BASIC POLYPEPTIDES AS HISTONE MODELS: SYNTHESIS AND CONFORMATION OF SEQUENTIAL POLYMERS POLY(LYSYL-AMINO ACYL-GLYCYL) AND THEIR COMPLEXES WITH DNA

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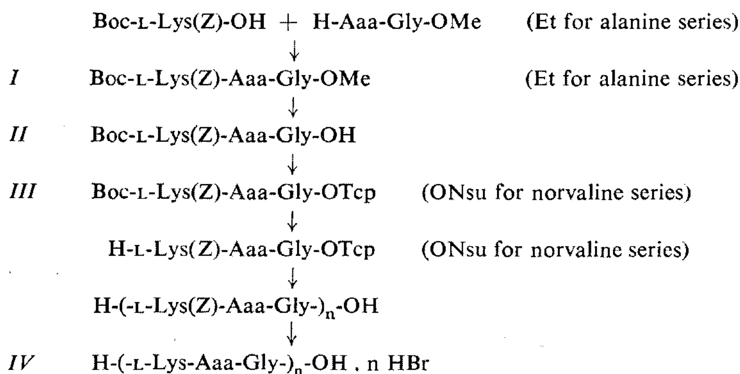
Polypeptides of molecular weight between 5000 and 10000 daltons were synthesized by polymerization of tripeptides Lys-X-Gly, where X represents amino acid residues Ala, Nva, Val and Leu. Circular dichroism and in some cases infra-red spectra were used to study the conformation of $(Lys\text{-}X\text{-}Gly)_n$ in aqueous solution as a function of pH and ionic strength, and also in 90% 2-propanol. With the exception of leucine derivative all the polymers in aqueous solution were in unordered conformation, the character of which depended to some degree on the nature of residue X. The polymer $(Lys\text{-}Leu\text{-}Gly)_n$ at neutral pH in high ionic strength, and also at alkaline pH, aggregates and takes on a conformation probably similar to that characteristic for polyglycine II or some collagen models. In 90% 2-propanol the polymers gave CD spectra typical for a mixture of unordered conformation and α-helix. CD spectra of complexes of polymers $(Lys\text{-}X\text{-}Gly)_n$ with DNA are characteristic for complexes of unordered lysine-containing polymers, with some differences in the case of $(Lys\text{-}Leu\text{-}Gly)_n$–DNA.

Previous studies have involved two series of synthetic lysine-containing polypeptides as models of natural histones[1–5]. Both the conformation of these polypeptides in solution as well as the character of their complexes with DNA were followed mainly by means of circular dichroism measurements. One series was formed by statistical copolymers containing lysine and alanine in various ratios[3], the second series involved sequential polypeptides containing alanine, lysine and proline[2]. Polypeptides in the first series assumed in aqueous solutions, at least partially, an α-helical structure and with DNA gave complexes which were markedly different from complexes with polypeptides of the second series. The latter polypeptides, because of a high proline content, were not able to assume an α-helical conformation and this fact influenced the character of their complexes with DNA (*cf.*[5]).

In the present work attention has been given to a similar series of sequential polypeptides, in which the helix-breaking proline residue was replaced by a glycine residue. As compared with proline the latter does not interfere so strongly with

an α-helical structure, but contributes to its destabilization. It was therefore of interest to study the conformation and formation of complexes of sequential polypeptides $(Lys\text{-}X\text{-}Gly)_n$, in which X represented amino acid residues with a hydrocarbon side-chain of various lengths and bulk. The conformation of one of these polypeptides, $(Lys\text{-}Ala\text{-}Gly)_n$, has been recently studied by Cernosek and coworkers[6].

The synthesis of sequential polypeptides was carried out by the standard procedure[2] shown in Scheme 1. For the preparation of the basic monomer units – protected tripeptide *I* – N^{α}-tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysine was condensed with the appropriate dipeptide ester (all previously reported on with the exception of the derivative of L-norvaline) using 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline or dicyclohexylcarbodiimide. Polycondensation involved the 2,4,5-trichlorophenyl esters *IIIa*, *IIIc*, *IIId*, in the case of the derivative of L-norvaline the 1-succinimidyl ester *IIIb*. The protected polymer was isolated without detailed characterization and deblocked with hydrogen bromide in acetic acid, isolated as a polyhydrobromide and purified from low molecular weight substances by dialysis of an aqueous solution. The lyophilizate was characterized by elemental and amino acid analyses. In substances *IVa*, *IVb* and *IVc* we compared optical rotations of mixtures of amino acids after hydrolysis with mixtures of the original amino acids. No racemization exceeding the error of measurement (±2%) was observed.



SCHEME 1*

Aaa indicates in series *a* L-Ala, *b* L-Nva, *c* L-Val, *d* L-Leu.

* Symbols according to published suggestions[7].

EXPERIMENTAL

Melting points were determined on a Kofler block and values are not corrected. Samples for elemental analysis were dried over phosphorus pentoxide for 12 h at room temperature and 1 Torr. The homogeneity of low-molecular-weight compounds was checked by thin-layer chromatography on silica gel plates (Kieselgel G, Merck) in systems 2-butanol–25% ammonia–water (85 : 7·5 : 7·5) and 2-butanol–90% formic acid–water (75 : 13·5 : 11·5). Detection was made by means of ninhydrin and chlorination. Samples for amino-acid analyses were hydrolysed for 20 h at 105°C in 6M-HCl (ampoules sealed at 1 Torr). The analyses were carried out on an automatic two-column analyser (type 6020, Development Workshops, Czechoslovak Academy of Sciences, Prague). A rotary evaporator was used to concentrate solutions (bath temperature 40°C). Solutions were dried with Na_2SO_4. The $[\alpha]_D^{25}$ values were estimated on a Perkin Elmer 141 Polarimeter in dimethylformamide, concentration about 0·5 g/100 ml.

2-Nitrobenzenesulphenyl-L-norvalyl-glycine Methyl Ester

To a solution of 2-nitrobenzenesulphenyl-L-norvaline dicyclohexylammonium salt (4·5 g, 10 mmol) in chloroform (50 ml) glycine methyl ester hydrochloride (1·25 g, 10 mmol) and at 0°C dicyclohexylcarbodiimide (2·3 g, 11 mmol) were added. The mixture was left standing overnight at 0°C, evaporated, the concentrate was suspended in ethyl acetate, the solid portion was filtered off, solution was shaken up with 0·5M-H_2SO_4, 0·5M-$NaHCO_3$ and water, dried and evaporated. The remnant was dissolved in ethyl acetate and induced to crystallize by adding light petroleum. The yield was 2·8 g (82%) of a substance with m.p. 91—92°C which did not change on subsequent crystallization; $[\alpha]_D$ −33·8°. For $C_{14}H_{19}N_3O_5S$ (341·4) calculated: 49·25% C, 5·60% H, 12·30% N; found: 49·53% C, 5·72% H, 12·22% N.

Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-leucyl-glycine Methyl Ester (*Id*)

The benzyloxycarbonyl-L-leucyl-glycine methyl ester[8,9] (3·0 g) was decarbobenzoxylated by 30% HBr in acetic acid, the precipitate was washed with ether, dried *in vacuo* over sodium hydroxide and dissolved in chloroform (100 ml). To this solution dicyclohexylammonium salt of N-tert-butyloxycarbonyl-N-benzyloxycarbonyl-L-lysine (5·15 g) and 2·1 g dicyclohexylcarbodiimide were added, the mixture was stirred for 1 h at −10°C, left overnight and evaporated. The remnant was covered with ethyl acetate, the solid portion filtered off, the solution washed with 20% citric acid, 0·5M-$NaHCO_3$ and water, dried and evaporated. To the remainder light petroleum was added and the insoluble portion was filtered off. The yield was 4·05 g, m.p. 91—93°C, after crystallization from ethyl acetate and light petroleum m.p. was 92—93°C, the yield was 3·55 g (69%); $[\alpha]_D$ −23·9°. For analysis see Table I. In an analogous manner we prepared also the alanine tripeptide *Ia*, starting from 2-nitrobenzenesulphenyl-L-alanyl-glycine ethyl ester[9].

Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-norvalyl-glycine Methyl Ester (*Ib*)

The 2-nitrobenzenesulphenyl-L-norvalyl-glycine methyl ester (1·4 g) was dissolved in 10 ml methanol, mixed with 4 ml of a methanolic solution of 3M-HCl, the mixture was left standing for 10 min and evaporated. The concentrate was mixed with ether, dried in desiccator (yield of the hydrochloride 1·1 g) and dissolved in 100 ml chloroform. To this solution dicyclohexylammonium salt of N^{α}-tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysine (2·8 g) and 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (1·3 g) were added. The mixture was stirred overnight at 20°C and evaporated, the remainder was suspended in ethyl acetate, the solution after filtra-

tion was washed with 20% citric acid, 0·5M-$NaHCO_3$, and water, dried and evaporated. The remainder was dissolved with heating in ethyl acetate, and after cooling, the gel which separated out was filtered off and ground up with ether and light petroleum. The yield was 2·0 g, m.p. 122—123°C; after recrystallization from a mixture of ethyl acetate, ether and light petroleum 1·8 g (68%) with the same m.p.; $[\alpha]_D$ −17·9°. For analysis see Table I. In an analogous manner we also prepared the valine tripeptide *Ic* starting from benzyloxycarbonyl-L-valyl-glycine methyl ester[10].

Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-leucyl-glycine (*IId*)

To a solution of tripeptide *Id* (3·0 g) in acetone (40 ml) 1M-NaOH (5·9 ml) was added. The mixture was stirred 2 h at room temperature, diluted with water, filtered, acetone was evaporated from the filtrate, the remaining aqueous solution was filtered with active charcoal, acidified with citric acid and extracted with ethyl acetate. The extract was dried and evaporated, the remnant was recrystallized from a mixture of ethyl acetate and light petroleum. The yield was 2·75 g (94%), m.p. 107—109°C; $[\alpha]_D$ −23·9°. In an analogous manner we also prepared substances *IIa*—*IIc*, *cf.* Table I.

Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-leucyl-glycine 2,4,5-Trichlorophenyl Ester (*IIId*)

To a solution of substance *IId* (1·65 g) in dimethylformamide (30 ml) 2,4,5-trichlorophenol (0·80 g) and at −30°C dicyclohexylcarbodiimide (0·87 g) were added. The mixture was stirred 1 h at −30°C, left standing overnight at 0°C and evaporated at a pressure of 1 Torr. To the remainder ethyl acetate and light petroleum were added, the crystals were filtered off, washed with 0·5M-$NaHCO_3$ and water, and dried. The yield was 1·77 g, m.p. 140—142°C; after recrystallization from a mixture of ethyl acetate and light petroleum the yield was 1·40 g (64%), m.p. 146—148°C; $[\alpha]_D$ −22·3°. In an analogous manner we also prepared substances *IIIa* and *IIIc*, see Table I.

Poly(L-lysyl-L-leucyl-glycyl Hydrobromide) (*IVd*)

Trifluoroacetic acid (5 ml) was added to the ester *IIId* (6·0 g). The mixture was left standing for 16 min at 22°C and then poured into ether. The precipitate which separated out was filtered off, dried, and dissolved in dimethylformamide (19·5 ml). To this solution triethylamine (1·47 ml) was added with stirring and the mixture was stirred for 10 days at room temperature. After diluting with water, the substance which separated out was filtered off, washed with water, ethanol and ether. The yield was 1·33 g (37%). 0·67 g of this material was added to 3 ml 30% HBr in acetic acid, after 2 h the mixture was poured into ether, the solid material was filtered off, washed with ether, dried and dissolved in water. The solution was filtered and placed in dialysis tubing (Serva, Heidelberg, GFR) previously treated with ethylenediaminetetraacetic acid, dialyzed against a volume of 1—2 l of water with several changes and freeze-dried. The yield was 0·35 g of polymer *IVd*; amino acid analysis: 1·00 Lys, 1·02 Leu, 1·00 Gly. The product did not contain material migrating on paper chromatography in the system 1-butanol–pyridine–acetic acid–water (15 : 10 : 2 : 12) on Whatman 3 MM paper in descending electrophoresis for 24 h. In an analogous manner we also prepared polymers *IVa* and *IVc*.

Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-norvalyl-glycin 1-Succinimidyl Ester (*IIIb*)

To a solution of substance *IIb* (0·80 g, 1·5 mmol) in dimethylformamide (10 ml) 1-hydroxysuccinimide (0·20 g) and at −5°C dicyclohexylcarbodiimide (0·34 g) were added. The mixture was stirred for 1 h at −5°C, left standing at 0°C overnight and filtered. From the filtrate dimethylformamide was evaporated at 40°C and 1 Torr. The remainder was dissolved in ethyl

TABLE I

Chemical Properties of Compounds *I*—*III* (*cf.* Scheme 1)

Compound (yield, %)[a]	M.p., °C [α]D	Formula (m.w.)	Calculated/Found			
			% C	% H	% N	% Cl
Ia[b] (68)	109—110 −13·9°	$C_{26}H_{40}N_4O_8$ (536·6)	58·19 58·05	7·51 7·57	10·44 10·63	— —
Ib (65)	122—123 −17·9°	$C_{27}H_{42}N_4O_8$ (550·6)	58·89 59·13	7·69 7·77	10·17 10·07	— —
Ic (67)	119—121 −17·7°	$C_{27}H_{42}N_4O_8$ (550·6)	58·89 58·49	7·69 7·68	10·17 9·99	— —
Id (69)	92—93 −23·9°	$C_{28}H_{44}N_4O_8$ (564·7)	59·56 59·28	7·85 7·92	9·92 9·84	— —
IIa (75)	122—124 −14·6°	$C_{24}H_{36}N_4O_8$ (508·2)	56·68 56·49	7·13 7·13	11·02 10·65	— —
IIb (72)	120—123 −17·1°	$C_{26}H_{40}N_4O_8$ (536·6)	58·19 58·57	7·51 7·64	10·44 10·29	— —
IIc (84)	150—151 −17·9°	$C_{26}H_{40}N_4O_8$ (536·6)	58·19 58·28	7·51 7·53	10·44 19·48	— —
IId (94)	107—109 −23·9°	$C_{27}H_{42}N_4O_8$ (550·6)	58·89 59·27	7·69 7·81	10·17 10·38	— —
IIIa (83)	141—144 −15·5°	$C_{30}H_{37}Cl_3N_4O_8$ (688·0)	52·37 52·50	5·42 5·40	88·14 8·29	15·46 15·26
IIIb[c] (75)	152—154 −18·1°	$C_{30}H_{43}N_5O_{10}$ (633·7)	56·85 57·58	6·83 6·90	11·05 11·12	— —
IIIc[d] (86)	145—148 −19°	$C_{32}H_{41}Cl_3N_4O_8$ (716·1)	53·68 53·84	5·77 6·08	7·82 8·39	14·85 —
IIId (64)	146—148 −22·3°	$C_{33}H_{43}Cl_3N_4O_8$ (730·1)	54·29 54·32	5·94 6·16	7·67 7·95	14·57 14·25

[a] Yield of product with the same melting point as analytically pure sample; [b] ethyl ester; [c] 1-succinimidyl ester; [d] a small amount of *IIc* is present.

acetate, the solution was washed twice with 0·5M-$NaHCO_3$, water, dried and evaporated. The residue was again dissolved in ethyl acetate, a small amount of N,N'-dicyclohexylurea was filtered off and on addition of ether and light petroleum the product precipitated out; yield was 0·81 g, m.p. 151—153°C. After recrystallization from a mixture of ethyl acetate, ether and light petroleum the yield was 0·70 g (75%) of a substance with m.p. 152—154°C, homogeneous on thin layer chromatography on silica gel in a system chloroform–methanol (9 : 1), $[\alpha]_D$ −18·1°.

Poly(L-lysyl-L-norvalyl-glycine Hydrobromide) (*IVb*)

Polymerization was carried out in the same manner as with substance *IVd* in dimethyl sulphoxide (2·3 ml/0·6 g active ester). After completing the polymerization (7 days) the product was deprotected and purified, with the yield of 90 mg of the polymer *IVb*.

Molecular Weight Determination

Molecular weights of the polypeptides dissolved in 0·15M NaCl and 0·013M sodium phosphate pH 6·8 were determined from the sedimentation coefficients $s_{20,w}$, as described previously[2,3]. In one case sedimentation equilibrium[3] was also used in order to obtain the molecular weight and to characterize the association behaviour of the polypeptide $(Lys\text{-}Leu\text{-}Gly)_n$ in 0·15M-NaCl. Approximate molecular weights obtained from the measurement of $s_{20,w}$: *IVa* 6000; *IVb* 10500; *IVc* 4500. Molecular weight of 10900 was obtained for *IVd* from sedimentation equilibrium measurement.

Preparation of DNA–Polypeptide Complexes

The DNA preparation was from calf thymus[2]. Complexes of polypeptides with DNA were prepared by mixing both components in a given ratio of the lysine residue to the nucleotide (Lys/DNA) in 2M-NaCl, buffered with 0·013M sodium phosphate, pH 6·8, followed by flow dialysis against a linear gradient of NaCl molarity[2,3,5,11] to 0·15M-NaCl and 0·013M phosphate, pH 6·8, in some cases with additional dialysis to 0·01M Tris buffer, pH 7·0. The fraction of DNA in aggregated complex, F_{ppt} was determined as described earlier[2].

Measurements of Circular Dichroism

The circular dichroism spectra of polypeptides were recorded with a Cary 61 apparatus in the range 260—195 nm in cells with optical path 0·01, 0·05, 0·1, 0·2 and 0·5 cm. The concentration of solutions was usually about 0·1% (w/v). For the concentration dependence of the CD spectra of the polymer $(Lys\text{-}Leu\text{-}Gly)_n$, the region between 0·04 and 0·01% (w/v) was covered. The pH adjusted by adding 0·5M-NaOH to the original solution (in 0·02M-NaF); pH was measured with an accuracy of 0·1 unit. Solutions with various ionic strengths were prepared by adding 4M-NaCl to a solution of polypeptide in water. Solutions in 90% 2-propanol were prepared by adding appropriate amounts of 2-propanol to a solution of polypeptide in water. The circular dichroism was expressed as molar ellipticity $[\Theta]$ (deg cm^2 $dmol^{-1}$) where the average molecular weight of the residue for the given polypeptide was used in the calculations. The concentrations of polypeptide solutions were taken from weighed amounts. The samples were dried *in vacuo* and kept in desiccators.

The circular dichroism spectra of DNA–polypeptide complexes in the region 300—200 nm were recorded with a Roussel Jouan CD 185 dichrographe in cells with optical path 0·5 cm,

at 22–24°C. The circular dichroism was expressed in specific ellipticity [Ψ] (deg cm^2 dg^{-1}) referred to the total weight concentration of DNA in the system.

Infra-Red Spectra Measurements

Infra-Red spectra of solutions of $(Lys\text{-}Leu\text{-}Gly)_n$ in D_2O and 2M-NaCl in D_2O were measured in a Perkin Elmer 621 spectrophotometer, at neutral pD, in a 0·03 mm KRS-5 cell. The concentrations of the solutions were about 3% (w/v).

RESULTS

Chiroptical Properties of Polypeptides in Solution

The CD spectra of sequential polypeptides of the series $(Lys\text{-}X\text{-}Gly)_n$ were measured in dependence on pH and ionic strength of the aqueous medium and also in 90% 2-propanol, as in previous studies[2,3]. Decisive for evaluation of the spectra was their comparison in a series of homologues with increasing side-chains of the amino acid residue X.

For the polymer with the smallest side chain, $(Lys\text{-}Ala\text{-}Gly)_n$ in aqueous solution, CD spectra characteristic for polypeptide in unordered conformation were obtained (Fig. 1). There was a strong negative maximum at 198 nm ([Θ] about −15000) and a weak negative maximum at 230 nm ([Θ] less than −1000) which were separated by a distinct negative minimum between 215 and 220 nm, indicating the presence

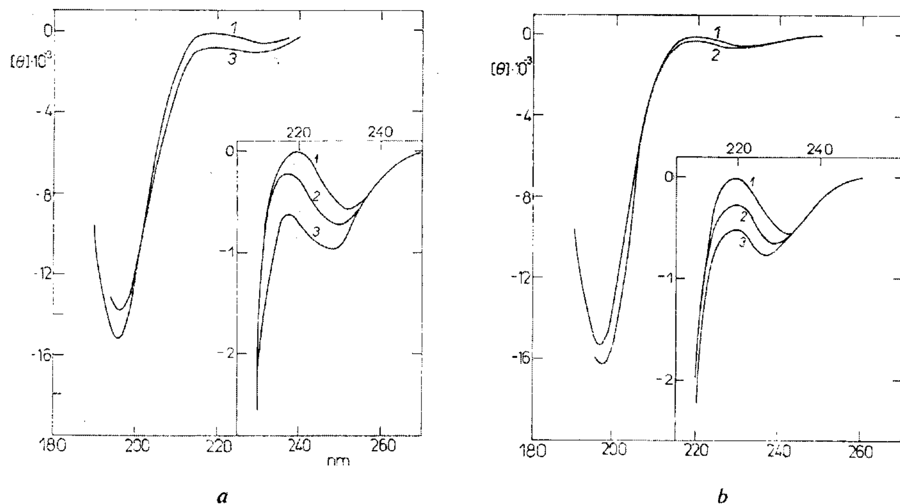


FIG. 1

CD Spectra of $(Lys\text{-}Ala\text{-}Gly)_n$

a) pH dependence in 0·02M-NaF: *1* pH 6·9, *2* pH 9·4, *3* pH 10·1; *b*) ionic strength (NaCl molarity) dependence at pH 7: *1* *I* 0·01, *2* *I* 0·15, *3* *I* 2·0.

of a positive dichroic band in this region. A similar type of spectrum was observed[2] also with the sequential polypeptides $(\text{Ala-Lys-Pro})_n$ and $(\text{Ala-Lys-Lys-Pro-Lys})_n$. Dependence on pH and ionic strength was manifest mainly in the long wavelength region of the spectrum. The maximum at 230 nm showed a hypsochromic shift, and its intensity increased somewhat with increasing pH and ionic strength (Fig. 1) at pH 7 and $I = 0{\cdot}01$ $[\Theta]_{max}$ was -580, at pH and $I = 2$ $[\Theta]_{max}$ was -780 and at pH 11 and $I = 0{\cdot}02$ $[\Theta]_{max}$ was -980.

The results obtained with the $(\text{Lys-Ala-Gly})_n$ in general corresponded to those reported by Cernosek and coworkers[6] with the same sequential copolymer, which differed somewhat from our own preparation in terms of molecular weight.

In the spectra of the next higher homologue, $(\text{Lys-Nva-Gly})_n$, smaller difference in ellipticity between the negative minimum at 217 nm and the negative maximum at 230 nm was observed (Fig. 2). Even in neutral medium at low ionic strength there is only a small difference which still decreases with increasing ionic strength and pH. The negative ellipticity of the band at 230 nm increased in the same sense: at pH 7 and $I = 0{\cdot}01$ $[\Theta]_{max}$ was -850, at pH 7 and $I = 2{\cdot}0$ the negative maximum changed into a shoulder with $[\Theta]$ about -1700. The strong $\pi - \pi^*$ band below 200 nm with $(\text{Lys-Nva-Gly})_n$ in neutral medium and low ionic strength was more intense than in the previous polypeptide ($[\Theta]_{max}$ is -19500 as compared with -15000). With in-

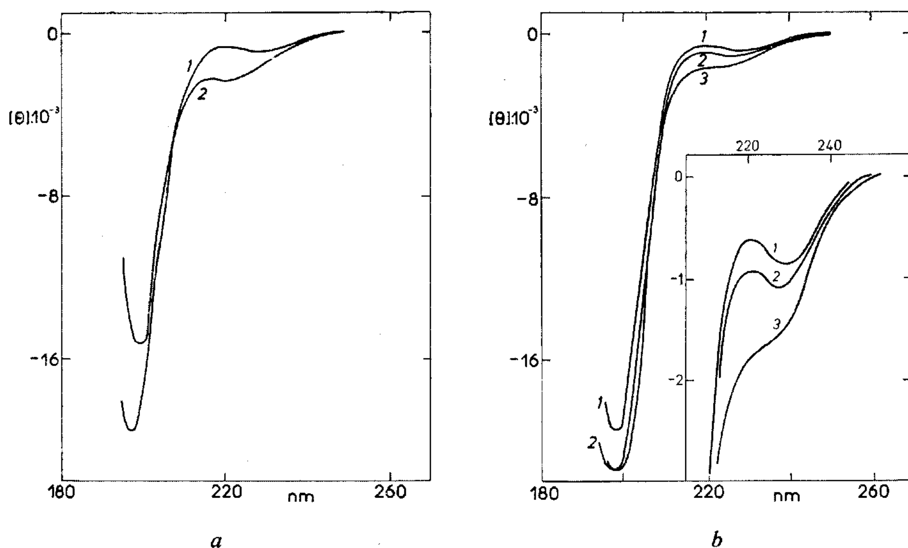
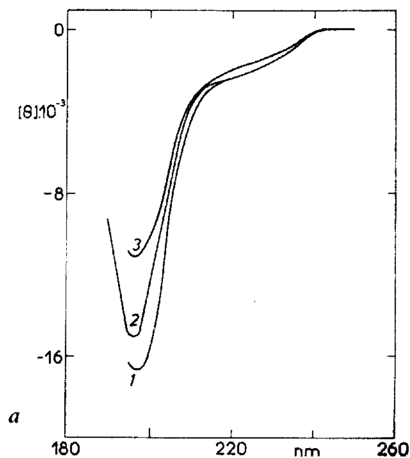


FIG. 2

CD Spectra of $(\text{Lys-Nva-Gly})_n$

a) pH dependence in 0·02M-NaF: 1 pH 6·5, 2 pH 11·9; *b*) ionic strength dependence at pH 7: 1 I 0·01, 2 I 0·15, I 2·0.

creasing pH the amplitude decreased (at pH 11·9 $[\Theta]_{max}$ was −15600) and was somewhat shifted to longer wavelengths. With increasing ionic strength the amplitude slightly increased.



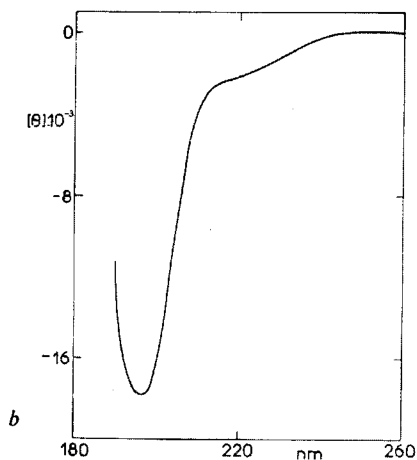
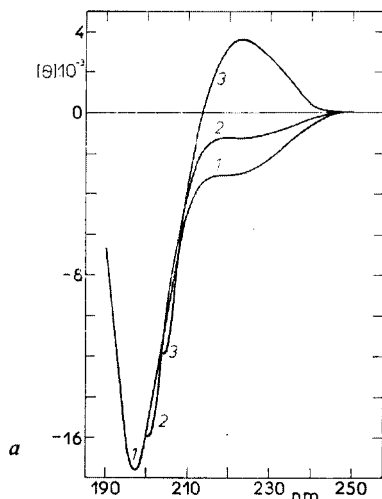


FIG. 3

CD Spectra of (Lys-Val-Gly)$_n$

a) pH dependence in 0·02M-NaF; *1* pH 6·7, *2* pH 9·1, *3* pH 10·8; *b*) only one curve obtained for ionic strengths 0·01, 0·15 and 2·0 at pH 7.



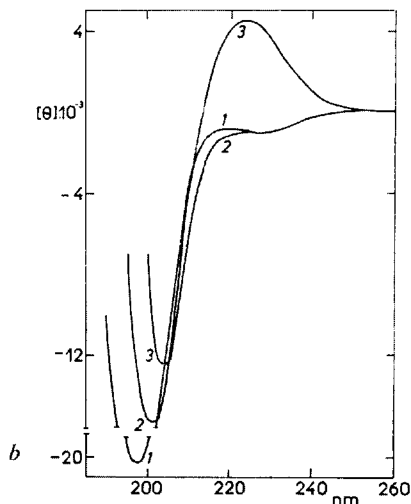


FIG. 4

CD Spectra of (Lys-Leu-Gly)$_n$

a) pH dependence in 0·02M-NaF; *1* pH 7·0, *2* pH 9·2, *3* pH 11·4; *b*) ionic strength dependence at pH 7: *1* *I* 0·01, *2* *I* 0·15, *3* *I* 2·0.

In the spectra of the copolymer with valine residue (Fig. 3) no positive maximum at 217 nm could be observed. The shoulder at about 225 nm did not change at all with a change in ionic strength and varied only slightly with a change in pH. The strong negative band at about 197 nm had, in neutral medium, a molar ellipticity $[\Theta]$ of -16700 to -17800. Neither position nor amplitude depended upon ionic strength. With increasing pH, however, amplitude markedly decreased (at pH 10·8 $[\Theta]_{max}$ was -11000).

$(Lys\text{-}Leu\text{-}Gly)_n$ showed a very unusual dependence of CD spectrum on ionic strength and pH (Fig. 4). CD spectra in 0·02M-NaF at neutral pH suggested a random coil conformation. With partial neutralization of the ε-amino groups of the lysine residues the negative maximum at 227 nm changed into a shoulder. At alkaline pH a marked positive maximum at 224 nm was formed the intensity of which is fairly high (at pH 11·4 $[\Theta]_{224} = 4500$). The negative maximum, which in neutral medium is at 197 nm with a molar ellipticity $[\Theta] = -20400$, decreased with increasing pH and shifted to longer wavelengths (at pH 9·2 λ_{max} was 202 nm and $[\Theta]_{max} = -15300$, at pH 11·4 $\lambda_{max} = 203{\cdot}5$ and $[\Theta]_{max} = -12500$). The dependence of the CD spectra on ionic strength was similar. In aqueous solution without electrolytes, the spectrum showed a shoulder at about 222 nm ($[\Theta]_{222} = -3000$). With increasing ionic strength the negative ellipticity in this region decreased and in 2M-NaCl as well as in alkaline pH a positive maximum at 223–224 nm was formed. The sign and intensity of this band depended upon time and concentration of the polymer (Table II). On addition of salt to the solution of the polymer in water the ellipticity of the shoulder at 222 nm at first decreased to zero and then a positive maximum was formed the amplitude of which gradually increased reaching a limiting value within 24 h. This end value of $[\Theta]_{max}$ depended on the concentration of the polymer in solution (Table II). On dilution $[\Theta]_{max}$ decreased but no time-dependent changes were observed. In more concentrated solutions an opalescence developed after some time. The negative maximum situated in neutral water solution at 197 nm decreased with increasing ionic strength, just as with increasing pH, and shifted to higher wavelengths.

TABLE II

Changes in Molar Ellipticity $[\Theta]_{224} \cdot 10^{-3}$ as a Function of Time and Concentration of $(Lys\text{-}Leu\text{-}Gly)_n$ in Solution in 2M-NaCl

Concentration mg ml^{-1}	Time, min			
	7	15	60	1 440
0·4	+0·58	+1·30	+2·37	+3·98
0·2	−0·08	+0·63	+1·67	+3·44
0·1	−0·76	−0·13	+1·04	+2·99

This observation has only a qualitative character because of the high absorption of 2M-NaCl in the 200 nm region.

In 90% 2-propanol, polymers $(Lys\text{-}Ala\text{-}Gly)_n$ and $(Lys\text{-}Nva\text{-}Gly)_n$ showed CD spectra typical for mixtures of an unordered form with a smaller amount of α-helical conformation, *cf.* Fig. 5. In the norvaline homologue, for example, $[\Theta]_{220} = -11000$, and the ellipticity of the negative maximum of the split $\pi - \pi^*$ band at 206·5 nm was $[\Theta]_{206.5} = -15800$. The valine copolymer also showed in 90% 2-propanol the presence of an α-helical structure which, however, rapidly changed to another conformation with a CD spectrum similar to that of a β-conformation. The amplitude of the negative maximum at 215 nm was low ($[\Theta]_{max} = -7200$). Some opalescence of the solution under these conditions suggested aggregation. The relatively highest content of α-helical conformation, according to measured CD spectra, was shown by the polymer $(Lys\text{-}Leu\text{-}Gly)_n$. The amplitudes of both negative maxima were substantially higher than in the CD spectra of previous polymers, $[\Theta]_{220} = -21700$ and $[\Theta]_{208} = -26000$.

The infra-red spectra of $(Lys\text{-}Leu\text{-}Gly)_n$ showed in D_2O solution at pH 7 amide-I′ band at 1646 cm^{-1}, amide-II′ band at 1461 cm^{-1}; in 2M-NaCl in D_2O the amide-I′ band shifted to 1635 cm^{-1} and the amide-II′ band to 1457 cm^{-1}.

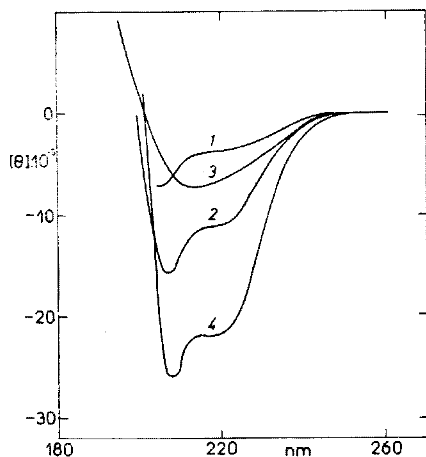


FIG. 5

CD Spectra of $(Lys\text{-}Ala\text{-}Gly)_n$ *1*, $(Lys\text{-}Nva\text{-}Gly)_n$ *2*, $(Lys\text{-}Val\text{-}Gly)_n$ *3* and $(Lys\text{-}Leu\text{-}Gly)_n$ *4* in 90% 2-propanol

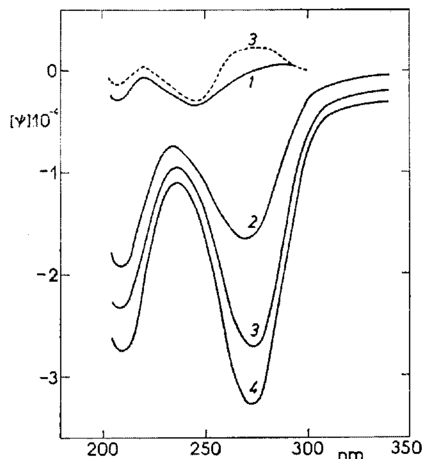


FIG. 6

CD Spectra of Complexes $(Lys\text{-}Ala\text{-}Gly)_n$–DNA

——— 0·15M-NaCl; ------ 0·01M Tris; Lys/DNA = 0·17 *1*, 0·32 *2*, 0·51 *3*, 0·64 *4*.

Chiroptical Properties of Complexes $(Lys\text{-}X\text{-}Gly)_n$–DNA

Circular dichroism was used to study complexes prepared by dialysis against a linear gradient of NaCl from 2M to 0·15M NaCl and to 0·01M Tris with various input ratios of Lys/DNA. CD spectra of complexes $(Lys\text{-}Ala\text{-}Gly)_n$–DNA are shown in Fig. 6. At higher Lys/DNA ratios the spectra showed two strong negative bands at about 210 and 270 nm, like the spectra of complexes of DNA with other lysine containing polypeptides in an unordered conformation[2,3,12] and histone H 1 (*cf.*[13], for histone symbols *cf.*[14]). Quantitatively, the ellipticity values in the maxima are in good agreement with those of complexes $(Ala\text{-}Lys\text{-}Pro)_n$–DNA (*cf.*[2]). On dialysis into a low ionic strength (0·01M Tris) in both cases formation of a soluble complex was observed with a spectrum similar to that of DNA itself (Fig. 6, *cf.*[2]). Also chiroptical properties of complexes of DNA with $(Lys\text{-}Val\text{-}Gly)_n$ and $(Lys\text{-}Nva\text{-}Gly)_n$ were similar (Figs 7, 8). The spectra of $(Lys\text{-}Nva\text{-}Gly)_n$–DNA differed somewhat in the lower ellipticity of the dichroic band of the complex in 0·15M-NaCl (Fig. 7) and also in the incomplete transition into a soluble complex (with a typical CD spectrum of DNA) in 0·01M Tris (Fig. 8).

The CD spectrum of $(Lys\text{-}Leu\text{-}Gly)_n$–DNA is also composed of two negative dichroic bands of about the same wavelength and ellipticity as spectra of the other sequential polymers with one residue of lysine in the monomeric unit. However, in

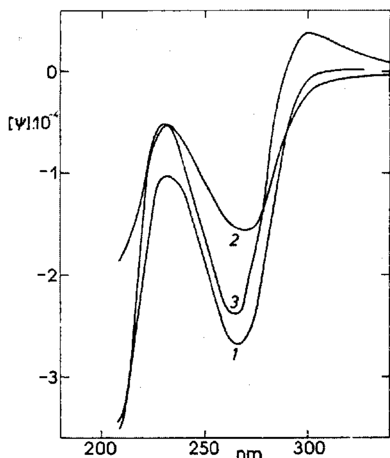


FIG. 7

CD Spectra of Complexes $(Lys\text{-}X\text{-}Gly)_n$–DNA in 0·15M-NaCl, Lys/DNA = 0·5
X = Val *1*, Nva *2*, Leu *3*.

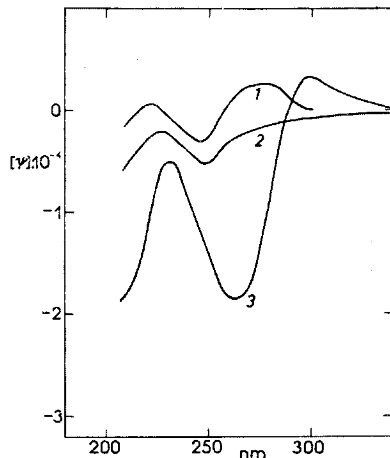


FIG. 8

CD Spectra of Complexes $(Lys\text{-}X\text{-}Gly)_n$–DNA in 0·01M Tris, pH 7; Lys/DNA = 0·5
X = Val *1*, Nva *2*, Leu *3*.

addition there is also a positive band in the long wavelength region at 300 nm (Figs 7 and 8). No transition to the soluble complex with DNA spectrum was observed in low ionic strength (Fig. 8).

DISCUSSION

The CD spectrum of the polymer $(Lys\text{-}Ala\text{-}Gly)_n$, as follows from dependence on pH and ionic strength, probably involves superposition of two types of unordered structures: *1.* that of polypeptide type (characteristic *e.g.* for poly-L-lysine with charged ε-amino groups) the CD spectrum of which has a positive maximum at about 217 nm and a negative maximum at about 198 nm, and *2.* another one typical for proteins the spectra of which show a negative shoulder at 230 nm replacing the positive maximum at 217 nm (*cf.*[15]). With neutralization or ionic shielding of the charged ε-amino groups of the lysine residues in the polypeptide $(Lys\text{-}Ala\text{-}Gly)_n$ the unordered conformation of the polypeptide type is obviously supressed in favour of that typical for proteins.

The conformational properties of the studied polymers $(Lys\text{-}X\text{-}Gly)_n$ change continuously with alteration in the second residue following the series Ala→Nva→→Val→Leu. In the first two members there is still a distinct formation of an unordered conformation of the polypeptide type which is no longer observed in the valine derivative. The existence of this form in the polymer $(Lys\text{-}Leu\text{-}Gly)_n$ is questionable; the features of the CD spectra which would suggest this could be due to a positive maximum of a different origin emerging at alkaline pH's and high ionic strength. Preference for the unordered conformation of the protein type increases with the increasing bulk of the side-chain of amino acid residue X. The ability to form an α-helix or a β conformation in 2-propanol also increases with increasing bulk of the X residue. The isopropyl side-chain of the valine polypeptide is bulky enough to eliminate practically any conformational variability of the polypeptide in various aqueous media.

The last member of the series, $(Lys\text{-}Leu\text{-}Gly)_n$, behaves in all of its observed properties with greater or lesser deviations from behaviour which could be expected from comparison with other polypeptides, taking into consideration only the steric requirements of the side-chains. Thus, at neutral pH and low ionic strength $(Lys\text{-}Leu\text{-}Gly)_n$ exists in unordered conformation, just as with the other polymers of this series. At neutral pH and high ionic strength (2M-NaCl), and at alkaline pH even at low ionic strength, there are changes in the CD spectra which suggest changes in conformation. Under the given conditions, these changes are relatively slow and are concentration dependent. This indicates the intermolecular nature of the conformational changes accompanying aggregation, which was distinctly manifested by opalescence of more concentrated solutions in 2M-NaCl and in pH 11. An aggregation could also be observed at 0·15M-NaCl in measurements of molecular weight

by sedimentation equilibrium. Molecular weight averages evaluated from the region at the bottom of the ultracentrifuge cell ($\overline{M}_w$ = 18300 and $\overline{M}_z$ = 28300) were higher than molecular weight of the non-aggregated sample (monomer, $\overline{M}_1$ = 10900), suggesting association.

The nature of the conformation assumed by (Lys-Leu-Gly)$_n$ under these conditions is not completely clear. The frequency of the amide-I′ band (in 2M-NaCl in D_2O 1635 cm^{-1}) could correspond to a β structure, but the absence of a second band at about 1680 cm^{-1}, characteristic for an antiparallel β conformation, practically excludes the presence of this form[16,17]. The frequency of the amide-I′ band could correspond theoretically to the calculated frequency for a parallel β conformation[16]. This form, however, has not yet been experimentally demonstrated in polypeptides. An important argument against β conformation are the CD spectra which fundamentally differ from the CD spectra found for various polypeptides in β conformation[17], and also from spectra theoretically calculated for antiparallel and parallel β conformations[18]. The frequency of the amide-I′ band is, however, also compatible with that of the same band of polyglycine II and some glycine-containing polytripeptides, considered as good models of collagen. The wave-number of the amide-I band in films of polyglycine II is 1641 cm^{-1} (ref.[19]) and, in the polytripeptides, it is 1640–1639 cm^{-1} (ref.[20,21]). After deuteration in a film of polyglycine II the wave-number of amide-I′ band is 1639–1632 cm^{-1} (ref.[22]). The CD spectra of polymer (Lys-Leu-Gly)$_n$ in 2M-NaCl or in alkaline pH both resemble in terms of general shape and the position of the separate bands the CD spectra of collagen and its models in solutions[20,21]. There is some difference quantitatively, particularly in the lower ellipticity of the negative maximum at about 200 nm.

In view of general agreement of results of the two independent methods, it is felt that the polypeptide chain of (Lys-Leu-Gly)$_n$ in the aggregated form is at least partly ordered into a conformation characteristic for polyglycine II and some synthetic models of collagen. A basic structural feature allowing formation of this conformation is the presence of the glycine residue. Since, however, neither (Ala-Ala-Gly)$_n$ (ref.[23]) nor the other polymers described in the present work, form a similar conformation, one can conclude that intra- and/or intermolecular hydrophobic interactions of the side-chains of leucine play a decisive role in conformation forming in the case of (Lys-Leu-Gly)$_n$. The exceptional properties of the leucine residue in polypeptide chains have already been noted[24–26]. One possible explanation for this anomalous behaviour can involve the greater number of non-covalent interactions of carbon atoms in the δ-position (and protons bound to it) with the polypeptide backbone and the effect on conformation of the polypeptide which follows from this. (The organic chemist is reminded of the old Newman "six-number" rule[27,28].)

The possibility of formation of a defined structure of the described type as a result of intermolecular interaction in synthetic histone models could be of interest in relation to the recent findings of stoichiometrically defined interactions of various histo-

nes[29] and the presence of defined aggregates of histones in chromatin[30]. Particularly histone H 4 (F2al), characterized by a high content of glycine and a fairly high content of leucine[31], is capable of very strong interaction with other histones[30] and itself forms defined dimers[32] and fibrous polymeric aggregates[33].

The complex $(Lys\text{-}Ala\text{-}Gly)_n$–DNA shows properties analogous to those of previously studied complexes of $(Ala\text{-}Lys\text{-}Pro)_n$–DNA, the basic tripeptide of which also contains one lysine residue. Both complexes differ only in the third amino acid residue, in one case glycine, in the other proline, both of which are not conductive to helix formation. Analogous is the complex $(Lys\text{-}Val\text{-}Gly)_n$–DNA and to some degree also $(Lys\text{-}Nva\text{-}Gly)_n$–DNA (Figs 7 and 8). On the other hand, the CD spectrum of the complex $(Lys\text{-}Leu\text{-}Gly)_n$–DNA in 0·15M-NaCl contains, in addition to the two negative bands, also a positive dichroic band in the region of 300 nm. A similar type of spectrum has been found in complexes of a statistical copolymer $(Lys_{47}, Ala_{53})_n$, ref.[3]. It would appear that this band is typical for complexes of polypeptides which, in addition to an unordered conformation, also take on at medium and high ionic strength some degree of ordered helical conformation[3].

In the case of the polymer $(Lys\text{-}Leu\text{-}Gly)_n$ we are apparently dealing with another ordered conformation than the α-helix. In all probability, as appears from studies of the conformation of the polypeptide in solution, the helix is of the polyglycine-II type. On dialysis into low ionic strength, the character of the spectra of the complex $(Lys\text{-}Leu\text{-}Gly)_n$–DNA does not change, there is only a slight decrease in amplitude of the negative bands. From this one can suggest that the structure of the complex $(Lys\text{-}Leu\text{-}Gly)_n$–DNA is different from that of the other complexes, probably due to the helical conformation of the polypeptide during complex formation.

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