Water-soluble Lysine-containing Polypeptides. I. The Synthesis and Characterization of Several Sequential Lysine–Glycine Polypeptides Including a Preliminary Study of their Interaction with DNA[1]

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The preparation and characterization of several sequential lysine–glycine polypeptides is described. A preliminary study of their interaction with DNA is also presented.

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La préparation et caractérisation de plusieurs lysine–glycine polypeptides d'une sequence régulière est décrite. De plus, une étude préliminaire sur leur association avec ADN est presentée.

Introduction

Chromosomal proteins, of which the basic amino acid containing histones constitute by far the largest class, play an important role in the determination of the morphology and metabolism of the eukaryotic cell (1, 2). The associations between these basic proteins and the cellular DNA (deoxyribonucleic acid) have been postulated to play an important role in the maintenance of the chromosomal architecture and may offer some degree of regulation during the various stages of cell development (2).

The formation and stability of these protein–DNA complexes is governed primarily by long and short range interactions. The longer range electrostatic interactions form the basis of histone–DNA association and are of prime importance in formation of histone–DNA complexes.

A firmer understanding of the various ways in which electrostatic interactions between the histones and DNA could regulate cell architecture and metabolism could be gained from experiments using models of the natural proteins. As models, synthetic polypeptides containing significant quantities of either of the basic amino acids, lysine or arginine, could be used. The homopolymers, poly-(L-lysine) and poly-(L-arginine), have been used as models in the study of DNA – basic protein (polypeptide) interactions (3, 4). A natural extension to these studies would be to use synthetic copolymers containing one or more of the common aliphatic amino acids as well as a basic amino acid. Incorporation of the amino acids in a sequential or known manner would facilitate the interpretation of experiments designed to investigate the interaction of these polypeptides with DNA. This should give some insight into the effect of chain sequence and solution conformation on the interaction of proteins with DNA.

We have synthesized several polypeptides having the amino acids lysine and glycine in a defined sequence. In this paper we wish to describe the synthesis of the monomer blocks used for the preparation of the polymers, the polymerization conditions used, and the spectroscopic characterization of the final polypeptide products. Preliminary results on the interaction of the polypeptides with DNA are also presented.

Methods and Materials

Experimental

All chemicals were of reagent grade and were used as received unless otherwise stated.

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TABLE 1. Molecular weights of lysine–glycine polypeptides

Polymer[a]	End-group analysis[b]	Gel filtration[c]
Poly-(L-lys)	10 250	—
Poly-(L-lys)	20 540	—
Poly-(L-lys-gly)	19 000	19 000 ± 2000
Poly-(L-lys-gly-gly)	12 500	12 000 ± 3000
Poly-(gly-L-lys-gly-gly)	7 410	7 000 ± 1000

[a]Final polypeptide product isolated as the hydrobromide salt.
[b]Measured on fully blocked polypeptide, perchloric acid procedure, ref. 9. All values given are ± 5% given value.
[c]Measured on unblocked polypeptide: Sephadex G-100, 0.002 *M* sodium phosphate, p*H* 7.0, refs. 10, 11. Distribution limits are given. Ribonuclease, chymotrypsinogen, and ovalbulmin were used as standards.

Optical rotations were measured on a Perkin-Elmer polarimeter, model 141 in a 1 dm microcell. Dilute hydrochloric acid (HCl), acetic acid (HOAc), or methanol (MeOH) were used as solvents as noted. Melting points were taken on a Fisher-Johns melting point apparatus (heated block) and are uncorrected. Combustion analyses were determined on a Perkin-Elmer C, H, and N analyzer, model 240. Amino acid analyses were done on a Beckman automatic analyzer, model 120 C. Amino acid hydrolyses were carried out at 110 °C for 16 h.

N^{ϵ}-Benzyloxycarbonyl(Cbz)-L-lysine m.p. 235–237 °C, $[\alpha]_D$ +14.1° (*c* 1.0, 0.1 *N* HCl) (lit. (5) m.p. 235 °C, $[\alpha]_D$ +14.0 (*c*, 2.6 dilute HCl)), *N*-hydroxysuccinimide (HOSu), dicyclohexylcarbodiimide (DCC), and glycylglycine were purchased from Fluka AG, Buchs, Switzerland. Triethylamine (TEA) (B.D.H. reagent grade) was used only if taken from a freshly opened bottle. For polymerizations it was purified by distillation from *p*-toluenesulfonyl chloride and stored under nitrogen atmosphere (6). Dimethylformamide (DMF) was purified by treatment with barium oxide, filtration, and distillation under reduced pressure (6). Dimethoxyethane (DME) was purified by distillation from lithium aluminum hydride (6).

N^{α}-*t*-Butyloxycarbonyl(Boc)-glycine, m.p. 85–86 °C, was prepard in 94% yield according to the procedure of Schnabel (7) from glycine and *t*-butyloxycarbonyl azide (Pierce Chemicals, Rockford, Illinois); reaction conditions: pH_{obs} 10.0, in water. N^{α}-*t*-Boc-N^{ϵ}-Cbz-L-lysine was prepared similarly (isolated in 92% yield as N^{α}-*t*-Boc-N^{ϵ}-Cbz-L-lysine·DCHA salt, m.p. 113 °C, $[\alpha]_D$ −9.3° (*c* 1.0, HOAC)); reaction conditions: pH_{obs} 10.2, in dioxane–water, 2:1, v/v, 50 g per 700 ml of solution, vigorous stirring.

Highly polymerized deoxyribonucleic acid (DNA) was obtained from Worthington Biochemicals (Freehold, N.J.). Stock solutions (approximately 1 mg/l) were prepared by suspension of the solid in cacodylate buffer, 0.01 *M*, p*H* 7.0, and dialysis overnight (4 °C) against this buffer. The solution was centrifuged before being diluted to the desired concentration. DNA-phosphate concentration was estimated from the absorbance at 260 nm (ε phosphate 6600(8)).

Polymer molecular weights were estimated on the blocked polypeptides by end-group titration (perchlorate procedure) (9) and on the unblocked polymers by thin-layer gel filtration on Sephadex G-100 (sodium phosphate, 0.002 *M*; sodium chloride, 0.05 *M*; p*H* 7.0) using ribonuclease, chymotrypsinogen, and ovalbumin. as standards (10, 11). Detection was by trinitrobenzenesulfonic acid treatment of a paper replica (12). The molecular weights are given in Table 1.

Circular dichroic spectra were taken on a Cary spectropolarimeter, model 61, in quartz cells (path length 0.1 cm). Reported ellipticities are based upon the lysine concentration (as determined by amino acid analysis). The ellipticities measured at 225 nm are given in Table 2.

Poly-(N^{ϵ}-Cbz-L-lysine) (13) of differing molecular weights was prepared by polymerizing N^{ϵ}-Cbz-L-lysine *N*-carboxyanhydride, m.p. 97–98 °C (lit. (14) m.p. 100 °C dec.) in DMF solution using diethylamine as initiator. The polymer was precipitated with acidified water (2.3 drops concentrated HCl per liter), washed with methanol–ether (2:1 v/v) to remove small molecular weight impurities, and dried under high vacuum.

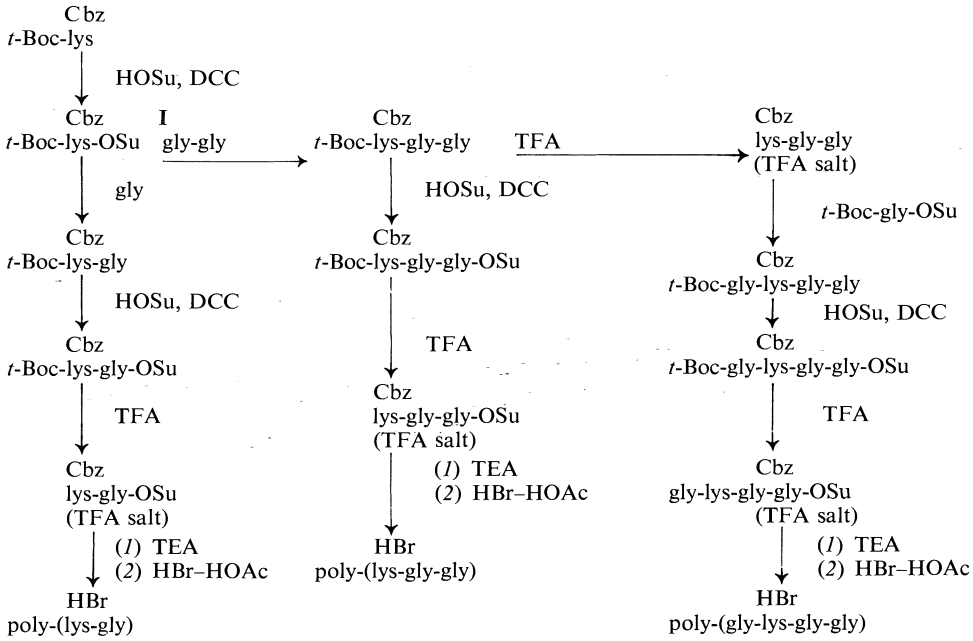
Monomer Preparation

The monomers used for the preparation of the polypeptides were prepared according to the procedures outlined in Scheme 1. The physical constants and elemental analyses are given in Table 3. The general procedure for the preparation may be exemplified by the preparation of N^{α}-*t*-Boc-N^{ϵ}-Cbz-L-lysylglycylglycine·DCHA salt. N^{α}-*t*-Boc-N^{ϵ}-Cbz-L-lysine (73.9 g, 0.195 *M*) and *N*-hydroxysuccinimide (22.4 g, 0.195 *M*) were dissolved in purified dimethoxyethane (750 ml). The solution was cooled to melting ice temperature and dicyclohexylcarbodiimide (DCC) (40.6 g, 0.205 *M*) was added. After stirring overnight, during which time the temperature was allowed to rise to ambient, the solution was filtered to remove the precipitated dicyclohexylurea. The filtrate was evaporated (93 g) and was taken up in 150 ml dimethoxyethane. This solution was added directly to a stirred solution containing the following: glycylglycine (27.1 g), sodium bicarbonate (65.5 g), and water (225 ml). Reaction was allowed to proceed with stirring for 20 h at room temperature. Water (2000 ml) was added and the solution carefully acidified with saturated citric acid solution to p*H* 3. The product was extracted into ethyl acetate, the organic phase was dried over sodium sulfate, and evaporated to dryness under reduced pressure (oil, 96 g). This oily product was taken up in ether (260 ml) and purified dicyclohexylamine (38.2 g, 0.211 *M*) was added. The solution was refluxed on a steam bath for 2 h during which time the salt precipitated. The solution was then allowed

TABLE 2. Circular dichroic ellipticities

	$\varepsilon_L - \varepsilon_R$ (225 nm)[a]			
	0.14 *M* NaF		1.0 *M* NaF	
	p*H* 7	p*H* 12	p*H* 7	p*H* 12
N^{α}-Acetyl-L-lysine amide[b]	−0.32	−0.18	—	—
Poly-(L-lys)[c]	+0.47	−6.36	+0.49	−6.54
Poly-(L-lys-gly)	−0.44	−0.39	−0.36	−0.36
Poly-(L-lys-gly-gly)	−0.34	−0.24	−0.43	−0.48
Poly-(gly-L-lys-gly-gly)	−0.24	−0.24	−0.21	−0.13

[a]All values of $\Delta\varepsilon$ ($\varepsilon_L - \varepsilon_R$) are ±5%. The calculations are based upon the number of moles of lysine in solution (approximately 5–10 × 10^{-4} *M*/l) as determined by hydrolysis and amino acid analysis. Spectra were first taken at p*H* 7 in Tris–HCl buffer, 0.01 *M*, at the salt concentrations noted. The solutions were then adjusted to p*H* 12 with concentrated sodium hydroxide (1 *N*) and the spectra measured. All compounds were as their hydrobromide salts.
[b]Prepared by HBr removal of the Cbz blocking group from *N*-α-acetyl-N^{ε}-Cbz-L-lysine amide (21).
[c]Molecular weight, 10 250 (end group titration). The lower $\Delta\varepsilon$ observed for this polymer may be related to effects of the lower molecular weight upon the dichroic properties (22, 23).



SCHEME 1

to cool overnight to room temperature. The salt was collected by filtration and crystallized from ethyl acetate (EtOAc) – petrol (30–60°) (107.5 g).

N^{α}-*t*-Boc-N^{ε}-Cbz-L-lysylglycine·DCHA salt was prepared in a similar manner from N^{α}-*t*-Boc-N^{ε}-Cbz-L-lysine succinimide ester and glycine.

N^{α}-*t*-Boc-glycyl-*N*-Cbz-L-lysylcylglycine was prepared as the free acid by coupling *t*-Boc-glycine *N*-hydroxysuccinimide ester (15) with N^{ε}-Cbz-L-lysylglycylglycine in aqueous sodium bicarbonate and working up as described above. The unblocked tripeptide used was prepared from N^{α}-*t*-Boc-N^{ε}-Cbz-L-lysylglycylglycine by deblocking with 1:1 (v/v) trifluoroacetic acid (TFA) – methylene chloride (0.5 h, room temperature) (16) and precipitating with ether.

Activation and Polymerization of the Monomer Blocks

N-Hydroxysuccinimide esters of the blocked peptides were prepared by coupling the free acid (liberated from its DCHA salt where necessary) with *N*-hydroxysuccinide using DCC. The coupling was done in ice cold dimethoxyethane–pyridine (95:5, v/v). After reaction (usually 6 h) the urea was filtered off and the solution evaporated. The oily residue was triturated several times with ether to remove excess pyridine. The washed residue was then thoroughly dried and treated with an excess of trifluoro-

acetic acid and methylene chloride (1:1, v/v) at room temperature (20 min). The TFA salt was precipitated by addition of 10–20 volumes of ether and then washed several times with ether by decantation.

The TFA-succinimide ester salt (approximately 1 g) was suspended in amine-free, purified DMF in a centrifuge tube (50 ml) to make the solution 1 *M* in monomer. A chain terminator (HCl·N^{ε}-Cbz-L-lysine methyl ester) (17) was added to give a monomer:terminator ratio of 50–100:1, depending on the molecular weight desired. Purified triethylamine (2–3 mol equiv. per mol of monomer) was added and the mixture rapidly stirred with a glass rod. No attempt was made to remove the heat of reaction. The polymerization was allowed to proceed overnight at ambient temperature. The gel or viscous solution which had formed was then diluted with purified DMF to give a monomer concentration of approximately 0.2 *M*. The polymerization was allowed to proceed for a further 2 or 3 days. The polymeric material was isolated by addition of the DMF solution to approximately 10 volumes of acidified water (1 drop concentrated HCl per liter). The resulting solid was collected by centrifugation and dried under vacuum. Small molecular weight fragments and cyclic materials were removed by washing the solid with methanol three times (15–25 ml/g). The insoluble residue was washed with ether and dried under vacuum. The blocked polymers could be generally prepared in 60–80% overall yield based upon the amount of monomer acid used to prepare the succinimide ester.

Blocking Group Removal

Benzyloxycarbonyl (Cbz) blocking groups were removed from all polymers with saturated HBr – acetic acid (18). The hydrobromide salt was isolated by precipitation with ether. The HBr – acetic treatment acid was repeated in order to ensure complete removal of all blocking groups. The ether insoluble salt was then washed with isopropanol (three times) (10 ml/g) and with ether and then thoroughly dried under high vacuum over KOH pellets. The extent of blocking group removal after two treatments with HBr – acetic acid was estimated from the residual benzyloxycarbonyl (Cbz) absorption at 254 nm (aqueous solution, approximately 2 mg/ml (ε_{254} 200 (19)). In all cases greater than 99.5% of all blocking groups were estimated to have been removed.

Precipitation Experiments

DNA precipitation experiments were performed by a method similar to that described by Leng and Felsenfeld (20). To prepare samples with equal lysine:DNA-phosphate ratios, 20 μl aliquots of a buffered stock solution of the polypeptide (cacodylate, 0.01 *M*, p*H* 7.0; polypeptide 1.0–2.5 mg per ml; lysine concentration approximately 4×10^{-4} *M*) were added sequentially to 3.0 ml of a buffered salt solution containing DNA (50 ± 5 μg per ml, phosphate concentration approximately 1.5×10^{-5} *M*). Each aliquot was added as the solution was vigorously mixed (Vortex mixer). After the final addition to obtain the desired ratio (5 × 20 μl), the solution was thoroughly mixed and allowed to equilibrate for 30 min at room temperature. At the end of the equilibrium period the solutions were again mixed. The tubes (15 ml Corex) were then centrifuged at 10 000 r.p.m. (Sorval, rotor SS-34, 12 000 g) at 4 °C for 20 min. The u.v. absorption at 260 nm (1 cm pathlength) of the top portion of the supernatant (approximately 1.5 ml) was then read to estimate the amount of DNA remaining in solution. A solution of DNA in the same buffered salt solution without added polypeptide was used as a control (100% soluble). It was thoroughly mixed to simulate conditions of addition, incubated, spun and read as above. The % DNA precipitated by each of the polymers under these standardized conditions is presented in Table 4.

Results

Synthesis of Monomers and Polypeptides

The synthetic scheme for preparation of the monomers and their polymerization is given in Scheme 1. All monomers were prepared from the common *N*-hydroxysuccinimide active ester **I** via a backing-off procedure (15) and led directly without racemization (24, 25) to the desired acid products. They were either purified and characterized directly or converted to their dicyclohexylamine (DCHA) salts for characterization (Table 1). For polymerization the acids were converted to their respective *N*-hydroxysuccinimide esters since it has been previously demonstrated that good yields of polymeric materials could be obtained using these esters (26–30). The *t*-Boc blocking group was removed and the resulting active ester trifluoroacetate (TFA) salt used for polymerization in DMF solution. During polymerization monomer concentration was kept high (approximately 1 *M*) in order to reduce the formation of cyclic and low molecular weight material and molecular weights were controlled by use of a chain terminator. The polymers were isolated by precipitation in acidified water and extracted with methanol to remove cyclic and low molecular weight material. The fully blocked polymers were generally isolated in 60–80% yield based upon the amount of free acid used to prepare the monomer active ester. Two treatments with HBr – acetic acid were then used to ensure complete removal of all the benzyloxycarbonyl blocking groups. The resulting hydrobromide salts were washed with isopropanol, rinsed with ether, and dried under high vacuum.

Polypeptide Molecular Weights

Molecular weight determinations of the polymers (Table 1) were performed on the fully blocked material by end-group titration using perchloric acid titration in acetic acid (9). After unblocking the molecular weights were again checked by gel filtration on Sephadex G-100 (10, 11). This latter determination allowed us to determine molecular weight distribution (Table 2)

TABLE 3. Physical constants and elemental analyses of lysine–glycine monomers

Compound	M.p. (°C) (crystallization solvent)	$[\alpha]_D$ (deg.)	Elemental analysis (%)					
			Calculated			Found		
			C	H	N	C	H	N
Cbz | *t*-Boc-lys-gly·DCHA	146–151 (EtOAc–petrol)	−3.39 (*c* 2.42, HOAc)	64.05	8.79	9.05	64.09	8.85	8.94
Cbz | *t*-Boc-lys-gly-gly·DCHA	128–130 (EtOAc–petrol)	−3.52 (*c* 2.21, HOAc) −2.52 (*c* 1.03, MeOH)	62.20	8.50	10.36	62.06	8.67	10.09
Cbz | *t*-Boc-gly-lys-gly-gly	73–75 (EtOAc–petrol)	−10.2 (*c* 1.00, HOAc)	54.43	6.76	12.70	54.30	6.85	12.52

TABLE 4. Amount (%) DNA precipitated by lysine–glycine polymers[a]

Polymer	Salt concentration (M)[b]				
	0.15	0.30	0.40	0.60	1.0
Poly-(L-lys)[c]	99	91	92	95	35(45[d])
Poly-(L-lys-gly)	63	32	12	0	0
Poly-(L-lys-gly-gly)	80	27	0	0	0
Poly-(gly-L-lys-gly-gly)	73	5	0	0	0

[a]Percentage precipitated was estimated at lysine – DNA phosphate ratio, 1.0 ± 0.05. All polypeptides were as their hydrobromide salts. All values are an average of three determinations. Estimated error in each value is ± 5%.
[b]Sodium chloride concentration in cacodylate buffer, 0.01 M, pH 7.0.
[c]Titrated molecular weight, 10 250.
[d]Titrated molecular weight, 20 540.

and determine if any degradation had occurred during the deblocking. As indicated the distributions were quite narrow and little degradation seemed to have occurred.

From the gel filtration results, one thing may be noted. At pH 7 all lysine–glycine polymers seemed to behave hydrodynamically very much like globular proteins, because the end-group titrated molecular weights and those determined by gel filtration analysis (when globular proteins were used as standards) were identical. This would indicate that the polypeptides were not adopting a rod shaped conformation but were completely disorganized in solution. This is in accord with following the circular dichroism results.

Circular Dichroic Spectra

Circular dichroic spectra have been used to investigate the conformation of polypeptides and proteins (31). Circular dichroic spectra of polypeptides in the various standard conformations are available (32, 33) and comparison of these with the circular dichroic spectra of the lysine–glycine polypeptides would indicate which, if any, of the standard conformations the synthetic polypeptides adopt.

Circular dichroic spectra under differing conditions of pH (7 and 12) and salt concentrations (0.14 and 1.0 M) have been taken of each of the polypeptides. The ellipticities at 225 nm, presented in Table 2, are based upon the concentration of lysine in solution. The ellipticity observed at 225 nm has been used to compare each of the polymers with poly-(L-lysine) in random and α-helical conformations (pH 7 and 12, respectively) and with N^{α}-acetyl-L-lysine amide under the same experimental conditions. The 225 nm band is sensitive to changes in environment (changes in salt concentration) and conformation (changes in pH) as is readily seen in the results obtained with poly-(L-lysine), molecular weight 10 250.

In general, as evidenced by the weak negative ellipticity at 225 nm, each one of the lysine–glycine polymers exhibited CD spectra at pH 7 (0.14 M NaF) which were apparently those of a random coil conformation. This may be contrasted with the poly-(L-lysine) spectrum which is that of an extended or disordered conformation under these conditions (34–36). When the pH of the solution was changed to 12 in order to neutralize the side-chain charges, the poly-(L-lysine) conformation shifted to that of the α-helix with the concomitant production of an intense negative band at 225 nm. With the lysine–glycine polymers, on the other hand, changing the salt concentration (0.14 to 1.0 M) or altering the charge on the side chains (pH 7 to 12) caused only slight changes in the shapes or intensity of the spectra, thus indicating that the insertion of one or more glycines into alternating positions along the lysine chain had hindered the formation of helical structure. In fact, the observed intensities of the n–π^* band at 225 nm were found to resemble those of N^{α}-actyl-L-lysine amide, again indicating that the lysine residues were behaving more or less independently and were not being influenced by their nearest neighbors. These observations on the influence of the glycine residue on helix formation are in accord with recent *ab initio* calculations made by Scheraga and co-workers (37).

In conclusion, circular dichroic spectra indicated that all lysine–glycine polymers were very flexible chains, lacking, for the most part, the rigidity observed in the polypeptide poly-(L-lysine).

DNA-Polypeptide Interactions

The ability of each of the lysine–glycine polymers to precipitate DNA from solution was determined in a manner similar to that used by Leng and Felsenfeld (20). Poly-(L-lysine) of two different molecular weights was used to allow comparison of our results (Table 4) with those previously reported. As can be seen, at a lysine: DNA-phosphate ratio of 1.0, poly-(L-lysine) was very effective in precipitating DNA from solution at salt concentrations below 1 *M*. In salt concentrations at or above 1 *M*, poly-(L-lysine) became decreasingly effective, as had previously been reported (20). At salt concentrations above 0.8 *M* the amount of DNA precipitated increased linearly with lysine – DNA-phosphate ratio. The amount precipitated also appeared to be dependent upon the molecular weight of the sample (see 1 *M* salt results).

Three things are immediately obvious when comparison is made of the abilities of the lysine-glycine polypeptides to precipitate DNA at lysine:DNA-phosphate ratios of 1.0: (*a*) all polymers are ineffective at precipitating DNA above sodium chloride concentrations of 0.40 *M*; (*b*) in 0.30 *M* salt solutions, their precipitating ability is apparently inversely proportional to either charge density or molecular weight; (*c*) the most striking difference among the polymers is seen under physiological conditions (*i.e.* p*H* 7.0, 0.15 *M* sodium chloride). Of all polymers, poly-(L-lysylglycine) precipitated the least at 0.15 *M* salt even though it has the highest molecular weight and charge density. Poly-(L-lysylglycylglycine) is almost as effective as poly-(L-lysine) under these conditions while poly-(glycyl-L-lysylglycylglycine), on the other hand, did not completely precipitate the DNA.

The differing abilities of the lysine–glycine polymers to precipitate DNA under the physiological conditions do not appear to be caused simply by changes in charge density along the peptide chain. It is unlikely to be a reflection of the initial conformational differences since the c.d. data indicates that each has roughly the same conformation. Rather, the differences seem to arise from the changing lysine–lysine spacings in the polymers.

Discussion

As can be seen from the c.d. spectra the lysine–glycine polymers exist in a random conformation. The insertion of an amino acid containing no side chain between the lysines seems to have caused a complete loss of the disordered or extended conformation of poly-(L-lysine) (34–36). These results seem to be in accord with recent *ab initio* calculations made on the conformational stabilities of polypeptides containing glycine residues (37) and support the fact that whenever one glycine residue, and most certainly whenever two are found in sequence, the surrounding polypeptide chain conformation will be broken.

The precipitation experiments show that considerable difference exists in the ability of the lysine–glycine polymers to precipitate DNA from solution, especially at physiological salt concentrations (0.15 *M*). These differences must reflect changes in primary sequence only and not changes in initial conformational states, since the c.d. spectra indicate that each has essentially the same conformation. The differences are not a reflection of changes in molecular weight since poly-(L-lysylglycine) with a molecular weight of 19 000 precipitated less DNA than poly-(L-lysylglycylglycine) with a molecular weight of 12 500. The cause of the differences must reside then in the changing lysine–lysine spacings which would lead to alterations in the degree of coincidence of lysines and DNA-phosphates in the complex.

The differences amongst the polymers can be satisfactorily explained by a modified version of a previous proposal (38). The distance between phosphate groups spacings along the sugar phosphate backbone in one strand of DNA (β-conformation) is 7.1 Å (39). In poly-(L-lysine) inter-lysine side chain distances depend upon helix geometry, but are approximately 3.5 Å in the disordered or zig-zag form (46). Thus, in poly-(L-lysine)-DNA complexes, every other lysine residue is out of phase with the phosphates, and thus is available to make not only inter-strand bonds which would cause helix disruption and lead to DNA aggregation but also bonds between two DNA helices and so crosslink the helices. This network would be more easily centrifuged from solution. In poly-(L-lysylglycine), the inter-lysine spacings are about 7 Å apart, depending again on the helix geometry. The random form, indicated by the c.d. results, could easily adopt a zig-zag form, and this would put the two side chains very nearly 7.2 Å apart (40). This distance would allow each lysine residue to be coincident with a DNA-phosphate within the same strand. The result would lead to little or no helix disruption and/or crosslinking

of the complex, and thus, a greater solubility. Similar arguments would explain the results obtained for the two other polypeptides; poly-(L-lysylglycylglycine) would have every second lysine out of phase, while each lysine in poly-(glycyl-L-lysylglycylglycine) could coincide with a DNA phosphate. The increased amount of cross-linking seen in going from poly-(L-lysylglycine) to poly-(glycyl-L-lysylglycylglycine) may be explained by a diminished cooperative factor by which one lysine is able to position the subsequent lysine for the making of the next lysine–phosphate bond.

The results we have obtained through the precipitation experiments may have relevance to the interaction of proteins, especially of lysine-rich histones, with DNA (41). Our observations on the interactions of the polypeptides with DNA indicate a similarity in behavior between the lysine-rich histone F1 and the polypeptides described here, since little or no complex formation was observed above 0.4 *M* salt. Histones F1 can be dissociated from the calf thymus chromatin complex quite readily with salt concentrations around 0.5 *M* (41). These observations might lead one to conclude that the electrostatic bonds between DNA and histone F1 are either very accessible to dissociation and the number of charged amino acid groups are spread along the chains. Examination of the primary sequence of several F1 fractions indicates that only two short blocks of basic amino acids exist in the molecule whereas the remainder of the basic groups are spread throughout the chain (42). This distribution of charges would make it more easily dissociated from complexes with DNA.

Experiments, now in progress, are aimed at examining the physicochemical nature of complexes formed between these lysine–glycine polypeptides and DNA's of various base compositions.

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