BASIC POLYPEPTIDES AS HISTONE MODELS: SYNTHESIS AND CONFORMATION OF (α,ω-DIAMINOACYL-ALANYL-GLYCYL) SEQUENTIAL POLYMERS AND THEIR COMPLEXES WITH DNA

K.Bláha[a], V.K.Burichenko[b], Š.Štokrová[c], J.Mikeš[c] and J.Šponar[a]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*
[b] *Institute of Chemistry, Academy of Sciences of the Tadjik SSR, Dushanbe, USSR and*
[c] *Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague 6*

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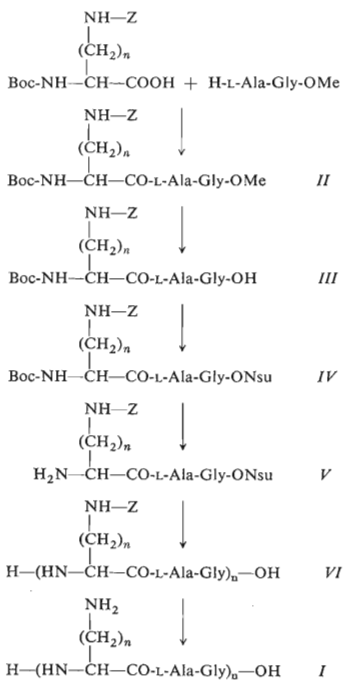
Polymers of the $(X\text{-Ala-Gly})_n$ type (X is equal to L-ornithine, L-2,4-diaminobutanoic acid or L-2,3-diaminopropanoic acid) were prepared *via* the polymerisation of 1-hydroxysuccinimidyl esters of the corresponding tripeptides. These polymers and the analogous L-lysine-containing polypeptide (molecular weight 6000—12000) were subjected to measurements of pK values of the ω-amino groups; the CD spectra were also examined in dependence on the pH value and ionic strength. In aqueous solutions, all these polymers display random conformation; the effect of shorter side chains of the basic amino acid is different in the neutral and alkaline pH region. The CD spectra of DNA complexes are characteristic of complexes of random lysine-containing polymers. Shortening of the side chain in a basic amino acid results in differences which could be connected with a closer distance between the polypeptide backbone and the polynucleotide moiety.

Sequential polytripeptides containing one residue of a basic amino acid, one residue of an amino acid with a hydrophobic side chain, and one residue of an amino acid not supporting formation of α-helical structures, proved to be suitable models for investigations on interactions of histones with DNA (ref.[1]) and interesting objects for conformational analysis of peptide chains in solutions[2]. Thus, attention was paid to the synthesis and properties of some sequential polymers containing the amino acids lysine, alanine, and proline[3]. Furthermore, a series of sequential polytripeptides $(\text{Lys-Y-Gly})_n$ was systematically examined[4]. In this series, the character of the side chain of the amino acid Y was modified and the effect of these modifications on conformation of the polypeptide and on interaction with DNA was studied[4]. Both hydrophobic and steric factors were observed to assert themselves, particularly with respect to the conformation of the polypeptide chain alone. Attention has been now focussed on the influence of the side chain of a basic amino acid, especially on the effect of its length (*i.e.*, the number of methylene groups separating the ω-ami-

no group from the backbone). The cation group, important for the electrostatic interaction with phosphate groups of DNA, has a various distance from the polypeptide backbone in the present series of compounds. Consequently, also the distance between the polypeptide chain and DNA in polypeptide–DNA complexes can be different and mutual interactions of both polymers can be modified.

In the present sequential polytripeptides $(X\text{-Ala-Gly})_n$, the basic diamino acid X was equal to L-lysine (Lys), L-ornithine (Orn), L-2,4-diaminobutanoic acid (A_2bu), and L-2,3-diaminopropionic acid (A_2pr).* The synthetic approach was similar to the



SCHEME 1

In formulae *I*—*VI*: *a*) $n = 4$ (L-Lys); *b*) $n = 3$ (L-Orn); *c*) $n = 2$ (L-A_2bu); *d*) $n = 1$ (L-A_2pr).

* Symbols and nomenclature according to published suggestions[5,6].

sequence of reactions applied to the preparation of $(Lys\text{-}Y\text{-}Gly)_n$ polypeptides[4] and is illustrated in Scheme 1. The thus-obtained polypeptides were purified in the final stage by dialysis and characterised by determination of the molecular weight and by the amino acid analysis. The polymer containing 2,4-diaminobutanoic acid did not exhibit any racemisation after hydrolysis with hydrochloric acid in determination of the optical rotation of the resulting mixture of amino acids.

EXPERIMENTAL

Melting points (uncorrected) were taken on a heated microscope stage (Kofler block). Samples for elemental analysis were dried at room temperature and 1 Torr over phosphorus pentoxide. Homogeneity of low-molecular compounds was checked by thin-layer chromatography on silica gel plates (Kieselgel G, Merck) in the solvent systems 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) and 2-butanol–90% aqueous acid–water (75 : 13·5 : 11·5). Spots were detected by ninhydrin and chlorination. Samples for amino acid analysis were hydrolysed in 6M-HCl at 105°C for 20 h in ampoules sealed at 1 Torr and analysed on a automatic two-column analyser (Developmental Workshops, Czechoslovak Academy of Sciences, Prague). Solutions were taken down on a rotatory evaporator under diminished pressure at bath temperatures below 40°C. Organic solutions were dried over anhydrous sodium sulfate. The $[\alpha]_D$ values were measured at room temperature on a Perkin Elmer 141 polarimeter in dimethylformamide (concentration about 0·5 g of the substance per 100 ml of the solvent), unless stated otherwise.

N^{α}-tert-Butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-2,4-diaminobutanoic Acid Dicyclohexylammonium Salt

To a suspension of N^{α}-benzyloxycarbonyl-L-2,4-diaminobutanoic acid (8·6 g) in water (40 ml) and dioxane (40 ml), tert-butyloxycarbonyl azide (6 ml) and 4M-NaOH were added, the pH value being maintained at 11·2 (pH-stat). After 24 h, the mixture was washed with ether, acidified with 20% aqueous citric acid, and extracted with ethyl acetate. The extract was washed 5 times with water, dried, and evaporated. The residue was dissolved in ethyl acetate and to the solution, dicyclohexylamine (6·4 ml) was added, followed by ether and light petroleum. The precipitate was recrystallised from ethyl acetate and ether. Yield, 13·86 g (75·5%), m.p. 102—104°C, $[\alpha]_D \pm 0°$ (dimethylformamide, methanol). For $C_{29}H_{47}N_3O_6$ (533·7) calculated: 65·26% C, 8·87% H, 7·87% N; found: 65·94% C, 9·02% H, 8·16% N.

N^{α}-tert-Butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-2,3-diaminopropanoic Acid Dicyclohexylammonium Salt*

The salt was prepared analogously to the preceding paragraph. M.p. 179—181°C, $[\alpha]_D \pm 0°$ (methanol). For $C_{28}H_{45}N_3O_6$ (519·7) calculated: 64·71% C, 8·73% H, 8·09% N; found: 65·05% C, 8·81% H, 8·38% N.

* Prepared in this Laboratory by the late Dr K. Poduška.

tert-Butyloxycarbonyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-2,3-diaminopropanoyl-L-alanyl-glycine Methyl Ester (*IId*)

N$^{\alpha}$-tert-Butyloxycarbonyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-2,3-diaminopropanoic acid dicyclohexylammonium salt (1 g) and L-alanyl-glycine methyl ester hydrobromide (0·5 g) were dissolved in chloroform (50 ml) and 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (0·6 g) was added. The mixture was stirred at 20°C overnight, evaporated, the residue triturated with ethyl acetate, the insoluble solid filtered off, the filtrate washed with 20% aqueous citric acid, 0·5M-$NaHCO_3$, and water, dried, and evaporated. The residue was dissolved in ethyl acetate and the solution precipitated with light petroleum. Yield, 0·61 g. The protected tripeptides *IIc* and *IIb* were prepared analogously (Table I).

tert-Butyloxycarbonyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-2,3-diaminopropanoyl-L-alanyl-glycine (*IIId*)

To a stirred solution of the tripeptide *IId* (1 g) in acetone (20 ml) and methanol (10 ml), 1M-NaOH (1 ml) was added and the stirring continued at 20°C for 90 min. The mixture was diluted with water, the organic solvents evaporated, the residual aqueous solution filtered with active charcoal, the filtrate acidified with 20% aqueous citric acid, and extracted with ethyl acetate. The extract was dried, evaporated, and the residue crystallised from ethyl acetate. Yield, 0·85 g. The derivatives *IIIc* and *IIIb* were prepared analogously (Table I).

tert-Butyloxycarbonyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-2,3-diaminopropanoyl-L-alanyl-glycine 1-Hydroxysuccinimidyl Ester (*IVd*)

A solution of the tripeptide *IIId* (0·5 g) in dimethylformamide (10 ml) was treated with 1-hydroxysuccinimide (0·15 g) and then at −10°C with N,N′-dicyclohexylcarbodiimide (0·23 g). The mixture was stirred at −10°C for 1 h, kept at 0°C overnight, and evaporated at 1 Torr. The residue was triturated with ethyl acetate, the N,N′-dicyclohexylurea filtered off, the filtrate washed with 0·5M-$NaHCO_3$, dried, and evaporated, The residue was dissolved in ethyl acetate and the solution precipitated with ether and light petroleum. Yield, 0·47 g of the ester *IVd*. The activated tripeptide esters *IVc* and *IVb* were prepared analogously (Table I).

tert-Butyloxycarbonyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-2,4-diaminobutanoyl-L-alanyl-glycine 2,4,5-Trichlorophenyl Ester

A solution of compound *IIIc* (1·4 g) in dimethylformamide (30 ml) was treated with 2,4,5-trichlorophenol (0·63 g) and then at −10°C with N,N′-dicyclohexylcarbodiimide (0·65 g). The mixture was stirred at −10°C for 1 h, kept at 0°C overnight, and evaporated at 1 Torr. The residue was triturated with ethyl acetate, the N,N′-dicyclohexylurea filtered off, the filtrate washed with 0·5M-$NaHCO_3$ and water, dried, and evaporated. After recrystallisation from ethyl acetate the yield was 1·43 g (74%), m.p. 165°C, $[\alpha]_D$ −7·3° (dimethylformamide). For $C_{28}H_{33}Cl_3N_4O_8$ (659·9) calculated: 50·96% C, 5·04% H, 8·48% N; found: 51·51% C, 5·27% H, 8·73% N.

Poly(L-2,3-diaminopropanoyl-L-alanyl-glycine) (*Id*)

A mixture of the activated ester *IVd* (0·28 g) and trifluoroacetic acid (2 ml) was kept at 20°C for 15 min and diluted with ether. The solid trifluoroacetate *Vd* was filtered off, washed with ether, dried in a desiccator, and dissolved in dimethyl sulfoxide (1·15 ml). Triethylamine (0·090 ml) was then added with stirring to the solution which solidified within 5 min. The mixture was kept at 20°C for 7 days, triturated with water, the N$^{\varepsilon}$-protected polymer *VId* collected with suction,

and washed with water, ethanol, and ether; yield, 0·145 g. A mixture of the polymer *VId* (0·120 g) and 30% hydrogen bromide in acetic acid (1 ml) was kept at room temperature for 30 min and diluted with ether. The solid was collected with suction, dried in a desiccator (yield, 0·114 g), and dialysed[4]. Lyophilisation yielded 45 mg of the $(A_2pr\text{-}Ala\text{-}Gly)_n$. n HBr copolymer. Amino acid analysis: A_2pr 1, Ala 1, Gly 1. The $(A_2bu\text{-}Ala\text{-}Gly)_n$. n HBr (*Ic*) and $(Orn\text{-}Ala\text{-}Gly)_n$. n HBr (*Ib*) polymers were prepared, purified, and characterised analogously. For molecular weights see below.

Sedimentation Analysis

Sedimentation measurements were carried out in an analytical centrifuge Spinco Model E. The sedimentation coefficients of polypeptides were determined in 0·15M-NaCl + 0·013M sodium phosphate (pH 6·8) in a synthetic boundary cell with an optical path of 12 mm in a titanium rotor An-H at 67770 rev/min, and recorded by schlieren optics. The molecular weights M_s were inferred from a calibration graph[7]. The molecular weight of $(Orn\text{-}Ala\text{-}Gly)_n$. n HBr (*Ib*) was also measured by a sedimentation equilibrium method according to Chervenka[8], with interference optics. The partial specific volume $\bar{v} = 0{\cdot}722$ ml/g was used in the molecular weight calculation.

TABLE I
Protected Tripeptides *II* to *IV*

Compound	M.p., °C[a] (Yield, %)	$[\alpha]_D$	Formula (M.w.)	Calculated/Found		
				% C	% H	% N
IIb	99—100	−8·0	$C_{24}H_{36}N_4O_8$	56·68	7·13	11·01
	(56)		(508·6)	56·82	7·19	11·24
IIc	139—140	−6·7	$C_{23}H_{34}N_4O_8$	55·86	6·92	11·32
	(82)		(494·5)	55·84	7·08	11·49
IId	144—145	−5·1	$C_{22}H_{32}N_4O_8$	54·99	6·71	11·66
	(67)		(480·5)	54·94	6·66	11·55
IIIb	151—152	−6·5	$C_{23}H_{34}N_4O_8$	55·85	6·92	11·32
	(87)		(494·6)	55·97	6·92	11·58
IIIc	90—91	−4·8	$C_{22}H_{32}N_4O_8$	54·99	6·71	11·65
	(88)		(480·5)	55·02	6·92	11·65
IIId	177—179	−8·4	$C_{21}H_{30}N_4O_8$	54·07	6·48	12·01
	(87)		(466·5)	54·36	6·58	12·00
IVb	156—158	−9·7	$C_{27}H_{37}N_5O_{10}$	54·81	6·30	11·83
	(66)		(591·6)	55·08	6·35	11·86
IVc	108—110	−7·6	$C_{26}H_{35}N_5O_{10}$	54·07	6·11	12·12
	(64)		(577·6)	54·06	6·43	11·90
IVd	110—114	—	$C_{25}H_{33}N_5O_{10}$	53·28	5·90	12·43
	(81)		(563·5)	54·07	6·18	12·10

[a] From ethyl acetate with the addition of ether and light petroleum.

Potentiometric Titrations

Potentiometric curves were measured on a Radiometer PHM 64/TTT 60/REC 61/ABU 12 automatic apparatus with a GK 2301 C combined electrode. The copolymers were dissolved in freshly distilled CO_2-free water and then the corresponding amount of sodium chloride was added. The concentration of copolymer solutions was 5 mg per 1 ml of water. The solutions were titrated with carbonate-free 0·15M-NaOH at 25 ± 0·1°C under nitrogen. The dilution during the titration was insignificant under the applied concentration conditions. The dissociation degree α in the equilibrium process $NH_3^+ \rightleftarrows NH_2 + H^+$ was calculated according to the formula $\alpha = (c_{Na^+} - c_{OH^-})/c^0_{NH_2}$, wherein c_{Na^+} is the stoechiometric concentration of Na^+ ions originating from the sodium hydroxide added, c_{OH^-} is the concentration of hydroxylic ions calculated from pH values under the assumption of a unit activity coefficient, and $c^0_{NH_2}$ is the concentration of all amino groups susceptible to the titration. The correction for the free hydroxylic ions asserts itself only with $(Orn\text{-}Ala\text{-}Gly)_n$ and $(Lys\text{-}Ala\text{-}Gly)_n$ polypeptides at pH > 9. The apparent dissociation constants of α,ω-diamino acids were measured in the concentration of 0·015 mol l^{-1} (2,3-diaminopropanoic acid and 2,4-diaminobutanoic acid) and 0·015 and 0·08 mol l^{-1} (ornithine and lysine). With ornithine and lysine, the dissociation constants were not concentration-dependent.

Measurement of Circular Dichroism

The circular dichroism spectra of polypeptides were recorded on a Cary 61 apparatus in the range of 260—195 nm in cells with optical paths 0·01 and 0·05 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 about 0·05—0·1% (w/v). Solutions with various ionic strengths were prepared by adding 4M-NaCl to a solution of the appropriate polypeptide in water. The pH was adjusted by adding 0·5M-NaOH to the original solution (in 0·02M-NaF). The pH was measured with an accuracy of 0·1 unit. Solutions in 90% 2-propanol were prepared by adding the appropriate amount of 2-propanol to the aqueous solution of the polypeptide. The circular dichroism is expressed in molar ellipticities $[\Theta]$ (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 molar ellipticity calculations were taken from weight amounts. The samples were dried under diminished pressure and kept in desiccators.

The CD spectra of complexes with DNA in the 300—200 nm region were measured on a Roussel Jouan Dichrographe CD 185 apparatus in cells with optical path 0·5 cm at 22—24°C. Circular dichroism was expressed in specific ellipticity values $[\Psi]$ (deg cm^2 dg^{-1}) referred to the total weight concentration of DNA in the system.

Preparation of Complexes

DNA was prepared from calf thymus[1,3]. A solution of the polypeptide hydrobromide and DNA in molar ratio of the basic residue to the nucleotide (X/DNA) equal to 0·5 in 2M-NaCl and buffered with 0·013M sodium phosphate (pH 6·8) was dialysed against a linear concentration gradient of NaCl solution according to Carroll[9]. To obtain a complex in 0·15M-NaCl, the sample was dialysed further against 0·15M-NaCl + 0·013M sodium phosphate. The low ionic strength was created by 0·01M-Tris buffer solution (pH 7·0); complexes were dialysed against several changes of this buffer.

RESULTS AND DISCUSSION

The molecular weights of $(Lys\text{-}Ala\text{-}Gly)_n$, $(A_2bu\text{-}Ala\text{-}Gly)_n$, and $(A_2pr\text{-}Ala\text{-}Gly)_n$ were determined from median $s_{20,w}$ values. The $\overline{M}_s$ values of 6000, 12000, and 10000, resp., were obtained. The average molecular weight of $(Orn\text{-}Ala\text{-}Gly)_n$ was calculated from a sedimentation equilibrium measurement $\overline{M}_e = 9000$.

Potentiometric curves were measured both in aqueous solutions of copolymers and in solutions in 2M-NaCl. Potentiometric data were expressed as a dependence of the apparent dissociation constant K (expressed as $pK = pH + \log[(1 - \alpha)\alpha]$) on the dissociation degree α (ref.[10]). In aqueous solutions, the pK values moderately increase with the increasing dissociation degree as expected. In 2M-NaCl solutions, the charged groups are shielded by ionic atmospheres; consequently, the pK values are roughly independent of the dissociation at medium dissociation degrees. In NaCl-containing solutions, the pK values do not increase due to the shielding of charges. In Table II, the pK value is shown and the course of curves is characterised by tangent of the pK *vs* α plot at the half-dissociation point. The curves do not exhibit any indication of a conformational change. The particular curves differ by mutual shifts on the pK coordinate; the basicity of the ω-amino group increases with increasing length of the side chain, analogously to the free α,ω-diamino acids[11]. Apparent dissociation constants of amino groups in these acids were determined from potentiometric curves of substances in the starting hydrochloride form. The pK values moderately decreased with the increasing dissociation degree. In Table II, pK values are shown for the half-dissociation degree of the corresponding amino group (these values are simultaneously medium values).

TABLE II

The pK ($\alpha = 0{\cdot}5$) and Tangent k ($\alpha = 0{\cdot}5$) Values of the pK *vs* α Dependence of the $(X\text{-}Ala\text{-}Gly)_n$ Polymer Solutions in Water and in 2M-NaCl and the pK_1 and pK_2 Values of α,ω-Diamino Acids X in Water

X	pK		k^a		pK_1	pK_2
	H_2O	2M-NaCl	H_2O	2M-NaCl		
Lys	9·92	10·14	0·34	0·003	9·19	10·80
Orn	9·58	9·90	0·38	0·005	8·79	10·61
A_2bu	8·85	9·39	0·58	0·04	8·14	10·36
A_2pr	7·49	8·02	0·52	0·04	6·65	9·55

[a] $k = dpK/d\alpha$.

Chiroptical Properties of Polymers

The CD spectra of sequential polymers $(X\text{-Ala-Gly})_n$ were measured in dependence on ionic strength and pH in aqueous medium and in 90% 2-propanol, analogously to earlier papers[3,4,7]. The spectra of homologues were evaluated with respect to the decreasing length of the chain bearing the basic amino acid residue. Since the characteristic changes in CD spectra of the particular polymers were observed to occur

TABLE III

The $[\Theta] \cdot 10^{-3}$ Molar Ellipticity Values of Sequential Polypeptides at Some Wavelengths as a Function of Ionic Strength I and pH

I^a	λ 217 nm	λ 230 nm	λ_{max}, nm	pH	λ 217 nm	λ 230 nm	λ_{max}, nm
			$(Lys\text{-}Ala\text{-}Gly)_n$				
0·02	−0·1	−0·5	−15·2 (197)	6·9	−0·1	−0·5	−15·2 (197)
0·15	−0·3	−0·6	−16·2 (197)	9·4	−0·2	−0·7	−15·3 (198)
2·00	−0·6	−0·7	—	10·1	−0·6	−1·0	−13·8 (198)
2·00[b]	−0·9	−0·9	—	11·0	−0·5	−0·8	—
			$(Orn\text{-}Ala\text{-}Gly)_n$				
0·00	0·1	−0·5	−19·5 (195)	6·8	−0·3	−0·7	−18·2 (195)
0·15	−0·3	−0·6	−20·0 (195)	8·9	−0·4	−0·7	—
2·00	−0·7	−0·6	—	9·3	−0·5	−0·6	−14·0 (195)
2·00[b]	−0·8	−0·8	—	10·3	−0·3	−0·4	−12·4 (195)
				11·0	−0·2	−0·6	—
			$(A_2bu\text{-}Ala\text{-}Gly)_n$				
0·00	−0·6	−0·7	−24·0 (196)	6·7	−0·5	−0·7	−21·0 (195)
0·15	−0·9	−0·8	−18·5 (196)	8·3	−0·4	−0·6	−19·7 (195)
2·00	−1·2	−0·8	—	10·1	0·0	−0·5	−16·3 (195)
2·00[b]	−0·3	−0·5	—	11·0	0·3	−0·4	—
			$(A_2pr\text{-}Ala\text{-}Gly)_n$				
0·00	−1·8	−0·9	−13·0 (195)	6·4	−1·8	−0·8	− 8·8 (195)
0·15	−2·5	−1·3	−10·5 (195)	8·4	0·2	−0·5	−12·4 (195)
2·00	−2·5	−1·2	—	10·1	0·6	−0·3	−13·2 (195)
2·00[b]	0·0	−0·5	—	11·0	0·5	−0·4	—

[a] Molarity of NaCl; [b] 2M-NaCl, pH 11·0.

in regions of about 217, 230, and 195 nm, the molar ellipticity values for these wavelengths are shown in Table III.

The CD spectra of the $(Lys\text{-}Ala\text{-}Gly)_n$ (*Ia*) polymer with the longest side chain of the basic amino acid residue were described in one of our earlier papers[4] and also by Cernosek and coworkers[12]. In an aqueous solution of neutral pH value, the spectrum exhibits a strong negative maximum at about 198 nm and a weak negative maximum at 230 nm, separated from each other by a negative minimum between 215 and 220 nm indicating the presence of a positive band in this region. With the increasing ionic strength and pH value, the maximum at 230 nm is hypsochromically shifted and its intensity moderately increases. Under the conditions stated, the spectra of the $(Lys\text{-}Ala\text{-}Gly)_n$ polymer are characteristic of the random conformation. The CD spectra of the next homologue $(Orn\text{-}Ala\text{-}Gly)_n$ (*Ib*) differ only a little from those of the preceding polypeptide. Only under extreme conditions (alkaline pH region and a high ionic strength), some changes can be observed, particularly in the 217–220 nm region (Fig. 1*a*,*b*). The $(A_2bu\text{-}Ala\text{-}Gly)_n$ (*Ic*) polypeptide differs from the two former homologues. With the increasing ionic strength, the intensity

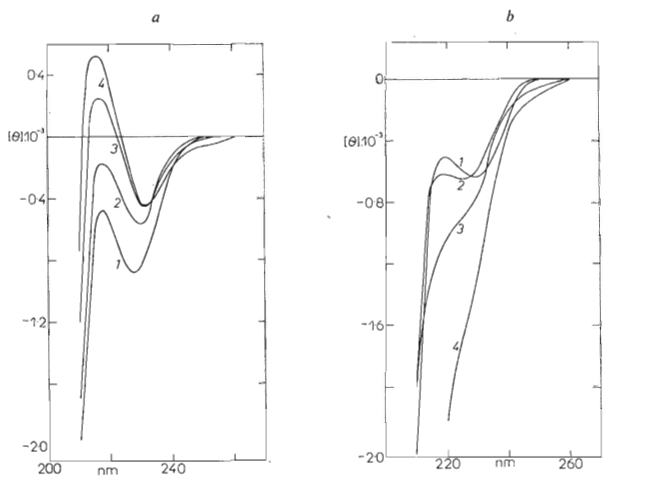


FIG. 1

Circular Dichroic Spectra of Sequential Polypeptides

a 0·02M-NaF, pH 11; *b* 2M-NaCl, neutral pH; *1* $(L\text{-}Lys\text{-}L\text{-}Ala\text{-}Gly)_n$; *2* $(L\text{-}Orn\text{-}L\text{-}Ala\text{-}Gly)_n$; *3* $(L\text{-}A_2bu\text{-}L\text{-}Ala\text{-}Gly)_n$; *4* $(L\text{-}A_2pr\text{-}L\text{-}Ala\text{-}Gly)_n$.

of the band centered at 196 nm is considerably lowered and in 2M-NaCl, the negative minimum at about 217 nm completely disappears (Fig. 1*b*). The most significant difference manifests itself in the pH-dependence in the region of about 220 nm. With the polypeptides *Ia* and *Ib*, the negative minimum (indicative of a positive band at about 220 nm) is shifted to more negative values with the increasing pH values; the flattening is greater with $(Lys\text{-}Ala\text{-}Gly)_n$ (*Ia*) than with $(Orn\text{-}Ala\text{-}Gly)_n$ (*Ib*). With the polypeptide *Ic*, the pH-dependence of the ellipticity at 217 nm is quite opposite. Thus, with increasing pH values, the negative minimum becomes deeper, attains more positive ellipticity values, and is shifted to lower wavelengths. This observation is indicative of the increasing intensity of the positive band in the region below 220 nm. A positive $n - \pi^*$ band is obviously involved, typical of the random conformation of homopolymers with an ionised side chain as observed in the case of polylysine and glutamic acid. The position of the negative minimum at 230 nm does not depend on the pH value but its intensity moderately decreases with the increasing pH value. The $(A_2pr\text{-}Ala\text{-}Gly)_n$ (*Id*) polypeptide differs from all preceding polymers in dependence of its behaviour on ionic strength. The differences between CD spectra in water and in 2M-NaCl are very small and both the negative minimum and negative maximum in the 215–230 nm are absent. Only

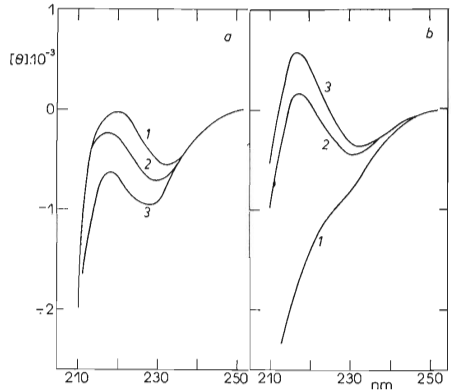


FIG. 2

Circular Dichroic Spectra of Sequential Polypeptides as a Function of pH in 0·02M-NaF
a $(L\text{-}Lys\text{-}L\text{-}Ala\text{-}Gly)_n$, pH: *1* 6·8; *2* 9·4; *3* 10·0; *b* $(L\text{-}A_2pr\text{-}L\text{-}Ala\text{-}Gly)_n$, pH *1* 6·4; *2* 8·4; *3* 10·0.

an insignificant inflex may be here observed. The negative band at about 196 nm is lower than with the other polymers. However, an increase of pH from 6·4 to 10·0 is accompanied by a significant change in the region of longer wavelengths. The pH-dependence of the CD spectrum of $(A_2pr\text{-}Ala\text{-}Gly)_n$ in this region is compared with that of $(Lys\text{-}Ala\text{-}Gly)_n$ on Fig. 2*a*,*b*. The behaviour of the other two polymers *Ib* and *Ic* in this region under analogous conditions may be regarded as a logical interstage between the two extrema *Ia* and *Id*.

The dependence of CD spectra on the ionic strength in the 215–230 nm region has been observed with several lysine-containing sequential copolymers[3,4] that exhibit random conformation in aqueous solutions. The changes in CD spectra of these substances were always parallel for the increasing pH value and ionic strength and qualitatively comparable with dependence of CD spectra of the $(Lys\text{-}Ala\text{-}Gly)_n$ (*Ia*) polypeptide on pH and ionic strength. The opposite effect of the increasing pH value and ionic strength on the intensity and/or the sign of bands in the 215–230 nm region may be for the first time observed with copolymers $(A_2bu\text{-}Ala\text{-}Gly)_n$ (*Ic*) and $(A_2pr\text{-}Ala\text{-}Gly)_n$ (*Id*). When the ellipticity at 217 nm is plotted versus the length of the side chain of the basic amino acid residue in the polypeptide under various conditions, curves are obtained which indicate that the effect of pH and ionic strength on the CD spectrum at 217 nm is opposite in the present series of polymers and that the pH-effect is superior to the effect of the ionic strength (Fig. 3).

The CD spectra of the present copolymers in 90% 2-propanol do not afford reproducible results, particularly those of copolymers with a shorter side chain. Except for $(Lys\text{-}Ala\text{-}Gly)_n$ (*Ia*), the solutions of polymers are turbid and the solubility decreases with the increasing number of methylene groups in the side chain of the basic amino acid residue.

As it may be inferred from titration curves, no cooperative transition occurs in the examined region of pH values and ionic strengths. All the obtained spectra with both

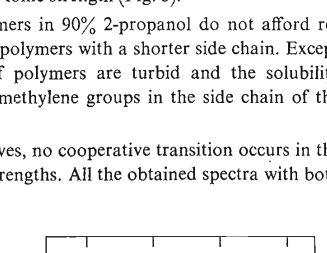


FIG. 3

The $[\Theta] \cdot 10^{-3}$ Molar Ellipticity Values (λ 217 nm) as a Function of n (Number of Methylene Groups in the Side Chain of the First Amino Acid Residue in the Monomer Unit)

1 H_2O; *2* 2M-NaCl; *3* H_2O, pH 11; *4* 2M-NaCl, pH 11.

the positive and negative $n - \pi^*$ band thus represent the random structure of the polypeptide backbone. The spectra of the particular polymers in the examined series differ mainly in the 215–230 nm region. The spectra are distinctly influenced by the presence of a conformation with a characteristic positive band in this region of wavelengths. The importance of this band has been object of controversy[13–15]. A contribution to this discussion has been recently presented by Mattice and Harrison[16] and their measurements of CD spectra of the alanine and lysine oligomers. On the basis of these measurements, the CD spectrum of poly-L-alanine and poly-L-lysine in random conformation was predicted in accord with the obtained spectrum of poly-L-lysine with the dissociated ε-amino group, *i.e.*, a positive $n - \pi^*$ band at about 217 nm. Diminuation of the positive maximum during neutralisation of the ε-amino group of poly-L-lysine is ascribed by the authors[16] to hydrophobic interactions between the long side chains of lysine residues which may result in a limited distribution of dihedral angles of the polypeptide chain. On the other hand, the electrostatic repulsion of side chains in the protonated form of poly-L-lysine interferes with these interactions and the spectrum of this protonated form thus resembles the spectrum of poly-L-alanine. Furthermore, the different stability of the α-helical conformation of polymeric diamino acids is attributed to the effect of the side-chain length on the occurrence of hydrophobic interactions[17,18]. In addition to electrostatic and hydrophobic interactions, the final conformation may be affected by interactions of the ω-amino group with the carbonyl group of the backbone. In this manner, Hatano and Yoneyama[19] explain the lesser ability of poly-L-diaminopropanoic acid in the deprotonated form to produce an α-helix when compared with the ability of poly-L-lysine or poly-L-ornithine.

With the $(X\text{-Ala-Gly})_n$ polymers, two different effects may be observed when the side chain is shortened. Thus, shortening of the deprotonated side chain (in alkaline pH region) results in a shift of the $[\Theta]_{217}$ value into the positive region (Fig. 3). This shift could be explained on the basis of ideas on dependence of hydrophobic interactions on the chain length[16] or by changes in conformation of the backbone due to hydrogen bonds with the ω-amino groups[19]. On the other hand, the presence of a protonated ω-amino group results in a marked shift of the $[\Theta]_{217}$ value to the negative region (Fig. 3), the character of the CD spectrum being changed to that typical of random conformation of the protein type with a shoulder in the 220–230 nm region (Fig. 1*b*, curves *3* and *4*). In the field of synthetic basic polypeptides, such a type of CD spectrum has been observed with the $(Lys\text{-}Val\text{-}Gly)_n$ polymer[4]. In the latter case, restriction of the conformational variability of the polypeptide back-bone by the bulky isopropyl group of the valine residue obviously plays an important role. An analogous effect could be ascribed to a nearer distance between the backbone and the positive charge of the ω-amino group.

Complexes of Polypeptides with DNA

The CD spectra of complexes of polypeptides with DNA in 0·15M-NaCl are shown on Fig. 4. In all cases, the spectra are of the same nonconservative type with strong negative bands in the 270 nm and 210 nm region, as characteristic of complexes of the mainly random lysine-containing polymers[3,4,7] or of histone H 1 complexes[20]. As inferred from Fig. 4, the intensity of negative bands in complexes of polymers of the $(X\text{-Ala-Gly})_n$ series increases with increasing length of the side chain of the basic amino acid, *i.e.*, in the order X = A_2pr, A_2bu, Orn, Lys. Since the nonconservative CD bands obviously reflect chiroptical properties of the aggregate superstructure[1], it may be inferred from the order of X that the ability of aggregated complexes to attain a certain degree of the ordered conformation depends on the length of the side chain on which the positive charge is placed.

Fig. 5 shows CD spectra of complexes dialysed to a low ionic strength (0·01M-Tris). While the spectra of complexes of the Lys- and Orn-containing polymers at wavelengths above 250 nm only very little differ from the spectrum of DNA (analogously to the majority of the other Lys-containing polymeric tripeptides[3,4]), the spectra of complexes of the A_2pr- and A_2bu-containing polymers are markedly different. The different behaviour of complexes is due to the different character of the interaction.

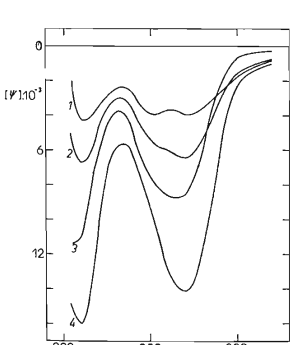


FIG. 4

Circular Dichroic Spectra of the $(X\text{-Ala-Gly})_n$–DNA Complexes in 0·15M-NaCl

X/DNA = 0·5; X: *1* A_2pr, *2* A_2bu, *3* Orn, *4* Lys.

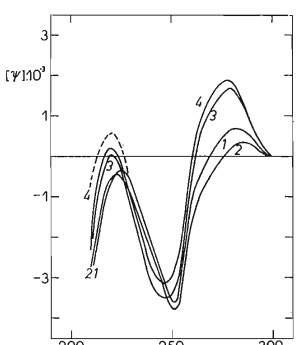


FIG. 5

Circular Dichroic Spectra of the $(X\text{-Ala-Gly})_n$–DNA Complexes in 0·01M-Tris

X/DNA = 0·5; X: *1* A_2pr, *2* A_2bu, *3* Orn, *4* Lys; ------ DNA.

A shorter side chain could favour a closer interaction between the polypeptide backbone and DNA which could result in the formation of further bonds (*e.g.*, hydrophobic) other than electrostatic ones that are affected by the change of ionic strength in a different way. Furthermore, the different conformation of the backbone of the A_2pr- and A_2bu-containing polypeptides could assert itself in the present case as mentioned above.

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