CONFORMATION OF BRANCHED POLYPEPTIDES BASED ON POLY(L-LYSINE): THE EFFECT OF TERMINAL AMINO ACIDS IN THE BRANCHES

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CD Spectra of branched polypeptides based on poly(L-lysine) and containing three DL-alanine residues and one to three other L- or D-amino acid residues in the branches were measured in water, water–methanol and water–trifluoroethanol mixtures. In aqueous solutions dependence of the CD spectra on pH and ionic strength was studied. The effect of branch elongation was followed mainly with compounds containing glutamic acid. One terminal D-amino acid residue and also an extension by two L- or D-amino acid residues does not hinder the α-helix formation in the backbone but affects the conditions of its formation. In polypeptides with three L- or D-amino acids additional α-helical segments in the branches are assumed to be formed. For branches with L-amino acids the CD curves express additively the contributions of both helical components, in the case of D-amino acids the increasing population of the ordered structure in branches is manifested by compensation of dichroic contribution of the L-amino acid backbone leading even to enantiomorphous curves.

In our previous papers[1,2] we have studied CD spectra of branched peptides in solutions of various ionic strength and pH. These polypeptides were based on poly(L-lysine) substituted at ε-amino groups by side chain composed of about 3 DL-alanine residues (the inside area) and one or more other amino acid residues (the outside determinant[3,4]). In the case of polypeptides containing one L-amino acid residue as the outside determinant the conformation is strongly dependent on the nature of the amino acid[2]. Polypeptides containing three lysine or glutamic acid residues as the outside determinant exhibit a clear dependence of conformational properties on the absolute configuration of the glutamic acid and lysine residues and also the length of side chain seems to be important[1].

In order to understand better the factors affecting the conformation of these polypeptides we investigated more systematically the effect of the branch length and of the absolute configuration of the branch terminating amino acids[5]. We have studied polypeptides containing one, two or three glutamic acid residues of L or D

configuration and analogous polypeptides containing lysine residues. The CD measurements performed in water solutions of various ionic strength and in water–alcohol mixtures have been interpreted in terms of ordered (helical) and unordered conformations.

As helical we denote such conformation whose CD spectrum is similar to that of helical poly(L-lysine)[6,7]. As CD spectra of the unordered form of polypeptide type are denoted spectra similar to that of charged poly(L-lysine)[6,7], as spectra of unordered form of protein type we designate spectra similar to those of denatured proteins[8].

EXPERIMENTAL

Polypeptides of the following composition were used (the ratio of Lys : m : i is given): poly(L-Lys.(L-Nle$_i$-DL-Ala$_m$)) (*I*) 1 : 3 : 0·95, poly(L-Lys(L-Ile$_i$-DL-Ala$_m$)) (*II*), 1 : 3 : 0·95, poly(L-Lys.(D-Leu$_i$-DL-Ala$_m$)) (*V*) 1 : 3 : 0·95, poly(L-Lys(D-His$_i$-DL-Ala$_m$)) (*VI*) 1 : 2·95 : 0·53, poly(L-Lys.(L-Glu$_i$-DL-Ala$_m$)), 1 : 2·9 : 0·81 (*III*), and 1 : 3·3 : 2·8 (*XIII*), poly(L-Lys((L-Glu-L-Glu)$_i$-DL-Ala$_m$)) (*IX*), 1 : 2·9 : 0·62, poly(L-Lys(D-Glu$_i$-DL-Ala$_m$)) 1 : 2·9 : 0·87 (*VII*) and 1 : 3·8 : 3·23. (*XVI*), poly(L-Lys((D-Glu-D-Glu)$_i$-Ala$_m$)) 1 : 2·9 : 0·46 (*XI*), poly(L-Lys(L-Lys$_i$-DL-Ala$_m$)) 1 : : 2·9 : 0·5 (*IV*) and 1 : 3·1 : 3·4 (*XIV*), poly(L-Lys((L-Lys-L-Lys)$_i$-DL-Ala$_m$)) (*X*) 1 : 2·9 : 0·45, poly(L-Lys(D-Lys$_i$-DL-Ala$_m$)) (*VIII*) 1 : 2·9 : 0·87, poly(L-Lys((D-Lys-D-Lys)$_i$-DL-Ala$_m$)) (*XII*) 1 : 2·9 : 0·2 and poly(L-Lys (DL-Lys$_i$-DL-Ala$_m$)) (*XV*) 1 : 3·1 : 3·1. Their synthesis and molecular weight estimations have been described elsewhere[3,5].

CD spectra were measured using a Roussel–Jouan CD 185/II Dichrographe in cells of optical path 1·0, 0·2, 0·1, 0·05 and 0·02 cm or a Jobin–Yvon Dichrographe III in 0·01 cm cells. The samples were dissolved in 0·02M-NaCl and pH was adjusted with 0·1M-NaOH or 0·1M-HCl. Polypeptide solutions in water–methanol and water–trifluoroethanol mixtures were prepared by dissolving the polypeptide in 0·02M-NaCl and adding the appropriate amount of alcohol. The pH was adjusted before the addition of alcohols. The $[\theta]$ values are related to one lysine residue in the main chain including whole side chain. The CD spectra are collected in Table I – III and Figs 1 – 6.

RESULTS AND DISCUSSION

The polypeptides containing branches with one terminal L-norleucine (*I*), L-isoleucine (*II*), L-glutamic acid (*III*) or L-lysine (*IV*) residue represent further members of the polypeptide series studied in our previous paper[2]. Their CD spectra are given in Table I.

The polypeptides *I* and *II* containing hydrophobic amino acids show a significant tendency to form α-helical structures. Like the polypeptide containing L-leucine[2], they are to a significant extent helical even at pH 7·4 in low ionic strength.

The polypeptide *III* (with L-glutamic acid as outside determinant) contains both an acid and a basic group and is thus charged throughout the whole pH region. According to the CD spectra on 0·02M-NaCl (Fig. 1), in the whole region of pH this polypeptide is in a random conformation. The spectra in acid and alkaline medium

TABLE I

Characteristic values of CD spectra of branched polypeptides containing at the end of branches one additional amino acid residue

pH	NaCl mol l^{-1}	λ, $[\Theta] \cdot 10^{-3}$				
		cross	max	cross	min	max
		Poly(L-Lys(L-Nle$_{0.95}$-DL-Ala$_3$)) (*I*)				
7·4	0·02	202	207 (−20·8)	—	212 (−17·9)	222 (−22·8)
		Poly(L-Lys(L-Ile$_{0.95}$-DL-Ala$_3$)) (*II*)				
7·4	0·02	202	209 (−17·9)	—	215 (−16·6)	222 (−18·7)
		Poly(L-Lys(L-Glu$_{0.81}$-DL-Ala$_{2.9}$)) (*III*)				
1·1[a]	0·02	207	215 (+3·14)	227	—	232·5 (−0·76)
1·1	2·0	202	208 (−14·0)	—	213·5 (−12·0)	222 (−16·2)
5·0[b]	0·02	208	212·5 (+1·41)	220	—	228 (−1·80)
7·4[a]	0·2	201	213·5 (+0·16)	215	—	227 (−2·29)
9·5[a]	0·02	211	215 (1·88)	224	—	231·5) (−0·92)
9·5	2·0	203	208 (−9·04)	—	213 (−6·33)	222·5 (−10·6)
1·1	0·02 + 75% methanol	202·5	211 (−22·5)	—	215·5 (−20·7)	222 (−23·9)
1·1	0·02 + 75% TFE[c]	201	208 (−22·2)	—	214 (−19·0)	221·5 (−22·6)
		Poly(L-Lys(L-Lys$_{0.5}$-DL-Ala$_{2.9}$)) (*IV*)				
2·8	0·02	207	214·5 (+4·8)	232	—	237 (−0·17)
2·8[a]	2·0	—	—	—	215 (−1·53)	225·5 (−3·61)
7·3[a]	0·2	—	—	—	216·5 (−1·92)	224 (−3·54)
12·1	0·02	—	207 (−9·53)	—	215 (−6·36)	222 (−7·67)
12·1	2·0	—	204 (−21·0)	—	216 (−15·0)	221 (−17·7)

TABLE I
(*Continued*)

pH	NaCl mol l^{-1}	λ, $[\Theta] \cdot 10^{-3}$				
		cross	max	cross	min	max
		Poly(L-Lys(D-$Leu_{0.95}$-DL-Ala_3)) (*V*)				
2·8[a]	0·02	—	—	—	222 (−0·98)	226·5 (−1·39)
2·8	2·0	<200	208·5 (−37·0)	—	212·5 (−25·9)	221 (−31·3)
7·3	0·2	—	203·5 (−24·6)	—	217·5 (−12·3)	222 (−12·5)
11·1	0·02	200	207·5 (−24·3)	—	214·5 (−20·3)	220·5 (−25·8)
11·1	0·02 + 75% methanol	200	207·5 (−31·6)	—	215 (−23·7)	221 (−25·8)
11·1	0·02 + 75% TFE[c]	200	207 (−26·7)		213·5 (−21·7)	221 (−26·8)
		Poly(L-Lys(D-$His_{0.53}$-DL-$Ala_{2.95}$)) (*VI*)				
2·8[a]	0·02	—	—	—	215 (−2·20)	220 (−2·22)
2·8	2·0	—	205·5 (−20·1)	—	215 (−15·9)	220 (−16·9)
7·4	0·2	—	205 (−15·4)	—	214·5 (−11·1)	225 (−12·6)
11·8	0·02	—	207 (−22·5)	—	214 (−18·8)	221 (−23·1)
11·8	2·0	—	208 (−26·9)	—	215 (−23·2)	221 (−23·7)
		Poly(L-Lys(D-$Glu_{0.87}$-DL-$Ala_{2.9}$)) (*VII*)				
1·2	2·0	<200	204·5 (−25·7)	—	213·5 (−19·1)	219·5 (−19·6)
10·2[a]	2·0	—	—	—	217 (−5·69)	221·5 (−5·92)
1·2	0·02 + 75% methanol	199	207 (−35·7)	—	215 (−28·3)	220 (−28·6)
1·2[a]	0·02 + 95% TFE[c]	—	207·5 (−32·1)	—	215 (−26·3)	221·5 (−26·4)

TABLE I
(*Continued*)

pH	NaCl mol l^{-1}	λ, $[\Theta] . 10^{-3}$				
		cross	max	cross	min	max
		Poly(L-Lys(D-$Lys_{0.87}$-DL-$Ala_{2.9}$)) (*VIII*)				
3[a]	0·02	—	—	210	—	215 (+3·15)
12[a]	0·02	—	—	—	220 (−5·0)	223 (−5·3)
12	2·0	<200	206 (−46·5)	—	214 (−35·0)	222 (−39·0)

[a] Additional $\lambda_{max} < 200$ nm; [b] $\lambda_{max} = 203$ mn, $[\Theta] = 3{\cdot}25 . 10^3$; [c] TFE 2,2,2-trifluoroethanol.

are almost identical; at pH 5·0 an increased intensity of the long-wave negative maximum and decreased intensity of the positive maximum at about 217 nm was observed. An increase in ionic strength to 0·2M-NaCl (data not shown) leads to a rather small change in the CD spectra. Under approximately physiological conditions (pH 7·4, 0·2M-NaCl) this polypeptide exists in a random conformation. The shape of the CD spectra in 2M-NaCl (Fig. 1) corresponds to the spectra of α-helix but the band intensity is low. The α-helix content is higher in water–methanol (Fig. 1) and water–trifluoroethanol mixtures. The behaviour of this peptide resembles that of linear polypeptides $(Lys\text{-}Ala\text{-}Glu)_n$ (ref.[9]) and $(His\text{-}Ala\text{-}Glu)_n$ (ref.[10]) in which also acid and basic groups prevent the α-helix formation. However, under favourable conditions, *i.e.* in high ionic strength and particularly in water–alcohol mixtures, all these polypeptides are able to form the α-helix.

The L-lysine containing polypeptide *IV* fits, according to its properties, the series of polypeptides with one L-amino acid[2]. This compound is random in the charged and partially helical in the uncharged state. Under physiological conditions it exists in the random conformation. Comparison with the polypeptide containing only DL-alanine in the side chain[2] shows that lysine as outside determinant partially restricts the formation of an ordered secondary structure of the backbone.

Polypeptides with One Terminal D-*Amino Acid in the Branch*

In the charged state (pH 2·8) and low ionic strength (0·02M-NaCl), the polypeptide *V* containing D-leucine (Table I) assumes a random coil conformation. Its CD spectrum is of the protein type[8], *i.e.* without the positive band at about 217 nm. This poly-

peptide is unordered also under approximately physiological conditions (contrary to the analogous polypeptide with L-leucine).

In the uncharged state (pH 11·2) even in low ionic strength and in the charged state in high ionic strength (2M-NaCl) its CD spectra are similar to those of α-helical conformation of polypeptides composed exclusively of L-amino acids[6,7]. In the uncharged state in high ionic strength the sample is aggregated and the spectra are not measurable.

Compound *VI*, containing D-histidine (Table I) is unordered in the charged state (protein-type spectrum) and partly helical in the uncharged state; however, under comparable conditions the intensities of both negative bands are lower than those of the D-leucine-containing peptide. Under physiological conditions the CD spectrum of this peptide resembles an α-helix spectrum but the intensity of both bands is low.

Similarly to the case of polypeptide *III* containing L-glutamic acid, the behaviour of the polypeptide *VII* (with D-glutamic acid, Table I) is influenced by the fact that the compound is charged in the whole pH region. Its CD spectra in low ionic strength (Fig. 2) correspond to a random coil form and are of the protein type except the

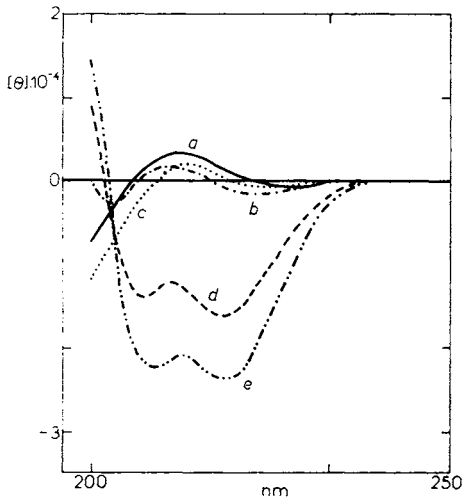

FIG. 1

CD Spectra of poly(L-Lys(L-$Glu_{0.81}$-DL-$Ala_{2.9}$)) (*III*). *a* 0·02M-NaCl, pH1·1, *b* 0·02M-NaCl, pH 5·0, *c* 0·02M-NaCl, pH 9·5, *d* 2·0M-NaCl, pH 1·1, *e* 0·02M-NaCl, pH 1·1, 75% methanol

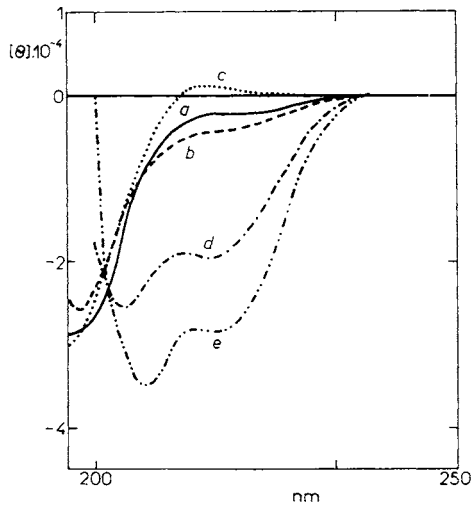

FIG. 2

CD Spectra of poly(L-Lys(D-$Glu_{0.81}$-DL-$Ala_{2.9}$)) (*VII*). *a* 0·02M-NaCl, pH 1·1, *b* 0·02M-NaCl, pH 5·4, *c* 0·02M-NaCl, pH 10·2, *d* 2·0M-NaCl, pH 1·1, *e* 0·02M-NaCl, pH 1·1, 75% methanol

spectrum at pH 10·2. The effect of pH on the CD spectrum in low ionic strength is analogous to that observed with the polypeptide containing L-glutamic acid. Under physiological conditions, compound *VII* is in a random coil conformation (data not shown) the CD spectrum being almost identical with that at pH 5·4 in 0·02M-NaCl (Fig. 2). In solutions of high ionic strength (2M-NaCl) the helical structure is formed at pH 7·4 (not shown) and 1·2 (Fig. 2) but not at pH 10·2. The α-helical structure exists also in the presence of methanol (Fig. 2) and trifluoroethanol.

The D-lysine-containing polypeptide *VIII* (Table I) is in a random conformation in low ionic strength (0·02M) both in the charged and uncharged state and also under physiological conditions (not shown). In the charged state, the compound exhibits CD spectrum of the unordered form of polypeptide type whereas in the other cases a protein-type spectrum is observed. Formation of the helical structure was observed only in the uncharged state (pH 12·0) in high ionic strength (2·0M-NaCl). In contrast to the L-lysine-containing polypeptide which forms an α-helix already in lower ionic strength, the presence of D-lysine has a helix-breaking effect.

The basic CD spectral features of polypeptides containing one D-residue as outside determinant correspond to those of peptides composed of L-residues. Although this behaviour is not surprising in the case of the D-histidine-containing polypeptide with only 50% substitution in the side chains, it was observed even in the case of the D-leucine-containing compound in which the side chain substitution is 95%. These results indicate that the L-residues in the backbone of these polypeptides contribute to the shape of the CD curves more than the D-residues in the side chains. Spectra of polypeptides with one D-amino acid as outside determinant have some common features which make them different from those of analogous polypeptides composed solely of the L-residues. The CD spectra of charged polypeptides containing one D-residue correspond to the unordered conformation of protein type (which is characterized by a negative CD maximum in the long-wavelength region) whereas the spectra of charged polypeptides with only L-residues are predominantly of the polypeptide type (*i.e.* they exhibit a positive maximum at about 217 nm). Under conditions favouring ordered structures, polypeptides of both types show spectra resembling that of α-helical conformation. The long-wavelength negative maximum of the D-residue-containing polypeptides is shifted by about 2 nm toward lower wavelength and the intensities of both negative bands are higher than for analogous compounds with L-residues. However, very probably, this difference cannot be interpreted as a difference in the content of the α-helical conformation.

The conditions under which the ordered structure is formed are affected by the absolute configuration of amino acid residues in the outside determinant. The D-enantiomers of leucine, lysine or glutamic acid in the outside determinant weaken the tendency to form an ordered structure. On the other hand, D-histidine-containing polypeptide forms the α-helical conformation under less favourable conditions than its L-analogue.

Polypeptides with Two Additional Amino Acid Residues at the Ends of the Branches

The effect of elongation of the branches was investigated with polypeptides containing lysine and glutamic acid in the L- or D-configuration. Polypeptides with one amino acid residue as the outside determinant were prepared *via* activated esters of protected amino acids, polypeptides with two amino acid residues were obtained similarly from activated esters of the corresponding dipeptides[5]. In both cases the length of the outside determinant in all branches is the same, its distribution on the poly(L-lysine) backbone being random. Polypeptides with an average of three amino-acid residues as outside determinant were prepared by reaction with N-carboxyanhydrides of lysine or glutamic acid[3] and their length varies. The pertinent CD data are given in Table II. Their interpretation is based on the measurements of glutamic acid-containing polypeptides because some of the analogous lysine-containing polypeptides are only sparingly soluble.

The CD spectra of the polypeptide *IX*, containing two L-glutamic acid residues, indicate a random coil conformation or a very low content of α-helix in 0·02M-NaCl. In contrast to the polypeptide *III* with only one L-glutamic acid residue, whose CD spectra corresponding to random conformation are of the polypeptide type at all pH values (Fig. 1), the polypeptide *IX* shows this type of spectrum only at pH 11·5; in an acidic medium the spectrum is of the protein type. Moreover, at pH 1·5 the spectrum is almost identical with that at pH 7·4 (not shown) whereas the polypeptide *III* has almost identical spectra in both acidic and alkaline medium. An increase of the ionic strength to 2·0M-NaCl or the presence of alcohols results in partial formation of an ordered α-helical conformation the α-helix content being higher in water–alcohol mixtures (Fig. 3, curve *b*). These results show that elongation of the branch by one L-glutamic acid residue is manifested mainly by its greater ability to form ordered structures in low ionic strength under conditions of minimum charge (acid medium). However, the maximum content of α-helix (maximum intensity of the bands) achievable in water–alcohol mixtures, is approximately the same as with the polypeptide *III* (Fig. 3, curves *a*, *b*).

The polypeptide *X* with two L-lysine residues is random in low ionic strength in the charged state. The CD spectrum of the uncharged compound corresponds either to random conformation of the protein type or to a small α-helix content. A similar type of spectrum was found also under approximately physiological conditions. An increase of ionic strength to 2M-NaCl leads to formation of the α-helical structure in the uncharged state and partly also in a neutral medium (not shown); in the charged state no ordered helical structure appears in high ionic strength. A comparison with the polypeptide *IV*, containing one L-lysine residue shows that in this case the elongation of the chain has little effect. The conditions under which the ordered helical structure is formed are the same in both cases and the difference is only

TABLE II

Characteristic values of CD spectra of branched polypeptides containing at the end of branches two additional amino acid residues

pH	NaCl mol l^{-1}	λ, $[\Theta] \cdot 10^{-3}$				
		cross	max	cross	min	max
		Poly(L-Lys(L-Glu-L-Glu)$_{0.62}$-DL-Ala$_{2.9}$) (*IX*)				
1·1	0·02	—	200·5 (−10·3)	—	214 (−0·93)	225·5 (−3·05)
1·5	2·0	202	207 (−11·9)	—	212·5 (−10·9)	224 (−14·4)
7·4	0·2	—	201·5 (−7·48)	—	211·5 (−2·83)	224 (−5·29)
11·5[a]	0·02	213·	214 (+0·34)	217	—	228·5 (−1·23)
11·5	2·0	—	207 (−15·1)	—	213·5 (−6·52)	222·5 (−7·90)
1·1	0·02 + 75% methanol	203	208·5 (−27·7)	—	213·5 (−17·6)	222 (−20·4)
1·1	0·02 + 95% TFE[b]	203	209 (−17·3)	—	214·5 (−14·4)	221·5 (−17·6)
		Poly(L-Lys(L-Lys-L-Lys)$_{0.45}$-DL-Ala$_{2.9}$) (*X*)				
2·0	0·02	208	216 (+5·71)	230	—	240 (−0·67)
7·3[c]	0·2	—	—	—	216 (−5·28)	220 (−5·87)
12	0·02	—	204·5 (−9·76)	—	220 (−5·92)	225 (−6·51)
12	2·0	—	208 (−17·2)	—	214 (−12·7)	220 (−14·2)
		Poly(L-Lys(D-Glu-D-Glu)$_{0.46}$-DL-Ala$_{2.9}$) (*XI*)				
1·1	0·02	195	203·5 (−10·1)	—	215 (−6·80)	220 (−6·81)
1·1	2·0	<200	208·5 (−14·5)	—	213 (−12·0)	220 (−12·2)
7·4	0·2	<195	205 (−12·1)	—	220 (−8·48)	225 (−9·74)
11[d]	0·02	192	205 (−12·2)	—	—	—
11	2·0	200	208 (−8·94)	—	215 (−6·27)	222·5 (−12·5)
1·1	0·02 + 75% methanol	199	208·5 (−19·9)	—	214 (−16·7)	220 (−17·6)
1·1	0·02 + 90% TFE[b]	200	207 (−23·9)	—	213·5 (−17·7)	218·5 (−19·7)

TABLE II
(*Continued*)

pH	NaCl mol l^{-1}	λ, $[\Theta] \cdot 10^{-3}$				
		cross	max	cross	min	max
		Poly(L-Lys(D-Lys-D-Lys)$_{0.2}$-DL-Ala$_{2.9}$) (*XII*)				
2·9	0·02	—	198 (−18·1)	—	215 (0)	222·5 (−1·95)
2·9	2·0	195	205 (−12·2)	—	213·5 (−7·29)	222·5 (−8·12)
7·4	0·2	—	200 (−7·33)	—	215 (0)	225 (−4·13)
11·6	0·02	198	207 (−14·1)	—	213·5 (−8·05)	220 (−10·3)
11·6	·2·0	200	210 (−24·2)	—	216 (−19·8)	222·5 (−25·1)

[a] Additional λ_{max} = 199 nm, $[\Theta] = 11{\cdot}3 \cdot 10^3$; [b] TFE 2,2,2-trifluoroethanol; [c] additional $\lambda_{max} <$ < 200 nm; [d] inflex at 220 nm, $[\Theta]_{215} = -8{\cdot}35 \cdot 10^3$, $[\Theta]_{220} = -7{\cdot}0 \cdot 10^3$.

quantitative, the apparent α-helix content being lower in the polypeptide *X* (under comparable conditions).

The spectrum of the polypeptide *XI*, containing two D-glutamic acid residues, in low ionic strength and at pH 11·6 corresponds to a protein-type random conformation, at pH 1·1 to a random conformation of the protein type or a small α-helix content. Under physiological conditions the peptide *XI* is in part helical. In a neutral medium its CD spectrum almost does not change (not shown) with increased ionic strength (2M-NaCl) whereas in acid or alkaline media the content of α-helix increases. A further increase of the α-helix content occurs in water–alcohol mixtures but the intensity of both negative bands is lower than in the sample with one D-glutamic acid residue (Fig. 4, curves *a*, *b*).

In low ionic strength, the polypeptide *XII* with two D-lysine residues is unordered in the charged state and partly helical when uncharged (pH 11·6). Under physiological conditions the CD spectrum corresponds to random conformation. An increase of ionic strength to 2M-NaCl increases the content of the α-helix throughout the whole pH region, its maximum being in the uncharged state.

In the D-series lengthening of the side chain by one amino acid has a slightly more pronounced effect than in the L-series. In low ionic strength under conditions of mini-

mum charge the CD spectra of polypeptides containing two D-residues show a higher content of α-helix than in the corresponding polypeptides with one D-amino acid. In polypeptides containing D-glutamic acid the ratio of D-glutamic acid residues in the branches to L-lysine residues in the backbone is approximately equal for the monosubstituted (*VII*) (0·92) and disubstituted (*XI*) compound (0·87). It is thus evident that the conformation depends on whether the D-glutamic acid residues in the branches are isolated or in the dipeptide form. In the polypeptide *XII* containing D-lysine the extent of substitution is very low, the D-Lys/L-Lys ratio being only 0·4, less than in the monosubstituted polypeptide *VIII* (0·87). Since it was observed that the presence of D-lysine reduces the helix-forming capacity we may assume that the higher ability to form α-helix in the peptide *XII* is caused by low degree of substitution. However, the maximum content of α-helix in water–alcohol mixtures is lower for disubstituted polypeptides than for the monosubstituted ones (Fig. 4).

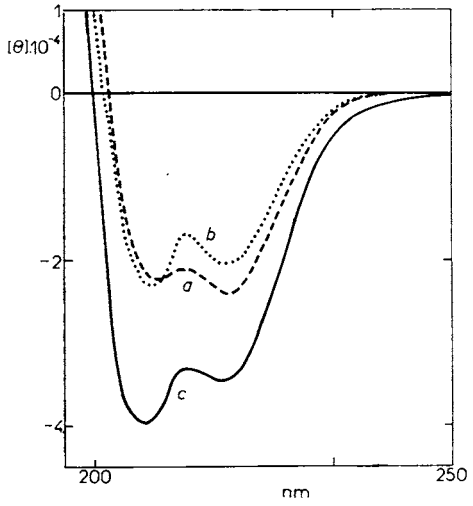

FIG. 3

The effect of the number of L-glutamic acid residues in the outside determinant on CD spectra in 0·02M-NaCl, pH 1, 75% methanol; *a* poly(L-Lys(L-$Glu_{0.81}$-DL-$Ala_{2.9}$)) (*III*), *b* poly(L-Lys(L-Glu-L-Glu)$_{0.62}$-DL-$Ala_{2.9}$)) (*IX*), *c* poly(L-Lys(L-$Glu_{2.8}$-DL-$Ala_{3.3}$)) (*XIII*)

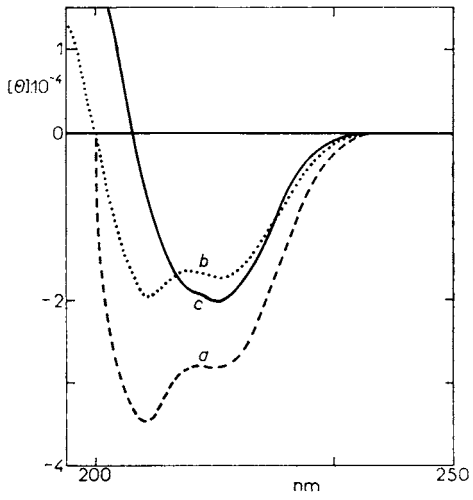

FIG. 4

The effect of the number of D-glutamic acid residues in the outside determinant on the CD spectra in 0·02M-NaCl, pH 1, 75% methanol; *a* poly(L-Lys(D-$Glu_{0.87}$-DL-$Ala_{2.9}$)) (*VII*), *b* poly(L-Lys((D-Glu-D-Glu)$_{0.46}$-DL-$Ala_{2.9}$)) (*XI*), *c* poly(L-Lys(D-$Glu_{3.23}$-DL-$Ala_{3.8}$)) (*XVI*)

Polypeptides with Three Additional Amino Acid Residues at the Ends of the Branches

On the average, these polypeptides contain six amino acid residues in the branches (three DL-Ala and three other D- or L-residues). Since the residues are randomly situated in the branches, we must assume that some of the branches are sufficiently long to be able to form an ordered helical structure. The CD spectral data are given in Table III.

In low ionic strength the polypeptide *XIII*, containing three L-glutamic acid residues, is in alkaline medium in the unordered conformation[1] whereas in acid medium[1] its CD spectrum corresponds to either the unordered conformation of protein type or to a small α-helix content. Under physiological conditions the peptide *XIII* is unordered (Fig. 5). The α-helix content in the acid medium can be increased by increasing the ionic strength to 2·0M-NaCl, however, even under these conditions the helix content is not too high. A greater effect can be achieved in water–alcohol mixtures (Fig. 3, curve *c*).

The polypeptide *XIV*, containing three L-lysine residues, behaves similarly to the polypeptide *XIII*. Under physiological conditions this compound exists in a random conformation; the same form exists in low ionic strength in the charged state[1] (pH 3). The CD spectrum in low ionic strength in the uncharged state[1] (pH 12) corresponds to a random conformation of protein type or a small α-helix content. However, the apparent content of α-helix is lower than that of the polypeptide *XIII* in acidic medium even though the compound *XIV* is uncharged in alkaline medium whereas in the polypeptide *XIII* the α-amino groups at the ends of branches are charged. The α-helix content in compound *XIV* with three L-lysine residues in uncharged state can be somewhat increased by increasing the ionic strength to 2M-NaCl; a larger increase is achieved in water–methanol mixtures. In both cases, however, the apparent content of α-helix is lower than that of the analogous polypeptide *XIII* containing L-glutamic acid residues under minimal charge conditions.

The polypeptide *XV*, containing three DL-lysine residues, has weak CD bands under all conditions indicating a random conformation. Upon increase of ionic strength or in water–methanol mixtures the CD spectra change, yet these changes are relatively small and in no case they correspond to formation of the ordered helical structure. These results show that DL-lysine residues in the branches hinder formation of the helical backbone structure.

The CD spectra of the polymer containing three L-lysine residues are in all ionic states similar to those of the polymer containing three L-glutamic acid residues. Under conditions of minimal charge the similarity of CD curves was observed only with trisubstituted polymers but not with mono- and disubstituted ones. In the monosubstituted polymer containing L-glutamic acid the formation of an ordered structure in low ionic strength is strongly suppressed by charged groups present even under

TABLE III

Characteristic values of CD spectra of branched polypeptides containing at the end of branches three additional amino acid residues

pH	NaCl mol l^{-1}	λ, $[\Theta] \cdot 10^{-3}$				
		cross	max	cross	min	max
		Poly(L-Lys(L-$Glu_{2.8}$-DL-$Ala_{3.3}$)) (*XIII*)				
1·0	0·02	<195	199 (−37·0)	—	216 (−4·36)	226 (−7·67)
1·0	2·0	<200	203·5 (−29·4)	—	215 (−12·4)	222·5 (−15·2)
7·35[a]	0·2	210	216 (+7·86)	229	—	235 (−1·03)
1·0	0·02 + 50% methanol	196	204 (−45·2)	—	215·5 (−25·3)	222·5 (−27·2)
1·0	0·02 + 75% methanol	200	207·5 (−40·2)	—	215·5 (−33·2)	221 (−34·7)
1·0	0·02 + 47·5% TFE[b]	196	204 (−48·5)	—	216·5 (−25·3)	222 (−26·0)
1·0	0·02 + 75% TFE[b]	<200	207 (−42·8)	—	215·5 (−27·5)	220 (−29·2)
1·0	0·02 + 95% TFE[b]	<200	209 (−33·5)	—	215·5 (−29·9)	221 (−35·7)
		Poly(L-Lys(L-$Lys_{3.4}$-DL-$Ala_{3.1}$)) (*XIV*)				
7·35[a]	0·2	211	217 (+7·80)	227	—	234 (−1·99)
12[a]	2·0	—	—	—	217 (−8·61)	225 (−10·9)
12[a]	0·02 + 50% methanol	—	—	—	216·5 (−14·5)	224 (−16·7)
12	0·02 + 75% methanol	—	203 (−50·6)	—	216 (−27·6)	222 (−29·7)
12	0·02	—	201 (−26·1)	—	217 (−2·66)	226 (−4·52)
		Poly(L-Lys(DL-$Lys_{3.1}$-DL-$Ala_{3.1}$)) (*XV*)				
7·35[c]	0·2	213	217·5 (+1·36)	226	—	233 (−0·65)
12	2·0	—	204 (−11·9)	—	214 (−1·09)	224·5 (−2·38)
12	0·02 + 50% methanol	—	205·5 (−7·31)	—	217 (−0·56)	228 (−1·81)
12	0·02 + 75% methanol	202	202 (−29·8)	—	216·5 (−2·92)	225 (−4·52)

TABLE III
(*Continued*)

pH	NaCl mol l^{-1}	λ, $[\Theta] . 10^{-3}$				
		cross	max	cross	min	max
		Poly(L-Lys(D-Glu$_{3\cdot23}$-DL-Ala$_{3\cdot8}$)) (*XVI*)				
1·0[d]	0·02	211	215 (−0·70)	221	—	231 (+1·23)
1·0	0·2	211	217 (−2·13)	226	—	233·5 (+1·07)
1·0	0·5	210	215 (−4·08)	234	—	239 (+0·42)
1·0	1·0	208	218 (−8·72)	239	—	243·5 (+0·19)
1·0	2·0	205	210 (−10·9)	—	211·5 (−9·97)	221 (−13·1)
1·0	0·02 + 10% methanol	209	218 (−4·55)	234	—	239 (+0·42)
1·0	0·02 + 20% methanol	206	218·5 (−10·3)	239	—	243 (+0·21)
1·0	0·02 + 50% methanol	207	219 (−16·6)	—	—	—
1·0	0·02 + 75% methanol	206	220 (−18·3)	—	—	—
1·0	0·02 + 6·3% TFE[b]	205	214 (−13·3)	—	215·5 (−13·2)	221 (−13·6)
1·0	0·02 + 12·5% TFE[b]	205	217·5 (−21·9)	—	—	—
1·0	0·02 + 25% TFE[b]	205	216·5 (−15·8)	—	—	—
1·0	0·02 + 50% TFE[b]	206	217·5 (−6·23)	—	—	—
1·0[d]	0·02 + 75% TFE[b]	<200	206·5 (−2·26)	229	—	236 (+0·72)
1·0[a]	0·02 + 85% TFE[b]	211	218 (+3·91)	—	—	—
1·0[a]	0·02 + 95% TFE[b]	211	217 (+7·33)	—	—	—
7·35[a]	0·2	208	215 (−10·4)	238	—	242 (+0·11)

[a] Additional $\lambda_{max} < 200$ nm; [b] TFE 2,2,2-trifluoroethanol; [c] additional $\lambda_{max} = 205{\cdot}5$ nm, $[\Theta] = -12{\cdot}4 . 10^3$; [d] additional $\lambda_{max} = 200$ nm, $[\Theta] = 11{\cdot}6 . 10^3$; [e] additional $\lambda_{max} = 203$ nm, $[\Theta] = -2{\cdot}28 . 10^3$, $\lambda_{min} = 105$ nm, $[\Theta] = -1{\cdot}84 . 10^3$.

conditions of minimal charge. The effect decreases with increasing length of the branch probably because of the helix-forming capacity of non-terminal glutamic acid residues. Elongation of branches by lysine residues results in an opposite effect, *i.e.* in suppressing the ordered structure formation. For this reason, the maximum content of α-helix (observed in water–alcohol mixtures) is higher in the polypeptide *XIII* containing three L-glutamic acid residues than in the corresponding L-lysine-containing polypeptide *XIV*. Since the maximum apparent α-helix content is also higher in the trisubstituted polymer *XIII* than in the corresponding mono- (*III*) and disubstituted (*IX*) polymer (Fig. 3) we assume that an ordered α-helical structure exists at least in part also in the branches. The behaviour of the polypeptide *XV* containing three DL-lysine residues shows that, unlike DL-alanine[2], DL-lysine in the branches restricts the formation of the backbone helix, probably because of its larger steric bulk. A similar restriction of helical backbone structure formation may be expected also in the polypeptide *XIV* containing L-lysine. The more pronounced content of the α-helical structure found in this compound under favourable conditions is probably due to formation of helical segments in the branches.

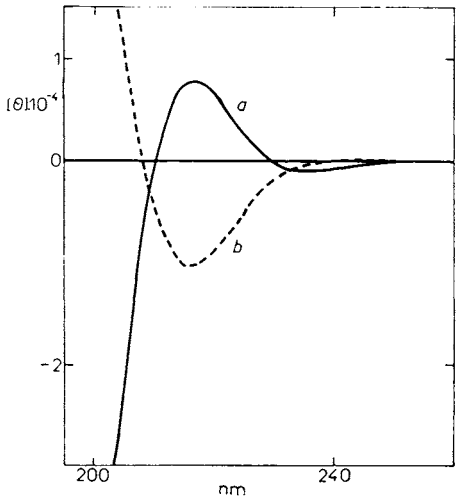

FIG. 5

CD Spectra of poly(L-Lys(L-$Glu_{2.8}$-DL-$Ala_{3.3}$)) (*XIII*), *a* and poly(L-Lys(D-$Glu_{3.23}$-DL-$Ala_{3.8}$)) (*XVI*), *b* in 0·2M-NaCl, pH 7·4

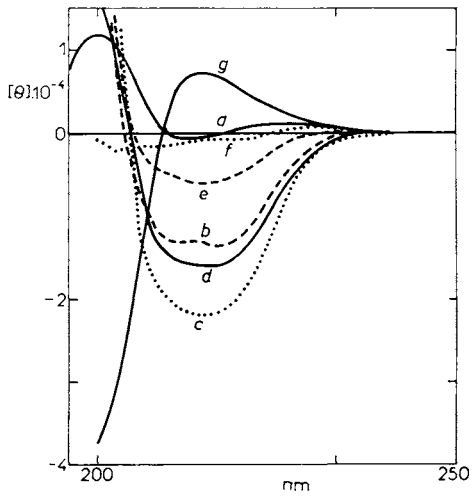

FIG. 6

CD Spectra of poly(L-Lys(D-$Glu_{3.23}$-DL-$Ala_{3.8}$)) (*XVI*) in the mixture water–trifluoroethanol (TFE), 0·02M-NaCl, pH 1, *a* 0% TFE, *b* 6·25% TFE, *c* 12·5% TFE, *d* 25% TFE, *e* 50% TFE, *f* 75% TFE, *g* 95% TFE

In the D-series we studied in detail only the polypeptide *XVI* with three D-glutamic acid residues since the polypeptide with three D-lysine residues[1] is very sparingly soluble; however, its CD spectra[1] in low ionic strength indicate features similar to those of polypeptide *XVI*. Under physiological conditions the CD spectrum of *XVI* corresponds to a random conformation of a polypeptide composed of D-residues (Fig. 5) and is almost independent of ionic strength (not shown). An analogous spectral type was found also in low ionic strength in an alkaline medium[1]. In acid medium the CD spectrum of *XVI* represents a transition between the polypeptide and protein random spectral types.[1] Increase of the ionic strength or methanol content in the water–methanol mixtures results in increasing negative ellipticity in the region 215–250 nm, the effect of methanol being more pronounced than that of higher ionic strength. The final CD spectrum is characterized by a negative maximum at about 220 nm (Fig. 4, curve c). One of the possible explanations of this change is the formation of α-helical structure of the backbone consisting of L-amino acids whereas the side-chains containing D-amino acids retain their unordered structure. A similar effect can be observed also in low concentrations of trifluoroethanol (up to 12·5%, Fig. 6). Increase of trifluoroethanol concentration to 75% leads to an opposite effect, *i.e.* to decrease in the intensity of the negative band. The CD spectrum in 75% trifluoroethanol is of very low intensity. A further increase of trifluoroethanol content to 85% and 95% makes the spectrum similar to that of the polypeptide type random conformation of a polypeptide consisting of L-residues but without the negative long-wavelength band. These changes reflect a complex conformational behaviour of the polypeptide in which the backbone is composed of L-residues and the branches contain several D-residues and in which conformational changes of the backbone and the branches cannot be independent. In polypeptides with three D- or L-amino acid residues in the branches the effect of the branches on the spectral shape is very marked. We assume that some of the branches are long enough to be capable of formation of ordered helical segments.

In the case of L-residues, the helical structure of both the backbone and the branches has the same sense so that the CD curves represent a sum of both contributions. On the other hand, the helical arrangement of branches composed of D-residues has an opposite sense than the helical arrangement of the backbone. This may lead to a compensation of the dichroic contributions or even to occurence of enantiomorphous curves.

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