SYNTHESIS AND CONFORMATION OF SEQUENTIAL BASIC POLYPEPTIDES WITH BRANCHED AND LINEAR SIDE CHAINS

Štěpánka ŠTOKROVÁ[a], Jan POSPÍŠEK[b], Jaroslav ŠPONAR[b] and Karel BLÁHA[b]

[a] *Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague 6 and*

[b] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

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Dedicated to Dr B. Sedláček on the occasion of his 60th birthday

Polypeptides $(Lys–X–Ala)_n$ and $(Lys–X–Gly)_n$ in which X represents residues of isoleucine and norleucine, respectively, and polypeptide $(Tle–Lys–Ala)_n$, were synthesized *via* polymerization of 1-hydroxysuccinimidyl esters of the appropriate tripeptides to complete previously studied series. Circular dichroism (CD) spectra of the respective polymers were measured as a function of pH and salt concentration of the medium. The results were correlated with those obtained previously with the same series containing different amino acid residues at the X position. The helix forming ability of the polypeptides $(Lys–X–Ala)_n$ with linear X side chain was found to be independent of the length. In the series $(Lys–X–Gly)_n$ the unordered conformation was the most probable one except $(Lys–Ile–Gly)_n$. This polymer assumed the β conformation even in low salt solution at neutral pH. An agreement with some theoretical work concerned with the restriction of conformational freedom of amino acid residue branching at C^β atom with our experimental results is evident.

The effect of amino acid side chains on the conformation of the polypeptide backbone has been discussed by several authors. Theoretical calculations have shown that the conformational freedom of the peptide molecule is dominated by the nature and conformation of the amino acid side chains[1] and that it is also restricted by the steric and energetic interactions with neighbouring residues[2]. The result of some experimental work dealing with conformational behavior of random copolymers as well as of sequential polypeptides lead to similar conclusions[3–6]. In our previous papers[7,8] the conformation was studied of synthetic polytripeptides $(Lys–X–Ala)_n$ and $(Lys–X–Gly)_n$, where X stands for an aliphatic hydrophobic residue of varying length and branching of the side chain (X = Ala, Nva, Val, Leu). It turned out that the structure of the X side chain considerably affects the degree and type of organization assumed by the polypeptide molecules. The purpose of this paper is to discuss the conformation and conformational changes of newly synthesized polymers (Lys–Ile–

$-Ala)_n$, $(Lys-Nle-Ala)_n$, $(Lys-Ile-Gly)_n$, $(Lys-Nle-Gly)_n$, and $(Tle-Lys-Ala)_n$*. These polymers make the previously discussed series $(Lys-X-Ala)_n$ and $(Lys-X-Gly)_n$ more complete in a sense that we have now polymers containing hydrophobic side chains with 2–5 carbon atoms, linear or branched at C^β or C^γ, in a combination with another helix forming (Ala) or helix breaking (Gly) amino acid residue. The synthesis of sequential polypeptides *Va–Vd* was carried out by the standard procedure (Scheme 1) using 1-succinimidyl esters in the polymerization step[8,10]. 2-Nitro-

$$\text{Nps-Aaa}_2\text{-OH} + \text{H-Aaa}_1\text{-OR}$$
$$\downarrow$$
$$\text{Nps-Aaa}_2\text{-Aaa}_1\text{-OR} \qquad I$$
$$\downarrow$$
$$\text{Nps-Lys}(Z)\text{-OH} + \text{H-Aaa}_2\text{-Aaa}_1\text{-OR}$$
$$\downarrow$$
$$\text{Nps-Lys}(Z)\text{-Aaa}_2\text{-Aaa}_1\text{-OR} \qquad II$$
$$\downarrow$$
$$\text{Nps-Lys}(Z)\text{-Aaa}_2\text{-Aaa}_1\text{-OH} \qquad III$$
$$\downarrow$$
$$\text{Nps-Lys}(Z)\text{-Aaa}_2\text{-Aaa}_1\text{-ONsu} \qquad IV$$
$$\downarrow$$
$$\text{H-Lys}(Z)\text{-Aaa}_2\text{-Aaa}_1\text{-ONsu}$$
$$\downarrow$$
$$\text{H-(Lys}(Z)\text{-Aaa}_2\text{-Aaa}_1)_n\text{-OH}$$
$$\downarrow$$
$$\text{H-(Lys-Aaa}_2\text{-Aaa}_1)_n\text{-OH . nHBr} \qquad V$$

In series *a*: Aaa_1 = Gly, Aaa_2 = Nle, R = Et
b: Aaa_1 = Ala, Aaa_2 = Nle, R = Me
c: Aaa_1 = Gly, Aaa_2 = Ile, R = Me
d: Aaa_1 = Ala, Aaa_2 = Ile, R = Me

$\text{Nps-Tle-Lys}(Z)\text{-Aaa}_1\text{-OY}$

VI, Y = Me
VII, Y = OH
VIII, Y = Nsu

$\text{H-(Tle-Lys-Aaa}_1)_n\text{-OH . } n\text{ HBr}$

IX

In formulae *VI–IX*: *a*, Aaa_1 = Gly, *b*, Aaa_1 = Ala.

SCHEME 1

* Amino acids used in the present work were all in the L-configuration. Nomenclature and symbols follow the published suggestions[9]. Other abbreviations: Tle tert-leucine (2-amino-3,3-dimethylbutanoic acid), Nle norleucine (2-aminohexanoic acid), Nsu 1-succinimidyl.

benzenesulfenyl group was used for the transient N^{α}-protection, benzyloxycarbonyl group for the permanent N^{ε}-protection. For the synthesis of polymers *IXa* and *IXb*, containing the very bulky tert-leucine residue, the Scheme 1 was modified and tripeptide derivative *VIII* was used as starting monomer. The protected polymers were isolated without detailed characterization, deblocked with hydrogen bromide in acetic acid and purified from low molecular weight substances by dialysis of an aqueous solution. Polyhydrobromides *V* and *IX* obtained by freeze-drying were characterized by amino acid analysis and bromide argentometric titration. The completeness of the splitting off of the benzyloxycarbonyl group was checked by UV spectroscopy.

EXPERIMENTAL

Melting points (uncorrected) were taken on a Kofler block. Samples for elemental analysis were dried at room temperature and 130 Pa over phosphorus pentoxide. Organic solutions were dried over anhydrous magnesium sulfate. Solutions were taken down on a rotatory evaporator under diminished pressure at bath temperature below 40°C. The $[\alpha]_D$ values were measured at room temperature on a Perkin–Elmer 141 polarimeter in N,N-dimethylformamide (concentration about 0·5 g of the substance per 100 ml of the solvent), unless stated otherwise. The purity of low-molecular-weight compounds was checked using TLC on silica gel plates (Kieselgel G, Merck) in systems 2-butanol–formic acid–water 75 : 12·3 : 12·7, 2-butanol–25% ammonia–water 85 : 7·5 : 7·5, 1-butanol–acetic acid–water 4 : 1 : 1, 1-butanol–pyridine–water–acetic acid 30 : 20 : 12 : 6. Detection was made by chlorination and ninhydrin.

2-Nitrobenzenesulfenyldipeptide Esters *Ia*—*Id*

A 10% excess (molar) of N,N′-dicyclohexylcarbodiimide was added to a solution of equimolar amount of amino acid ester (glycine or alanine) and dicyclohexylammonium salt of 2-nitrobenzenesulfenyl amino acid in dichloromethane (25 ml) at −10°C with stirring. The mixture was stirred at −10°C for 15—20 min and left to stand over 24 h in the refrigerator. The separated solid was filtered off (N,N′-dicyclohexylurea), the filtrate washed with 0·5M-H_2SO_4, 0·5M-$NaHCO_3$ and water, dried and evaporated. The residue was purified by crystallization from diethylether–light petroleum mixture 1 : 1. The experimental data are presented in Table I.

2-Nitrobenzenesulfenyl-N^{ε}-benzyloxycarbonyllysyldipeptide Esters *IIa*—*IId*

The protected dipeptides *Ia*—*Id* were dissolved in a minimum amount of methanol (of about 10 mmol) and then methanolic solution of HCl (3 ml, 2 mol l^{-1}) was added. After 5 min the mixture was evaporated, the residue washed with diethylether and light petroleum, dried and taken to dryness over solid KOH. Equimolar amount of the dipeptide ester hydrochloride (yielded by this procedure) and N^{α}-2-nitrobenzenesulfenyl-N^{ε}-benzyloxycarbonyllysine was dissolved in dichloromethane and 10% (molar) excess of N,N′-dicyclohexylcarbodiimide was added with stirring at −10°C. The mixture was left to stand for 20 min at −10°C and then for 24 h in a refrigerator. The separated N,N′-dicyclohexylurea was filtered off, the filtrate washed with 0·5M-H_2SO_4, 0·5M-$NaHCO_3$ and water, dried and taken to dryness; results are presented in Table I.

TABLE I

Chemical properties of compounds *I*—*IV*

Compound	M.p., °C (yield, %)	$[\alpha]_D$	Calculated/Found		
			% C	% H	% N
Nps–Nle–Gly–OEt	88—90	−26·6	52·02	6·27	11·37
Ia	(78)		52·31	6·72	11·27
Nps–Nle–Ala–OMe	118—120	−22·9	52·02	6·27	11·37
Ib	(89)		52·45	6·55	11·16
Nps–Ile–Gly–OMe	111—112	−38·0	50·69	5·95	11·94
Ic	(94)		50·98	6·00	11·94
Nps–Ile–Ala–OMe	144—145	−47·6	52·02	6·27	11·37
Id	(78)		51·99	6·41	11·46
Nps–Lys(*Z*)–Nle–Gly–OEt	117—118	0	57·04	6·54	11·08
IIa	(92)		57·40	6·96	11·35
Nps–Lys(*Z*)–Nle–Ala–OMe	110—112	−10·2	57·04	6·54	11·08
IIb	(95)		57·45	6·84	10·90
Nps–Lys(*Z*)–Ile–Gly–OMe	177—178	3·8	56·38	6·36	11·33
IIc	(82)		57·85	6·85	11·59
Nps–Lys(*Z*)–Ile–Ala–OMe	150—152	−6·1	57·04	6·54	11·08
IId	(90)		57·56	6·80	11·41
Nps–Lys(*Z*)–Nle–Gly–OH	116—118	0	55·70	6·17	11·60
IIIa	(98)		55·85	6·26	11·45
Nps–Lys(*Z*)–Nle–Ala–OH	118—120	−7·8	56·39	6·36	11·33
IIIb	(85)		56·86	6·46	11·20
Nps–Lys(*Z*)–Ile–Gly–OH	178—180	6·9	55·70	6·17	11·60
IIIc	(84)		55·66	6·52	11·29
Nps–Lys(*Z*)–Ile–Ala–OH	163—165	0	56·39	6·36	11·33
IIId	(99)		56·15	6·62	11·25
Nps–Lys(*Z*)–Nle–Gly–ONsu	90—92	−6·2	54·85	5·75	12·00
IVa	(98)		55·22	6·37	11·72
Nps–Lys(*Z*)–Nle–Ala–ONsu	102—104	−7·0	55·45	5·92	11·75
IVb	(98)		54·94	6·05	11·47
Nps–Lys(*Z*)–Ile–Gly–ONsu	135—137	−7·0	54·85	5·75	12·00
IVc	(82)		54·71	5·58	11·75
Nps–Lys(*Z*)–Ile–Ala–ONsu	152—154	−8·7	55·45	5·92	11·75
IVd	(90)		55·51	5·97	12·03

2-Nitrobenzenesulfenyl-N^{ε}-benzyloxycarbonyllysyl Dipeptides *IIIa–IIId*

The protected tripeptide *IIa–IId* (5 mmol) in 50 ml 1,4-dioxane and 10 ml of 1M-NaOH was mixed, the mixture was stirred 1 h at room temperature, diluted with water and evaporated. The solid material was dissolved in a minimum amount of water, acidified with 1M-H_2SO_4 and solution extracted with ethyl acetate. The extract was dried and evaporated, the solid washed with ether and crystallized from ethyl acetate–light petroleum. Results are presented in Table I.

Tripeptide 1-Hydroxysuccinimidyl Esters *IVa–IVd*

To a solution of 2-nitrobenezenesulfenyltripeptide (1·75 mmol) in dichloromethane (50 ml) 3·55 mmol of 1-hydroxysuccinimide and 2 mmol N,N′-dicyclohexylcarbodiimide was added with stirring at −10°C. The mixture was stirred at 10°C for 15 min and left to stand for 24 h in a refrigerator. N,N′-Dicyclohexylurea was filtered off, the filtrate washed with 0·5M-$NaHCO_3$ and water, dried and evaporated. The residue was crystallized at *IVa, c, d* from diethylether–light petroleum, at *IVb* from dichloromethane–light petroleum. Results are presented in Table I.

2-Nitrobenzenesulfenyl-N^{ε}-benzyloxycarbonyllysyl-glycine Ethyl Ester (*VIa*)

Dicyclohexylammonium salt of N^{α}-2-nitrobenzenesulfenyl-N^{ε}-benzyloxycarbonyllysine (6·14 g, 10 mmol) dissolved in 50 ml dichloromethane was mixed at −10°C with 1·4 g (10 mmol) of glycine methyl ester hydrochloride and 2·1 g (10·5 mmol) of N,N′-dicyclohexylcarbodiimide. The mixture was stirred at −10°C for 15 min and left to stand overnight in a refrigerator. The separated N,N′-dicyclohexylurea was filtered off and the filtrate washed with 0·5M-H_2SO_4, 0·5M-$NaHCO_3$ and water, dried and evaporated. The residue was crystallized from ethyl acetate–diethyl ether–light petroleum 2 : 1 : 1; yield 4·8 g (92%); m.p. 94–95°C; $[\alpha]_D$ −7·7 (*c* 0·2). For $C_{24}H_{30}N_4O_7S$ (518·6) calculated: 55·59% C, 5·83% H, 10·80% N; found: 55·80% C, 5·97% H, 10·93% N.

2-Nitrobenzenesulfenyl-tert-leucyl-N^{ε}-benzyloxycarbonyllysyl-glycine Ethyl Ester (*VIIa*)

To a solution of 2·08 g (4 mmol) of *VIa* in 15 ml of methanol 2 ml of 2M-HCl in methanol was added. After 5 min methanol was removed by distillation and the residue ground with ether, filtrated off and dried in a dessicator over solid KOH. The hydrochloride yielded by this procedure was dissolved in 25 ml of dichloromethane and dicyclohexylammonium salt of 2-nitrobenzenesulfenyl-tert-leucine (2·1 g, 4·8 mmol) was added to the solution[11]. Thereafter, N,N′-dicyclohexylcarbodiimide (1·0 g, 5 mmol) was added with stirring. The mixture was stirred for 30 min and left to stand for 4 days in a refrigerator. Separated N,N′-dicyclohexylurea was filtered off and the filtrate washed with 0·5M-H_2SO_4, 0·5M-$NaHCO_3$ and water, dried and evaporated. The residue was chromatographed on a column packed with silicagel, eluted with 2% ethanol in dichloromethane. Yield 1·35 g (53·5%), m.p. 110–111°C (ethyl acetate–diethyl ether with light petroleum (10%); $[\alpha]_D$ −35·0. For $C_{30}H_{41}N_5O_8S$ (631·7) calculated: 57·03% C, 6·54% H, 11·08% N; found: 56·67% C, 6·57% H, 10·99% N.

2-Nitrobenzenesulfenyl-tert-leucyl-N^{ε}-benzyloxycarbonyllysyl-glycine (*VIIIa*)

A solution of *VIIa* (2·3 g, 3·6 mmol) in 50 ml, 1,4-dioxane and 5 ml 1M-NaOH was mixed. The mixture was stirred for 1 h at room temperature, diluted with 20 ml H_2O and the solvent evaporated. The water solution was acidified with 0·5M-H_2SO_4 and extracted with ethyl acetate. Extract was dried and evaporated, the residue crystallized from ethyl acetate–diethyl ether. Yield 2·1 g (95%), m.p. 136–137°C; $[\alpha]_D$ −40·3 (*c* 0·2). For $C_{28}H_{37}N_5O_8S$ (603·7) was calculated: 55·0% C, 6·17% H, 11·65% N; found: 55·41% C, 6·14% H, 11·28% N.

2-Nitrobenzenesulfenyl-tert-leucyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-glycine 1-Hydroxysuccinimidyl Ester (*IXa*)

N,N'-dicyclohexylcarbodiimide (1·0 g, 5 mmol) and 1-hydroxysuccinimide (0·46 g, 4 mmol) was added to a solution of 2·1 g (3·5 mmol) of *VIIIa* in 25 ml dichloromethane at −10°C. The mixture was left to stand overnight in a refrigerator. Separated N,N'-dicyclohexylurea was filtered off, filtrate washed with 0·5M-$NaHCO_3$ and evaporated. The residue was purified by crystallization from ethyl acetate–diethyl ether; yield 2·4 g (98%), m.p. 99–102°C, $[\alpha]_D$ −21·7 (*c* 0·3). For $C_{32}H_{40}N_6O_{10}S$ (700·7) was calculated: 54·90% C, 5·75% H, 11·99% N; found: 54·88% C, 5·91% H, 12·48%,N.

2-Nitrobenzenesulfenyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-alanine Methyl Ester (*VIb*)

The compound was prepared in the same way as *VIIb*, yield 1·85 g (95%), m.p. 124–125°C, $[\alpha]_D$ −54·0 (*c* 0·2). For $C_{30}H_{41}N_5O_8S$ (631·7) calculated: 57·03% C, 6·54% H, 11·08% N; found: 57·38% C, 6·73% H, 11·13% N.

2-Nitrobenzenesulfenyl-tert-leucyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-alanine (*VIIIb*)

To a solution of 1·35 g (2·1 mmol) of *VIIb* in 50 ml acetone 10 ml 4M-NaOH was added. The mixture was stirred 1 h at room temperature, diluted with 20 ml water and organic solvent removed *in vacuo*. Water solution was acidified with 0·5M-H_2SO_4 and extracted with ethyl acetate. The extract was dried, evaporated and the residue crystallized from ethyl acetate–light petroleum, yield 1·2 g (93%), m.p. 148–150°C, $[\alpha]_D$ −56·0 (*c* 0·2). For $C_{29}H_{39}N_5O_8S$ (617·7) calculated: 56·39% C, 6·36% H, 11·33% N; found 56·12% C, 6·50% H, 11·31% N.

2-Nitrobenzenesulfenyl-tert-leucyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-alanine 1-Hydroxysuccinimidyl Ester (*IXb*)

By the same procedure as described for *IXa* compound *IXb* was prepared from the acid *VIIIb* (0·86 g, 1·4 mmol), yield 0·9 g (91%), m.p. 96–97°C. For $C_{33}H_{42}N_6O_{10}S$ (714·8) calculated: 55·45% C, 5·92% H, 11·75% N; found 55·37% C, 6·05% H, 11·67% N.

Polymerization

2 mmol solution of active ester (*IVa–IVd*, *IXa*, *IXb*) in a minimum amount of methanol was treated with a solution of hydrogen chloride in methanol (2 mol l^{-1}). After 5 min (ninhydrin positive reaction) the mixture was evaporated, ground with ether, dried and dissolved in N,N-dimethylformamide (4·5 ml). Triethylamine (0·3 ml) was added to the mixture with stirring until it solidified. The mixture was left to stand at room temperature for 1 week, then ground with water, the polymer filtered off and dried, dissolved in a minimum amount of dichloroacetic acid and 2 ml 33% hydrogen bromide in acetic acid was added. After 15 min the mixture was washed with ether and dried. The rough polymer hydrochloride was dissolved in 15 ml water and dialyzed against water in a dialysis tubing (Serva, Heidelberg, FRG) previously treated with ethylenediaminetetraacetic acid. The solution was then lyophilized. Yield 40–70 mg.

Sedimentation Analysis

Sedimentation measurements were carried out in an analytical ultracentrifuge Spinco Model E. The sedimentation coefficients of polypeptides were determined in 0·15M-NaCl buffered with 13 mmol l^{-1} sodium phosphate buffer pH 6·8, in cells with an optical path of 12 mm in an An–H rotor at 68 000 rev/min and recorded by schlieren optics. Molecular weights M_s were inferred

from a calibration graph[12,13]. Molecular weight averages $\bar{M}_w$ were measured by high speed sedimentation equilibrium method according to Chervenka[14] using interference optics.

Molecular weights of the polymers obtained from the sedimentation analysis of the polymers dissolved in 0·15M-NaCl: $(Lys–Ile–Ala)_n$ 5 200 ($\bar{M}_w$), $(Lys–Nle–Ala)_n$ 8 500 (M_s), $(Lys–Ile–Gly)_n$ 4 600 ($\bar{M}_w$), $(Lys–Nle–Gly)_n$ 4 700 ($\bar{M}_w$), $(Tle–Lys–Ala)_n$ 4 200 (M_s).

Measurement of Circular Dichroism

The circular dichroism spectra of polypeptides were recorded with a Cary 61 apparatus within the range 260—195 nm in cells with optical paths 0·05 and 0·01 cm. The circular dichroism system was calibrated with a 0·1% (w/v) aqueous solution of (+)-10-camphorsulfonic acid in a cell of optical path 1·0 cm by checking the ellipticity of the band centered near 290 nm. The concentration of solutions was usually 0·1% (w/v). pH was adjusted by adding 0·1M-NaOH to the original solution and measured with an accuracy of 0·1 unit. The circular dichroism is expressed in molar ellipticities [θ] (deg cm^2 $dmol^{-1}$), where the average molecular weight of the residue for the given polypeptide was used in the calculation. The concentrations of polypeptide solutions used for the molar ellipticity calculations were taken from weighed amounts. The samples were dried *in vacuo* and kept in desiccator.

The relative amounts of α helix, β conformation and unordered conformation was calculated using the component analysis of the CD spectra by the method of linear programming[8]. The molar ellipticity values of poly(L-lysine) under appropriate conditions[15] were taken as standard values for all the types of conformations.

RESULTS AND DISCUSSION

Circular Dichroism of Polymers with Residue X Branched at C^β

According to the CD results the polymer $(Lys–Ile–Ala)_n$ was in an aqueous solution of low ionic strength at neutral pH (0·02M-NaCl) essentially unordered, assuming a random coil conformation of the protein type. Component analysis revealed some 10% α helix, 18% β conformation and 72% random coil. On increasing the salt concentration to 0·15M-NaCl no substantial changes were observed. In 2M-NaCl negative ellipticity at 220 nm increased slightly and the component analysis indicated 29% α helix and 71% random coil. The results obtained in a slightly alkaline solution (pH = = 9) were similar (Fig. 1*a*, *b*; Table II). At pH ≧ 10 the amount of the α helix increased sharply, but a high residual spectrum was revealed by component analysis, probably indicating aggregation. A relative insensitivity of the conformation both to salt concentration and to pH changes is obviously shared by all the polymers of this series containing a C^β branched X residues. Conformational changes of the polymer $(Lys–Val–Ala)_n$ are very similar to those of $(Lys–Ile–Ala)_n$ and according to the component analysis its tendency to form ordered structures in high salt and high pH solutions is even lower.

The lowest conformational variability among all the polymers containing C^β branched X residue was observed with $(Tle–Lys–Ala)_n$. CD spectra of this polymer

virtually do not depend on salt concentration (not shown) or pH (Fig. 2) and indicate an unordered conformation of the protein type.

Our experimental results strongly support the conclusions of theoretical calculations of the effect of amino acid residues with short C^{β} branched side chains on peptide conformation[1]. They are consistent with the notion that the presence of another methyl group at C^{β} entails a severe restriction on the conformational space available and that in the presence of three methyl groups at C^{β} only a very extended peptide chain conformation is possible[16,17], at least in the homopolymer case. Although only one third of the amino acid residues in our polymers contain a C^{β} branched side chain, this conformational limitation is very pronounced especially in the case of neighbouring methyl group of Ala.

Different results were obtained with the polymer $(Lys–Ile–Gly)_n$. According to component analysis of the CD spectra this is the only polymer of polypeptides studied which assumes a β conformation even in a low salt concentration at neutral pH. With increasing salt concentration or pH the β structure content increases up to 84% (Table III, Fig. 3). In the whole range of NaCl concentrations a small amount of α helix is indicated by the component analysis. However, since polylysine standard CD spectra are used for component analysis[8] such results must be taken with caution. Nevertheless it is obvious that compared to $(Lys–Val–Gly)_n$[7] the conformation of the polymer $(Lys–Ile–Gly)_n$ is more variable, again in accordance with the theoretically calculated limited conformational freedom of the valine residue[1].

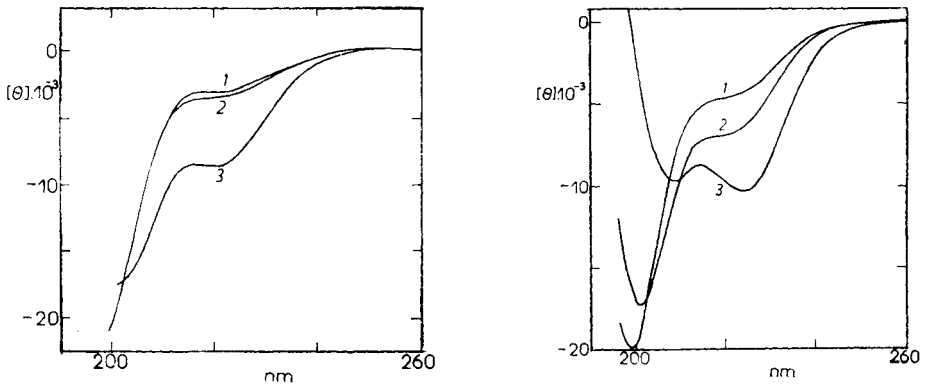

FIG. 1

Circular dichroic spectra of $(Lys–Ile–Ala)_n$ at different NaCl concentrations (*a*) and pH (*b*). [NaCl], mol l^{-1}: *1* 0·02, *2* 0·15, *3* 2·0. pH (0·02M-NaCl): *1* 9·1, *2* 9·4, *3* 10·0

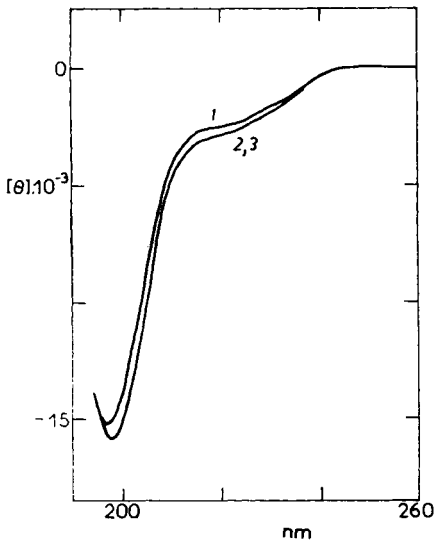

FIG. 2

Circular dichroic spectra of $(Tle-Lys-Ala)_n$ in 0·02M-NaCl at different pH: *1* 7·4, *2* 8·6, *3* 9·5

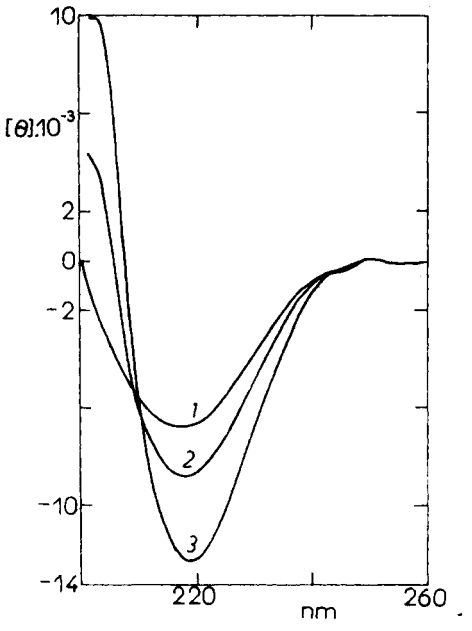

FIG. 3

Circular dichroic spectra of $(Lys-Ile-Gly)_n$ at different NaCl concentration (mol l^{-1}): *1* 0·15, *2* 1·0, *3* 2·0

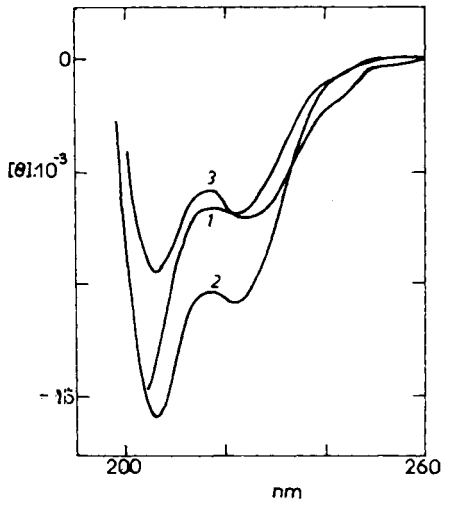

FIG. 4

Circular dichoric spectra of $(Lys-Nle-Ala)_n$ at different NaCl concentration (mol l^{-1}): *1* 0 (H_2O), *2* 0·15, *3* 2·0

Polymers without Branching in X Residues

The norleucine residue in $(Lys–Nle–Ala)_n$ is characterized by a five-carbon-atoms-long unbranched aliphatic side chain. The conformational variability of this polymer (Table II, Fig. 4) is practically identical with that of $(Lys–Nva–Ala)_n$ (ref. 8). In a low NaCl concentration at neutral pH about 30% of the chain assumes α-helical conformation the rest being unordered. As compared with $(Lys–Ala–Ala)_n$ the polymers containing norvaline or norleucine display a slightly stronger tendency for α-helix

TABLE II

Results of the component analysis of CD spectra of polypeptides as a function of pH and salt concentration. For max. ellipticity, $[\theta]_{max}$, wavelength given (nm). The content of α helix, β conformation and random conformation in a unit volume of the polypeptide solution; relative amount of respective conformation in percent not including residual spectrum (its presence or absence is indicated)

NaCl mol l^{-1}	$[\theta]_{220}$ 10^3 deg cm^2 $dmol^{-1}$	$[\theta]_{max}$	Conformation (content, %)			Residual spectrum
			α	β	ran.	
			$(Lys–Ile–Ala)_n$			
0·02	−3·23	−21·03 (199)[a]	0·10 (10)	0·17 (18)	0·69 (72)	−
0·15	−3·66	−21·85 (199)	0·12 (12)	0·15 (15)	0·73 (73)	−
2·0	−8·70	−17·52 (201)	0·29 (29)	−	0·71 (71)	+
9·0[a]	−4·64	−19·44 (197)	0·14 (14)	0·17 (17)	0·69 (69)	−
9·4[a]	−6·96	−17·33 (201)	0·22 (25)	−	0·66 (75)	−
10·0[a]	−9·81	−10·32 (224)	0·30 (69)	−	0·13 (31)	+
			$(Lys–Nle–Ala)_n$			
0·0(H_2O)	−7·85	−17·85 (201)	0·26 (28)	−	0·65 (72)	−
0·15	−10·73	−15·91 (206)	0·34 (34)	0·09 (9)	0·57 (57)	−
2·0	−6·63	−9·45 (206)	0·23 (45)	−	0·27 (55)	+[b]
			$(Lys–Ile–Gly)_n$			
0·02	−6·25	−6·54 (216)	0·11 (20)	0·22 (39)	0·23 (41)	−
0·15	−6·57	−6·78 (216)	0·11 (21)	0·22 (44)	0·18 (35)	−
0·20	−6·35	−6·72 (216)	0·09 (15)	0·27 (48)	0·21 (37)	
1·0	−8·67	−8·87 (218)	0·11 (28)	0·29 (72)	−	+
2·0	−12·20	−12·30 (219)	0·03 (5)	0·65 (95)	−	+

[a] Values of pH. [b] Precipitation.

formation[8]. Under conditions of shielding or neutralization of the charged lysyl ε-amino group some tendency of the $(Lys–Nle–Ala)_n$ polymer to aggregation was observed resulting in a higher residual spectrum in the component analysis.

CD spectrum changes of the polymer $(Lys–Nle–Gly)_n$ are also very similar to those of the previously studied $(Lys–Nva–Gly)_n$ and are slightly more pronounced than in the case of $(Lys–Ala–Gly)_n$[7]. In neither case any ordered conformation could be detected in high salt concentration or at high pH. With norleucine and norvaline containing polymers an increase of salt concentration up to 2M-NaCl results in a slight increase of the negative ellipticity at 220 nm and 195 nm.

General Considerations

We can conclude that within the series $(Lys–X–Ala)_n$ the tendency to form an α helix increases slightly with increasing side chain length assuming that there is no branching on C^β. An exceptional ability to form organized structures was observed with $(Lys–Leu–Ala)_n$. In this case an association of α helices due probably to hydrophobic interactions takes place in high salt concentrations[18]. When C^β branched X residues are involved, a limited tendency for α helix formation was observed only with the isoleucine containing polymer. CD spectra of valine and tert-leucine containing polymers are typical for random coil conformation and show only small or negligible dependence on salt concentration and pH conditions. These experimental findings agree with theoretical calculation[2] showing that the conformational freedom is about the same in all residues with a side chain longer than that of alanine, provided there is no C^β branching.

For the series $(Lys–X–Gly)_n$ similar conclusions can be made. However, α helix formation is not favored by the glycine residue. Therefore unordered conformation prevails throughout the whole series showing only small changes of the CD spectra with ionic strength and pH. A notable exception are the polymers containing residues with bulky side chains (isoleucine and leucine) that affect the conformation in a specific way. β Structure formation in the case of the isoleucine containing polymer may be due to specific combination of isoleucine and glycine residues. On the other hand leucine containing polymers appear to prefer the formation of a multichain structure of the collagen or polyglycine II type[18], the necessary conditions for which are created by the regular repeat of the glycine residues at every third position.

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