BASIC POLYPEPTIDES AS MODELS OF HISTONES: SYNTHESIS, CONFORMATION AND INTERACTION WITH DNA OF SEQUENTIAL POLYPEPTIDES CONTAINING LYSINE, ALANINE, AND PROLINE

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By means of polymerisation of pentachlorophenyl esters of the corresponding oligopeptides with side chains protected by benzyloxycarbonyl groups, and subsequent removal of the blocking groups, the following sequential polymers were synthesized: poly(L-Ala-L-Lys-L-Pro) (*Ic*), poly(L-Ala-L-Pro-L-Lys) (*IIc*), poly(L-Ala-L-Lys-L-Pro-L-Lys) (*IIIc*), and poly(L-Ala-L-Lys-L--Lys-L-Pro-L-Lys) (*IVc*). The molecular weights of substances *Ic* and *IVc* were about 10 000, those of substances *IIc* and *IIIc* were lower. The chiroptical properties of these polymers were measured in dimethylformamide, in 90% 1-propanol and in aqueous solutions with varied ionic strength and pH. The results did not reveal any markedly ordered conformation under the conditions of measurement. Polymers *Ic* and *IVc* interact selectively with DNA with a higher content of adenine and thymine. Complexes of DNA with *Ic* and *IVc* have similar properties to complexes of DNA with histone F1, more marked in the case of polymer *Ic* where similar properties with histone F1 were observed over the whole range of ionic strengths.

The carrier of genetic information in higher organisms (eukaryots) is the DNA molecule. At the present time the organisation of these extremely large linear macromolecules[1] has been studied. This organisation is related in all probability to expression of the genetic information contained in the active parts of the DNA molecule. The most important elements of this organisation are molecules of basic proteins — histones — found in the nucleus in close interaction with DNA. It has been shown that there exist only several types of histone molecules which differ in their amino-acid sequence and in other properties[2]. There are a number of published observations on the behaviour of native, partially dehistonised and reconstituted nucleohistones which suggest that different types of histones play different roles in the organisation of genetic material[3]. Since the properties of individual histones, their interaction with DNA and the function of histones in nuclear material appear to be very complex, an attempt has been made to study simplified model systems in order to acquire information to assist interpretation of experimental findings. Model polypeptides were synthesized which simulate some of the properties of histones. Further, their conformation and interactions with DNA were studied and compared with data obtained from reconstituted or partial nucleohistones. This approach would appear to be justifiable since histone

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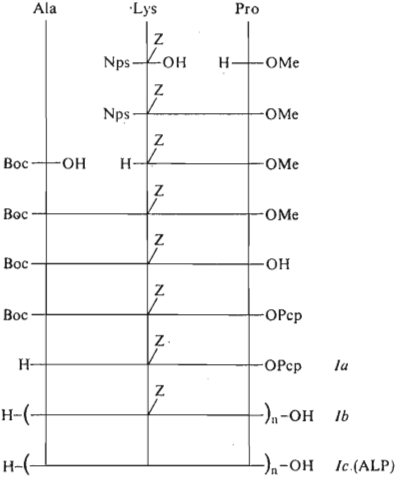
molecules do not form any rigid globular structures but consist of a single conformationally flexible polypeptide chain. Similarly as with synthetic polypeptides, their conformation in solution is strongly dependent upon the environment[3].

Our approach to the design of models of histones arises from the amino-acid composition and sequence which at present is completely, or almost completely, known in all basic histone types[2,4] and from the conformational properties of histones in solution as studied by NMR, CD, hydrodynamic methods, *etc.* From the results of these observations it would appear that the distribution of amino-acid residues in the histone chain is not regular and the chain is divided in several parts with varied properties[5]. These individual parts of the chain obviously have different functions in interaction with DNA and organisation of the structure of chromatin. Histone F1 (also labelled I) is in many respects different from other types of histones. It contains a high percentage of lysine (27%), alanine (24%), and proline (9%) residues which together form more than 60% of the 216 amino-acid residues in the entire molecule. Of the 40 first amino-acid residues from the N-terminal and from the further half of the molecule (residues 107—216) 87—88% of the chain is formed by residues of lysine (51 residues), alanine (47 residues) and residues of helix breaking amino acids (35 residues) the most pronounced of which is proline (22 residues)[6]. Despite the high content of basic amino acids histone F1 dissociates from native nucleohistone in a solution 0·4—0·5M-NaCl much more easily than other histones[7], and does not aggregate at higher ionic strengths, although a smaller portion of the chain would appear to be immobilised by inter- or intramolecular interactions[5]. At approximately physiological ionic strength (0·15—0·20M-NaCl) histone F1 selectively



SCHEME 1

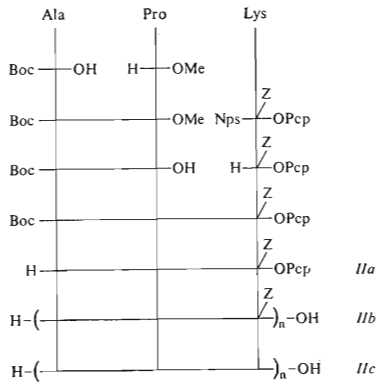
binds to regions of DNA richer in adenine and thymine[8]. Complexes in this solution show characteristic CD spectra dependent not only on the concentration of the complex, ionic strength, the presence of various ions and organic solvents [8–10] but also on molecular weight and the average composition of DNA (ref.[11]). The presence of histone F1 in nucleohistones has only a small effect on the X-ray diffraction of fibres of nucleoprotein gel[12], but its presence is necessary for condensation of nucleohistones[13] and chromosomes[14] at approximately physiological ionic strength.

In the present work we have attempted to simulate characteristic amino-acid composition of the major portion of the histone F1 chain as a sequence composed of residues of lysine, alanine, and proline. The synthesis of several sequential polypeptides containing these amino acids is described and the conformation of the synthesized polypeptides in solution has been studied as a function of the environement along with the interaction of these polypeptides with DNA and their effect on the arrangement of DNA.

A preliminary communication dealing with the behaviour of some lysine-containing synthetic models and of their complexes with DNA has already been reported[15].

SYNTHESES

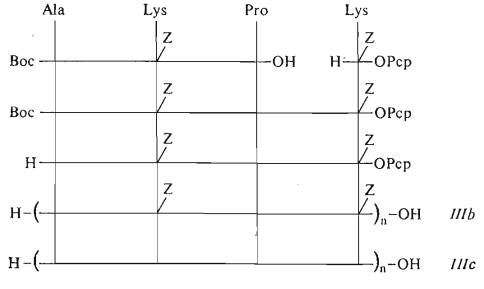
The preparation of sequential polymers is usually carried out by polymerisation of active esters of monomeric oligopeptides[16–18]. One disadvantage of this approach in comparison with the preparation of random copolymers by means of N-carboxy-



SCHEME 2

anhydrides is the relatively low molecular weight of the products, ranging from 4000 to 20000. For the present purposes it was not necessary to produce a high molecular weight polymer. A molecular weight range of 10000–20000, comparable to those of histones or the characteristic parts of their chain[3], was sufficient. However, it proved to be far from simple to attain this molecular size. Molecular weight of prepared polymers is apparently dependent upon *a*) the character of the activating group, *b*) the amino-acid sequence of the monomer and its length, *c*) the solubility of the monomer and the polymer in the solvent used. According to Johnson[17] the highest molecular weights are attainable with the use of pentachlorophenyl esters, and these were used in the present work. The presence of residues of proline in the monomeric unit can result in difficulties in polymerisation to the required degree[19,20]. This difficulty was dealt with by a change in the amino-acid sequence in the basic tripeptide. We therefore prepared derivatives of two tripeptides with sequences* L-Ala-L-Lys-L--Pro and L-Ala-L-Pro-L-Lys. The synthetic procedure is shown in schemes 1 and 2. Polymerisation of the trifluoroacetates of pentachlorophenyl esters of tripeptides *Ia* and *IIa*, carried out in dimethylformamide in the presence of triethylamine, yielded in the first case polymer *Ib* with utilisable properties. In the second case product *IIb* contained on paper chromatography a large fraction of low molecular weight oligomers. Differences in the results of polymerisation can be explained by the steric consequences of changes in position of the proline residue in the monomeric unit *IIa*.

In an attempt to increase the content of lysine residues in the polypeptide chain we further prepared derivatives of the tetrapeptide L-Ala-L-Lys-L-Pro-L-Lys (*IIIa*) and the pentapeptide L-Ala-L-Lys-L-Lys-L-Pro-L-Lys (*IVa*) (see schemes 3 and 4).



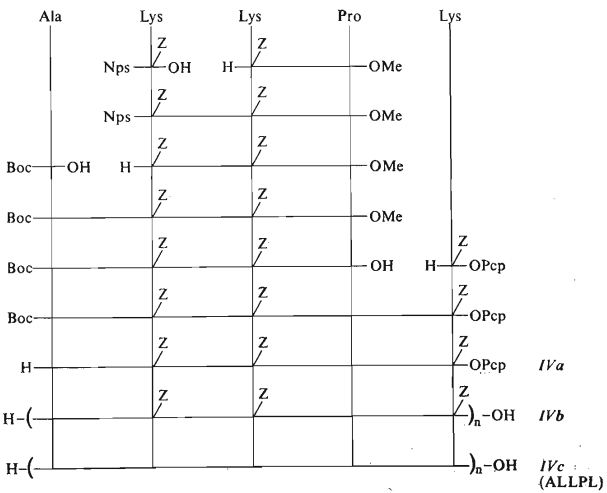
SCHEME 3

* The nomenclature and symbols comply with published suggestions[21].

Material *IIIc*, obtained by polymerisation of tetrapeptide *IIIa* similarly as in the case of tripeptide L-Ala-L-Pro-L-Lys, was for the most part formed of low molecular weight oligopeptides. Obviously in this case the proline residue in the sequence of the monomeric unit makes polymerisation difficult. The pentapeptide monomer *IVa*, the longer chain of which is no longer deformed unsuitably, gave a satisfactory polymer. It should be stressed that because of the poor solubility of this substance at room temperature, the monomer was dissolved at increased temperature. It would not appear, however, that this latter factor influenced polymerisation in a decisive manner, since an attempt at polymerisation of the L-alanyl-L-prolyl-N^{ε}-benzyloxycarbonyl--L-lysine pentachlorophenyl ester (*IIa*) under the same conditions (heating to 80°C) yielded material of the same properties as with polymerisation at room temperature.

EXPERIMENTAL

Melting points were determined on a Kofler block and were not corrected. Optical rotations were determined on a photoelectric polarimeter at 25°C, concentration 0·5% in dimethylformamide, unless otherwise stated. Samples for analysis were dried at 0·5—1 Torr over P_2O_5 (or KOH in the



SCHEME 4

case of a salt) at room temperature for at least 24 h. Paper chromatography was carried out in the system 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 12) on Whatman 3MM paper in a descending manner for 24 h. The electrophoresis was carried out in a wet chamber[22] at a potential gradient of 20 V/cm on Whatman 3MM paper in 1M acetic acid (pH 2·4) and in pyridinium acetate buffer (pH 5·7). Substances with a free amino group were detected by ninhydrin, others by *o*-tolidine after exposure to chlorine (on paper[23], in thin layer[24]). Amino-acid analyses were carried out after hydrolysis (6M-HCl, 105°C, 20 h) on an automatic Amino acid analyser 6020 A (Developmental Workshops, Czechoslovak Academy of Sciences). Evaporation of dimethylformamide was carried out at 2—3 Torr, other solvents were evaporated at 15—20 Torr at a temperature which did not exceed 40°C.

With standard condensation using N,N'-dicyclohexylcarbodiimide the condensation reagent was added to a solution of both components in chloroform (5 ml per mmol) at —15°C. After 60 min the temperature was increased to 0°C, after a further 120 min to room temperature. The next day the reaction mixture was evaporated, the residue was dissolved in ethyl acetate (0°C) and the N,N'-dicyclohexylurea which separated out was filtered off. The filtrate was washed with 0·2M-H_2SO_4, water, 5% sodium hydrogen carbonate, and water, dried and evaporated. The residue usually crystallised after grinding with light petroleum or ether.

Bis(4-toluenesulphonyl)imide Salt of the L-Proline Methyl Ester

A suspension of L-proline (11·5 g; 0·1 mol) in methanol (300 ml) was saturated with HCl. The solution was refluxed for 3 h and evaporated. The residue was dissolved in chloroform, a saturated solution of ammonia in chloroform was added and the ammonium chloride which separated out was filtered off. The filtrate was evaporated and the residue dissolved in ether (500 ml) and mixed with a saturated solution of bis(4-toluenesulphonyl)imide (30·9 g) in ethyl acetate. The product was filtered off: yield 41·8 g (92%), m.p. 157—158°C, $[\alpha]_D$ —12·6° (methanol). For $C_6H_{11}NO_2 \cdot C_{14}H_{15}NO_4S_2$ (454·6) calculated: 52·85% C, 5·77% H, 6·16% N; found: 52·61% C, 5·83% H, 6·08% N.

Bis(4-toluenesulphonyl)imide Salt of the N^{ε}-Benzyloxycarbonyl-L-lysyl-L-proline Methyl Ester

A protected dipeptide was prepared from the dicyclohexylammonium salt of 2-nitrobenzenesulphenyl-N^{ε}-benzyloxycarbonyl-L-lysine (18·44 g, 30 mmol), the bis(4-toluenesulphonyl)imide salt of the proline methyl ester (13·63 g, 30 mmol) and N,N'-dicyclohexylcarbodiimide (6·81 g, 33 mmol). To a solution of the crude product in boiling methanol (100 ml) bis(4-toluenesulphonyl)imide (9·76 g, 30 mmol) was added and the mixture was refluxed for 90 min and evaporated. The residue was ground with ether. Crystallisation from methanol–ether yielded 15·71 g (73%) of a substance with m.p. 138—140°C, $[\alpha]_D$ —20,0°. For $C_{20}H_{29}N_3O_5 \cdot C_{14}H_{15}NO_4S_2$ (716·9) calculated: 56·97% C, 6·19% H, 7·82% N; found: 57·07% C, 6·29% H, 7·91% N.

Tert-butyloxycarbonyl-L-alanyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-proline

The protected tripeptide was prepared from the bis(4-toluenesulphonyl)imide salt of the N^{ε}-benzyloxycarbonyl-L-lysyl-L-proline methyl ester (7·17 g, 10 mmol), tert-butyloxycarbonyl-L-alanine (1·89 g, 10 mmol) and N,N'-dicyclohexylcarbodiimide (2·27 g, 11 mmol) with addition of N-ethylpiperidine (1·37 ml). To a solution of the crude product in methanol (10 ml) 5 ml of 1M-NaOH were added. After 90 minutes the methanol was evaporated, the remaining aqueous solution was extracted with ethyl acetate, acidified with 0·2M-H_2SO_4 and the product was extracted twice into

ethyl acetate. Both extracts were pooled, washed with water, dried and evaporated, giving a foamy residue (4·90 g, 83·9%). For $C_{27}H_{40}N_4O_8$ (548·6) calculated: 59·11% C, 7·35% H, 10·21% N; found: 58·87% C, 7·49% H, 10·02% N.

L-Alanyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-lysyl-L-proline Pentachlorophenyl Ester Trifluoroacetate (*Ia*)

To a solution of tert-butyloxycarbonyl-L-alanyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-lysyl-L-proline (2·67 g, 4·9 mmol) and pentachlorophenol (1·33 g, 5 mmol) in ethyl acetate (25 ml) N,N′-dicyclohexylcarbodiimide (1·03 g, 5 mmol) was added. The mixture was worked up and the protected active ester was dissolved in trifluoroacetic acid (2 ml). After 15 min standing the solution was added to ether (250 ml) and the separated solid dried in an exsiccator. The yield was 0·99 g (41%), m.p. 96–100°C, $[\alpha]_D$ −20·2°. For $C_{28}H_{31}Cl_5N_4O_6 \cdot C_2HF_3O_2$ (810·9) calculated: 44·44% C, 3·98% H, 6·91% N; found: 44·35% C, 4·23% H, 7·15% N.

Poly(L-alanyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-lysyl-L-proline) (*Ib*)

To a solution of *Ia* (0·99 g, 1·2 mmol) in dimethylformamide (0·4 ml) triethylamine was added (0·16 ml) and the mixture was stirred until it gelled. After five days the gel was ground up with water, the polymer which separated out was decanted, dissolved in ethanol, evaporated with benzene three times and the residue was extracted with boiling 2-propanol. The insoluble fraction was washed with hot 2-propanol and dried. The yield was 0·31 g (59%) with m.p. 153–157°C, $[\alpha]_D$ −70·4°. For $(C_{22}H_{30}N_4O_5)_n$ (n. 430·5) calculated: 61·38% C, 7·02% H, 13·01% N; found: 60·61% C, 7·10% H, 12·64% N. Amino-acid analysis: Lys 1·01, Pro 1·01, Ala 0·98.

Poly(L-alanyl-L-lysyl-L-proline) (*Ic*)

A solution of polymer *Ib* (0·18 g) in trifluoroacetic acid (1 ml) was added to 35% HBr in acetic acid (3 ml). After 30 min the mixture was diluted with ether, decanted several times with ether and centrifuged. After drying, the hydrobromide of the polymer was dissolved in water and filtered through a column of Zerolite FF (OH^- form). The pH of the eluate was adjusted to 3 with HCl, evaporated and the residue was dried. Reprecipitation from a methanolic solution with ether gave a yield of 0·11 g (78%) of the hydrochloride of polymer *Ic* which did not melt up to 260°C, $[\alpha]_D$ −206·8° (*c* 0·2, water).

2-Nitrobenzenesulphenyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-lysine Pentachlorophenyl Ester

A suspension of the dicyclohexylammonium salt of 2-nitrobenzenesulphenyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-lysine (9·22 g, 15 mmol) in 0·2M-H_2SO_4 (50 ml) was shaken up with ethyl acetate (100ml) until it dissolved. The organic layer was washed with water, dried and evaporated. The residue was dissolved in ethyl acetate (75 ml) and to the solution pentachlorophenol (5·0 g; 16·9 mmol) and at −20°C N,N′-dicyclohexylcarbodiimide (3·15 g, 17·5 mmol) were added succesively. Crystallisation from ethanol gave 5·48 g (87%) of a substance with m.p. 118–119°C, $[\alpha]_D$ −32·8°. For $C_{26}H_{22}Cl_5N_3O_6S$ (681·8) calculated: 45·80% C, 3·25% H, 6·16% N; found: 45·80% C, 3·46% H, 6·14% N.

N$^{\varepsilon}$-Benzyloxycarbonyl-L-lysine Pentachlorophenyl Ester Hydrochloride

To a solution of the 2-nitrobenzenesulphenyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-lysine pentachlorophenyl ester (21·75 g, 31·9 mmol) in dioxane (150 ml) a solution of 96 mmol HCl in ether was added. The

product which separated out was filtered, washed with ether and dried. The yield was 17·65 g (98%) of a substance with m.p. 146—148°C, $[\alpha]_D$ +12·5°. For $C_{20}H_{19}Cl_5N_2O_4$. HCl (565·1) calculated: 42·51% C, 3·57% H, 4·96% N; found: 42·76% C, 3·61% H, 4·98% N.

Tert-butyloxycarbonyl-L-alanyl-L-proline

The bis(4-toluenesulphonyl)imide salt of the L-proline methyl ester (4·55 g, 10 mmol) was added to a saturated solution of ammonia in chloroform. The salt which separated out was filtered off and the filtrate evaporated. The residue was dissolved with tert-butyloxycarbonyl-L-alanine (1·60 g, 9 mmol) in chloroform and the synthesis was carried out with N,N'-dicyclohexylcarbodiimide (2·06 g, 10 mmol) with addition of 1-hydroxybenzotriazole (1·35 g, 10 mmol). To a solution of the crude product in acetone (30 ml) 1M-NaOH (10 ml) was added. After 1 h the mixture was diluted with the same volume of water, acetone was evaporated off, the remaining aqueous solution was extracted with ethyl acetate and acidified with 0·2M-H_2SO_4. The crystals which separated out were filtered off, washed with water and ether and dried. Crystallisation from ethyl acetate–light petroleum gave 1·54 g (54%) of a substance with m.p. 156—157°C (decomposition), $[\alpha]_D$ —74·5°. For $C_{13}H_{22}N_2O_5$ (286·3) calculated: 54·53% C, 7·74% H, 9·78% N; found: 54·60% C, 7·82% H, 9·91% N.

Tert-butyloxycarbonyl-L-alanyl-L-prolyl-N^{ε}-benzyloxycarbonyl-L-lysine Pentachlorophenyl Ester

The active ester of the tripeptide was obtained from the pentachlorophenyl ester of N^{ε}-benzyloxycarbonyl-L-lysine (2·83 g, 5·0 mmol), tert-butyloxycarbonyl-L-alanyl-L-proline (1·05 g, 5·1 mmol) and N,N'-dicyclohexylcarbodiimide (1·13 g, 5·5 mmol) with addition of N-ethylpiperidine (0·7 ml). The yield was 3·47 g (87%), m.p. 113—115°C (chloroform–light petroleum), $[\alpha]_D$ —36·4°. For $C_{33}H_{39}Cl_5N_4O_8$ (797·0) calculated: 50·81% C, 6·26% H, 8·01% N; found: 50·56% C, 6·10% H, 7·88% N.

L-Alanyl-L-prolyl-N^{ε}-benzyloxycarbonyl-L-lysine Pentachlorophenyl Ester Trifluoroacetate (*IIa*)

The tert-butyloxycarbonyl group was split off from the tert-butyloxycarbonyl-L-alanyl-L-prolyl--N^{ε}-benzyloxycarbonyl-L-lysine pentachlorophenyl ester (2·0 g, 2·5 mmol) by the same procedure as with compound *Ia*. The yield was 0·87 g (43%) of a substance which gradually melted up to 115°C, $[\alpha]_D$ —29·4° (*c* 0·16). For $C_{28}H_{31}Cl_5N_4O_6$. $C_2HF_3O_2$ (810·9) calculated: 44·44% C, 3·98% H, 6·91% N; found: 44·08% C, 4·05% H, 6·68% N.

Poly(L-alanyl-L-prolyl-N^{ε}-benzyloxycarbonyl-L-lysine) (*IIb*)

To a solution of the activated monomer *IIa* (0·89 g, 1·1 mmol) in dimethylformamide (0·7 ml) triethylamine (0·17 ml) was added. The viscous mixture was stirred and after 5 days diluted with water. The matter which separated out was ground with water, dissolved in ethanol, evaporated three times with benzene and three times precipitated with ether from a chloroform solution, The yield was 0·24 g (56%) of a polymer of m.p. 125—127°C, $[\alpha]_D$ —30·4°. For $(C_{22}H_{30}N_4O_5)_n$ (n. 430·5) calculated: 61·38% C, 7·02% H, 13·01% N; found: 60·85% C, 6·94% H, 12·52% N. Amino-acid analysis: Lys 1·10, Pro 0·94, Ala 0·95.

Poly(L-alanyl-L-prolyl-L-lysine) (*IIc*)

The benzyloxycarbonyl group was split off in the same manner as with the preparation of polymer *Ic*. From 132 mg of protected polymer *IIb* we got a yield of 91·5 mg (89%) of the hydrochloride of polymer *IIc*, m.p. 246—247°C, $[\alpha]_D$ —113·2° (*c* 0·2, water).

Tert-butyloxycarbonyl-L-alanyl-N$^\varepsilon$-benzyloxycarbonyl-L-lysyl-L-prolyl-N$^\varepsilon$-benzyloxycarbonyl-L-lysine Pentachlorophenyl Ester

The protected tetrapeptide was obtained from tert-butyloxycarbonyl-L-alanyl-N$^\varepsilon$-benzyloxycarbonyl-L-lysyl-L-proline (2·33 g, 4·06 mmol), the hydrochloride of N$^\varepsilon$-benzyloxycarbonyl-L-lysine pentachlorophenyl ester (2·29 g, 4·06 mmol) and N,N′-dicyclohexylcarbodiimide (0·93 g, 4·5 mmol) with the addition of N-ethylpiperidine (0·56 ml). The yield was 1·84 g (43%) of a substance with m.p. 110—112°C (ethyl acetate–ether–light petroleum), $[\alpha]_D$ —28·3°C. For $C_{47}H_{57}Cl_5N_6O_{11}$ (1059) calculated: 53·29% C, 5·42% H, 7·93% N; found: 53·53% C, 5·62% H, 8·21% N.

L-Alanyl-N$^\varepsilon$-benzyloxycarbonyl-L-lysyl-L-prolyl-N$^\varepsilon$-benzyloxycarbonyl-L-lysine Pentachlorophenyl Ester Trifluoroacetate (*IIIa*)

A mixture of the previous ester (2·0 g, 1·9 mmol) and trifluoroacetic acid (2 ml) was left for 15 min and then poured into ether (300 ml). The precipitate was decanted three times with ether and dried. The yield was 1·41 g (70%) of a substance with m.p. 92—94°C, $[\alpha]_D$ —18·6°. For $C_{42}H_{49}Cl_5N_6O_9 . C_2HF_3O_2$ (1073) calculated: 49·25% C, 4·70% H, 7·83% N; found: 49·55% C, 4·79% H, 8·03% N.

Poly(L-alanyl-N$^\varepsilon$-benzyloxycarbonyl-L-lysyl-L-prolyl-N$^\varepsilon$-benzyloxycarbonyl-L-lysine) (*IIIb*)

To a solution of the trifluoroacetate of *IIIa* (0·80 g, 0·75 mmol) in dimethylformamide (0·5 ml) triethylamine (0·11 ml) was added. After 5 days the mixture was diluted with ether, the product which separated out was ground with ether and then water, dissolved in a mixture of ethanol–benzene and evaporated. The residue was ground with ether, the powder which resulted was filtered off, extracted with hot ethyl acetate and dried. The yield was 0·39 g (74%) of a product with m.p. 122—127°C (with softening from 119°C). The sample for analysis was dried 72 h at 60°C and 0·5 Torr, the m.p. did not change, $[\alpha]_D$ —19·3°. For $(C_{36}H_{48}N_6O_8 . 1/2\ H_2O)_n$ (n. 701·8) calculated: 61·61% C, 7·04% H, 11·97% N; found: 61·48% C, 6·95% H, 11·89% N. Amino-acid analysis: Lys 1·97, Pro 1·01, Ala 1·02.

Poly(L-alanyl-L-lysyl-L-prolyl-L-lysine) (*IIIc*)

The protected polymer *IIIb* (0·34 g) yielded, with the same procedure as described for polymer *Ic*, 0·26 g (92%) of product *IIIc* as the hydrochloride, m.p. 223—224°C; $[\alpha]_D$ —102·5° (*c* 0·2,water).

Tert-butyloxycarbonyl-L-alanyl-N$^\varepsilon$-benzyloxycarbonyl-L-lysyl-N$^\varepsilon$-benzyloxycarbonyl-L-lysyl-L-proline

The protected tripeptide was prepared from the dicyclohexylammonium salt of 2-nitrobenzenesulphenyl-N$^\varepsilon$-benzyloxycarbonyl-L-lysine (3·1 g, 5 mmol), bis(4-toluenesulphonyl)imide salt of the N$^\varepsilon$-benzyloxycarbonyl-L-lysyl-L-proline methyl ester (3·6 g, 5 mmol) and N,N′-dicyclohexylcarbodiimide (1·13 g, 5·5 mmol). The oily product was dissolved in methanol and the 2-nitrobenzenesulphenyl group was split off with HCl (6 mmol) in methanol. After evaporation the crude hydro-

chloride of the tripeptide ester was chromatographed on a column of silica gel (60—120 μ; Service Laboratories, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague - Lysolaje; column 80 × 6 cm). Benzene was used at first to wash out a yellow methyl 2-nitrobenzenesulphenate and then a mixture of benzene and methanol, 10 : 1 (about 500 ml), eluted the product. The ninhydrin positive fractions were pooled and evaporated, the residue was dissolved in chloroform saturated with ammonia, the ammonium chloride which separated out was filtered off and the filtrate was evaporated. After drying there was a yield of 1·14 g (35%) of an oily N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-proline methyl ester. This ester (1·75 mmol) was condensed with tert-butyloxycarbonyl-L-alanine (0·32 g, 1·75 mmol) by the action of N,N′-dicyclohexylcarbodiimide (0·40 g, 1·94 mmol) with addition of 1-hydroxybenzotriazole (0·27 g, 2 mmol). The crude product was dissolved in acetone (10 ml) and treated with 1M-NaOH (2 ml). The solution was stirred for 90 min, acidified with 0·2M-H_2SO_4 and the product which separated out was extracted into ethyl acetate. After drying and evaporating the yield was 2·16 g (56%) of a substance of m.p. 57—59°C, $[\alpha]_D$ −27·6°. For $C_{41}H_{58}N_6O_{11} \cdot 1/2\ H_2O$ (820·0) calculated: 60·06% C, 7·25% H, 10·02% N; found: 59·90% C, 7·34% H, 10·02% N.

Tert-butyloxycarbonyl-L-alanyl-N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-prolyl-N^{ε}-benzyloxycarbonyl-L-lysine Pentachlorophenyl Ester

From the previous tetrapeptide (1·11 g, 1·35 mmol), the hydrochloride of the pentachlorophenyl ester of N^{ε}-benzyloxycarbonyl-L-lysine (0·76 g, 1·35 mmol) and N,N′-dicyclohexylcarbodiimide (0·31 g, 1·50 mmol) with the addition of N-ethylpiperidine (0·185 ml) we got a yield of 1·58 g (88%) of a product with m.p. 116—118°C, $[\alpha]_D$ −29·0°. For $C_{61}H_{75}Cl_5N_8O_{14}$ (1322) calculated: 55·44% C, 5·72% H, 8·48% N; found: 55·26% C, 5·51% H, 8·61% N.

L-Alanyl-N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-prolyl-N^{ε}-benzyloxycarbonyl-L-lysine Pentachlorophenyl Ester Trifluoroacetate (*IVa*)

The pentapeptide ester from the previous preparation (1·53 g, 1·15 mmol) was treated with trifluoroacetic acid (5 ml). After 15 min the mixture was poured into ether (300 ml), the precipitate was filtered off and the product was dried. The yield was 0·91 g (59%) of a product with m.p. 93—94°C, $[\alpha]_D$ −30·0°. For $C_{56}H_{67}Cl_5N_8O_{12} \cdot C_2HF_3O_2$ (1335) calculated: 52·16% C, 5·13% H, 8·39% N; found: 52·41% C, 5·32% H, 8·64% N.

Poly(L-alanyl-N^{ε}-benzyloxycarbonyl-L-lysyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-prolyl-N^{ε}-benzyloxycarbonyl-L-lysine) (*IVb*)

The trifluoroacetate of *IVa* (150 mg, 0·11 mmol) was dissolved at 80°C in dimethylformamide (0·15 ml) and to this solution triethylamine (0·025 ml) was added. After 3 days standing at room temperature the resulting gel was ground up several times with water and extracted repeatedly with boiling acetone. The resulting powder was centrifuged and dried. The yield was 106 mg (98%) of a product with m.p. 235—240°C, $[\alpha]_D$ −33·4° (*c* 0·14). For $(C_{50}H_{66}N_8O_{11})_n$ (n. 955·1) calculated: 62·88% C, 6·96% H, 11·73% N; found: 62·55% C, 6·89% H, 11·85% N. Amino-acid analysis: Lys 3·07, Pro 0·94, Ala 1·01.

Poly(L-alanyl-L-lysyl-L-lysyl-L-prolyl-L-lysine) (*IVc*)

From protected polymer *IVb* (69 mg) we achieved, using the same procedure as in preparing substance *Ic* a yield of 43 mg (90%) of the hydrochloride of substance *IVc* which did not melt up to 260°C, $[\alpha]_D$ −140·9° (*c* 0·2, water).

Preparation of Complexes

The DNA and histone F1 used have been described elsewhere[8,11]. A solution of the polypeptide hydrochloride and DNA with a given molar ratio of the lysine residue to the nucleotide (Lys/DNA) in 2M-NaCl buffered with 0·013M sodium phosphate at pH 6·8 was flow-dialysed against a linear gradient of NaCl solution molarity. The arrangement suggested by Carroll[25] was used, consisting of solution 1, against which the sample is dialysed and the volume of which, V_1, is kept constant during the dialysis, and solutions 2 and 3, the mixture of which gives the required gradient. The initial volume and molarity of all solutions were calculated in such a way that the molar gradient would be linear assuming a constant flow[25]: V_1 equals 300 ml, $c_1^0 = 1{\cdot}5$M-NaCl + 0·013M sodium phosphate; $V_2^0 = 1420$ ml, $c_2^0 = 1{\cdot}35$M-NaCl + 0·013M sodium phosphate; $V_3^0 = 1420$ ml, $c_3^0 = 0{\cdot}013$M sodium phosphate (the superscript ^{0} means initial volume or concentration). The flow rate was about 400 ml/h, the final NaCl molarity was 0·2M — 0·15M. In order to follow complex formation we withdrew individual samples during flow dialysis and the NaCl molarity of the sample was determined by refractometry; to obtain a complex in 0·15M-NaCl the sample was dialysed 15—18 h against 0·15M-NaCl + 0·013M sodium phosphate after the end of the flow dialysis. The low ionic strength was created by 0·01M Tris buffer, pH 7·0; complexes were dialysed against several exchanges of this buffer for a further 15—18 h. Along with the sample of a complex we always dialysed the DNA alone with a known initial concentration, in order to correct for possible volume and concentration changes.

The fraction of DNA in aggregated complex (f_{ppt}) was determined by centrifugation of the sample 30 min at 10 000 *g* and measurement of absorbance of the supernatant at 260 nm. Concentration of DNA was determined on the basis of values of $A_{1\,cm}^{1\%} = 200$ and the molar concentration of nucleotides was calculated using the mean molecular weight of the nucleotide residue 330.

Melting Curves

Melting curves of DNA and supernatants after centrifuging aggregated complexes were measured in 4M-$NaClO_4$ into which the measured sample was introduced by dialysis. The measurement was carried out in a Uvispek spectrophotometer in 1 cm cells. Cell temperature was changed continuously and the temperature was recorded by means of a thermocouple.

Sedimentation Analysis

Sedimentation measurements were carried out in an analytical ultracentrifuge Spinco model E. Sedimentation coefficients of the polypeptides were determined in dimethylformamide or in 0·15M-NaCl + 0·013M sodium phosphate in a synthetic boundary cell with an optical pathway 12 mm using maximum speed, and photographs were taken by schlieren optics. The sedimentation coefficients of DNA were measured by UV absorption systems in cells of optical path length 30 mm using solutions of concentration 10—15 μg/ml. Values of sedimentation coefficients were corrected for viscosity and density of the solvent. The molecular weight of DNA was calculated according to Crothers and Zimm[26].

Measurement of the molecular weight of the polypeptides by sedimentation equilibria was carried out by the method of Yphantis[27] using an interference optical record. The measurements were made in solutions of 0·03% polypeptide dialysed against 0·15M NaCl + 0·013M sodium phosphate and centrifuged at 47 660 r.p.m. at 20°C. The time required for attaining equilibrium was 15 h. Molecular weight was calculated using the value of partial specific volume $\bar{v} = 0{\cdot}722$ ml/g.

Measurement of Circular Dichroism and Optical Rotatory Dispersion

Circular dichroism spectra of polypeptides were measured on a spectropolarimeter Cary 61 in the region 260—195 nm in cells of optical pathways 0·05 and 0·01 cm. The concentrations of the solutions were about 0·1%. Correction of the pH of the solutions was made by adding the appropriate amount of 0·1M-NaOH to the original neutral solution (0·01M Tris, pH 7), the final pH was then measured with an accuracy of 0·1 units. Circular dichroism was expressed in molar ellipticities $[\Theta]$ (deg. cm^{-2} . $dmol^{-1}$) taking into account the average molecular weight of the amino-acid residues for the given peptide.

Optical rotatory dispersion in the region 550—300 nm was measured on a spectropolarimeter Jasco-ORD/UV-5 in a cell of optical pathway 10 cm at 27°C. The concentration of the sample in dimethylformamide was about 0·2%. The data were analysed with the Moffitt–Yang equation[28] using parameter b_0 as a measure of the helix content.

The CD spectra of the complexes in the region 300—200 nm were measured on a Roussel Jouan Dichrograph CD 185 in cells of optical pathway 0·5 cm at 22—24°C. The concentration of the

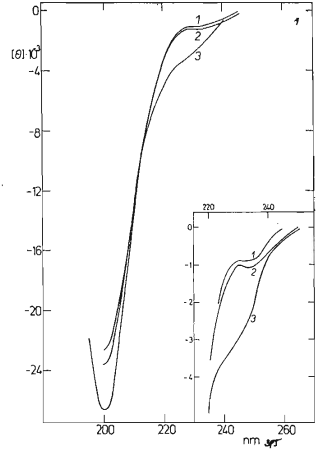


FIG. 1

CD Spectra of Polymeric Tripeptide *Ic* (ALP) as a Function of Ionic Strength

Inserted figure: $n \rightarrow \pi^*$ transition with scale expansion. *1* 0·01M Tris, pH 7·3; *2* 0·15M-NaCl; *3* 2M-NaCl.

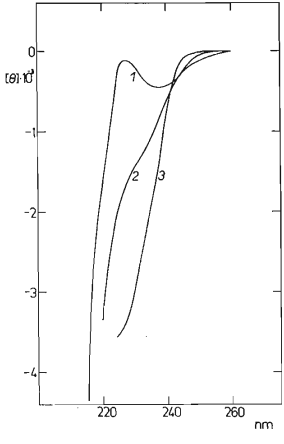


FIG. 2

CD Spectra of Polymeric Tripeptide *Ic* (ALP) as a Function of the Medium, Region of $n \rightarrow \pi^*$ Transition

1 90% 1-propanol; *2* 0·01M Tris, pH 11; *3* 2M-NaCl.

complex was selected so that A_{260}^{1cm} would be between 1·5—1·8. CD was expressed in specific ellipticity [ψ] (deg. cm^2. dg^{-1}) which was related to the total weight concentration of DNA in the system.

RESULTS

Molecular Weight of the Polypeptides

The sedimentation coefficients of protected polypeptides in dimethylformamide were determined. For polymer *Ib* we measured a value of $s^\circ = 1{\cdot}6$ S which according to the relation derived for poly(N$^\varepsilon$-benzyloxycarbonyl-L-lysine) in the same solvent[29] corresponds to a molecular weight of 13 800, *i.e.* 96 amino-acid residues. Polymeric tripeptide *IIb* and tetrapeptide *IIIb* gave very low values of sedimentation coefficients, from which it was impossible to determine molecular weights. Polymeric pentapeptide *IVb* apparently did dissolve in dimethylformamide, but remained obviously as an aggregate or a microgel, since the measured substance sedimented to the bottom of the cell during the initial increase of the ultracentrifuge speed.

The polymer hydrochlorides were dialysed against solvent (0·15M-NaCl) before measurement. The major portion of polymeric tripeptide *IIc* and the larger part of polymeric tetrapeptide *IIIc* escaped during dialysis. The remnant of tetrapeptide after dialysis (about 1/3 of the weighed amount) gave values of *s* about 0·6 S, which would roughly correspond to a molecular weight of about 5000, *i.e.* up to 40 amino-acid residues.

The molecular weights of a polymeric tripeptide *Ic* (ALP) and pentapeptide *IVc* (ALLPL) were determined by sedimentation equilibrium. The polymeric tripeptide revealed some polydispersity and fractionation in the ultracentrifuge cell resulted in two values of molecular weight: 9070 and 12 000 corresponding to 82 and 109 amino-acid residues. The polymeric pentapeptide did not show polydispersity and from the measured molecular weight of 12 800 the incorporation of 96 amino-acid residues can be calculated.

Conformation of Polymers in Solution

The conformation of protected polypeptides *Ib*, *IIb*, and *IIIb* in dimethylformamide were measured by ORD. In all measurements b_0 was zero or very low and $a_0 = -200$ to -500. These values clearly exclude conformation of an α-helix[28] and values of $[\alpha]_{550}$ in the range -30° to -90° exclude also a helix of the polyproline-II type (ref.[30]). All polypeptides were probably in random conformation.

CD of the polypeptide hydrochlorides was studied in aqueous solution as a function of ionic strength (molarity of NaCl) and pH, and in a solution of 90% 1-propanol. Fig. 1 shows the CD spectra of polymeric tripeptide ALP as a function of ionic

strength of the solution. The shape of the spectra is typical for polymers in random-coil conformation[31] (a strong negative band about 200 nm and a shoulder in the region above 220 nm, corresponding to an $n \rightarrow \pi^*$ transition). A slight ionic strength dependence was found. Deepening of the $n \rightarrow \pi^*$ band in 2M-NaCl (inserted portion of Fig. 1) was more marked than the effect of an alkaline pH (Fig. 2) whereas the action of 1-propanol was in the opposite direction, *i.e.* a spectrum which revealed the presence of a positive band in a region about 220 nm (Fig. 2) was obtained. This type of spectrum can be found also in the case of the polymeric pentapeptide ALLPL (Fig. 3) where the positive band is more marked and is suppressed by ionic strength, high pH, as well as by the presence of 1-propanol (inserted portion of Fig. 3). Conformation characterised by this positive band (which is attributed by some investigators to a partly ordered conformation, the extended helix) arises as the result of interaction of the positive lysine charges and is suppressed by all of the factors listed above. If we compare the conformation changes of polymers ALP and ALLPL in

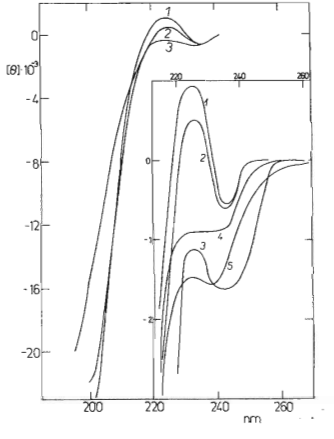


FIG. 3

CD Spectra of Polymeric Pentapeptide *IVc* (ALLPL) as a Function of the Medium

Inserted figure: $n \rightarrow \pi^*$ transition with scale expansion. *1* 0·01M Tris, pH 7·3; *2* 0·15M-NaCl; *3* 2M-NaCl; *4* 0·01M Tris, pH 11; *5* 90% 1-propanol.

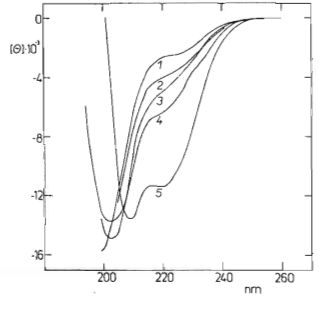


FIG. 4

CD Spectra of Histone F1 as a Function of the Medium

1 0·01M Tris, pH 7·3; *2* 0·01M Tris, pH 11; *3* 0·15M-NaCl; *4* 2M-NaCl; *5* 90% 1-propanol.

relation to environment, we observe a difference due mainly to a higher charge density in the pentapeptide. In both polymers we can see a greater dependence of the CD spectra on ionic strength than on pH. The effect of 1-propanol on ALLPL spectrum is in the expected direction, on the other hand it is not completely clear why there is a positive band in ALP in this solvent. It is possible that in this case a certain role is played by the relatively high proline content, since the polyproline-II helix is also characterised by a positive band in this region of wavelengths[32].

Fig. 4 shows a comparison of the CD spectra of histone F1 in aqueous medium depending on ionic strength and in 90% 1-propanol. Whereas the dependence on ionic strength is only slightly more marked than in the case of both synthetic polypeptides, 1-propanol induces such changes in the CD spectra which could only be explained on the assumption that about 1/4 of the histone chain is in α-helical conformation.

Complexes of Polypeptides with DNA

In order to compare the formation of complexes of DNA with polypeptides of varied composition which differ in charge density we used for the preparation of complexes

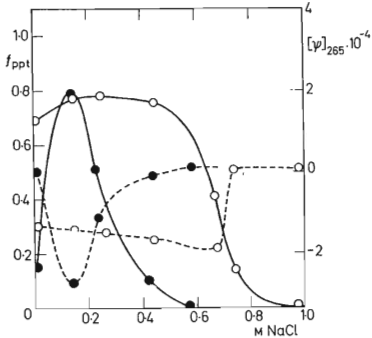


FIG. 5

Formation of Complexes as a Function of the NaCl Molarity

——— fraction of DNA in aggregated complex (left scale); – – – specific elipticity $[\psi]_{265}$ (right scale); ● ALP–DNA, Lys/DNA = 0·5; ○ ALLPL–DNA, Lys/DNA = = 0·5.

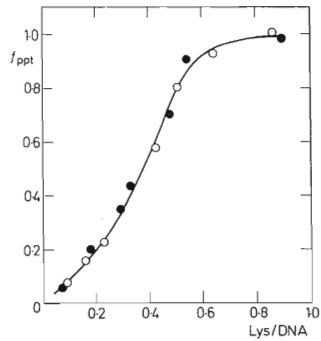


FIG. 6

Dependence of the Fraction of DNA in Aggregated Complex on the Lys/DNA Ratio

● ALLPL–DNA; ○ ALP–DNA.

dialysis against a linear gradient of NaCl molarity[25]. This guarantees the same conditions of annealing for complexes, the formation of which occurs in various regions of NaCl molarity. The formation of complexes with a gradual decrease in NaCl molarity was followed by determining the fraction of total DNA present in aggregated complex (f_{ppt}) and by changes in CD spectra in the region of the absorption band of DNA between 260 and 270 nm. Values of f_{ppt} and specific elipticity $[\psi]_{265}$ as a function of NaCl molarity for complexes characterised by the same input ratio of basic lysine residues to acid phosphate groups in DNA (Lys/DNA = 0·5) are shown in Fig. 5. The formation of complexes of both types occurs cooperatively in a relatively narrow region of NaCl molarity which for complex ALLPL–DNA is shifted to higher values of molarity in comparison with the complex ALP–DNA. A further significant difference in the behaviour of both complexes can be observed in the region of low ionic strength. Whereas for the complex ALLPL–DNA in this region there are only slight changes in the measured parameters, the measured values for complex

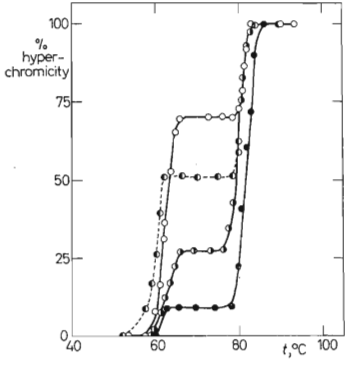


FIG. 7

Melting Curves of Supernatant DNA after Sedimentation of Aggregated Complexes

○ original mixture of DNA: DNA *Staphylococcus aureus*, 31% (G + C) + + DNA *Streptomyces chrysomallus*, 70% (G + C), 2 : 1; Supernatants: ◐ ALP–DNA, Lys/DNA = 0·3; ● ALP–DNA, Lys/DNA = 0·5; ◑ ALLPL–DNA, Lys/DNA = = 0·4.

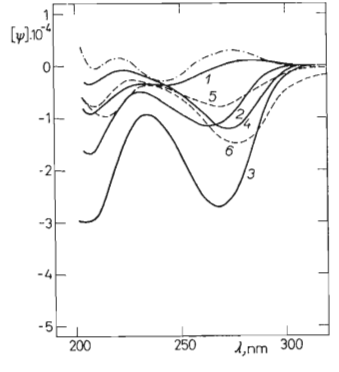


FIG. 8

CD Spectra of Complexes in 0·15M-NaCl

–·–·– DNA; ——— ALP–DNA, Lys/DNA = 0·08 (*1*), 0·22 (*2*), 0·49 (*3*), 0·64 (*4*); – – – ALLPL–DNA, Lys/DNA = 0·33 (*5*), 0·54 (*6*).

ALP–DNA exhibit a maximum in the region of about 0·15M-NaCl and at lower ionic strength there is a further transition which can be detected also visually as a decrease in turbidity of the solution. In this transition we are dealing apparently with a change of the aggregated complex to a complex which is molecularly soluble.

In further observations we were concerned mainly with the properties of complexes in 0·15M-NaCl, *i.e.* approximately physiological ionic strength. In this region both of the studied complexes are in aggregated form; the solution is opalescent and the aggregated complex can be separated by centrifugation at low *g*. Fig. 6 shows the dependence of the fraction of DNA in aggregated complex (f_{ppt}) on the input ratio Lys/DNA. It is clear that the curve normalised to the ratio Lys/DNA is the same for both complexes and that the shape and slope suggest some degree of cooperativity in complex formation.

A cooperative mechanism of complex formation is also obvious from experiments summarised in Fig. 7. In the latter we used a mixture of DNA composed of two components: DNA with a high content of adenine and thymine (*Staphylococcus aureus* DNA, 31% G + C) and DNA with a high guanine and cytosine content (*Streptomyces chrysomallus* DNA, 70% G + C) in a weight ratio 2 : 1. Since the thermal stability of these two DNAs is very different, the melting curve of the mixture displays two steps, the heights of which correspond approximately to the ratio of both components. Fig. 7 also shows melting curves of supernatant DNA which remains in solution after centrifuging the aggregated complex in 0·15M-NaCl. From these curves it would appear that after removing the complex by centrifugation the supernatant is relatively rich in DNA with a high G + C content, and that therefore a binding of polypeptide occurs selectively and cooperatively to DNA rich in A + T. From the results in Fig. 7 it would appear that binding of polymeric tripeptide *Ic* (ALP) has a higher degree of selectivity than binding of polymeric pentapeptide *IVc* (ALLPL). It is, however, possible that the final ionic strength into which the complexes were dialysed (0·015M--NaCl) is not optimal for the second complex (Fig. 5).

Fig. 8 shows changes in CD spectra of solutions containing a polypeptide and DNA in a given ratio of Lys/DNA after gradual dialysis into 0·15M-NaCl. With an increasing ratio Lys/DNA the spectrum changes from one typical for DNA by superposition of two negative bands in the region of 270 and 210 nm. Maximal intensity of both bands is reached under otherwise constant conditions with a Lys/DNA ratio approx. 0·5. In spectra of the complex ALP–DNA maximal ellipticity reaches higher values as compared with the spectra of ALLPL–DNA. With ratios of Lys/DNA higher than 0·5 (in the complex ALLPL–DNA with the same ratio) one can observe a shift of the maxima to longer wavelengths, accompanied also by a decreased intensity of bands, this being relatively more significant in the band at 210 nm. This change in the character of the spectrum with higher Lys/DNA ratios is obviously a result of optical artefacts arising from gradual increase in the particle size of the aggregates[33].

In Fig. 9 it can be seen how the CD spectra of complexes ALP–DNA in 0·15M-NaCl (Lys/DNA = 0·5) depend on the molecular weight and the composition of the DNA used. A decreased ellipticity of the negative CD bands in cases in which DNA of high mean molecular weight ($12 . 10^6$) was used for complex formation in comparison with complexes of DNA of lower molecular weight is apparent. In the latter region we found an order of magnitude difference in the molecular weight of DNA was without any effect on the CD spectra. From further CD spectra presented in Fig. 9 it would appear that the specific ellipticity of both negative CD bands is dependent upon the chemical composition of the DNA used and increases with the average value of G + C in a number of DNA preparations. The average molecular weight of these preparations varied in a range of values in which this parameter will have no effect on the CD spectrum.

DISCUSSION

Poly(N^ε-benzyloxycarbonyl-L-lysine) is in α-helical conformation in dimethylformamide solution[34]. In aqueous solution poly-L-lysine takes on an α-helix conformation in alkaline pH (ref.[34]) and the same applies also to random copolymers of lysine and alanine[35] and to a sequential polymer $(Lys\text{-}Ala)_n$ (see [36]). Poly-L-lysine transforms into helical conformation also at neutral pH in the presence of a higher concentration of some salts[37] and in 2-propanol and methanol[38] and there is a similar behaviour for statistical copolymers of lysine and alanine[39]. On the other hand sequential polymers containing residues of lysine, alanine, and proline in blocked state in solu-

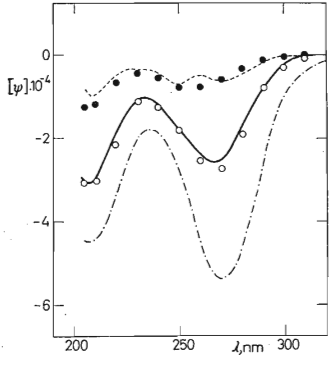


FIG. 9

CD Spectra of Complexes of ALP–DNA in 0·15M-NaCl as a Function of Molecular Weight and Composition of DNA

——— DNA from calf thymus, 42% (G + C), $M = 6·8 . 10^6$; ○ $M = 0·68 . 10^6$; ● $M = 12·0 . 10^6$. – – – crab poly d(A – T). poly d(A – T), 3% (G + C), $M = 0·94 . 10^6$. —·—·— satellite DNA from calf thymus, 55–60% (G + C), $M = 2·7 . 10^6$.

tion in dimethylformamide show properties corresponding to random coil arrangement and their hydrochlorides in aqueous solution and in 1-propanol do not show a tendency to take on an α-helical or other ordered conformation. An exception to this might be the extended helix reported by Tiffany and Krimm[40], the existence of which remains a matter of controversy[41].

The analogous conformational properties of the sequential polypeptides and histone F1 are best demonstrated by the insignificant effect of changes in pH and ionic strength on the conformation of the chain, due obviously to the high content of proline in the molecule. A more significant difference can be detected only in the certain tendency to formation of an α-helix shown by histone F1 in 1-propanol. Here we are apparently dealing with the fact that sequential polypeptides simulate only a part of the chain of histone F1 (about 3/4) and not the entire molecule. After replacing the proline residue with glycine in the polymeric tripeptide (the helix breaking ability of the latter being much weaker) a tendency to the formation of an α-helix similar to that of histone F1 can be observed in 1-propanol solution[42].

The preferential interaction of polypeptide molecules with DNA rich in A + T is analogous to the selectivity observed[8] in the case of histone F1. It would appear, however, that this selectivity is the property of all polymeric polypeptide chains which contain a larger amount of lysine, such as poly-L-lysine[43], the alternating polypeptide $(Lys\text{-}Ala)_n$ (ref.[44]) and statistical copolymers of lysine and alanine[39].

The behaviour of complexes ALP-DNA and ALLPL–DNA as a function of ionic strength is of interest when compared with the behaviour of complexes histone F1–DNA under similar conditions. The region of NaCl molarities in which binding or dissociation of complexes occurs is the same for complexes ALP–DNA and histone F1–DNA and both show the same kind of conformational transition at low ionic strength. On the other hand, the complex ALLPL–DNA behaves in a different manner. Since the conformational properties of both synthetic polypeptides are not markedly different it would appear probable that the agreement in the behaviour of polymeric tripeptide ALP and histone F1 is given by the comparable charge density determined primarily by the content of basic amino acids amounting in both cases to about 33%.

Agreement in the properties of complexes ALP–DNA with previously described properties of complexes histone F1–DNA (see[11]) is very striking. This includes the general character of the CD spectra of complexes and the dependence of these spectra on the ratio of both components, the molecular weight of DNA and the composition of the DNA. From this it is obvious that the fundamental properties of complexes of basic polypeptides with DNA are given by interaction of a rather unspecific character, dependent primarily on the average properties of the polypeptide chain (conformation in particular[15]) which in their turn depend mainly upon the average amino-acid composition. In order to detect specific interactions requiring a specific amino-acid sequence and conformation of binding centres a more detailed level of analysis

would be necessary. A study of such problems will further require the preparation of other sequential alterations in polymers, their fractionation, their further characterisation and the use of other methods of study of complexes.

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