CONFORMATION OF BRANCHED POLYPEPTIDES BASED ON POLY(L-LYSINE): CIRCULAR DICHROISM STUDY

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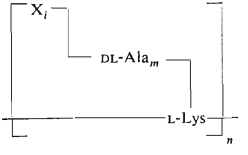
Circular dichroism (CD) spectra of branched (multichain) polypeptides based on poly(L-lysine) with DL-alanine and other amino-acid residues in the side chain have been measured under various conditions of pH. The spectra were interpreted using spectra of poly(L-lysine) as standard. No straightforward correlation between polypeptide CD spectra and their structure could be detected. The contribution of the side chain CD to that of the main chain is additive in some cases, but in other cases qualitatively different CD spectra arise. The reason for this CD behavior is discussed.

In our laboratory the conformation of random amino-acids copolymers as well as sequential polypeptides has been studied, especially in connection with their interaction with DNA. Up to now polypeptides with one linear peptidic chain have been investigated using several approaches[1–5]. Branched polypeptides (named also multichain polypeptides, *cf.*[6]) were shown to be useful as protein models in immunochemistry[7] and in studies on the molecular mechanism of protein–drug interactions[8,9], but only limited information on the conformation of these polypeptides is available in the literature[10].

This paper deals with CD spectra of selected branched polypeptides[9] in dependence on pH and protonation of basic and acidic groups. The branched polypeptides are based on a chain of poly(L-lysine). To the ε-amino group of every lysine residue about 3 DL-alanine residues and one or more other amino-acid residues have been added according to the following scheme:



Although complicated interactions between the main chain and side chains and between the side chains themselves can be expected, in our interpretation we must mainly rely on the CD spectra of well characterized conformations of linear homopolymers.

EXPERIMENTAL

Material

The composition of branched polypeptides used in this study is given in Table I. Their syntheses and molecular weights (ranging from 75000 to 225000) are presented in the preceding paper[9].

Circular Dichroism Measurements

The CD spectra were recorded on a Roussel Jouan Dichrographe CD 185/II apparatus in cells with optical path 1·0, 0·2 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 concentration of solutions was about 0·5 mg per 1 ml and was calculated from weight amounts. For very poorly soluble poly(L-Lys-(D-Lys_i-DL-Ala_m)) saturated solution was used. The excess solid material was separated, the absorbance of the solution measured at 212 nm and compared with the absorbance value for poly(L-Lys(L-Lys_i-DL-Ala_m)) which is well soluble. The $[\Theta]$ values refer to one lysine residue in the main chain including the whole peptidic side chain.

RESULTS AND DISCUSSION

A survey of the CD parameters of the branched polypeptides under variable pH conditions is given in Tables I and II.

The interpretation of these spectra is based on the well characterized CD spectra of poly(L-lysine) from which all the branched polypeptides have been derived. The dependence of the poly(L-lysine) CD spectra on conformation has been extensively studied[11,12]. In the uncharged state I (*i.e.* in alkaline medium, pH about 12) this polypeptide assumes α-helical conformation. The respective CD spectrum is characterized by two negative maxima at 221 nm and 208 nm of about the same intensity (Fig. 1*A*). In acidic and neutral media this polypeptide is in a charged state II and the interaction of charged groups interferes with the formation of α-helical conformation. The respective CD spectrum is characterized by a weak negative maximum at 234 nm, positive maximum at 218 nm and negative maximum at 199 nm (Table II, Fig. 1*B*). According to some authors this spectrum is due to a partly ordered conformation of a 3_1 lefthanded helix type. A careful analysis shows, however, that this spectrum belongs to an essentially unordered conformation, and is most probably due to a certain redistribution of the conformational angles as a consequence of charge interactions[12].

Polypeptides based on poly(L-lysine) with 3 to 8 DL-alanine residues in the side chains display CD spectra similar to respective poly(L-lysine) spectra, but quantitati-

vely some differences are observed. In the charged state II (acidic pH) the weak negative maximum is shifted from 239 to 233 nm and its intensity is slightly increased, the intensity of both positive and negative maxima at 218 nm and 199 nm, respecti-

TABLE I

Characteristic CD Parameters of the Branched Polypeptides in the Lowest Charge State I, λ in nm, $[\Theta] \cdot 10^{-3}$ Given in Parentheses

Polypeptide[a]	Ratio L-Lys : m : i	pH	λ_{max}	λ_{min}	λ_{max}	λ_{cross}[b]
Poly(L-Lys)	—	12·4	221 (—24·0)	212·5 (—21·3)	208 (—23·1)	201 (0)
Poly(D-Lys)	—	12	221 (+17·8)	213 (+16·1)	208 (+17·7)	200 (0)
Poly(L-Lys(DL-Ala_m))	1 : 3·1	9	222 (—11·5)	216 (—10·2)	205 (—17·7)	<200
Poly(L-Lys(DL-Ala_m))	1 : 8·56	9	222 (—13·0)	215·5 (—11·0)	208 (—14·6)	201 (0)
Poly(L-Lys(L-Lys_i-DL-Ala_m))	1 : 3·1 : 3·4	12	227 (—4·53)	214 (—2·66)	201 (—26·1)	<200
Poly(L-Lys(D-Lys_i-DL-Ala_m))	1 : 3·4 : 3·1	12	224 (+5·65)	214·5 (+4·86)	— (—)	<200
Poly(L-Lys(DL-Lys_i-DL-Ala_m))[c]	1 : 3·1 : 3·12	11·4	232 (—0·72)	— (—)	206 (—4·19)	203 (0)
Poly(L-Lys(L-Glu_i-DL-Ala_m))	1 : 3·3 : 2·8	1·1	226 (—7·67)	216 (—4·36)	199 (—37·0)	<195
Poly(L-Lys(D-Glu_i-DL-Ala_m))[d]	1 : 3·8 : 3·23	1·1	231 (+1·23)	— (—)	200 (+11·6)	<195
Poly(L-Lys(L-Leu_i-DL-Ala_m))	1 : 3 : 0·7	10·7	221·5 (—28·5)	214 (—25·5)	209 (—27·2)	202 (0)
Poly(L-Lys(L-Pro_i-DL-Ala_m))	1 : 3·1 : 0·53	12·0	221·5 (—22·6)	212 (—18·8)	207 (—23·4)	<200
Poly(L-Lys(L-His_i-DL-Ala_m))	1 : 2·9 : 0·6	9	224 (—7·40)	216 (—6·11)	204 (—15·3)	199 (0)
Poly(L-Lys(L-His_i-DL-Ala_m))	1 : 3·4 : 0·8	9	229 (—2·78)	217 (—1·16)	201 (—10·7)	<195
Poly(L-Lys(L-Phe_i-DL-Ala_m))	1 : 3·4 : 0·66	7·2	223 (—17·9)	217 (—16·6)	208 (—22·6)	200 (0)
Poly(L-Lys(L-Tyr_i-DL-Ala_m))[e]	1 : 3·66 : 0·82	10·5	220 (—17·4)	214 (—16·2)	208 (—19·4)	<200

[a] For symbolics see preceding paper[9]; [b] crossing of the CD curve with zero-line; [c] λ_{cross} 223 nm, λ_{max} 218 nm, ($[\Theta] = +1\,060$), λ_{cross} 211 nm; [d] λ_{cross} 221 nm, λ_{max} 215 nm; ($[\Theta] = -703$), λ_{cross} 212 nm; [e] λ_{max} 248 nm, ($[\Theta] = +515$), λ_{cross} 241.

vely, being decreased (Fig. 1*B*). Assuming that the net contribution to the CD spectrum of DL-alanine residues is zero because of compensation of both contributions (D and L), the differences observed suggest that DL-alanine residues affect somewhat the most probable distribution of conformational angles, the overall conformation remaining unordered. In uncharged state I (alkaline pH) the type of the CD spectra corresponds to a partly helical conformation (Fig. 1*A*). Obviously the tendency to form an α-helix is negatively affected by the presence of DL-alanine residues in the chains, possibly on steric grounds.

The addition of a single residue of another L-amino acid (L-Pro, L-Leu, L-Phe) at the end of the side chain somewhat increases the apparent α-helix content of the polypeptides in the uncharged state I, the greatest effect being found in the case of L-leucine (Fig. 1*A*, Table I). The polypeptides containing L-histidine, the CD spectrum of which corresponds to less helical conformation (Table I), are exceptions. It is not sure, however, whether all ionizable groups at pH 9 are uncharged, and the samples are insoluble at higher pH values.

The polypeptides with L-amino acid residues at the side chain ends show, in the charged state II (acidic pH), CD spectra similar to those of polylysine under similar conditions (Table II, Fig. 1*B*). Hence they are probably also in unordered conforma-

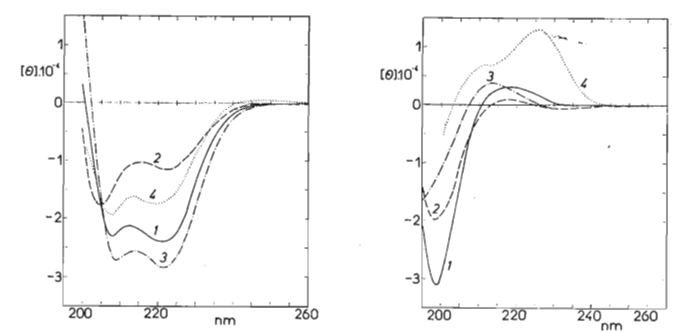


FIG. 1 *A* *B*

CD Spectra of Branched Polypeptides

A In the lowest charge state I: *1* poly(L-Lys) pH 12·4, *2* poly(L-Lys(DL-Ala_m)) with 3·1 DL-Ala per 1L-Lys pH 9, *3* poly(L-$Lys(L\text{-}Leu_i\text{-}DL\text{-}Ala_m)$) pH 10·7, *4* poly(L-$Lys(L\text{-}Tyr_i\text{-}DL\text{-}Ala_m)$) pH 10·5; *B* in the highest charge state II: The same polypeptides, *1*, *2* and *4* at pH 3, *3* at pH 2·8. All measurements in 0·02M-NaCl.

tion. However, in comparison with these polypeptides in α-helical or partly α-helical conformation, a higher variability of the CD spectra is observed. This is partly due to the presence of aromatic CD bands in the case of Tyr and Phe containing polypeptides. In the case of the tyrosine containing polymer two positive maxima

TABLE II

Characteristic CD Parameters of the Branched Polypeptides in the Highest Charge State II, λ in nm, $[\Theta] \cdot 10^{-3}$ Given in Parentheses

Polypeptide[a]	pH	λ_{max}	λ_{cross}[b]	λ_{max}	λ_{cross}[b]	λ_{max}
Poly(L-Lys)	3	239 (−0·11)	234 (0)	218 (+3·12)	211 (0)	199 (−31·2)
Poly(D-Lys)	3	239 (+0·15)	234 (0)	218 (−3·36)	210 (0)	196 (+36·5)
Poly(L-Lys(DL-Ala_m))[c]	3	233 (−0·59)	225 (0)	218 (+0·98)	213 (0)	198 (−19·7)
Poly(L-Lys(DL-Ala_m))[d]	3	233 (−0·83)	223 (0)	216 (+0·93)	213 (0)	199 (−14·8)
Poly(L-Lys(L-Lys_i-DL-Ala_m))	3	236 (−1·36)	229 (0)	216 (+11·1)	209 (0)	<200 (—)
Poly(L-Lys(D-Lys_i-DL-Ala_m))	1·8	238 (0·18)	234 (0)	216 (−4·4)	209 (0)	200 (33·2)
Poly(L-Lys(DL-Lys_i-DL-Ala_m))	3	235 (−0·45)	229 (0)	217 (+2·50)	212 (0)	196 (−31·2)
Poly(L-Lys(L-Glu_i-DL-Ala_m))	9	237 (−0·93)	231 (0)	217 (+10·2)	209 (0)	<195 (−)
Poly(L-Lys(D-Glu_i-DL-Ala_m))	9	242 (+0·05)	239 (0)	217 (−9·37)	206 (0)	<195 (+)
Poly(L-Lys(L-Leu_i-DL-Ala_m))	2·8	233 (−0·75)	227 (0)	213 (+3·75)	208 (0)	<195 (—)
Poly(L-Lys(L-Pro_i-DL-Ala_m))	3	234 (−0·66)	226 (0)	217 (+2·35)	210 (0)	<200 (—)
Poly(L-Lys(L-His_i-DL-Ala_m))[e]	3	240 (−0·16)	235 (0)	216 (+7·21)	208 (0)	<200 (—)
Poly(L-Lys(L-His_i-DL-Ala_m))[f]	3	240 (−0·23)	235 (0)	216 (+9·91)	208 (0)	<200 (—)
Poly(L-Lys(L-Phe_i-DL-Ala_m))	3	240 (−0·25)	235 (0)	218·5 (+12·5)	208 (0)	<195 (—)
Poly(L-Lys(L-Tyr_i-DL-Ala_m))[g]	3	247 (−0·26)	244 (0)	226 (+12·9)	203 (0)	<200 (—)

[a,b] See Table I; [c] 3·1 DL-Ala per one L-Lys; [d] 8·56 DL-Ala per one L-Lys; [e] 0·6 L-His per one L-Lys; [f] 0·8 L-His per one L-Lys; [g] λ_{min} 214 nm ($[\Theta] = +6560$), λ_{max} 212 nm ($[\Theta] = +6880$).

at 226 nm and 212 nm are observed instead of one maximum at 216–218 nm (Fig. 1*B*). The latter maximum is present in the CD spectrum of all other polypeptides with L-amino acid residues at the side chain ends and is of about the same intensity as in the polylysine case.

Another type of branched polypeptides has a side chain containing about 3 DL-alanine residues and additional three residues of lysine or glutamic acid with variable absolute configuration. CD spectra of polypeptides containing L, DL and D lysine residues in the side chain will be discussed first. In uncharged state I (alkaline pH) they differ from the spectra of other polypeptides measured. Their most striking feature is the dependence of the general spectral pattern on the absolute configuration of the lysine residue present in the side chain. The CD spectrum of the polypeptide containing L isomer (Fig. 2*A*) can be interpreted as similar to poly(L-lysine) CD spectra with a slight amount of α-helical conformation. An alternative interpretation can be the presence of an essentially unordered polypeptide chain of the type often found in globular proteins[13]. In any case, the degree of ordering in terms of linear polypeptide conformations appears to be low, which is at variance with our findings with other branched polymers in comparable media. In the case of the D isomer containing polypeptide the CD spectrum observed can be at least partly

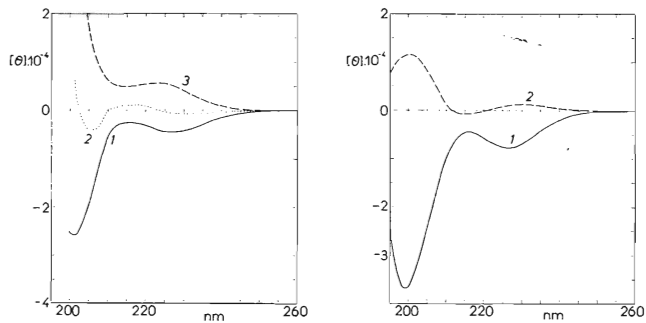


FIG. 2 *A* *B*

CD Spectra of Branched Polypeptides in the Lowest Charge State I

A With branches containing lysine: *1* poly-L-Lys (L-Lys_i-DL-Ala_m)) pH 12; *2* poly(L-Lys-(DL-Lys_i-DL-Ala_m)) pH 11·4, *3* poly(L-Lys (D-Lys_i-DL-Ala_m)) pH 12; *B* with branches containing glutamic acid: *1* poly(L-Lys(LGlu_i-DL-LAla_m)) pH 1·1, *2* poly(L-Lys(D-Glu_i-DL-Ala_m)) pH 1.1. All measurements in 0·02M-NaCl.

regarded as mirror image of that of the L isomer containing analogue. The CD spectrum of DL-lysine containing polypeptide is characterized by an extremely low intensity of the bands. The comparison of the results obtained for polypeptides containing different optical isomers in their side chains suggests that the most important CD determining factor is the absolute configuration of the side chain amino acids. The reason for the difference found between CD spectra of polypeptides containing 3–8 DL-alanine residues (Table I) and those containing 3 DL-Ala + 3 DL-Lys in the uncharged form (state I, Fig. 2*A*, Table I), is not clear at present. Some explanation can perhaps be found in considering different side chain sterical requirements in the presumably tightly packed conformation, in the possible role of hydrophobic forces among uncharged side chains, and in the effect of both these factors on the main chain conformation.

Analogous polypeptides containing glutamic acid residues in the side chains somewhat differ in their titration behavior. They are negatively charged in alkaline pH (state II corresponding to state II of lysine containing polymers in acidic pH) but in acidic pH (state I, lowest charge state) they are still positively charged due to protonated α-amino groups at the side chain ends (in contrast to the fully uncharged state I of lysine containing polypeptides). In spite of this difference an analogous

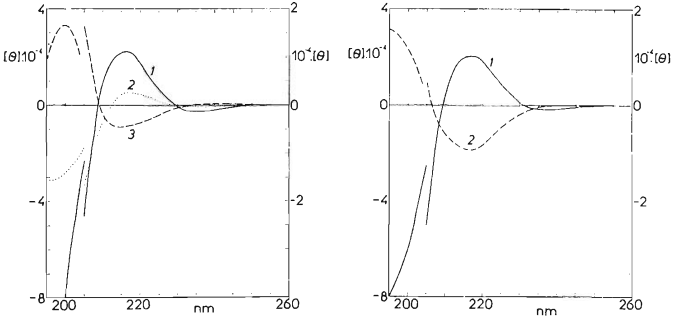


FIG. 3

CD Spectra of Branched Polypeptides in the Highest Charge State II

A With branches containing lysine: *1* poly(L-Lys(L-Lys_i-DL-Ala_m)) pH 3, *2* poly-(L-Lys(DL-Lys_i-DL-Ala_m)) pH 3, *3* poly-(L-Lys(D-Lys_i-DL-Ala_m)) pH 1·8; *B* with branches containing glutamic acid: *1* poly-(L-Lys(L-Glu_i-DL-Ala_m)) pH 9, *2* poly(L-Lys-(D-Glu_i-DL-Ala_m)) pH 9. All measurements in 0·02M-NaCl.

behavior is observed for both types of polymers (Fig. 2*A*, 2*B*), the most striking feature being again the dependence of the general CD pattern on the absolute configuration of the residues present in the side chains.

Assuming additivity of the CD contribution of the main and side chains and assuming the same contribution of both types of side chains containing L and D enantiomers except for the sign, a hypothetic contribution of the main chain can be calculated from both types of spectra (not shown). In the case of lysine containing side chains, the calculated main chain spectrum is very similar to that of the polymer containing DL lysine residues in the side chain (Fig. 2*A*), which suggest that this might be the side chain independent contribution of the main chain to the total CD spectrum. The spectrum calculated by the same procedure for the polypeptides containing glutamic acid residues in the side chains is of higher intensity indicating that the side chain independent contribution of the main chain is higher in this case.

CD spectra of the polypeptides containing 3 DL-Ala + 3 Lys or 3 DL-Ala + 3 Glu side chains (Fig. 3*A*, 3*B*) show in the highest charge state II (*i.e.* in acidic pH for lysine and alkaline pH for glutamic acid) similar dependence on the absolute configuration of the side chain residues as in the previously discussed state I. The spectra of polypeptides containing L or DL isomers resemble in their general pattern to those of charged poly(L-lysine), their intensity depending on the amount of L residues present in one monomer unit (Fig. 3*A*, 3*B*). Quantitatively the band ellipticities for the polypeptide with DL-lysine residues are comparable with those of poly(L-lysine) (compare Figs 3*A* and 1*B*) suggesting that in this case the side chains do not substantially affect the conformation of the main chain. This contrasts with the behavior of the same polypeptide in the uncharged state I. The spectra of D enantiomer containing polypeptides resemble in their general patterns to those of charged poly(D-lysine). Their intensity, however, is lower than that of corresponding L enantiomer containing analogues reflecting thus the independent contribution of the main polypeptide chain to the CD spectrum (Fig. 3*A*, 3*B*).

From our CD measurements on branched polypeptides it follows that no straightforward correlation exists between the structure of the polypeptides on one hand and their CD spectra on the other hand. In some cases nearly additive contributions of the side chain CD values to those of the main chain could be observed (Fig. 3*A*, 3*B*), but in other cases qualitatively new spectra appeared which had no alliance to the main chain and/or side chain spectra under comparable conditions (Fig. 2*A*, 2*B*). Presumably, sterical requirements (length and bulkiness) and the presence of specific intramolecular interactions between chains (hydrogen bonding and hydrophobic interactions) may result in various types of more or less rigid conformations.

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