SYNTHESIS OF THE POLYPEPTIDES $(Ala\text{-}Orn\text{-}Orn)_n$ AND $(Ala\text{-}Orn\text{-}Lys)_n$ AND THEIR STUDY AS MODELS OF HISTONES

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UDC 547.466.1

The regular polypeptides $(Ala\text{-}Orn\text{-}Orn)_n$ and $(Ala\text{-}Orn\text{-}Lys)_n$ have been synthesized by the polycondensation of the 2,4,5-trichlorophenyl esters of the corresponding tripeptides, which were obtained by the mixed-anhydride method. Benzyloxycarbonyl and tert-butoxycarbonyl groups were used for blocking the amino groups. The polycondensation was performed in dimethylformamide in the presence of triethylamine at room temperature for 6 days. The benzyloxycarbonyl groups were eliminated from the side chains of the polypeptides with 35% hydrogen bromide in glacial acetic acid. It has been shown by the method of circular dichroism that the adoption of the conformation of an extended left-hand helix is characteristic for both polypeptides, which model the terminal sections of histones.

In order to understand the working of the mechanisms of the genetic apparatus of animals and plants, it is necessary to elucidate the functional role of the nuclear proteins, histones, in the regulation of gene activity, and, in connection with this, to study the potential structural possibilities of various polypeptide chains of histones, to which is ascribed the capacity either for interacting with DNA or for histone-histone interactions, or interaction with other components of chromatin. The polypeptide chains of histones contain sections with the conformational potentials of three regular structures: α-helix, β form, and the conformation of an extended left-handed helix (ELH), between which conformational transitions may take place with a change in the conditions of the environment [1, 2].

Since individual sections of histones possess a completely determined secondary structure, it is possible to demonstrate concrete conformations that are characteristic of these sections with the aid of polypeptides of regular structure. An analysis of amino acid sequences [3] has shown that at the ends of histone molecules, which are characterized by a high density of positive charges because of their content of basic amino acids, the polypeptide chains are capable of adopting the ELH conformation. This can be seen clearly in models of polypeptides enriched with basic amino acids. We have synthesized the regular polypeptides $(Ala\text{-}Orn\text{-}Orn)_n$ and $(Ala\text{-}Orn\text{-}Lys)_n$, modeling the terminal sequences of various fractions of histones enriched with alanine and basic amino acids, and have investigated them by the circular dichroism (CD) method. The polypeptides were obtained by polymerizing the corresponding activated 2,4,5-trichlorophenyl esters of the tripeptides with the δ- and ε-amino groups of the side chains of the ornithine and lysine residues protected with the benzyloxycarbonyl (Z) groups. The α-amino group of alanine was blocked with a tert-butoxycarbonyl (BOC) protection, which was readily removed in the presence of the benzyloxycarbonyl group. The tripeptides were synthesized according to the scheme, using the mixed-anhydride method.

The polycondensation of the trifluoroacetates of the 2,4,5-trichlorophenyl esters of the tripeptides was carried out in dimethylformamide in the presence of triethylamine at room temperature for 6-8 days.

Analysis of the CD spectra of the polypeptides $(Ala\text{-}Orn\text{-}Orn)_n$ and $(Ala\text{-}Orn\text{-}Lys)_n$ shows that they are practically analogous and in aqueous solution at pH 5.8-6.5 give a spectrum indicating a more perfect ELH conformation than the CD spectra of $(Ala\text{-}Orn\text{-}Ala)_n$ [4], with a

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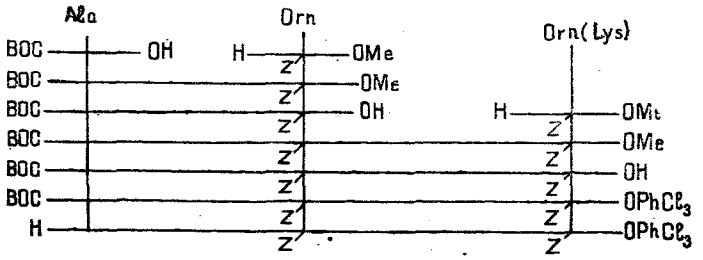
0009-3130/81/1702-0163$07.50



characteristic positive CD band in the 215-220 nm region, a strong negative short-wave band at 196 nm, and a weak negative long-wave band at 230-235 nm (Fig. 1). At pH 3, when the charge of the polypeptide $(Ala\text{-}Orn\text{-}Orn)_n$ does not change, the CD spectrum remains practically unchanged; the change in the CD spectra when the charged groups are neutralized (pH 11.5) shows a possible destabilization of the ELH. The polypeptide $(Ala\text{-}Orn\text{-}Ala)_n$ behave differently (Fig. 2), forming under these conditions a considerable amount of α-helical sections, i.e., a potential capacity for forming α-helices appears as soon as the charged groups are neutralized. On heating, the α-helical sections break down, and at 80°C the CD spectra in both cases show a destablized ELH. The screening of the charges by ions of salts in 1 M NaF does not effect the CD spectra of $(Ala\text{-}Orn\text{-}Orn)_n$, while under these conditions $(Ala\text{-}Orn\text{-}Ala)_n$ forms a small α-helical section. In a 1% solution of sodium dodecyl sulfate (SDS) the ELH is stabilized to some degree and $(Ala\text{-}Orn\text{-}Ala)_n$ forms approximately as large an amount of α-helical sections as at alkaline pH values. The formation of α-helical sections is observed in 80% trifluoroethanol (TFE) solutions of both polymers. The CD spectra of the polypeptide $(Ala\text{-}Orn\text{-}Glu)_n$ (for the synthesis, see [5]) (Fig. 3), which differs from $(Ala\text{-}Orn\text{-}Orn)_n$ both in amino acid composition and in the distribution of charges in the chain, behaves somewhat differently. While at neutral pH values this difference is not very pronounced, neutralization of the glutamic acid residues (at pH 2.8) and neutralization of the ornithine residues (at pH 11.5) leads to the stabilization of the ELH in the case of $(Ala\text{-}Orn\text{-}Glu)_n$ through the remaining other charges and to the destabilization of $(Ala\text{-}Orn\text{-}Orn)_n$, in which no such charges remain. Consequently, the "repulsion" of the charges makes the ELH conformation more perfect, since it promotes the formation of a more extended structure, and, possibly prevents the bending of the polypeptide chain.

The screening of the charges (in 1 M NaF) has little effect on the behavior of the polypeptide $(Ala\text{-}Orn\text{-}Glu)_n$, while in 1% SDS this polypeptide reveals the formation of a certain amount of α-helical sections, i.e., the negative band of the CD spectrum in the 222 nm region becomes stronger, and the short-wave band shifts somewhat in the red direction, while the band at 222 nm also becomes less intense when the solution is heated, which leads to the breakdown of α-helices. In 80% TFE solution, the tendency to the formation of an α-helix is greater for the glutamyl-containing polymer than for $(Ala\text{-}Orn\text{-}Orn)_n$. In a 4.8 M solution of guanidine hydrochloride the ELH conformation is stabilized for all the polymers considered.

Thus, in the charged state these polypeptides show in their CD spectra a tendency to the formation of the ELH conformation, which is more perfect and more heat-stable for the polypeptides $(Ala\text{-}Orn\text{-}Orn)_n$ and $(Ala\text{-}Orn\text{-}Lys)_n$, while the polypeptide $(Ala\text{-}Orn\text{-}Ala)_n$ has a greater tendency to the formation of an α-helical conformation. Some tendency to the formation of an α-helical conformation appears in the polypeptide $(Ala\text{-}Orn\text{-}Glu)_n$.

The differences in the conformational potentials of polypeptides enriched with basic acids as compared with polypeptides where the proportion of alanine residues is increased or there are glutamic acid residues in equal proportion to the alanine residues, modeling the terminal sections of various histones, are apparently connected with the biological functions that they fulfill in the chromatin. The considerable amounts of ELH conformation in histones are apparently explained by its biological function, since sections with such a conformation are, as a rule, localized in the terminal sections of the histone molecules responsible for their interaction with DNA.

EXPERIMENTAL

In the synthesis we used amino acids of the L form. Melting points were determined on a Boëtius instrument. For all the products obtained the results of elementary analysis for C, H, and N, which was carried out on a Hewlet-Packard CHN analyzer 185B, corresponded to

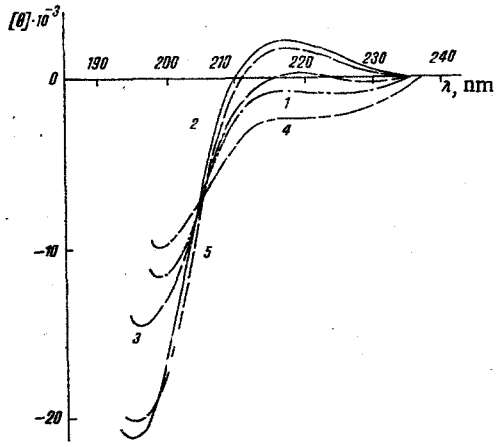


Fig. 1

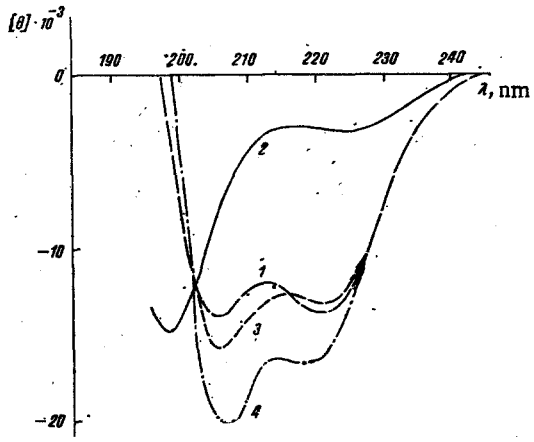


Fig. 2

Fig. 1. CD spectra of the polypeptide $(Ala\text{-}Orn\text{-}Orn)_n$ under various conditions: 1) pH 11.5; 2) 1 M NaF; 3) 1% SDS; 4) 80% TFE; 5) pH 5.8.

Fig. 2. CD spectra of the polypeptide $(Ala\text{-}Orn\text{-}Ala)_n$ under various conditions (symbols the same as for Fig. 1).

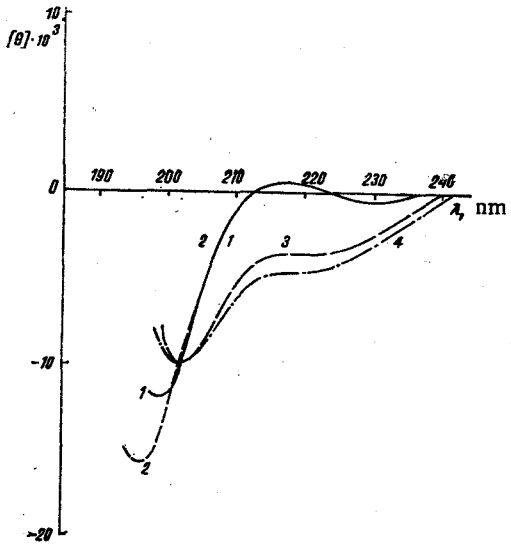


Fig. 3. CD spectra of the polypeptide $(Ala\text{-}Orn\text{-}Glu)_n$ under various conditions (symbols the same as for Fig. 1).

the calculated figures. The course of the reactions and the purity of the products were monitored chromatographically in a thin layer of silica gel fixed with gypsum in the systems: 1) benzene–ethanol (2:0.4), and 2) butan-1-ol–acetic acid–water (100:10:30). Substances with free amino groups were revealed on the chromatograms with ninhydrin, and the others with iodine vapor.

The completeness of the elimination of the protective groups from the polypeptides was checked spectrophotometrically on a Hitachi EPS-3T spectrophotometer.

In the synthesis of the tripeptides, the reaction mixtures in organic solvents were washed with water, 10% citric acid solution, and 5% sodium bicarbonate solution and were dried over anhydrous sodium sulfate. The values of $[\alpha]_D^T$ were measured on a polarimeter of domestic manufacture. No racemization exceeding the errors of measurement (±2%) was observed for either polypeptide. The mean molecular weights of the polypeptides $(Ala\text{-}Orn\text{-}Orn)_n$ and $(Ala\text{-}Orn\text{-}Lys)_n$ determined by the Van Slyke method and by gel filtration were 6500 and 7500, respectively.

The CD measurements were made on a Cary-6003 spectropolarimeter with an attachment for measuring circular dichroism in the 270-190 nm region in thermostated quartz cells with a path length of 1 mm. THF stands for tetrahydrofuran, DMFA for dimethylformamide, TEA for triethylamine, and IBCF for isobutyl chloroformate.

Methyl Esters of tert-Butoxycarbonylalanyl-N^{δ}-benzoxycarbonylornithyl-N^{δ}-benzoxycarbonylornithine (II). With stirring, 0.63 ml of TEA was added to a solution of 2 g of tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithine (I) [5] in 20 ml of THF, and then the solution was cooled to −(13-15)°C, and 0.6 ml of IBCF was added to the reaction mixture with constant stirring. After 10 min, a suspension containing 1.44 g of the hydrochloride of the methyl ester of N^{δ}-benzyloxycarbonylornithine, 0.63 ml of TEA, and 15 ml of absolute THF was added at −15°C. The mixture was stirred at room temperature for 12 h, and then the THF was evaporated off in vacuum and the residue was mixed with a 10% solution of citric acid (150 ml). The oily precipitate that deposited was separated off and dissolved in ethyl acetate, and the solution was washed and dried. Yield 2.8 g (87.5%), mp 106-108°C (ethyl acetate–ether), R_f 0.82 (system 1), 0.87 (system 2) $[\alpha]_D^{23}$ −28° (c 1; chloroform).

The methyl ester of tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithyl-N^{ε}-benzyloxycarbonyllysine (III) was obtained similarly to compound (II) from 2.1 g of (I) and 1.54 g of the hydrochloride of lysine methyl ester. Yield 2.71 g (79.4%), mp 113-115°C, R_f 0.78 (system 1), 0.73 (system 2); $[\alpha]_D^{23}$ −23.5° (c 1; chloroform).

tert-Butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithyl-N^{δ}-benzyloxycarbonylornithine (IV). At room temperature, 4.4 ml of 1 N caustic soda solution was added to a solution of 2.8 g of (II) in 4.4 ml of acetone. After 3.5 h, part of the acetone was evaporated off and the remaining mixture was diluted with water. To eliminate unsaponified ester, the reaction mixture was extracted with chloroform (2 × 15 ml). The aqueous layer was acidified with citric acid to pH 2, and the saponified product (IV) was extracted with chloroform (4 × 20 ml). The extract was washed with water, dried, and evaporated. On the addition of absolute ether, the oily product was converted into an amorphous white precipitate. Yield 2.74 g (95%), mp 111-113°C, R_f 0.21 (system 1), 0.56 (system 2).

tert-Butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithyl-N^{ε}-benzyloxycarbonyllysine (V) was obtained and isolated in a similar manner to substance (IV). Yield 1.7 g (94%), mp 126-128°C, R_f 0.3 (system 1), 0.58 (system 2).

2,4,5-Trichlorophenyl Ester of tert-Butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithyl-N^{δ}-benzyloxycarbonylornithine (VI). With stirring, 0.54 ml of TEA was added to a solution of 2.74 g of (IV) in 35 ml of THF, and then the mixture was cooled to −(13-15)°C and 0.5 ml of IBCF was added. After 10 min, 1.28 g of 2,4,5-trichlorophenol was added. The mixture was stirred at room temperature for 12 h. The THF was evaporated off in vacuum, the residue was dissolved in ethyl acetate, and the solution was washed, dried, and evaporated. Yield 2.79 g (81%), mp 122-123°C (ethyl acetate–petroleum ether), R_f 0.62 (system 1), 0.83 (system 2); $[\alpha]_D^{23}$ −23° (c 1; chloroform).

The 2,4,5-trichlorophenyl ester of tert-butoxycarbonylalanyl-N^{δ}-benzyloxycarbonylornithyl-N^{ε}-benzyloxycarbonyllysine (VII) was obtained in a similar manner to (VI) from 2.8 g of (V) and 0.8 g of 2,4,5-trichlorophenol. Yield 3.3 g (94%), mp 105-107°C (ethyl acetate–ether); R_f 0.80 (system 1); $[\alpha]_D^{23}$ −26° (c 0.58; chloroform).

2,4,5-Trichlorophenyl Ester of the Trifluoroacetate of Alanyl-N^{δ}-benzyloxycarbonylornithyl-N^{δ}-benzyloxycarbonylornithine (VIa). A solution of 1 g of (VI) in 1.2 ml of trifluoroacetic acid was kept at room temperature for 60 min. Then the solvent was eliminated by evaporation in vacuum. The residue was washed with ether and was reprecipitated with ether from methanol. Yield 0.85 g (84.3%), mp 118-120°C, R_f 0.65 (system 2).

Polycondensation. A solution of 0.4 g of (VIa) in 0.34 g of absolute DMFA was treated with 0.077 g of TEA and the mixture was left at room temperature for 6 days. Then it was treated with water, and the resulting precipitate was filtered off and was washed with methanol and ether. Yield $\simeq$52%. The polycondensation of (VIIa) was performed similarly. The yield at the polycondensation stage was $\simeq$46%.

The benzyloxycarbonyl groups were eliminated from the side chains of the polypeptides with an excess of hydrogen bromide in glacial acetic acid at room temperature for 2 h. The polypeptides were purified as described previously [6].

SUMMARY

The polypeptides of regular structure $(Ala\text{-}Orn\text{-}Orn)_n$ and $(Ala\text{-}Orn\text{-}Lys)_n$ modeling the terminal sections of various histones enriched with alanine and basic amino acids, have been synthesized. Analysis of the CD spectra of both polypeptides has shown that a tendency to the formation of the ELH conformation is characteristic for them, and this apparently is explained by the biological function of the terminal sections of histones which are responsible for their interaction with DNA.

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A STUDY OF THE INTERACTION OF THE CYTOTOXIN OF THE VENOM OF THE CENTRAL ASIAN COBRA WITH LIPOSOMES BY THE SPIN PROBE METHOD

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UDC 547.993.615:577.3

It has been shown by the spin probe method that the action of the cytotoxin of the venom of the central asian cobra on artifical liposomes extends at least to the depth of localization of the iminoxyl head of stearic acid spin-labeled at C-16. The greatest effect of the cytotoxin is observed on liposomes with a negative surface charge.

The cytotoxin (CT) of the venom of the cobra *Naja naja oxiana* forms a complex with natural and model phospholipid membranes through the interaction of the positive charges of the amino groups of the lysine residues with the negatively charged groups of the membranes and the hydrophobic sections immersed in them [1, 2]. However, the depth of the structural changes in the membranes caused by the action of CT is not clear.

In the present paper we consider the results of an investigation by the spin probe method of the structural changes in the hydrophobic region of liposomes caused by the action of CT. As the spin probe we used stearic acid spin-labeled at C-16 (Fig. 1). After its introduction into the membrane, the probe is orientated with its fatty chain parallel to the tails of the phospholipids of the membrane [3]. Consequently, a change in the electron spin resonance (ESR) spectrum of the probe will indicate changes in the membrane in the region in which its iminoxyl fragment is localized. For a quantitative estimate of the dependence of the structural changes in the membrane taking place under the action of CT on the surface charge of the membrane, we used liposomes prepared from neutral phophatidylcholine (PC), a mixture of the negatively charged phosphatidic acid (PA) and PC in a ratio by weight of 1:1, and also sonicated suspensions of PC which, together with liposomes, form lamellar structures.

The ESR spectrum of the probe in ethanol consists of a triplet signal with narrow components (Fig. 2, curve 1). When the probe is added to samples of suspensions of liposomes,

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Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 211-215, March-April, 1981. Original article submitted September 16, 1980.

0009-3130/81/1702-0167$07.50