(5 ml). The reaction mixture was stirred vigorously for 8 min, acidified with acid to pH 5.0, and concentrated in vacuum, and then methanol was added to the residue in portions and the boric acid was evaporated off in vacuum in the form of methyl borates. The residue was dissolved in 20 ml of 0.5 N hydrochloric acid and, after 20 min, it was passed through a column of Polikhrom as described above. The progenin (VIII) that was eluted with 50% ethanol was chromatographed on a column of silica gel in system 1. This gave 23 mg of (VIII), mp 238-240°C, $[\alpha]_D^{20}$ −23.8° (c 0.12; pyridine).

Methylation. A solution of 20 mg of a desulfated glycoside or progenin in 1 ml of dry dimethyl sulfoxide (DMSO) was added to a solution of the methylsulfinyl anion (prepared from 300 mg of sodium hydride and 7 ml of DMSO), and the mixture was stirred at 60°C in an atmosphere of dry argon for 2 h. Then, at 0°C, 2 ml of methyl iodide to the reaction mixture and it was left at room temperature for 2 h and was then diluted with water (10 ml) and extracted with CH_3I (2 × 2 ml). The extract was washed with 2 ml of a saturated solution of sodium thiosulfate and with 2 ml of water and was evaporated in vacuum. The residue so obtained was boiled in 2 ml of anhydrous methanol saturated with hydrogen chloride for 2 h, the solvent was driven off in vacuum, and the residue was dissolved in 2 ml of pyridine–acetic anhydride (1:1) to give a reaction mixture which was heated at 100°C for 1 h. The mixture of derivatives of methyl α- and β-glycosides obtained was analyzed by GLC and by GLC-MS.

LITERATURE CITED

1. S. A. Avilov, L. Ya. Tishchenko, and V. A. Stonik, Khim. Prir. Soedin., 799 (1984).
2. G. B. Elyakov, V. A. Stonik, Sh. Sh. Afiyatullov, A. I. Kalinovskii, V. F. Sharypov, and L. Ya. Korotkikh, Dokl. Akad. Nauk SSSR, 1363 (1981).
3. V. F. Sharypov, A. I. Kalinovskii, V. A. Stonik, S. A. Avilov, and G. B. Elyakov, Khim. Prir. Soedin., 55 (1965).
4. A. S. Shashkov and O. S. Chizhov, Bioorgan. Khim., 2, 437 (1976).
5. S. A. Avilov, A. I. Kalinovskii, and V. A. Stonik, Khim. Prir. Soedin., 53 (1990).
6. A. I. Kalinovskii, I. I. Mal'tsev, A. S. Antonov, and V. A. Stonik, Bioorg. Khim., 10, 1655 (1984).
7. V. I. Kalinich, V. R. Stepanov, and V. A. Stonik, Khim. Prir. Soedin., 789 (1983).

CONFORMATIONAL FEATURES OF PEPTIDE FRAGMENTS OF THE C-END OF HISTONE H1

R. R. Kamilova, E. I. Ramm, G. S. Ivanov, L. I. Mar'yash, and V. K. Burichenko

UDC 541.65+547.466.1

The conformational possibilities of four synthesized oligopeptides with the amino acid sequences 165–172, 173–184, 152–172, and 152–184 of the C-end of histone H1 of calf thymus above been studied in solution under various conditions by the method of circular dichroism.

Histone H1 is ascribed a particularly important role in the structure of chromatin [1, 2]. This fraction, bound with the linker section of DNA, is responsible for maintaining the higher levels of its structural organization and it also, apparently, participates in the processes of compactization–decompactization of chromatin during its functioning. The elucidation of the structural possibilities of the polypeptide chain of this histone is extremely important for understanding the mechanism of such processes.

Histone H1 has a three-domain organization [3, 4]. The N-terminal domain from the 1st to the 35th amino acid residue is the most variable and is highly charged, and in it are con-

V. I. Nikitin Institute of Chemistry, Academy of Sciences of the Tadchik SSR, Dushanbe. Institute of Cytology, Academy of Sciences of the USSR, Leningrad.

Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 793-798, November-December, 1990. Original article submitted May 23, 1989; revision submitted January 29, 1990.

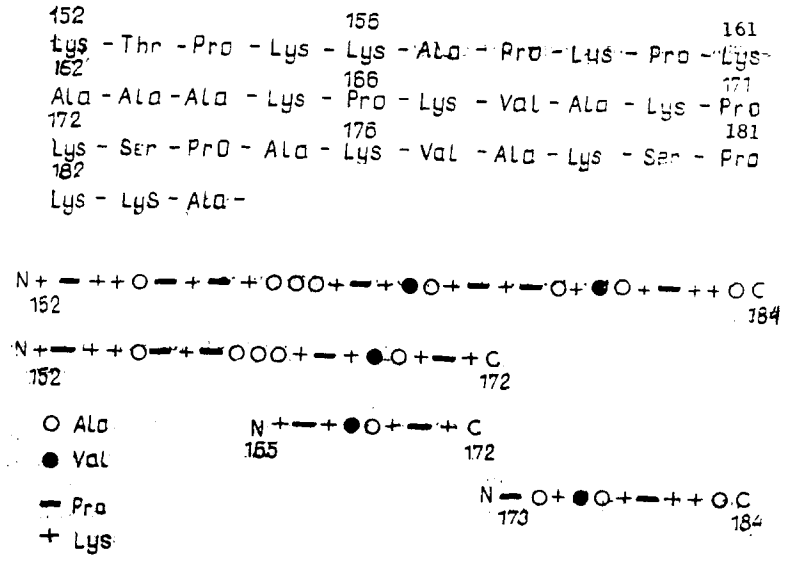

Fig. 1. Amino acid sequence of the peptide fragment 152–184 of the C-end of histone H1, consisting of 33 amino acids, and schematic illustration of all the peptide fragments studied: 152–184, 152–172, 165–172, and 173–184.

centrated the main sites of the modification of this protein. The central section of H1, approximately from the 36th to the 121st amino acid residues, is capable of forming a globular structure in a solution with a high ionic strength [4-6]. On interacting with the DNA of the core, this section fixes in place two complete turns of the DNA superhelix on the surface of the nucleosome, stabilizing the structure of the chromatosome. The third domain [the long highly charged C-terminal fragment of histone H1 (from the 122nd to the 215th amino acid)] consists mainly of alternating lysine, alanine, and proline residues. It is the longest of the terminal sections ("tails") of the histones and its conformational possibilities have scarcely been studied. Model investigations have shown that it is just this section of histone H1 in the chromatin of sea urchin spermatozoa that may be responsible for the supercompact state of this chromatin [6, 7]. All the above-mentioned facts have impelled us to undertake a more detailed investigation of the conformational features of the C-fragment of histone H1.

There have been repeated previous attempts at modeling the conformational properties of this fragment by synthetic polypeptides – copolymers of lysine and alanine amino acid residues [8, 9]. It has been shown, that under various conditions they are capable of forming considerable amounts of α-helical or β-folded structures. However, the large number of proline residues present in the C-terminal domain of histone H1 throws doubt upon the possibility of transferring the properties of these model polypeptides to the conformation of this fragment, since, because of its steric characteristics, proline is not incorporated into an α-helix or a β-structure. Serving as a basis for such a hypothesis are investigations of polypeptides including proline residues together with lysine and alanine residues [10].

We have synthesized oligopeptides with the following amino acid sequences: 1) 165–172; 2) 173–184; 3) 152–172; and 4) 152–184 of the C-end of calf thymus histone H1 (Fig. 1) and have studied their conformational possibilities in solution by the method of circular dichroism (CD).

We have shown previously that in water at acid and neutral pH values all histone fractions, and H1 in particular, adopt mainly the conformation of an extended left-handed helix of the type of polyproline II (Pro-II) [11]. In the CD spectra, this conformation is characterized by a deep negative band at ~197 nm and a weak positive band in the ~220 nm region. Destabilization of the Pro-II structure is shown in the appearance of a negative CD at 230 nm and a lowering of the amplitude of the positive band at 215 nm with its passage into the region of negative values of the ellipticity [12]. A study of the CD spectra of synthetic peptide fragments of the N-ends of histones H2B and H4 [13, 14] and of polypeptide models of the terminal sections of histones [15] and a comparison of them with the CD spectra of whole

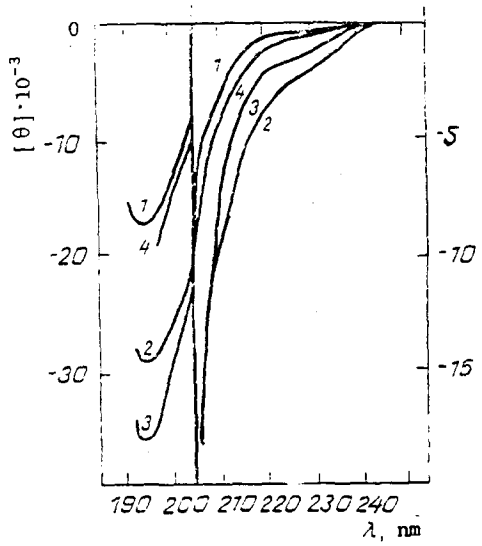

Fig. 2. CD spectra of synthetic peptide fragments of the C-end of histone H1 in deionized H_2O, pH 3.5-4.0, 20°C: 1) 165–172; 2) 175–184; 3) 152–172; 4) 152–184.

fractions of histones has shown that sections with the Pro-II conformation are localized in the histones mainly in the terminal sections. Being an extremely labile structure it is apparently most favorable for interaction with DNA.

The presence of a large number of charges and proline residues in our peptide fragments (Fig. 1) must also promote the stabilization of the Pro-II structure in them. However, the presence of hydrophobic amino acid residues under the conditions of neutralization of the ε-amino groups of the lysine residues at alkaline pH values or in solution at a high ionic strength may lead to the formation of α-helical structures or β-structures in the sections 160–166 or 174–181 between proline residues.

The CD spectra of all four peptide fragments in H_2O at pH 3.5-4.0 given in Fig. 2 show features of a left-handed helical conformation. They all have a deep negative CD band close to 195 nm and a shoulder in the long-wave part of the spectrum at 215-240 nm. The magnitude of the optical effect in this region characterizes the degree of defectiveness of the left-handed helical conformation. The mutual repulsion of a large number of positively charged side chains of the lysine residues and the cumbersome side chains of the proline residues (Fig. 1) in solution in deionized water are factors stabilizing an extended structure of the oligopeptides under investigation. However, the presence of other amino acid residues such as those of alanine, valine, etc., leads to destabilization (an increase in fluctuations, breaks, and degects) of the regular left-handed helix of the Pro-II type. Thus, the CD spectra shown in Fig. 2 of all four oligopeptides demonstrate features characterisitc for a somewhat destabilized left-handed helical conformation.

Figure 3a-d, shows the spectra in the 210-250 nm region of all four oligopeptides studied, under various conditions. In concentrated solutions of urea and of guanidine chloride, the positive contribution in the 220 nm region characteristic for a regularly left-handed conformation is intensified in all cases. However, it is greatest for the 152-172 oligopeptide and least for the 173–184 oligopeptide. Apparently, the amino acid sequence of the terminal part of the 152–172, of the oligopeptide 152-184 (Fig. 1) is more favorable for the formation of a left-handed helical conformation under conditions stabilizing it (10 M urea and 6 M guanidine chloride) [16].

Section 173–184 is apparently capable, under favorable conditions (80% ethanol, 1% SDS, 80% TFE) of forming a turn of an α-helix between two prolines (174 and 181) while under other conditions it is subject to a different type of deformation to a greater extent than the other sections. The peptide 152–184 (Fig. 3a), which contains both these fragments, possesses an intermediate capacity for the formation of an extended left-handed helix in concentrated urea and guanidine chloride solutions.

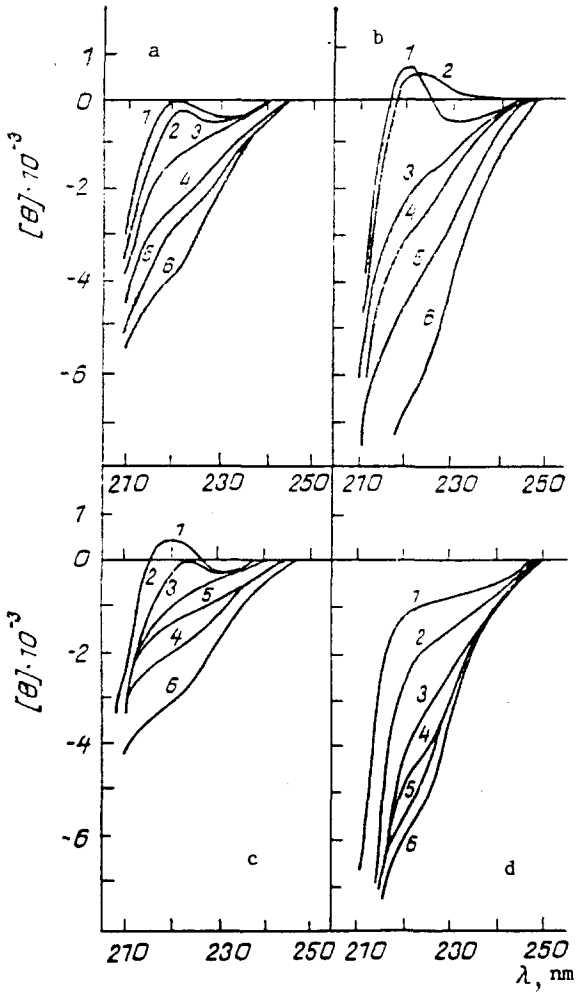

Fig. 3. CD spectra of synthetic peptide fragments of the C-end of histone H1: 152–184 (a); 152–172 (b); 165–172 (c); and 173–184 (d). Under various conditions: 1) 6 M guanidine chloride; 2) 10 M urea; 3) H_2O (pH 1.0-10; $\mu = 10^{-3}$-1.0 M NaCl); 4) 80% ethanol; 5) 1% SDS; 6) 80% trifluoroethanol.

An increase in the ionic strength of the solution (from 10^{-3} to 1.0 M NaCl) and in the pH (from 1.0 to 10) had little effect on the conformational properties of the peptides (Fig. 3a-d). On the basis of the results obtained, it is possible to put forward the hypothesis that the main factor in the stabilization of a left-handed helical conformation of the Pro-II type in the peptide fragments under investigation is a relatively high content of regularly distributed proline residues, although the influence of the mutual repulsion of the positively charged lateral ε-amino groups of lysine residues is not excluded.

An investigation of the CD spectra of the peptide in solutions of a detergent (1% SDS) or in helicalizing solvents (80% ethanol and 80% trifluoroethanol) (Fig. 3) showed that the most considerable increase in the contribution to the negative value of the CD close to 220 nm relating to the first longest-wave CD band of the α-helical conformation (222 nm) was observed for peptides 152–172 and 173–184. Each of these fragments is capable of forming one turn of an α-helix – between prolines 166 and 171 in the 152–172 peptide and between prolines 174 and 181 in the 173–184 peptide (Fig. 1). It is possible that the observed changes in CD are connected with an increase in the defectiveness of the left-handed helical conformation.

EXPERIMENTAL

The synthesis of the peptide fragments of the C-end of histone H1 was carried out by classical methods of peptide synthesis, as described in [17, 18]. After the elimination of the protective groupings, the peptides were completely soluble in water. The amino acid compositions of the oligopeptides obtained were confirmed by amino acid analysis. The concentra-

tions of the peptide solutions were determined by weight. The peptides were initially dissolved in water and the resulting solutions were then diluted with solutions of sodium dodecyl sulfate, urea, guanidine chloride, and ethanol to the necessary concentration, or microamounts of NaOH were added to bring them into the region of alkaline pH values.

The CD measurements were performed on a Varian Cary-60CD recording spectropolarimeter with an attachment for measuring circular dichroism in the 200-320 nm region in quartz cells with an optical path length of 1 cm at 20°C. The absorption of the cells with the solution did not exceed a unit of optical activity in all cases. The value of the molar ellipticity in deg·cm^2/dmole was calculated to the mean weight of an amino acid residues. The accuracy of the measurements was ±0.0002°. The instrument was calibrated with a solution of d-camphor-10-sulfonic acid.

LITERATURE CITED

1. B. O. Glotov and L. G. Nikolaev, Mol. Biol. (Moscow), 17, 891 (1983).
2. R. Losa, F. Thoma, and T. Koller, J. Mol. Biol., 175, 529 (1984).
3. R. D. Cole, Anal. Biochem., 136, No. 1, 24 (1984).
4. J. Allan, T. Mitchell, and N. Harborne, J. Mol. Biol., 187. No. 4, 591 (1986).
5. C. Crane-Robinson, D. Z. Staynov, and J. P. Baldwin, Commun. Mol. Cell. Biophys., 2, 219 (1984).
6. V. Giancotti, E. Russo, S. Cosimi, P. D. Cary, and C. Crane-Robinson, Biochem. J., 197, 655 (1981).
7. R. Misselwitz, D. Z. Zirwer, H. Damashun, G. Damashun, H. Welfle, I. A. Zalenskaya, E. I. Ramm, and V. I. Vorobjev, Int. J. Biol. Macromol., 8, 194 (1986).
8. S. Stokrova, K. Zimmerman, J. Sponar, and K. Blaha, Collect. Czech. Chem. Commun., 43, 2341 (1978).
9. J. Votavova, J. Pirkova, V. Gut, and K. Blaha, Collect. Czech. Chem. Commun., 53, No. 2, 389 (1988).
10. J. Sponar, S. Stokrova, I. Koruna, and K. Blaha, Collect. Czech. Chem. Commun., 39, 1625 (1974).
11. N. G. Esipova, E. I. Ramm, V. M. Lobachev, and V. I. Vorob'ev, Biofizika, 21, No. 3, 582 (1976).
12. Yu. A. Lazarev, V. M. Lobachev, N. G. Esipova, V. S. Grechishko, and V. A. Shibnev, in: Proceedings of the IIIrd All-Union Symposium on the Chemistry of Peptides and Proteins [in Russian], Kiev (1974), p. 81.
13. E. I. Ramm, N. I. Koryakina, A. I. Pisachenko, V. M. Lobachev, N. G. Esipova, V. K. Burichenko, Yu. A. Lazarev, and V. I. Vorob'ev, Biofizika, 21, 32 (1977).
14. E. I. Ramm, G. S. Ivanov, V. I. Vorob'ev, N. G. Esipova, M. V. Grigolava, V. S. Grechishko, and V. K. Burichenko, Biofizika, 28, No. 1, 35 (1983).
15. E. I. Ramm, R. R. Kamilova, O. L. Polozova, V. I. Vorob'ev, and V. K. Burichenko, Biofizika, 24, No. 5, 815 (1979).
16. M. L. Tiffany and S. Krimm., Biopolymers, 11, 2309 (1972).
17. L. I. Mar'yash, V. K. Burichenko, and V. A. Shibnev, Bioorg. Khim., 10, No. 4, 459 (1984).
18. L. I. Mar'yash, V. K. Burichenko, and V. A. Shibnev, Bioorg. Khim., 10, No. 4, 467 (1984).