SEQUENTIAL OLIGOPEPTIDES CONTAINING LYSINE: SYNTHESIS, CIRCULAR DICHROISM, CONFORMATION AND COMPLEXES WITH DNA

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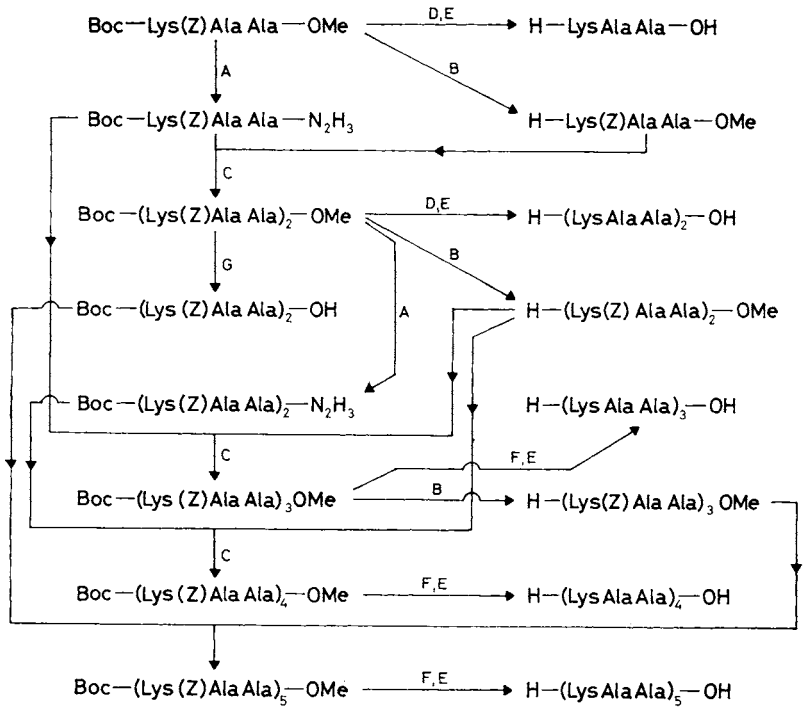
Received July 4th, 1987

Oligomeric (Lys-Ala-Ala)$_{1-5}$ and (Lys-Leu-Ala)$_{1-4}$ were synthesized by fragment condensation in solution, (Lys-Ala-Ala)$_{10}$Ape was obtained by stepwise synthesis from the tripeptide monomer on a polymeric carrier. Conformation of the compounds in water, aqueous methanol and trifluoroethanol was studied by CD spectroscopy. The dimer and trimer in both the structural series show no signs of α-helix formation. The higher oligopeptides exist in random conformation in aqueous solutions, whereas in the presence of alcohols they are partly in the α-helical conformation, depending on the chain length and the character and concentration of the alcohol. However, the ability of α-helix formation is substantially lower even for the studied decamer than for polytripeptides of DP ~30 and higher. According to the CD spectra, the conformational changes resulting from interaction of the oligopeptides with DNA are generally small for both components. The changes of DNA structure may be interpreted as winding of the double helix. The oligopeptide conformation is very little affected by the complexation and is invariably random, contrary to that of the corresponding polypeptides which in complexes with DNA are at least partially α-helical.

In interactions of sequential polymers containing basic amino acids (lysine) with polyanions[1-4] the ability of the polypeptide chain to assume regular conformations (e.g. α-helix) is of great importance. Necessarily, a dependence on the polypeptide chain length should be observed[5]. Therefore, we prepared two series of oligomers with sequences corresponding to the most widely studied polypeptides*, i.e. Lys-Ala-Ala and Lys-Leu-Ala (see Scheme 1 and 2). The synthesis of oligomers (Lys-Ala-Ala)$_{1-5}$ and (Lys-Leu-Ala)$_{1-4}$ was performed using the classical chemical procedure in solution. The preparation of higher oligomers by this method was not successful, mainly for difficulties with the solubility of intermediates. For this reason, we also tried to prepare higher-molecular oligotripeptides (Lys-Ala-Ala)$_{10}$-Ape and (Lys-Ala-Ala)$_{12}$-Ape by stepwise condensation of monomeric tripeptide units on an insoluble carrier. Some of the mentioned oligomers of the Lys-Ala-Ala series have already been described in the literature as products obtained by chromato-

* The nomenclature and symbols of the amino acids and peptides obey the published IUPAC-IUB recommendations[6]. All the amino acids mentioned in this paper are of L-configuration. Ape is used for norvaline (2-aminopentanoic acid).

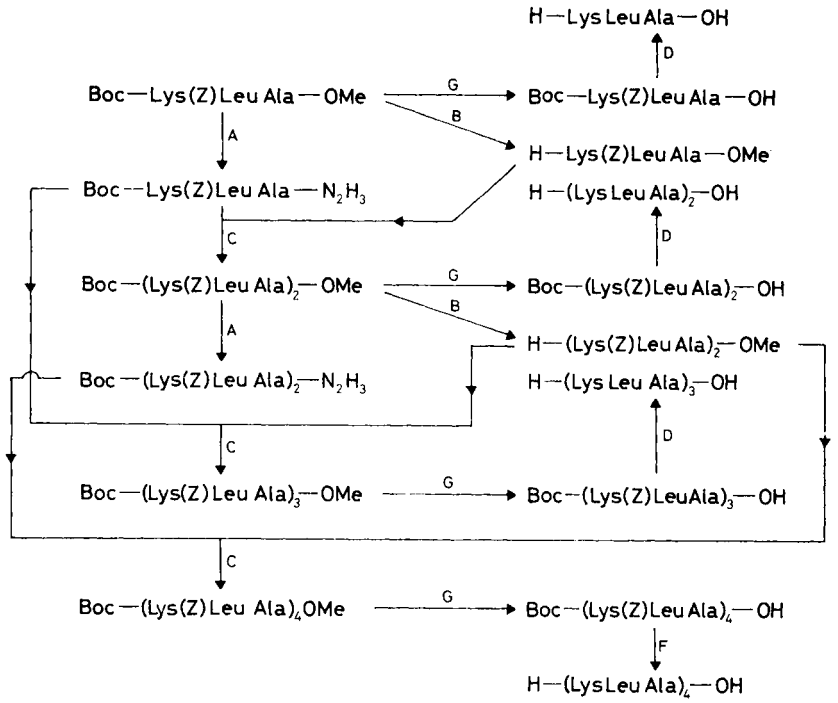


SCHEME 1

graphic separation of polymerization products[7]. We investigated the solution conformation of the prepared oligomers by CD spectroscopy. We also studied the CD spectra of their complexes with DNA. From the difference spectra we were able to estimate the conformational changes due to interaction of both components. In all cases, the behaviour of the oligopeptides was compared with that of the corresponding polypeptide.

EXPERIMENTAL

Melting points were determined on a Kofler block and are not corrected. Samples for elemental analyses were dried over phosphorus pentoxide for 12 h at room temperature, unless stated otherwise. The homogeneity of compounds was checked by thin-layer chromatography (TLC) on silica gel-coated plates (Silufol, Kavalier, Czechoslovakia) in the following systems: 2-butanol–98% formic acid–water 75 : 13·5 : 11·5, 2-butanol–25% ammonia–water 85 : 7·5 : 7·5, 1-butanol–acetic acid–water 4 : 1 : 1, 1-butanol–acetic acid–water–pyridine 15 : 3 : 6 : 10. Paper electrophoresis was performed in a moist chamber in 1 mol l^{-1} acetic acid (pH 2·4) and in a pyridine–acetate buffer (pH 5·7) on Whatman 3MM paper at 20 V/cm for 60 min. Spots in TLC and electrophoresis were detected with ninhydrin or by the chlorination method. High performance liquid chromatography (HPLC) was carried out on an SP-8 700 instrument and SP-8 400 detector



SCHEME 2

(Spectra Physics, Santa Clara, U.S.A.). All substances were pure according to these criteria. A rotatory evaporator was used to concentrate solutions (bath temperature 40°C). The $[\alpha]_D$ values were estimated on a Perkin–Elmer 141 polarimeter in a concentration about 0·5 g per 100 ml. Samples for amino acid analyses were hydrolyzed with 6M-HCl at 105°C for 20 h. The analyses were performed on a Durrum D-500 analyzer.

N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine Hydrazide (Procedure *A*)

Hydrazine hydrate (1·5 ml) was added to a solution of N^{α}-tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine methyl ester (4·5 g) in methanol (50 ml) and the mixture was refluxed for 6 h. Another portion (1 ml) of hydrazine hydrate was added and the reflux was continued for another 6 h. After standing at 20°C for 40 h, the solvent was evaporated, the residue triturated with water, collected on filter, washed with water, dried and crystallized from methanol–diethyl ether; yield 3·4 g (76%); see Table I.

N^{ε}-Benzyloxycarbonyllysyl-alanyl-alanine Methyl Ester Trifluoroacetate (Procedure *B*)

Trifluoroacetic acid (4 ml) was added to N^{α}-tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine methyl ester (0·3 g). After standing for 12 min with intermittent stirring, the mixture was poured into diethyl ether (200 ml), the solid was filtered and washed with diethyl ether; yield 2·6 g (94%); see Table I.

TABLE I

Protected intermediates, X = Lys(Z)-Ala-Ala; Y = Lys(Z)-Leu-Ala

Compound Procedure	M.p., °C $[\alpha]_D$[a]	Formula (mol. w.)	Calculated/found		
			% C	% H	% N
$Boc(X)_1N_2H_3$ *A*	138—140 −13·6°	$C_{25}H_{33}N_4O_7$ (536·6)	55·96 56·06	7·51 7·46	15·66 15·44
$H(X)_1OMe$[b] *B*	166—168 −4·1°	$C_{23}H_{33}F_3N_4O_8$ (550·5)	50·18 50·51	6·04 6·14	10·18 10·17
$Boc(X)_2OMe$ *C*	224—227 −20·9°	$C_{46}H_{68}N_8O_{13}$ (941·1)	58·71 58·50	7·28 7·27	11·91 11·91
$Boc(X)_3OMe$[c] *B, C*	246—248 −17·4°	$C_{66}H_{96}N_{12}O_{18}$ (1 345 + H_2O)	58·14 57·98	7·24 7·00	12·32 12·28
$Boc(X)_2N_2H_3$[c] *A*	242—244 −15·9°	$C_{45}H_{68}N_{10}O_{12}$ (941·1)	57·43 57·43	7·28 7·23	14·88 14·78
$Boc(X)_4OMe$[c] *B, C*	261—265 [d]	$C_{86}H_{124}N_{16}O_{23}$ (1 750 + 2 H_2O)	57·84 57·88	7·22 7·14	12·55 12·84
$Boc(X)_2OH$ *G*	177—181 −13·3°	$C_{40}H_{56}N_8O_{10}$ (927·1)	58·30 58·68	7·18 7·44	12·10 11·89
$Boc(X)_5OMe$[e] *B*	250 (decomp.) [f]	$C_{106}H_{152}N_{20}O_{28}$ (2 154 + 2 H_2O)	58·12 57·90	7·18 6·96	12·78 12·88
$Boc(Y)_1OH$[g] *G*	85—87 −23·7°[h]	$C_{28}H_{44}N_4O_8$ (564·7 + 0·5 H_2O)	58·73 58·62	7·92 7·72	9·78 9·67
$Boc(Y)_1N_2H_3$[i] *A*	161—163 −44·1°	$C_{28}H_{46}N_6O_7$ (578·7)	58·11 58·30	8·01 8·02	14·52 14·67
$Boc(Y)_2OMe$ *B, C*	202—204 −34·3°[h]	$C_{52}H_{80}N_8O_{13}$ (1 025)	60·92 61·10	7·87 7·58	10·93 10·87
$Boc(Y)_2N_2H_3$ *A*	214—217 −23·5°	$C_{51}H_{80}N_{10}O_{12}$ (1 035)	59·75 59·59	7·87 8·01	13·66 13·54
$Boc(Y)_2OH$ *G*	240—244 −27·2°	$C_{51}H_{78}N_8O_{13}$ (1 011 + H_2O)	59·50 59·73	7·83 7·65	10·89 10·86
$Boc(Y)_3OMe$[c] *B, C*	254—257 −26·7°	$C_{75}H_{114}N_{12}O_{18}$ (1 472)	61·21 60·89	7·81 7·61	11·42 11·34
$Boc(Y)_3OH$ *G*	316 (decomp.) −24·6°	$C_{74}H_{112}N_{12}O_{18}$ (1 458 + H_2O)	60·22 60·35	7·79 7·60	11·38 11·38
$Boc(Y)_4OMe$[c] *B, C*	290—293 −19·4°	$C_{98}H_{148}N_{16}O_{23}$ (1 918)	61·36 61·27	7·78 7·69	11·68 11·43
$Boc(Y)_4OH$[c] *G*	[j] −14·1°	$C_{97}H_{146}N_{16}O_{23}$ (1 904 + 4 H_2O)	58·95 58·80	7·85 7·60	11·49 11·49

N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-
-alanyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine Methyl Ester (Procedure *C*)

A solution of hydrogen chloride in ethyl acetate (4·4 mol l^{-1}; 4 ml) was added at −30°C to a solution of N^{α}-tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine hydrazide (3·8 g) in dimethylformamide (50 ml). Tert-butyl nitrite (1·62ml) was added during 1 min and the mixture was stirred for 4 min. After addition of N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine methyl ester (3·8 g) in dimethylformamide (15 ml), the mixture was adjusted to pH 8·5 with N-ethylpiperidine, set aside at 0°C for 2 days and taken down. The residue was triturated with 20% (w/v) citric acid, filtered, washed with water, 0·5M-$NaHCO_3$, water, and dried. Crystallization from methanol–diethyl ether gave 4·7 g (73%) of the product (see Table I).

Lysyl-alanyl-alanyl-lysyl-alanyl-alanine Trihydrochloride

Procedure *D*: A solution of hydrogen bromide (33% w/v) in acetic acid (1·5 ml) was mixed with N^{α}-tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanyl-N^{ε}-benzyloxycarbonyllysyl-
-alanyl-alanine methyl ester (0·2 g). The mixture was set aside for 30 min with intermittent stirring, the solid was filtered, washed with diethyl ether and dried in an desiccator over solid sodium hydroxide; yield 0·14 g (83%) of electrophoretically pure hydrobromide.

Procedure *E*: The salt obtained in the preceding experiment (0·14 g) was dissolved in water (20 ml), stirred with Amberlite IRA 410 ($OH^{(-)}$ cycle) for 2 h, filtered, acidified with 1M-HCl and taken down. The residue was coevaporated with benzene (2×) and diethyl ether (2×). The obtained compound was triturated with diethyl ether, filtered and dried in a desiccator (hygroscopic!). Yield 65 mg (66% overall yield for procedures *D* and *E*), see Table II.

Lysyl-alanyl-alanyl-lysyl-alanyl-alanyl-lysyl-alanyl-alanine Tetrahydrochloride (Procedure *F*)

N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanyl-N^{ε}-benzyloxycarbonyllysyl-
-alanyl-alanyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine methyl ester was dissolved in warm dichloroacetic acid (2 ml), cooled and 33% (w/v) hydrogen bromide in acetic acid (2 ml) was added. After standing for 45 min at room temperature, the mixture was poured into diethyl ether, the solid was filtered, washed with diethyl ether and dried in a desiccator over solid sodium hydroxide, affording 0·22 g of the electrophoretically pure hydrobromide which was converted (Procedure *E*) into the title compound. Yield 0·13 g (59% overall yield for procedures *F* and *E*); see Table II.

N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-
-alanyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine (Procedure *G*)

2-Propanol (40 ml) and 2M-NaOH (7·5 ml) were added to N^{α}-tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine methyl ester (0·94 g). The solid gradually dissolved. After standing for 1·5 h, the mixture was diluted with water, 2-pro-

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[a] In dimethylformamide, *c* 0·2–0·5 g per 100 ml; [b] trifluoroacetate; [c] crystallized from dimethylformamide–methanol–diethyl ether; [d] CD spectrum (*c* 0·05 g/100 ml, hexafluoro-2-propanol): λ_{max} 220 nm ($[\Theta] = -4{\cdot}88 \cdot 10^3$), λ_{max} 201 nm ($[\Theta] = -13{\cdot}2 \cdot 10^3$); [e] crystallized from dimethylformamide–methanol; [f] CD spectrum (*c* 0·05, hexafluoro-2-propanol): λ_{max} 220 nm ($[\Theta] = -6{\cdot}11 \cdot 10^3$), λ_{max} 202 nm ($[\Theta] = -13{\cdot}2 \cdot 10^3$); [g] set to crystals under light petroleum; [h] in methanol; [i] crystallized from ethyl acetate–light petroleum; [j] does not melt up to 300°C.

panol was evaporated, the residue was acidified with 20% (w/v) citric acid and set aside at 0°C for 2 h. The solid was filtered, washed with water, dried in a desiccator and crystallized from methanol–ether; yield 0·67 g (73%), see Table I. In the preparation of BocLys(Z)-Leu-Ala-OMe the solution after acidification was extracted with ethyl acetate, the organic extract washed with water, the solvent evaporated and the residue crystallized; see Table I.

N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine Methyl Ester

Pentafluorophenol–dicyclohexylcarbodiimide (3 : 1) complex (0·26 g) was added to a solution of N^{α}-tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine (0·27 g) in dimethylformamide–dioxane (1 : 3; 40 ml). The mixture was stirred

TABLE II

Free peptides, X = Lys-Ala-Ala; Y = Lys-Leu-Ala

Compound Procedure	k'[a] solvent	Formula (mol. w.)	Calculated/found		
			% C	% H	% N
$H(X)_1OH.2\ HCl$	1·66	$C_{12}H_{25}Cl_2N_4O_4$	38·10	7·19	14·89
D, E	water	(378·3 + H_2O)	38·53	7·06	14·79
$H(X)_2OH.3\ HCl$	6·7	$C_{24}H_{48}Cl_3N_8O_7$	42·08	7·36	16·36
D, E	water	(667·1 + H_2O)	42·28	7·49	16·06
$H(X)_3OH.4\ HCl$	5·2	$C_{36}H_{72}Cl_4N_{12}O_{10}$	41·30	7·70	16·05
E, F	5% methanol	(974·9 + 4 H_2O)	41·00	7·63	15·70
$H(X)_4OH.5\ HCl$	4·5	$C_{48}H_{94}Cl_5N_{16}O_{13}$	42·05	7·64	16·35
E, F	10% methanol	(1 280 + 5 H_2O)	42·07	7·58	16·51
$H(X)_5OH.6\ HCl$	4·0	$C_{60}H_{117}Cl_6N_{20}O_{16}$	42·50	7·66	16·52
E, F	10% methanol	(1 587 + 6 H_2O)	42·73	7·13	16·80
$H(Y)_1OH.2\ HCl$[b]	1·8	$C_{15}H_{30}Cl_2N_4O_4$	42·96	7·69	13·36
D	20% methanol	(401·3 + 2 H_2O)	43·10	8·09	13:54
$H(Y)_2OH.3\ HCl$[b]	3·4	$C_{30}H_{58}Cl_3N_8O_7$	45·89	7·96	14·46
D	30% methanol	(749·2 + 2 H_2O)	45·73	7·90	14·46
$H(Y)_3OH.4\ HCl$[b]	3·6	$C_{45}H_{86}Cl_4N_{12}O_{10}$	46·95	8·05	14·80
D	40% methanol	(1 061 + 3 H_2O)	46·56	7·85	14·56
$H(Y)_4OH.5\ HCl$[b]	2·2	$C_{60}H_{114}Cl_5N_{16}O_{13}$	46·95	8·14	14·59
F	70% methanol	(1 445 + 5 H_2O)	46·53	7·78	14·40

[a] HPLC capacity factor; [b] the hydrobromide obtained by procedure *C* was converted into the hydrochloride on Amberlite IRA 410.

at room temperature for 1 h and then left in a refrigerator for 1 h. The solvents were evaporated, the residue was triturated with light petroleum and crystallized from methanol–diethyl ether; yield 0·25 g (78%), m.p. 190–194°C.

A solution of the thus-obtained activated ester (0·16 g) in dimethylformamide (3 ml) and dioxane (6 ml) was mixed with N^{ε}-benzyloxycarbonyllysyl-alanyl-alanyl-N^{ε}-benzyloxycarbonyl-lysyl-alanyl-alanyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-alanine methyl ester trifluoroacetate (0·2 g; prepared from the N^{α}-tert-butyloxycarbonyl derivative by Procedure *B*) and with triethylamine (0·02 ml). The mixture was stirred at room temperature for 6 h and set aside at 0°C overnight. The solvents were evaporated and the residue was triturated with 20% (w/v) citric acid, filtered, washed with water, 0·5M-$NaHCO_3$ and water, dried and crystallized from dimethylformamide–methanol to give 0·2 g (65%) of the title product, see Table I.

(Lysyl-alanyl-alanyl)$_n$-norvaline (n = 10, 12)

Chloromethylated polystyrene (cross-linked with 1% divinylbenzene; 1·17% Cl; 4 g) was acylated with N^{α}-2-nitrobenzenesulfenyl-2-aminopentanoic acid (4·68 mmol; 1 equivalent), liberated from its dicyclohexylammonium salt (2·11 g) and converted into tetramethylammonium salt, (see ref.[8]) in sulfolane (12 ml) and dioxane (40 ml) at 90°C for 33 h. After filtration and washing with dioxane, methanol, 2-propanol and water, the weight increase of the dried resin was 0·79 g (i.e. substitution 0·70 mmol/g). The title peptide was synthesized by stepwise acylation with Nps-Lys(Z)-Ala-Ala-OH. The 2-nitrobenzenesulfenyl group was removed in each step by shaking with thiosemicarbazide hydrochloride[5] (0·63 g) in hexamethylphosphoric triamide (10 ml) and dichloromethane (40 ml) for 20 min, the polymer was washed three times with dichloromethane, dimethylformamide, methanol and the amino group was liberated from the salt by treatment with triethylamine (4 ml) in dimethylformamide (40 ml). After 12 min the polymer was washed with dimethylformamide and dichloromethane. The condensation of the protected tripeptide was carried out under shaking for 18 to 24 h. After each reaction the polymer was washed three times with dichloromethane, methanol, dimethylformamide and toluene. The reacylation was carried out on the basis of spectrophotometric determination of the 2-nitrobenzenesulfenyl group after cleavage with hydrogen chloride in 2-propanol from a carrier sample. Data on the individual condensation steps are given in Table III. The substitution dropped after the first step to 0·4 mmol/g. Its further slow decrease is characterized in Table III by the ratio of amino acids as found by amino acid analysis of a carrier sample.

The cleavage of the peptide from the carrier and removal of the protecting groups were effected by stirring with liquid hydrogen fluoride with 10% of anisole at 0°C for 30 min. The hydrogen fluoride was evaporated at 0°C (bath) during 30 min, the residue was triturated three times with dry ether and decanted. The crude decamer was chromatographed on a column of Fractogel TSK HW-40(S) (Merck) 2·5 × 80 cm in 0·05 mol l^{-1} HCl using recycling technique. The crude dodecamer was not processed further.

Complexes of DNA with Oligopeptides

Calf thymus DNA was isolated according to ref.[10], fractionated as described by Mandell and Hershey[11] and fractions containing no satellite components were used in the experiments. Complexes were prepared by mixing solution of DNA (60 μg/ml) in buffer A (1·5 mmol l^{-1} NaCl, 0·2 mmol l^{-1} Na_2HPO_4, 0·05 mmol l^{-1} Na_2EDTA) with appropriate amount of the peptide solution (2 mg/ml) in water. The Lys/DNA ratio was 0·5, in the case of complexes with polymer (Lys-Ala-Ala)$_n$ 0·25 and 0·5.

Spectral Measurements

The CD spectra were measured on Jobin-Yvon Dichrographe Mark V using software Dichrosoft Version A written by Dr P. Maloň, Laboratory of Peptide Chemistry, Institute of Organic

TABLE III

The course of solid phase synthesis of (Lys-Ala-Ala)$_n$Ape-OH by stepwise condensation of Nps-Lys(Z)-Ala-Ala-OH using dicyclohexylcarbodiimide (DCCI)

Step, n[a]	Amount, equiv.		Ratio[d] Ala/(n Ape)
	of acyl component[b]	of DCCI[c]	
1	2·6	2·6	0·98
1·1	2·6	3·5[e]	1·01
2	2·6	3·5	0·95
2·1	1·3	1·75	0·92
3	2·6	3·5	0·91
3·1	1·75	2·45	0·82
3·2	1·75	2·45	0·89
3·3	1·75	2·6[e]	0·78
4	3·5[f,g]	3·5	0·80
4·1	5·25[f,g]	3·5	0·85
4·2	3·5[f,h]	3·5	0·89
4·3	3·5[f,h]	3·5	0·87
4·4	1·75[g,i]	2·45	0·85
5	3·5[j]	3·5	0·81
5·1	0·4[k]	1·3	0·81
6[l]	3·5	3·5	0·77
7	3·5	3·5	0·83
8	3·5	3·5	0·77
9	3·5	3·5	0·79
9·1	0·9	1·3	0·79
9·2	2·6	3·8	0·79
10	3·5	3·5	0·79
10·1	2·6	3·5	0·81
11[m]	3·5	3·5	0·81
12	3·5	3·5	0·73

[a] The second number denotes repeated condensation; [b] based on substitution by the first amino acid (Ape) 0·7 mmol/g; [c] based on substitution by the tripeptide 0·4 mmol/g; [d] from amino acid analysis; [e] 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline used instead of dicyclohexylcarbodiimide; [f] adsorption of Nps tripeptide for 4 h prior to reaction (ref.[9]); [g] in dichloromethane–dimethylformamide; [h] in chloroform, [i] adsorption at −12°C, reaction at +4°C; [j] in dichloromethane–chloroform; [k] in dimethylformamide; [l] 1/3 of polymer withdrawn; [m] 1/2 of the remaining polymer withdrawn.

Chemistry and Biochemistry, Prague. CD spectra of the peptides were measured in cells of 1 mm and 0·2 mm optical path at concentrations 0·2–0·3 mg/ml. Solutions of the peptides in diluted buffer–methanol and water–trifluroethanol mixtures were prepared by dissolving the samples in water or diluted buffer and then adding alcohol. CD spectra of the DNA-peptide complexes were measured in 5 mm and 1 mm cells. The difference spectra were calculated by subtracting the CD spectrum of DNA from CD spectrum of the DNA–peptide complex using Silex microcomputer.

The circular dichroism was expressed in molar ellipticities [Θ] where the average molecular weight of the amino acid residue for a given peptide was used in the calculation of the CD spectra of peptides and the difference CD spectra, and the average molecular weight of the nucleotide (M = 330) in the calculation of the CD spectra of DNA and DNA–peptide complexes.

RESULTS

CD *Spectra of Oligopeptides*

Characteristic data on CD spectra of the Lys-Ala-Ala oligomers in aqueous medium and in solutions containing methanol and trifluoroethanol are given in Table IV and V. For comparison we also included the CD spectra of a polymer of the same type whose molecular weight corresponded to 34 tripeptide units (Table V). The CD spectral data for the oligomers and the polymer of the Lys-Leu-Ala series are given in Table VI.

In aqueous solutions of low ionic strength (buffer A, 1·5 mmol l^{-1} NaCl, 0·02 mmol l^{-1} Na_2HPO_4, 0·05 mmol l^{-1} Na_2EDTA) the Lys-Ala-Ala oligomers show a negative π–π^* band at about 195 nm. Two bands occur in the n–π^* transition region: a positive one at 217–219 nm whose intensity decreases with increasing number of monomeric units, and a negative one at 231–236 nm. The wavelength of this band decreases and its intensity increases with increasing number of the monomeric units. In the spectra of the Lys-Leu-Ala trimer and tetramer the intensity of the positive band in the n–π^* region is so low that it appears only as a negative minimum.

The CD spectra of $(Lys\text{-}Ala\text{-}Ala)_{10}Ape$ in pure water and in a solution of low ionic strength (buffer A) (Fig. 1) are practically identical. In this respect, the decamer differs from the polymer whose CD spectra are markedly dependent on the ionic strength just in the region of low ionic strengths (Table V).

In both the studied series, CD spectra of the dimer and trimer change only little with increasing amount of methanol. There is a very small decrease in the intensity of the negative π–π^* band and a change of the ratio of both bands in the n–π^* region. The positive band becomes weaker whereas the negative band grows stronger and its maximum shifts to shorter wavelengths. Under the same conditions, the leucine-containing oligopeptides (Lys-Leu-Ala series) show lower intensity of the negative π–π^* band and of the positive band in the n–π^* region and higher intensity of the negative band in the n–π^* region than the corresponding oligomers of the Lys-Ala-Ala

series. For the Lys-Ala-Ala oligomers at higher concentrations of methanol, and for the Lys-Leu-Ala oligomers already in 30% methanol, the positive CD band in the n–π^* region appears only as a negative minimum.

Compounds $(Lys\text{-}Ala\text{-}Ala)_4$, $(Lys\text{-}Ala\text{-}Ala)_5$ and $(Lys\text{-}Leu\text{-}Ala)_4$ show larger changes of the CD spectra with increasing methanol concentration than the shorter

TABLE IV
Characteristic values of CD spectra of low-molecular-weight oligomers of Lys-Ala-Ala

Solvent[a]	λ, nm ([Θ] . 10^{-3})				
	max	cross	max	cross	max
			Lys-Ala-Ala		
90% TFE	188 (−14·1)	204	214·5 (+4·28)	— —	— —
			$(Lys\text{-}Ala\text{-}Ala)_2$		
Buffer A	195 (−20·7)	212	217 (+0·96)	229	235·5 (−0·20)
30% CH_3OH	195·5 (−20·1)	213	219 (+0·73)	227·5	234·5 (−0·25)
60% CH_3OH	196 (−19·0)	216	219 (+0·15)	221·5	233 (−0·58)
90% CH_3OH	196·5 (−17·8)	— —	218·5[b] (−0·50)	— —	229 (−1·0)
90% TFE[c]	196 (−10·7)	— —	215·0[b] (−1·32)	— —	224·5 (−1·52)
			$(Lys\text{-}Ala\text{-}Ala)_3$		
Buffer A	195·5 (−21·8)	212·5	217·5 (+0·78)	227·5	236 (−0·24)
30%CH_3OH	195·5 (−21·4)	212·5	218 (+0·44)	225	234·5 (−0·37)
60% CH_3OH	196 (−20·1)	— —	218·5[b] (−0·15)	— —	230 (−0·74)
90% CH_3OH	196 (−19·1)	— —	218·5[b] (−1·01)	— —	228 (−1·39)
90% TFE[d]	197 (−10·1)	— —	215·0[b] (−2·0)	— —	223 (−2·21)

TABLE IV
(*Continued*)

Solvent[a]	λ, nm ([Θ] . 10^{-3})				
	max	cross	max	cross	max
			(Lys-Ala-Ala)$_4$		
Buffer A	195 (−24·1)	214·5	218·5 (+0·42)	224	233·5 (−0·39)
30% CH_3OH	195·5 (−21·0)	216	218·5 (+0·10)	221	231 (−0·63)
60% CH_3OH	197 (−18·4)	— —	218·5[b] (−0·96)	— —	226·5 (−1·15)
90% CH_3OH	198 (−14·7)	— —	216·5[b] (−2·67)	— —	223 (−2·78)
90% TFE[e]	201·5 (−10·8)	— —	217·0[b] (−4·14)	— —	220 (−4·19)
			(Lys-Ala-Ala)$_5$		
Buffer A	196 (−24·0)	213·5	219 (+0·68)	226·5	234 (−0·42)
30% CH_3OH	216·5 (−21·3)	216·5	219 (+0·09)	221	231·5 (−0·59)
60% CH_3OH	197·5 (−16·2)	— —	219·5[b] (−1·17)	— —	228 (−1·32)
90% CH_3OH[f]	202 (−11·0)	— —	218·0[b] (−3·94)	— —	221 (−3·96)
90% TFE[g]	202 (−10·9)	— —	217·5[b] (−4·86)	— —	220·5 (−4·80)

[a] Solutions containing methanol are mixtures of methanol with buffer A (1·5 mmol l^{-1} NaCl, 0·02 mmol l^{-1} Na_2HPO_4, 0·05 mmol l^{-1} Na_2EDTA), solutions containing trifluorethanol, (TFE) are mixtures of trifluorethanol with water; [b] negative minimum; [c] crossover at 185 nm; [d] crossover at 186·5 nm; [e] positive maximum at 187·5 nm, $[\Theta] = +6{\cdot}50 \cdot 10^3$, crossover at 193 nm; [f] crossover at 192·5 nm; [g] positive maximum at 187·5 nm, $[\Theta] = +6{\cdot}59 \cdot 10^3$, crossover at 193 nm.

peptides. With increasing concentratin of methanol there is a substantial decrease in intensity of the negative π–π^* band which is shifted to longer wavelengths. At the same time, the n–π^* negative band becomes stronger and is shifted to shorter wave-

lengths. Except the CD spectra of $(Lys\text{-}Ala\text{-}Ala)_2$ and $(Lys\text{-}Ala\text{-}Ala)_3$ in 30% methanol, which exhibit a positive band at 217–219 nm, spectra of all the other compounds

TABLE V
Characteristic values of CD spectra of high-molecular-weight oligomers of Lys-Ala-Ala

Solvent[a]	λ, nm, ([Θ] . 10^{-3})						
	max	cross	max	cross	max	cross	max
			$(Lys\text{-}Ala\text{-}Ala)_{10}Nva$				
Water	—	—	195·5	215·5	219	221·5	231
	—	—	(−15·2)		(+0·17)		(−0·42)
Buffer A	—	—	196	215·5	219	221·5	231
	—	—	(−15·2)		(+0·17)		(−0·42)
30% CH_3OH	—	—	198	—	217·0[b]	—	224·5
	—	—	(−11·1)	—	(−1·19)	—	(−1·46)
60% CH_3OH	—	—	203·5	—	216·5[b]	—	222
	—	—	(−9·35)	—	(−4·50)	—	(−4·54)
90% CH_3OH	190·0[c]	199	206·5	—	215·5[b]	—	220
	(24·8)		(−11·7)	—	(−8·32)	—	(−8·56)
30% TFE	189·5	194·5	203	—	215·5[b]	—	220
	(+7·66)		(−8·62)	—	(−4·11)	—	(−4·28)
60% TFE	189·5	196·5	205	—	215·5[b]	—	220
	(+13·4)		(−9·41)	—	(−5·89)	—	(−5·99)
90% TFE	189	198	205·5	—	216·0[b]	—	219
	(+19·2)		(−11·9)	—	(−7·75)	—	(−7·87)
			$(Lys\text{-}Ala\text{-}Ala)_n$				
Water	—	—	197	—	216·5[b]	—	226
	—	—	(−23·8)	—	(−1·11)	—	(−1·92)
Buffer A	188	194	202·5	—	214·5[b]	—	222
	(+13·5)		(−18·1)	—	(−9·10)	—	(−10·2)
90% TFE[d]	189·5	199	206·5	—	214·0[b]	—	219·5
	(+59·4)		(−29·7)	—	(−23·2)	—	(−23·8)

[a] Solutions containing methanol are mixtures of methanol with buffer A (1·5 mmol l^{-1} NaCl, 0·02 mmol l^{-1} Na_2HPO_4, 0·05 mmol l^{-1} Na_2EDTA), solutions containing trifluorethanol (TFE) are mixtures of trifluorethanol with water; [b] negative minimum; [c] limit of wavelengths; [d] CD spectrum in 30% and 60% methanol is not significantly different from that in 90% methanol.

show a negative minimum in this region. The spectra in 90% methanol are already similar to those of a mixture of α-helix and random conformation.

The changes in the CD spectra of $(Lys\text{-}Ala\text{-}Ala)_{10}Ape$ due to increasing amount of methanol (Fig. 1) are of the same character as those observed for the shorter peptides, however, they are greater in magnitude. In 60% methanol, the CD spectrum resembles that of a mixture of random conformation and α-helix and in 90% methanol its shape (but not intensities) is close to that of a spectrum of α-helix.

TABLE VI
Characteristic values of CD spectra of oligomers of Lys-Leu-Ala

Solvent[a]	λ, nm, ([Θ] . 10^{-3})		
	max	min	max
		$(Lys\text{-}Leu\text{-}Ala)_2$	
Buffer A[b]	196 (−16·9)	218·5[c] (+0·34)	234 (−0·34)
30% CH_3OH	196 (−15·5)	217·5 (−0·37)	229·5 (−0·73)
60% CH_3OH	196 (−14·7)	217·5 (−0·97)	225·5 (−1·25)
90% CH_3OH	196 (−14·2)	217·5 (−1·39)	225·5 (−1·64)
90% TFE	196 (−13·2)	216·0 (−1·69)	225·5 (−2·09)
		$(Lys\text{-}Leu\text{-}Ala)_3$	
Buffer A	196 (−19·6)	218·0 (−0·20)	229 (−0·72)
30% CH_3OH	196 (−17·2)	218·0 (−0·95)	227·5 (−1·17)
60% CH_3OH	196·5 (−15·8)	218·0 (−1·72)	225 (−1·87)
90% CH_3OH	197 (−13·7)	218·0 (−2·17)	223·5 (−2·42)
90% TFE[d]	198 (−10·5)	216·0 (−2·90)	220 (−2·95)

TABLE VI
(*Continued*)

Solvent[a]	λ, nm ([Θ] . 10^{-3})		
	max	min	max
	(Lys-Leu-Ala)$_4$		
Buffer A	196·5 (−20·5)	218·0 (−0·25)	230 (−0·84)
30% CH_3OH	196·5 (−18·9)	217·5 (−1·20)	226 (−1·49)
60% CH_3OH	197·5 (−15·3)	217·5 (−2·42)	224 (−2·61)
90% CH_3OH	202 (−13·0)	218·5 (−4·03)	221·5 (−4·08)
90% TFE[e]	203·5 (−12·4)	218·5 (−5·38)	221 (−5·42)
	(Lys-Leu-Ala)$_n$		
Water[f]	203 (−15·6)	215·5 (−7·94)	222 (−8·48)
Buffer A[g]	205·5 (−18·3)	214 (−14·9)	221·5 (−16·2)
90% TFE[h]	206·5 (−29·9)	215 (−22·6)	220·5 (−23·0)

[a] Solutions containing methanol are mixtures of methanol with buffer A (1·5 mmol l^{-1} NaCl, 0·02 mmol l^{-1} Na_2HPO_4, 0·05 mmol l^{-1} Na_2EDTA), solutions containing trifluoroethanol (TFE) are mixtures of trifluoroethanol with water; [b] crossover at 214 nm and 224 nm; [c] positive maximum; [d] crossover at 187·5 nm; [e] positive maximum at 189 nm, $[\Theta] = +10{\cdot}5 \cdot 10^3$, crossover at 195 nm; [f] positive maximum at 187 nm, $[\Theta] = +11{\cdot}8 \cdot 10^3$, crossover at 194 nm; [g] positive maximum at 190 nm, $[\Theta] = +33{\cdot}0 \cdot 10^3$, crossover at 198·5 nm; [h] positive maximum at 190·5 nm, $[\Theta] = +58{\cdot}2 \cdot 10^3$, crossover at 199·5 nm.

The dependence of CD spectra on the percentage of trifluoroethanol in the solvent was measured for the decamer (Lys-Ala-Ala)$_{10}$Ape; spectra of other peptides were determined only in 90% trifluoroethanol (Fig. 2). The spectral changes resulting from addition of trifluoroethanol are analogous to those caused by addition of methanol. Spectra of Lys-Ala-Ala dimer, trimer, tetramer and pentamer and Lys-Leu-Ala dimer, trimer and tetramer in 90% trifluoroethanol exhibit weaker negative π–π^* band and stronger negative band in the n–π^* region than in 90% methanol.

On the other hand, CD spectra of the decamer $(Lys\text{-}Ala\text{-}Ala)_{10}Ape$ in 90% methanol and in 90% trifluoroethanol are practically identical. However, the different influence of methanol and trifluoroethanol manifests itself at lower concentrations: at the same concentration of alcohol, the spectra of the decamer exhibit a stronger negative band in the n–π^* region in solutions containing trifluoroethanol. Analogously as in methanol-containing solutions, spectra of the Lys-Leu-Ala peptides have a stronger negative band in the n–π^* region than those of the corresponding Lys-Ala-Ala peptides.

CD *Spectra of Complexes of Oligopeptides with* DNA

Complexes of DNA with oligopeptides were studied in the region 190–320 nm. An example of the CD spectrum (complex DNA–$(Lys\text{-}Ala\text{-}Ala)_3$) is given in Fig. 3. At wavelengths longer than about 245 nm only the polynucleotide component contributes to the CD spectrum whereas below 245 nm the spectrum is a sum of the DNA and peptide contributions. The DNA–peptide interaction reduces the intensity

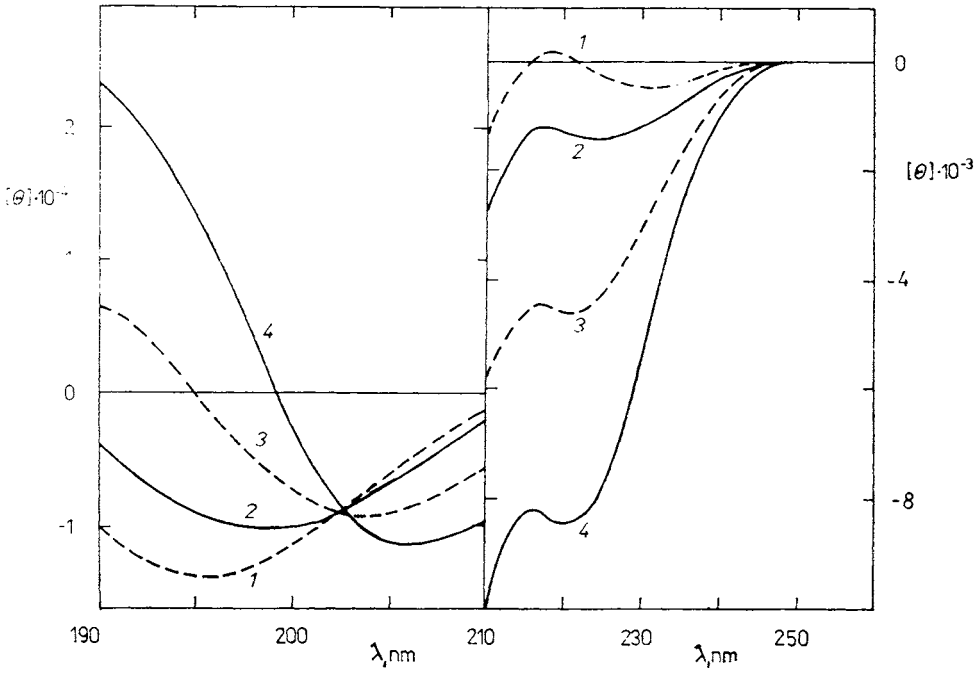

FIG. 1

CD spectra of $(Lys\text{-}Ala\text{-}Ala)_{10}$ in methanol–buffer solutions; *1* buffer A, *2* 30% methanol, buffer A, *3* 60% methanol, buffer A, *4* 90% methanol, buffer A

of the long-wavelength positive DNA band, the reduction depending on the peptide length (Table VII). In both series, the highest intensity changes were found for the dimers ($[\Theta]$ at 276 nm: from $9{\cdot}80 \cdot 10^3$ to $8{\cdot}31 \cdot 10^3$ for $(Lys\text{-}Ala\text{-}Ala)_2$ and to $7{\cdot}87 \cdot 10^3$ for $(Lys\text{-}Leu\text{-}Ala)_2$). The intensity change decreases with longer peptide chain and for $(Lys\text{-}Ala\text{-}Ala)_{10}$Ape the $[\Theta]$ value at 276 nm ($9{\cdot}48 \cdot 10^3$) is already very close to that of DNA. With the polymer $(Lys\text{-}Ala\text{-}Ala)_n$, measured for comparison, the positive DNA band is only negligibly stronger (at 276 nm $[\Theta] = 10{\cdot}35 \cdot 10^3$). Interaction with peptides has no effect on the negative DNA band at about 248 nm (Fig. 3).

A more exact information about changes of the peptide spectra caused by their binding to DNA is gained from difference CD spectra obtained by subtracting the spectrum of DNA from that of the complex. This procedure assumes that the short-wavelength CD spectrum of DNA is not altered by the interaction. It is known[12-14] that the positive long-wavelength band is very sensitive to even small conformational changes that have no effect on the intensity of the band at shorter wavelengths (down to 190 nm). Changes of the long-wavelength positive DNA band, comparable

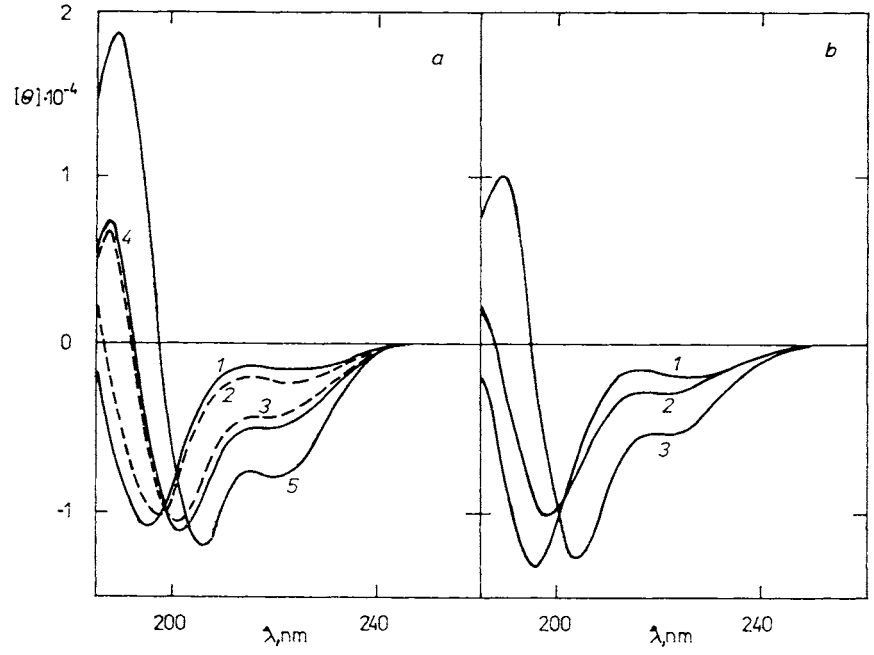

FIG. 2

CD spectra of $(Lys\text{-}Ala\text{-}Ala)_n$ (*a*) and $(Lys\text{-}Leu\text{-}Ala)_n$ (*b*) in 90% trifluoroethanol; *a* *1* n = 2, *2* n = 3, *3* n = 4, *4* n = 5, *5* n = 10, *b* *1* n = 2, *2* n = 3, *3* n = 4

with those resulting from the interaction with the oligomers, may also be obtained by action of divalent cations. As shown by comparison of CD spectrum of DNA

TABLE VII

The value of $[\Theta]$ at 276 nm of complexes of DNA with Lys-Ala-Ala and Lys-Leu-Ala oligomers

DNA-(Lys-Ala-Ala)$_n$							
n	0	2	3	4	5	10	34
$[\Theta]_{276} \cdot 10^{-3}$	9·80	8·31	8·56	9·16	9·35	9·48	10·35
DNA–(Lys-Leu-Ala)$_n$							
n	0	2	3	4			
$[\Theta]_{276} \cdot 10^{-3}$	9·80	7·87	8·18	8·58			

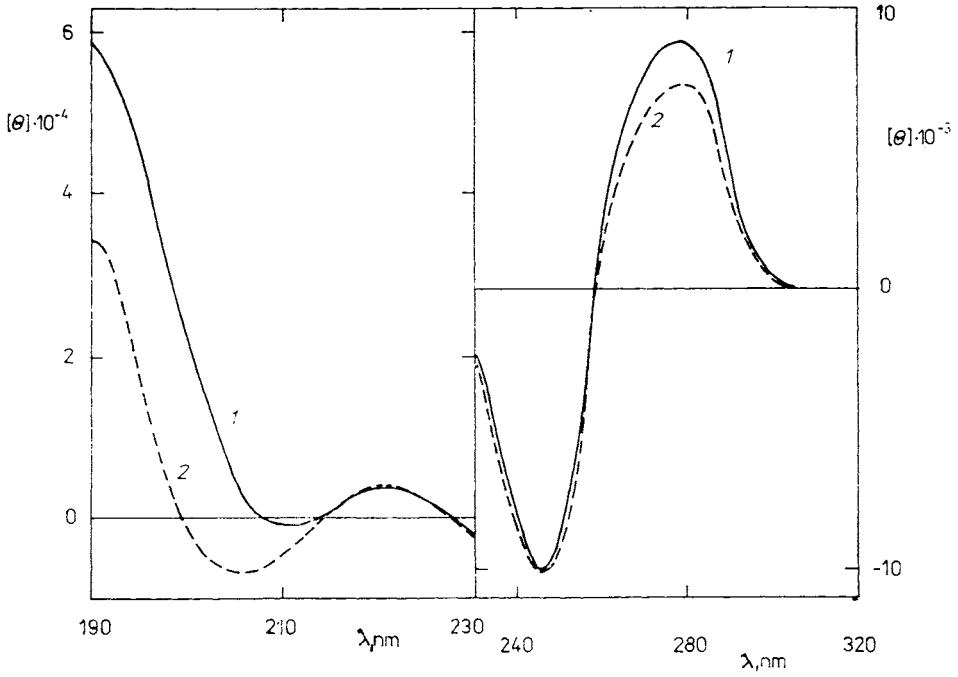

FIG. 3

CD spectrum of DNA (*1*) and DNA–(Lys-Ala-Ala)$_3$ complex (*2*) in buffer A

in 0·01 mol l^{-1} $MgCl_2$ with that in buffer A, both spectra differ only in the region of the long-wavelength DNA band; thus the assumption that an interaction between DNA and oligopeptides has no effect on the short-wavelength spectrum of DNA is probably correct.

For the Lys-Ala-Ala dimer, trimer ad tetramer and for the Lys-Leu-Ala dimer and trimer, the difference CD spectrum is within the limits of experimental error identical with that of the corresponding peptide measured under the same conditions. Differences between the CD spectrum of the peptide in solution and in the complex have been found for longer peptides. These changes are of the same character for all the oligomers: relative intensity decrease of the positive band and relative intensity increase of the negative band in the n–π^* region. Except for $(Lys\text{-}Ala\text{-}Ala)_5$ (Fig. 4), an intensity decrease of the negative π–π^* band was also observed. The largest changes of the CD spectra resulting from binding to DNA were found for

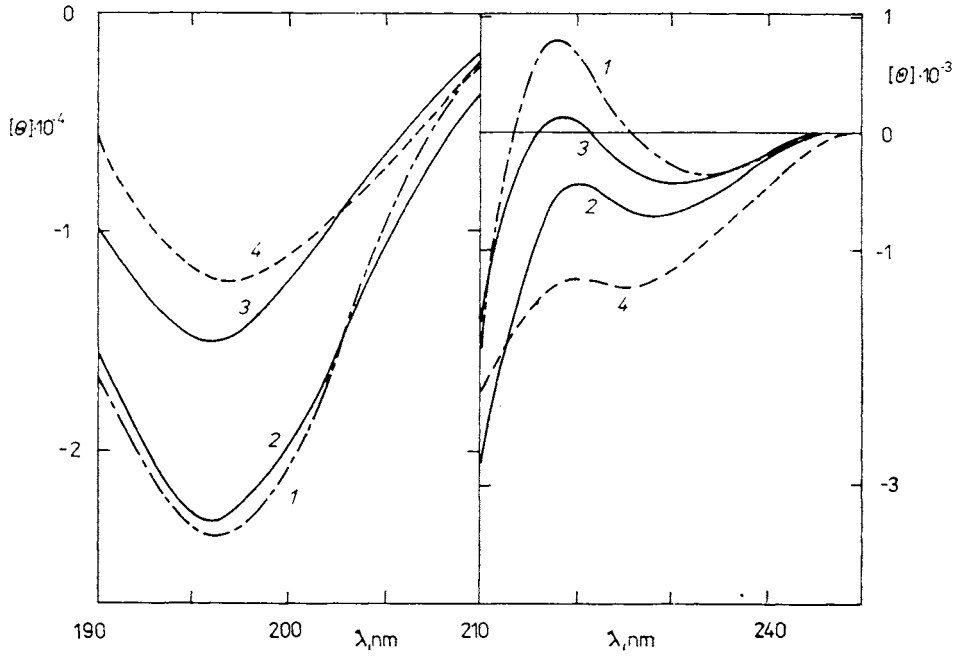

FIG. 4

CD spectra and difference CD spectra of $(Lys\text{-}Ala\text{-}Ala)_{5,10}$ in buffer A. Difference CD spectra were calculated by subtracting CD spectra of DNA from CD spectra of DNA–peptide complexes. The values of $[\Theta]$ were recalculated using molecular weights of pure peptides. *1* $(Lys\text{-}Ala\text{-}Ala)_5$, CD spectrum of peptide, *2* $(Lys\text{-}Ala\text{-}Ala)_5$, difference CD spectrum, *3* $(Lys\text{-}Ala\text{-}Ala)_{10}$, CD spectrum of peptide, *2* $(Lys\text{-}Ala\text{-}Ala)_{10}$, difference CD spectrum

$(Lys\text{-}Ala\text{-}Ala)_{10}Ape$ (Fig. 4). For comparison, we also measured spectra of the complexes DNA–$(Lys\text{-}Ala\text{-}Ala)_n$ (Lys/DNA = 0·25 and 0·5) and DNA–$(Lys\text{-}Leu\text{-}Ala)_n$ (Lys/DNA = 0·5). The difference CD spectra of $(Lys\text{-}Ala\text{-}Ala)_n$ and $(Lys\text{-}Leu\text{-}Ala)_n$ differ from each other and from those of the corresponding peptides in solution (Fig. 5).

DISCUSSION

The CD spectra show (Tables IV–VI) that in aqueous solutions of low ionic strength all the studied oligopeptides exist in a random conformation. If we consider a random conformation of short peptides as a mixture of various conformers, the differences in CD curves of the individual peptides may be explained by a different population of these conformers. In the same way one can also explain the spectral changes for the dimer and trimer in both the studied series on addition of methanol or trifluoro-

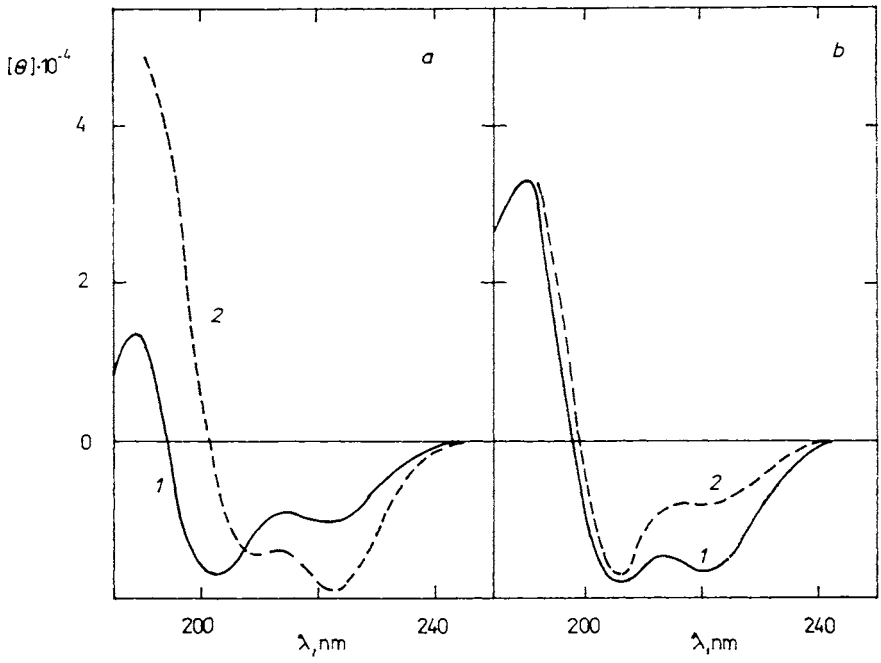

FIG. 5

CD spectra and difference CD spectra of $(Lys\text{-}Ala\text{-}Ala)_n$ and $(Lys\text{-}Leu\text{-}Ala)_n$ in buffer A; *a* $(Lys\text{-}Ala\text{-}Ala)_n$ *1* CD spectrum of peptide, *2* difference CD spectrum, *b* $(Lys\text{-}Leu\text{-}Ala)_n$ *1* CD spectrum of peptide, *2* difference CD spectrum

ethanol (Tables IV, VI, Fig. 2). Because of the length of the peptide chain, formation of an ordered α-helical structure in these peptides is not very probable.

Spectral changes, observed for the Lys-Leu-Ala tetramer and Lys-Ala-Ala tetramer, pentamer and decamer upon addition of methanol or trifluoroethanol, indicate a partial formation of the α-helical structure (Table IV–VI, Fig. 1, 2). As seen from the properties of the tetramers, leucine-containing peptides show a greater ability to form α-helical structure, in accord wiht our previous results with polymers[2] as well as with the results of other authors[15]. Trifluoroethanol is invariably more efficient than methanol in the formation of α-helical structure, the only exception being the decamer in 90% alcoholic solution where the effect of both solvents is practically the same. This result shows that the decamer is much more able to adopt α-helical structure than shorter peptides. However, compared with the polymer $(Lys\text{-}Ala\text{-}Ala)_n$, containing in average 34 monomeric units, the α-helix-forming ability of the decamer is substantially lower. This is illustrated already by the different behaviour of both peptides in an aqueous medium (Table V) where the CD spectra of the decamer are identical in pure water and in a solution of low ionic strength whereas the spectra of the polymer show that even a partial shielding of electrostatic repulsion by addition of small amount of salt is sufficient to promote formation of a certain amount of α-helical structure. Differences in behaviour of decamer and polymer can be observed also in a water–trifluoroethanol mixture (Table V). For the polymer the CD spectra corresponding to the α-helical structure were found already at trifluoroethanol concentration 30% whereas for the decamer the necessary concentration is 90%. In both cases further addition of trifluoroethanol has almost no effect. The CD spectra corresponding to maximum α-helicity for the decamer and polymer differ both in the intensities and in the intensity ratio of the negative bands at 205·5 to 206·5 nm and 219–219·5 nm. It follows from these results that the limit of chain lengths beyond which a linear polypeptide begins to behave as a polymer is higher than 31 amino acid units.

The CD spectra of oligopeptide–DNA complexes enabled us to evaluate conformational changes, accompanying the complex formation. The results show that interaction with oligomers of both the studied series brings about no substantial DNA conformational changes: the long-wavelength positive CD band of DNA does not change (for decamer and polymer) or its intensity decreases (for shorter peptides) at most by 20% (Table VII). As shown by Baase and Johnson[14], this band is sensitive to very small changes of the average rotation per base pair. The decrease in the intensity of this band is probably due to a small increase in the winding angle per base pair which corresponds to the winding of the B DNA helix and thus reduction of the number of base pairs per turn. Analogous changes in the CD spectra were also found for lysine[16,17] and arginine[17] oligomers.

The conformation of the oligopeptides in complex with DNA is reflected by difference CD spectra in the short-wavelength region. No conformational change has

been found for the dimer, trimer and tetramer of Lys-Ala-Ala and the dimer and trimer of Lys-Leu-Ala. In the pentamer and decamer of Lys-Ala-Ala (Fig. 4) and tetramer of Lys-Leu-Ala, the binding to DNA is accompanied by conformational changes that can be formally interpreted by formation of a small amount of α-helical structure. Nevertheless, we may say that in their complexes with DNA all the studied oligomers exist in a random conformation.

In the polymers of both the studied series the greater ability of longer chains to assume an ordered structure is also reflected in the properties of the complexes. The binding of polymers to DNA is accompanied by greater conformational changes than in the case of oligomers and the CD spectra of polymers in the complexes indicate higher population of ordered structures (Fig. 5). The difference in binding of the Lys-Ala-Ala polymer and decamer, particularly as concerns its reversibility has been already pointed out in one of our previous papers[5]. Whereas the binding of the decamer to DNA is reversible, that of the polymer is not. CD measurements in the region of shorter wavelengths proved that both complexes differ also in the conformation of the peptide component. The CD difference spectrum of the polymer resembles a spectrum of α-helix (Fig. 5), however, the intensity ratio for the negative π–π^* and n–π^* bands differs from that in a typical α-helix spectrum. We suppose that this spectrum might represent a mixture of α-helix and a small amount of β-conformation. The difference CD spectrum of $(Lys\text{-}Leu\text{-}Ala)_n$ (Fig. 5) differs from that of $(Lys\text{-}Ala\text{-}Ala)_n$ and is similar to that of the aggregated peptide in solution[18] where α-helical conformation is expected. Subirana and coworkers[19] studied complexes of DNA with polymers $(Lys\text{-}Ala\text{-}Ala)_n$ and $(Lys\text{-}Leu\text{-}Ala)_n$ by X-ray diffraction in fibers. According to these authors, the complex DNA-$(Lys\text{-}Leu\text{-}Ala)_n$ is composed of parallel layers consisting of double helices of DNA and α-helices of the polypeptide, whereas in the DNA-$(Lys\text{-}Ala\text{-}Ala)_n$ the polypeptide is probably wound around the DNA double helix. In accord with Subirana and coworkers[19], our results show that $(Lys\text{-}Leu\text{-}Ala)_n$ exists in α-helical conformation; contrariwise, however, we found the α-helix also in the complex of DNA with $(Lys\text{-}Ala\text{-}Ala)_n$. This difference may be due to the fact that the complexes are not entirely identical because in our case they were prepared by direct mixing whereas Subirana and coworkers[19] used equilibrium dialysis. We cannot exclude even the possibility that the complex could consist of DNA double helices around which α-helices of the polypeptide are wound, which represents one of the possible explanations given by Subirana and coworkers[19] for their results. Both complexes also differ substantially in their melting curves[20,21] which indicates differences in stabilization of the DNA secondary structure by the polypeptides $(Lys\text{-}Ala\text{-}Ala)_n$ and $(Lys\text{-}Leu\text{-}Ala)_n$.

Our results show that, as in solution, the peptide conformation in its DNA–-complexes depends on its length. Also from these measurements it follows that the limit beyond which properties of a linear peptide are independent of its length is higher than 31 amino acid units.

We are indebted to Mrs H. Janešová for the extraordinarily effective help in the synthesis and characterization of the prepared compounds. Our thanks are also due to Dr I. Frič for useful discussion and valuable comments concerning interpretation of the CD *spectra.*

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Translated by M. Tichý.