in the monomer mixture, at room temperature: (▲) β sheet fraction after heating the samples at 60 °C for 48 h and cooling down to room temperature, (dashed line) β sheet fraction from statistical analysis. Concentrations 0.2 mg mL^{-1} in 0.03 M $NaClO_4$.

Table IV. Percent of the Three Conformations Exhibited by Sample 70:30[a]

con-	time, h									
form	0.1	1	3	6	12	36	120	336	504	840
β	15	13	17	21	23	27	29	32	35	35
rc[b]	18	21	20	18	18	16	17	17	16	17
α	67	66	63	61	59	58	55	51	49	48

[a] In 0.03 M $NaClO_4$ at room temperature as a function of time. Polymer concentration: 0.2 mg mL^{-1}. [b] rc = random coil.

Table V. Evolution of the Percents of the Three Conformations for Sample 70:30[a]

	time, h							
conform	0	0.1	3	6	12	20	28	34
β	13	29	41	45	50	52	53	51
rc[b]	21	16	13	11	11	8	13	15
α	66	55	46	44	41	40	34	34

[a] In 0.03 M $NaClO_4$ at 60 °C as a function of time. The solutions were cooled down just before the spectra were run. Polymer concentration: 0.2 mg mL^{-1}. [b] rc = random coil.

agreement was obtained between recombined and experimental spectra in most of the cases. The data given in Figure 2 show a sharp transition when the fraction (1 − 3α) reaches 75%, where α helices and β structures coexist in a comparable amount, while the random-coil contribution is still low. At about 80%, the β-sheet structure dominates. The β fraction (R_β), estimated by statistical analysis as explained above on a model in which the sheet structure is generated by those segments containing seven or more residues, was expressed also as a function of (1 − 3α) and is represented as a dashed line in Figure 2. The experimental curve approaches the calculated one, but a β sheet structure deficiency can be noted. This departure was reduced, on the average, when the samples were heated at 60 °C for 48 h and cooled down to room temperature.

The salt-induced transitions were followed as a function of time at room temperature. The coil → helix transition for the sample 40:60 was achieved within the 6 min necessary to run the spectrum and no evolution of the spectrum could be noticed after 5 weeks in 0.03 M $NaClO_4$. For alternating poly(Leu-Lys), the coil → β transition required 2 h to be complete under the same conditions. The transition of sample 70:30 is given in Table IV. During the first minutes, structuration via α-helical nuclei seems to take place, confirming previous data on poly(Leu^{32}, Glu^{68}) published by Ptitsyn's group.[13] Then, a slow α → β rearrangement occurs with time.

(13) Anufrieva, E. V.; Bychkova, V. E.; Krakovyak, M. G.; Pautov, V. D.; Ptitsyn, O. B. *FEBS Lett.* **1975**, *55*, 46–49.

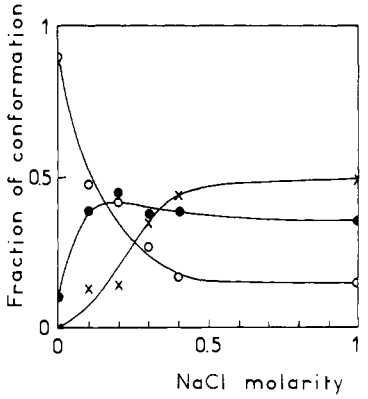

Figure 3. Fraction of random coil (○), α helix (●), and β sheet (×) as a function of NaCl molarity for sample 70:30 (2 mg mL^{-1}) after standing one night at room temperature.

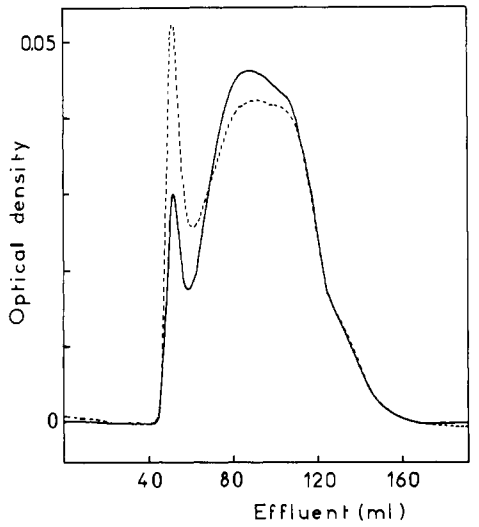

Figure 4. Elution profile of sample 70:30 at 210 nm from a Sephadex G 200 (2, 5 × 30 cm) with 0.2 M NaCl after 1 h 30 (—) and after 24 h (---) aging in 0.2 M NaCl. Applied solution: 1 mg in 0.5 mL of 0.2 M NaCl.

When raising the temperature to 60 °C, the evolution with time is indicated in Table V and a much faster α → β transition is observed. Such an α → β transiton induced by elevation of temperature has already been briefly reported by Welch and Fasman[14] for random copoly(Glu^{77}, Val^{23}) which exhibits the three conformations simultaneously, like our samples. Both transitions, at room temperature and at 60 °C, do not obey any simple kinetic order. No drastic changes were observed when the polymer concentration was changed from 0.02 to 2 mg mL^{-1} in 0.05 M $NaClO_4$.

When $NaClO_4$ was replaced by NaCl, the proportions of α helices and β sheet structure were decreased for the benefit of the random-coil contribution. As expected, the β sheet structure was found to be more sensitive to ion-shielding effects by salts than was the α helix. This is examplified in Figure 3 representing the variation of the fraction of the conformations for increasing NaCl molarities observed after one night for sample 70:30. The β fraction varies from 13 to 49%, while the α fraction stays constant around 39% when the NaCl molarity varies from 0.1 to 1.

In order to know if the multiconformational state of the 70:30 sample in the presence of salt is intramolecular (globular) or of an intermolecular type of aggregation, the sample was subjected to gel chromatography in 0.2 M NaCl at various times after salt addition. The elution profiles are given in Figure 4. The noticeable increase of the excluded high molecular weights peak after

(14) Welch, W. H., Jr.; Fasman, G. D. *Biochemistry* **1974**, *13*, 2455–2466.

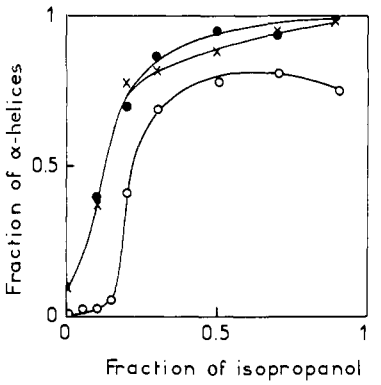

Figure 5. Fraction of α helix as a function of the percent of added 2-propanol to aqueous solutions of alternating poly(Leu-Lys) (○) of sample 40:60 (×) and of sample 70:30 (●). Polymer concentration: 0.2 mg mL^{-1}.

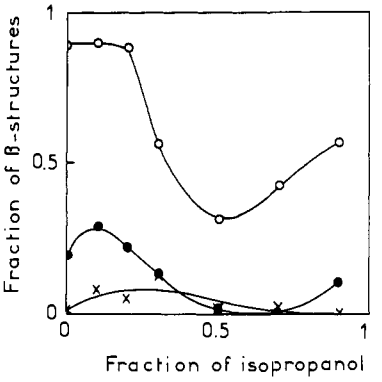

Figure 6. Fraction of β structure as a function of the percent of added 2-propanol to aged (one night) 0.03 M $NaClO_4$ solutions of sample poly(Leu-Lys) (○), sample 40:60 (×), and sample 70:30 (●). Polymer concentration: 0.2 mg mL^{-1}.

24 h, while the amount of β structure determined by analysis of the CD spectra remains almost constant (from 18 to 20%), indicates that the β fraction rearranges into an intermolecular form.

Effect of Alcohols. Addition of increasing amounts of 2-propanol to aqueous solutions of the polymer induces a sharp coil $\rightarrow \alpha$ transition, as already reported for other polypeptides.[15] The results are illustrated in Figure 5. The transition occurs at low percentages of 2-propanol, indicating that the amount of α helix is very sensitive to the hydrophobicity of the milieu. A similar transition, however, less sharp, is observed with ethanol while methanol induces the transition when its percentage is about 30%. Interestingly, when 2-propanol is added to samples previously transconformed into β sheets by addition of $NaClO_4$, a $\beta \rightarrow \alpha$ transition also occurs (Figures 6 and 7).

Infrared Spectroscopy. The conformation of sample 70:30 was also analyzed by infrared spectroscopy at the amide I absorption band, utilizing for the random coil the standard published elsewhere.[10] For the α helix standard the sample 40:60 was taken in 0.03 M $NaClO_4$ at 20 mg mL^{-1} concentration in D_2O, for which the circular dichroism indicated 30% of random coil. For the β form, the sample 100:0 was chosen under the same conditions, taking off 10% of random coil (Figure 8). The latter standard has an optical density at 1610 cm^{-1} lower than the β standard determined by Chirgadze[16] by substracting more contribution of the random coil, but is was used as such for its reliability to the CD spectrum taken under the same conditions of concentration.

Thus, the results for the sample 70:30 in 0.03 M $NaClO_4$ in D_2O were 25% β form, 20% random coil, and 55% α helix, with a rather large uncertainty on the two last contributions because of the overlapping of the standards. These values are to be compared with the values obtained by circular dichroism: 28, 25, and 47%, respectively.

(15) Barteri, M.; Pispisa, B. *Biopolymers* **1973**, *12*, 2309–2327.
(16) Chirgadze, Yu, N.; Shestpalov, B. V.; Venyaminov, S. Yu. *Biopolymers* **1973**, *12*, 1337–1351.

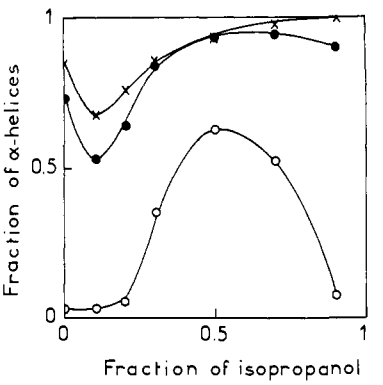

Figure 7. Fraction of α helix obtained as for Figure 6.

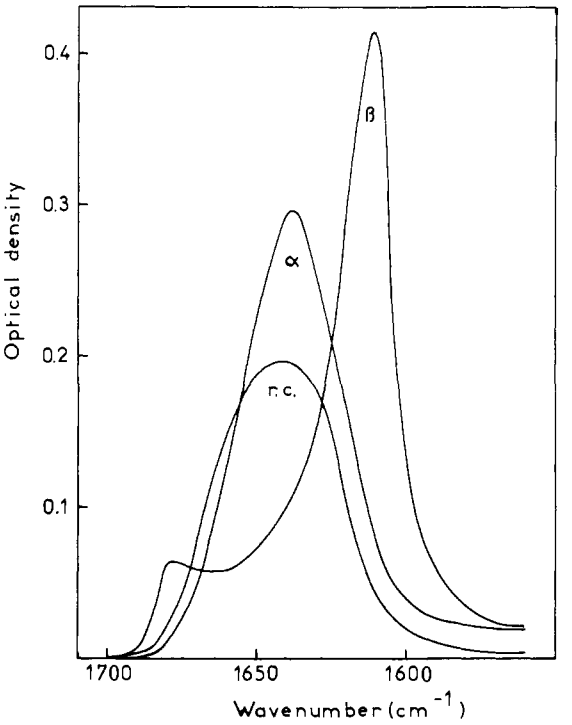

Figure 8. Infrared standards: random coil (rc), poly(Leu-Lys) in pure D_2O (20 mg mL^{-1}, cell path 0.05 mm). The spectra of the β structure (β) and of the α helix (α) are corrected as explained in the text.

Discussion

The synthetic polypeptides described in this paper can take simultaneously three different conformational states, namely, the α helix, the β sheet, and the random-coil structure, although they are built up with only two amino acid residues, one hydrophobic, L-leucine, and the other hydrophilic, L-lysine. This is obtained by playing with the sequence of the two residues, the alternating sequence favoring the formation of β sheets and the random one promoting the α helix. These polypeptides could be useful for the study of the nucleation and self-organization of the ordered structures and their mutual transconformation as a function of temperature or hydrophobicity of the milieu. Although some discrepancies still exist between our polypeptides and globular proteins—such as the presence of intermolecular β-sheet structures and some long-lasting rearrangement of the conformations—the former still may be considered as a starting material for further research on globular protein models.

Finally, their behavior shed some light on the importance of the sequence at long distance which is usually not taken into account when prediction of the secondary structures of proteins are planned. Indeed, an α helix is predicted for those segments which have alternating as well as random Leu and Lys residues.

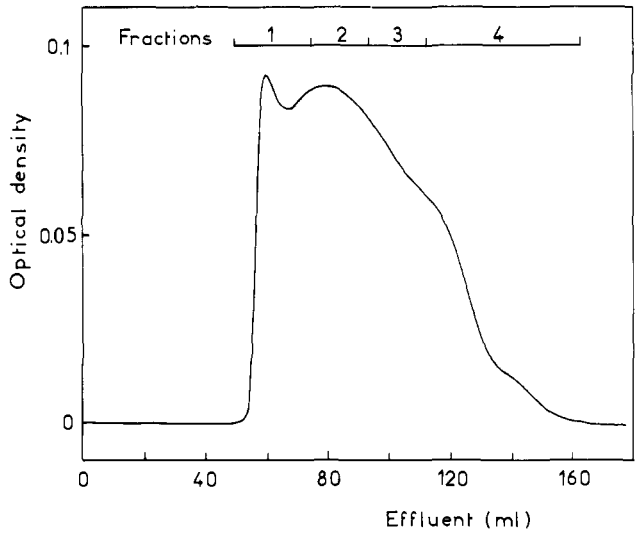

Figure 9. Elution profile of sample 40:60 at 210 nm from Sephadex G 200 (2, 5 × 30 cm) with NaCl (0.2 M) after 1 night at room temperature. The four fractions were subjected separately to hydrolysis and to amino acid analysis (see Experimental Section). Applied solution: 2 mg in 1 mL of 0.2 M NaCl.

Experimental Section

Amino acid analysis were performed by Miss A. Chabin, Orléans University, on a Beckman amino acid analyzer 118 BL. The purity of the compounds was checked by TLC using Merck precoated plates 60 F.254 (silica gel). Optical rotations were determined using a Perkin-Elmer 141 M polarimeter (1-dm cell). UV spectra were recorded on a Beckman Acta III spectrophotometer, while infrared spectra were run on a Perkin-Elmer 297 spectrophotometer. CD spectra were recorded on a Roussel Jouan 185 Model II dichrograph at 22–24 °C. Viscosity was measured with an Ubbelhode viscosimeter Cannon CUSMU, size 75, for trifluoroacetic acid and size 150 for dichloroacetic acid. Melting points were determined with a hot-plate Leitz microscope. Solvents and triethylamine used for polycondensation reactions were distilled once from benzyloxycarbonylglycine *p*-nitrophenyl ester and then redistilled.

HCl, H-Leu-ONp. A suspension of 18.2 g of Nps-Leu-OH, DCHA[17] (4×10^{-2} mol) in 220 mL of ethyl acetate was vigorously shaken with 300 mL of 0.2 M sulfuric acid until dissolved. The organic layer was washed with water and dried over sodium sulfate. The solution was cooled to −10 °C at which time 5.6 g of *p*-nitrophenol (4×10^{-2} mol) and 8.2 g of dicyclohexylcarbodiimide (4×10^{-2} mol) were added with stirring. After 2 h at −10 °C and one night at room temperature, the mixture was filtered and evaporated to give the product as an oil which was dissolved in acetone and precipitated with *n*-hexane. It could not be crystallized and was used without further purification.

The oil was dissolved in 200 mL of ether and treated with a saturated solution of 8×10^{-2} mol of hydrogen chloride in ether. After 15 min the precipitate was filtered off and thoroughly washed with ether. The product was purified by crystallization from acetone followed by addition of ether: yield 6.85 g (59%); mp 178–180 °C; $[\alpha]^{25}_{546}$ −7.0°(*c* 1, AcOH); chloride argentometric titration, 99%. Anal. ($C_{12}H_{17}ClN_2O_4$)C, H, Cl, N.

Dipeptides. The activated dipeptides were prepared according to the same general procedure. Their characteristics are given in Table I. (HCl, H-Leu-Lys(Z)-ONp from Brack and Caille[6]). The standard procedures are as follows on a 10-mmol scale.

Coupling Procedure. Dicyclohexylcarbodiimide (2.06 g) was added to a stirred solution (cooled at −10 °C) of the Nps-amino acid dicyclohexylamine salt (from Zervas et al.[17] in chloroform (110 mL). Afterwards, the amino acid ester (HCl,H-Lys(Z)-ONp from Brack and Caille[6]) was added. After 2 h, the mixture was allowed to reach room temperature and was left overnight. Chloroform was evaporated off and the residue was stirred with ether (procedure 1a), ethyl acetate (procedure 1b), or acetone (procedure 1c). The insoluble matter was filtered, the filtrate evaporated, and the residue purified by crystallization in solvents as given in Table I.

Removal of the *O*-Nitrophenylthio Group. To a solution of the protected peptide ester in the required amount of tetrahydrofuran–ether (1:1) (procedure 2a), ethyl acetate (procedure 2b), or acetone (procedure 2c), a saturated solution of hydrogen chloride in ether (20 mmol) was added. After 15 min, the precipitate was filtered off, thoroughly washed with ether, and purified by crystallization in solvents as given in Table I.

Polycondensation. A mixture (750 mg, 1.36 mmol) of the four peptide ester hydrochlorides (α HCl, H-Lys(Z)-Leu-ONp, α HCl,H-Leu-Leu-ONp, α HCl,H-Lys(Z)-Lys(Z)-ONp, (1 − 3α) HCl,H-Leu-Lys(Z)-ONp) was thoroughly triturated with 0.4 mL of dimethylformamide until a homogeneous solution was obtained. Triethylamine (0.21 mL, 15 mmol) was then added. After 4 days, more dimethylformamide was added (5 mL) and the reaction mixture was homogenized. Isopropylamine (0.12 mL, 1.4 mmol) was added with stirring for 15 min. The polymer was precipitated with water. It was purified by dissolution in dimethylformamide and precipitation with water. The characteristics of the protected polymers are given in Table II.

Free Polypeptides. Chloroform (100 mL) was added to a solution of the protected polypeptide (1 mmol) in dichloroacetic acid (5 mL). Hydrogen chloride was bubbled through for 30 min, and then hydrogen bromide for 1 h. The turbid solution was concentrated under vacuum to ca. 5 mL. The crude polymer was precipitated with acetone, dissolved in water, and dialyzed against 0.01 N HCl and water for 3 days. The freeze-dried samples were hydrolyzed by treating in 5.6 N HCl at 110 °C for 2 days. Cleavage of the side-chain protecting groups was controlled by UV absorption 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. The characteristics of the free polymers are given in Table III.

Fractionation of the Samples. The polymers were fractionated by gel filtration on a Sephadex G 200 column equilibrated with 0.2 M NaCl. The flow rate of the 2.5 × 30 cm column averaged 45 mL h^{-1}. The effluent was monitored at 210 nm (Gilson Spectrochrom MD, 1-cm path length). The sample 40:60 was fractionated in four fractions, each of which was dialyzed against 0.01 N HCl and water and freeze-dried. Hydrolysis in 5.6 N HCl at 110 °C for 2 days gave Leu:Lys ratios of 1.03, 0.97, 0.98, and 1.02 for fractions 1, 2, 3, and 4, respectively (see Figure 9).

Preparation of the Solution. The polymers isolated as hydrochlorides are readily soluble in water. However, they cannot be dissolved directly in aqueous solution 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 3200 per mean residue for the disordered conformation.[18] The measurements were in good agreement for poly(Leu-Lys) (sample 100:0) with those obtained from the weight of the sample and its elemental analysis.

Infrared spectra were run on 20 mg mL^{-1} D_2O solutions in CaF_2 cells. Solid $NaClO_4$ was added to aliquots of polymer solutions.

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