CONFORMATION OF BRANCHED POLYPEPTIDES BASED ON POLY(L-LYSINE). EFFECT OF THE IONIC STRENGTH AND OF THE PRESENCE OF ALCOHOLS

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CD spectra of branched polypeptides based on poly(L-lysine) containing in the side chains approximately 3, 5 and 8·5 DL-alanine residues or 3 DL-alanine residues and another terminal L-amino acid were measured at pH 7.4, and in the uncharged state in various ionic strengths, and also in water–methanol and water–trifluoroethanol mixtures. At pH 7·4 and in low ionic strength (0·02M and 0·2M-NaCl) the polypeptides assume an unordered conformation, except for the L-leucine containing polypeptide, which is helical to a considerable extent. Increase of the ionic strength to 2·0M-NaCl leads to the formation of the α-helical structure. In the uncharged state all polypeptides are at least partly helical. The content of the α-helix increases with increasing ionic strength and depends also on the nature of the side chain. Formation of the α-helix is supported by the presence of L-leucine and L-proline, some limitation of the α-helix formation by histidine is manifested mainly in low ionic strength. The presence of methanol and trifluoroethanol has an effect similar to the increase of ionic strength, *i.e.* increases the α-helix content.

In a preceding paper[1] we studied CD spectra of branched polypeptides in solutions of low ionic strength at pH values corresponding either to the charged or to the uncharged state. In view of the fact that the branched polypeptides can be used as model compounds in biology[2–4], mainly in immunology, it was found useful to extend the experimental conditions to the physiological pH and ionic strenth, *i.e.* to pH 7·4 and 0·2M-NaCl. It follows further from our previous paper[1] that, compared with poly(L-lysine), the formation of α-helical structure in branched polypeptides is rather limited depending on the structure of the side chains. In the present paper we studied the content of the α-helix in these polypeptides under conditions which can be assumed favorable to its formation, *i.e.* in high ionic strength (2M-NaCl) and in water–methanol and water–trifluoroethanol mixtures. Polypeptides containing in the side chains 3, 5 or 8 residues of DL-alanine and polypeptides containing 3 DL-alanine residues and another terminal L-amino acid (histidine, proline or leucine) were studied.

EXPERIMENTAL

Polypeptides

Polypeptides of following composition were used (the ratio L-Lys : m : i is given): Poly(L-Lys.(DL-Ala_m)) 1 : 3·1, 1 : 4·78, and 1 : 8·56; poly(L-Lys(L-Leu_i-DL-Ala_m)) 1 : 3 : 0·7; poly(L-Lys.(L-His_i-DL-Ala_m)) 1 : 2·9 : 0·6; poly(L-Lys(L-Pro_i-DL-Ala_m)) 1 : 3·1 : 0·53. The composition was determined by amino-acid analysis. Synthesis and molecular weight determination of the polypeptides used were described earlier[5].

CD Measurements

CD spectra were measured in a Roussel–Jouan CD 185/II dichrographe in cells of optical path 1·0, 0·2, 0·1 and 0·02 cm. The samples were dissolved in 0·02M-NaCl and the pH was adjusted by adding 0·1M-NaOH or 0·1M-HCl. The ionic strength was adjusted by adding 5M-NaCl. Polypeptide solutions in 50% and 75% methanol and trifluoroethanol were prepared by mixing the polypeptide solutions in 0·02M-NaCl with the appropriate amounts of alcohols. The pH of the solutions was measured before the alcohol was added. The $[\theta]$ values are related to one lysine residue in the basic chain including whole side-chain.

RESULTS

Side chains of the polypeptides studied in this paper contain either DL-alanine residues (approximately 3, 5 and 8·5) only, or a sequence of 3 DL-alanine residues and of another L-amino acid (His, Pro or Leu) at its amino terminal. In this case no ordered conformation of side chains can be expected. Thus by the term "helical conformation" we mean mainly the conformation of the backbone. As a basis for the calculation of $[\theta]$ the number of lysine residues in the main chain was considered. It is assumed that in the case of α-helical conformation DL-alanine residues do not contribute to the overall optical activity and the contribution of terminal L-amino-acid residues is (with regard to their unordered structure) much smaller than that of the ordered lysine residues of the main chain. The contribution of L-amino acid residues in side chains to the total $[\theta]$ values of the random conformation may be somewhat higher and can at least partly cause the differences found among the CD spectra of individual polypeptides.

CD spectra of the polypeptides studied in 0·02M, 0·2M, and 2·0M-NaCl, pH 7·4, are shown in Table I and Fig. 1 and 2. The CD spectra in 0·02M-NaCl correspond to the unordered conformation in all polypeptides except for poly(L-Lys(L-Leu_i-DL-Ala_m)) which is partly helical. On increasing the NaCl concentration to 0·2 mol l^{-1} the α-helix content of poly(L-Lys(L-Leu_i-DL-Ala_m)) increases (Fig. 2). In the case of other polypeptides no important conformational change takes place and their CD spectra correspond to those of the unordered conformation. However, further increase of NaCl concentration to 2·0 mol l^{-1} promotes the formation of α-helical structure in all cases. The polypeptides containing only DL-alanine in their side

TABLE I

Characteristic values of CD spectra of polypeptides at pH 7·4

Concentration NaCl (mol l^{-1})	λ_{max} ($[\theta] . 10^{-3}$)	λ_{min} ($[\theta] . 10^{-3}$)	λ_{max} ($[\theta] . 10^{-3}$)	λ_{cross}
	Poly(L-Lys(DL-Ala_m)), 1 : 3·1			
0·02	227 (−1·79)	216 (−0·55)	198 (−18·7)	<195
0·2	225 (−2·69)	216 (−2·03)	203 (−13·8)	<200
2·0	221 (−16·1)	214 (−14·5)	208 (−17·8)	<200
	Poly(L-Lys(DL-Ala_m)), 1 : 4·78			
0·02	227 (−1·76)	215 (−0·79)	202 (−7·15)	<200
0·2	224 (−3·59)	216 (−3·08)	201 (−8·04)	<200
2·0	220 (−14·6)	214 (−13·3)	206 (−18·2)	201
	Poly(L-Lys(DL-Ala_m)), 1 : 8·56			
0·02	227 (−2·16)	215 (−1·17)	<200	—
0·2	225 (−4·47)	214 (−3·83)	201 (−17·8)	<200
2·0	221 (−16·7)	212 (−13·2)	205 (−20·3)	<200
	Poly(L-Lys(L-Leu_i-DL-Ala_m))			
0·02	222 (−17·8)	214 (−15·3)	208 (−16·8)	201
0·2	222 (−26·7)	212 (−21·5)	209 (−24·2)	201
2·0	222 (−17·7)	210 (−19·7)	208 (−14·4)	202
	Poly(L-Lys(L-His_i-DL-Ala_m))			
0·02	228 (−3·03)	215 (−0·30)	204 (−76·8)	<200
0·2	225 (−5·61)	216 (−2·53)	204 (−13·5)	<200
2·0	222 (−16·8)	214 (−14·6)	207 (−17·6)	<200

TABLE I
(*Continued*)

Concentration NaCl, mol l^{-1}	λ_{max} $[\theta] \cdot 10^{-3}$	λ_{min} $[\theta] \cdot 10^{-3}$	λ_{max} $[\theta] \cdot 10^{-3}$	λ_{cross}
		Poly(L-Lys(L-Pro_i-DL-Ala_m))		
0·02[a]	232 (−1·02)	—	215 (+1·39)	<200
0·2[b]	229 (−1·85)	—	216 (−0·25)	<200
2·0	222 (−21·6)	214 (−19·4)	207 (−24·2)	<200

[a] Additional λ_{cross} values 223 and 212 nm; [b] additional λ_{cross} values 218 and 214 nm.

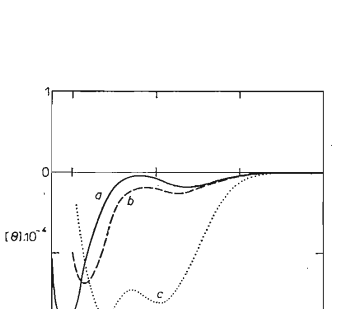

FIG. 1

CD Spectra of poly(L-Lys(DL-Ala_m)) (m = = 3·1) at pH 7·4 in *a* 0·02M-NaCl, *b* 0·2M--NaCl, *c* 2·0M-NaCl

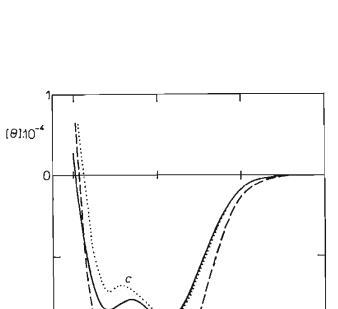

FIG. 2

CD Spectra of poly(L-Lys(L-Leu_i-DL-Ala_m)) at pH 7·4 in *a* 0·02M-NaCl *b* 0·2M-NaCl, *c* 2·0M-NaCl

chains display CD spectra characteristic for about the same helix content in all three cases, *i.e.* independently of whether 3, 5 or 8 DL-alanine residues are present in the side chain. Nearly the same α-helix content is shown by poly(L-Lys(L-His_i-DL-Ala_m)), a higher helix content by poly(L-Lys(L-Pro_i-DL-Ala_m)). L-Leucine containing polypeptide is turbid in 2M-NaCl and a CD spectrum of low amplitude is found (Fig. 2).

CD spectra of polypeptides in the uncharged state (pH 10·7–11·9) in 0·02M-NaCl, 0·2M-NaCl (only some polypeptides) and 2·0M-NaCl are shown in Table II and Fig. 3. CD spectra of L-proline and L-leucine containing polypeptides in 0·02M-NaCl were published in the previous paper[1] and are given here for the sake of comparison. CD spectra of all polypeptides in 0·02M-NaCl show the presence of α-helical conformation. The L-leucine containing polypeptide has the highest α-helix content, followed by the L-proline containing polypeptide. Next are the three polypeptides containing DL-alanine only, and the L-histidine containing polypeptide has the lowest α-helix content. The α-helix content of the polypeptide containing DL-alanine only is higher at pH 11 (Table II) than at pH 9 (ref.[1]). No difference on comparing

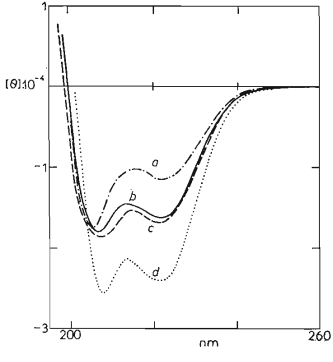

FIG. 3

CD Spectra of poly(L-Lys(DL-Ala_m)) (m = = 3·1) in *a* 0·02M-NaCl, pH 9, *b* 0·02M--NaCl, pH 11, *c* 0·2M-NaCl, pH 11, *d* 2·0M--NaCl, pH 11

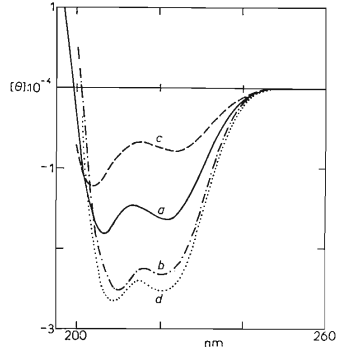

FIG. 4

CD Spectra of poly(L-Lys(DL-Ala_m)) (m = = 3·1) in *a* 0·02M-NaCl, pH 11, *b* 75% methanol, pH 11·7, and poly(L-Lys(L-His_i--DL-Ala_m)) in *c* 0·02M-NaCl, pH 11·7, *d* 75% methanol, pH 11·7. pH was measured before the addition of alcohol

TABLE II

Characteristic values of CD spectra of polypeptides in non-charged state

Concentration NaCl (mol l^{-1})	λ_{max} ($[\theta] . 10^{-3}$)	λ_{min} ($[\theta] . 10^{-3}$)	λ_{max} ($[\theta] . 10^{-3}$)	λ_{cross}
	Poly(L-Lys(DL-Ala_m)), 1 : 3·1, pH 11			
0·02[a]	221 (−16·3)	213 (−14·6)	207 (−18·1)	199
0·2	221 (−16·8)	215 (−15·3)	207 (−18·6)	199
2·0	221 (−24·0)	214 (−21·3)	208 (−25·6)	201
	Poly(L-Lys(DL-Ala_m)), 1 : 4·78, pH 11·9			
0·02	221 (−10·8)	215 (−9·35)	206 (−14·7)	200
0·2	221 (−11·9)	213 (−10·2)	206 (−15·3)	198
2·0	221 (−20·1)	215 (−18·9)	210 (−21·4)	201
	Poly(L-Lys(DL-Ala_m)), 1 : 8·56, pH 11·7			
0·02[a]	222 (−15·4)	215 (−13·0)	207 (−17·3)	<200
2·0	222 (−22·7)	213 (−20·2)	208 (−21·7)	202
	Poly(L-Lys(L-Leu_i-DL-Ala_m)), pH 10·7			
0·02[b]	221 (−28·5)	212 (−25·5)	208 (−27·2)	202
0·2	221 (−29·9)	215 (−27·2)	210 (−28·5)	201
2·0	225 (−8·16)	211 (−4·93)	209 (−5·26)	204
	Poly(L-Lys(L-His_i-DL-Ala_m)), pH 11·7			
0·02[a]	224 (−7·88)	215 (−6·72)	204 (−12·3)	<200
2·0	222 (−21·1)	214 (−19·3)	203 (−21·7)	<200

TABLE II
(*Continued*)

Concentration NaCl (mol l^{-1})	λ_{max} ($[\theta] . 10^{-3}$)	λ_{min} ($[\theta] . 10^{-3}$)	λ_{max} ($[\theta] . 10^{-3}$)	λ_{cross}
	Poly(L-Lys(L-Pro_i-DL-Ala_m)), pH 11·8			
0·02[a]	221 (−22·3)	213 (−19·7)	207 (−22·1)	<200
2·0	221 (−29·9)	214 (−27·4)	208 (−31·2)	<200

[a] Measurement at pH 9 in 0·02M-NaCl was published[1]; [b] measurement at pH 12·0 in 0·02M-NaCl was published[1].

the spectra at those pH values was found in the case of L-histidine containing polypeptide (Table II, ref.[1]).

CD spectra in 0·2M-NaCl were measured in the case of two polypeptides containing DL-alanine only (approximately 3 and 5 residues) (Table II) and in the case of L-leucine containing polypeptide. The spectra are nearly identical with those in 0·02M-NaCl.

The effect of the increase of NaCl concentration to 2·0 mol l^{-1} is significant. Except for poly(L-Lys(L-Leu_i-DL-Ala_m)), where the situation is similar to that found at pH 7·4 (turbidity and a decrease of the CD spectra amplitude), an increase of the α-helix content takes place. This increase is particularly notable in the case of the L-histidine containing sample, where the helix formation in low ionic strength is largely limited. Similarly as at pH 7·4 also in the alkaline medium in 2M-NaCl, the highest α-helix content was found with the L-proline containing polypeptide. In the case of other polypeptides the differences are within the limits of experimental error.

With poly(L-Lys(DL-Ala_m)) (m = 3·1), poly(L-Lys(L-His_i-DL-Ala_m)), poly(L-Lys(L-Pro_i-DL-Ala_m)) and poly (L-Lys(L-Leu_i-DL-Ala_m)), also the effect of methanol and trifluoroethanol on the CD spectra in uncharged state was studied. The results are shown in Table III and Fig. 4. CD spectra of all polypeptides in 50% methanol and 50% trifluoroethanol indicate the presence of α-helical conformation. An increase of methanol concentration from 50% to 75% results in a small increase of the α-helix content with all polypeptides with the exception of that containing L-leucine (in this case spectra in both methanol concentrations are identical). The effect of the increase of trifluoroethanol concentration to 75% is negligible. Amplitude of the CD bands at corresponding alcohol concentrations is somewhat higher with methanol than with trifluoroethanol. L-Leucine containing polypeptide shows the highest

TABLE III

Characteristic values of CD spectra of polypeptides in solvent mixtures containing alcohols

Alcohol %[a]	λ_{max} ($[\theta] . 10^{-4}$)	λ_{min} ($[\theta] . 10^{-4}$)	λ_{max} ($[\theta] . 10^{-4}$)	λ_{cross}
	Poly(L-Lys(DL-Ala_m)), 1 : 3·1, pH 11·7			
Methanol, 50%	220 (−2·20)	215 (−2·08)	209 (−2·43)	201
Methanol, 75%	221 (−2·32)	216 (−2·26)	210 (−2·54)	201
Trifluoroethanol, 50%	220 (−2·10)	215 (−2·03)	210 (−2·17)	201
Trifluoroethanol, 75%	221 (−2·06)	217 (−1·99)	208 (−2·24)	201
	Poly(L-Lys(L-His_i-DL-Ala_m)), pH 11·7			
Methanol, 50%	219 (−2·29)	215 (−2·21)	209 (−2·47)	201
Methanol, 75%	220 (−2·43)	216 (−2·38)	208 (−2·72)	200
Trifluoroethanol, 50%	220 (−2·09)	216 (−2·04)	207 (−2·33)	200
Trifluoroethanol, 75%	219 (−1·96)	215 (−1·87)	208 (−2·49)	200
	Poly(L-Lys(L-Pro_i-DL-Ala_m)), pH 11·7			
Methanol, 50%	220 (−2·53)	215 (−2·40)	209 (−2·65)	201
Methanol, 75%	221 (−2·69)	215 (−2·53)	211 (−2·89)	203
Trifluoroethanol, 50%	220 (−2·43)	214 (−2·24)	209 (−2·68)	202
Trifluoroethanol, 75%	219 (−2·39)	215 (−2·33)	210 (−2·67)	202

TABLE III
(*Continued*)

Alcohol % [a]	λ_{max} ($[\theta] . 10^{-4}$)	λ_{min} ($[\theta] . 10^{-4}$)	λ_{max} ($[\theta] . 10^{-4}$)	λ_{cross}
	Poly(L-Lys(L-Leu$_i$-DL-Ala$_m$)), pH 10·9			
Methanol, 50%	221 (−2·96)	215 (−2·67)	209 (−2·95)	202
Methanol, 75%	220 (−2·99)	213 (−2·72)	211 (−2·80)	202
Trifluoroethanol, 50%	220 (−2·81)	214 (−2·57)	209 (−3·07)	201
Trifluoroethanol, 75%	220 (−2·67)	215 (−2·52)	208 (−2·32)	201

[a] Volume percent of alcohol in the measured mixture.

α-helix content in both alcohol solutions, followed by the L-proline containing polypeptide and finally by the polypeptides containing DL-alanine only and L-histidine containing polypeptide, the spectra of which are virtually identical (Table III, Fig. 4).

DISCUSSION

It was demonstrated in our previous paper[1] that the capacity of branched polypeptides to assume the α-helical structure depends strongly on the nature of the side chains. This is also true if all ionizable amino groups of the side chains are uncharged. In low ionic strength (0·02M-NaCl) and uncharged state the amount of the α-helix increased in the order poly(L-Lys(L-His$_i$-DL-Ala$_m$)), poly(L-Lys(DL-Ala$_m$) (all three samples), poly(L-Lys(L-Pro$_i$-DL-Ala$_m$)) (CD spectra approximately identical with those of poly(L-lysine)) and poly(L-Lys(L-Leu$_i$-DL-Ala$_m$). Hence the side chain containing DL-alanine residues only decreases the α-helix forming capacity as compared with poly(L-lysine). Another L-amino acid residue at the amino terminus of the 3 DL-alanine residues containing side chain contributes in a specific way. The effect of mere side-chain elongation can be excluded because in the case of polypeptides with side chains containing only DL-alanine residues the α-helix content is independent of the number of these residues (within the limits of 3 to 8 residues). The α-helix forming capacity is further decreased by histidine. On the other hand proline and especially leucine increase this capacity. The α-helix forming character of leucine

is also reflected by the fact that the leucine containing polypeptide is the only one which is helical in low ionic strength at pH 7·4. It appears that the helix forming capacity of leucine is due to its hydrophobic nature, because similar somewhat less distinct effect was observed also with aromatic amino acids (unpublished results). Our results agree with those of Goodman and coworkers[6] who found similar effects with poly(L-lysine) substituted at the ε-position by leucine and phenylalanine.

The α-helix content can be increased by increasing the ionic strength (2M-NaCl). Under these conditions the polypeptides studied are helical even at pH 7·4 their α-helix content being, however, lower than in the uncharged state (alkaline medium). Observed spectra of the L-leucine containing polypeptide indicate a maximum helicity already in 0·2M-NaCl at both pH values. This polypeptide aggregates in 2·0M-NaCl and its CD spectra of low amplitude do not allow unequivocal interpretation. The effect of the side chain on the helix content is smaller in 2·0M-NaCl than in low ionic strength. The highest helix content was found with L-proline containing polypeptide, the difference in the helicity between the polypeptides containing DL-alanine only (independently of the number of DL-alanine residues) and the L-histidine containing polypeptide being very small.

The presence of alcohols has an effect similar to that of high ionic strength. Using trifluoroethanol a maximum effect is observed at lower concentrations (except for the L-leucine containing polypeptide). Using methanol, however, somewhat higher amplitudes of the negative CD bands are found. We are not able to decide unequivocally whether this is due to a higher α-helix content or to the side chain contributions which may differ in both solvents. The helix-forming effect of L-leucine and partly also of L-proline is observed in both alcohols. In the case of leucine the maximal effect is obtained at lower methanol concentrations than in other cases. On the other hand the helix-breaking effect of L-histidine observed in aqueous solutions in low ionic strength (and partly also in 2·0M-NaCl) was not found in the presence of alcohols.

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