COMPARATIVE INVESTIGATION OF THE STRUCTURE OF COMPLEXES OF DNA WITH THE POLYPEPTIDE FRAGMENT 152–184 OF HISTONE H1 AND THE POLYPEPTIDE $(Lys\text{-}Ala\text{-}Ala)_n$

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The interactions with DNA of a synthetic peptide fragment of histone H1 33 amino acid residues long the sequence of which corresponds to fragment 152–184 and of a synthetic polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ modeling the properties of the C-terminal fragments of histone H1 have been studied by the CD method. The use of the CD method for recording the spectra of the polypeptides under investigation in the free state and of the artificial complexes of DNA formed with them has permitted the characterization of different structural possibilities of the polypeptides in solution and of their complexes with DNA, and the conformational changes of the components of the complex under various ionic conditions.

One of the main functions of the histones of the H1 family in chromatin is the participation in the mechanism of its condensation [1, 2]. Histones H1 have a three-domain structure – a central, globular, domain and two terminal, highly charged, sections with extended structures [3]. Apparently, each of the domains is characterized by definite conformational possibilities and binds to different sections of the DNA in the chromatin, fulfilling functions distinct from those of the other domains. A considerable number of investigations has been devoted to the study of the interaction of histone H1 and its individual structural domains with DNA [4-8]. A definite role has been revealed for the C-terminal domain of histone H1 in the formaiton of the condensed form of DNA in vitro, which is shown in the form of a characteristic ψ-type of CD spectra of the DNA, reflecting a regular packing of its molecules [4].

The C-terminal fragment of histone H1 (122–215), consisting mainly of alternating lysine, alanine, and proline residues, is the longest of the terminal sections ("tails") of the histones and is characterized by a stable conformation of and extended left-handed helix of the type of polyproline II (Pro-II) [9]. The conformational flexibility and lability of such a structure and the high density of the positive charge in this domain, which are responsible for its affinity with DNA, are important factors for the fulfillment of such a function as the condensation of DNA. Model investigations of the interaction of a number of synthetic polypeotides of different compositions with DNA [10, 11] have shown that the mechanism of their interaction and their action on the structure of DNA for polypeptides characterized by different conformational possibilities are different. Therefore, to understand the situation in chromatin it is important to investigate the interaction of histone fragments with DNA and to study the structure of the complexes obtained.

We have studied the interaction with DNA of a synthetic peptide containing 33 amino acid residues the sequence of which corresponded to the 152–184 fragment of the C-end of histone H1: ...Lys-Thr-Pro-Lys- Lys-Ala-Pro-Lys-Pro-Lys-Ala-Ala-Ala-Lys-Pro-Lys-Val-Ala-Lys-Pro-Lys- Ser-Pro-Ala-Lys-Val-Ala-Lys-Ser-Pro-Lys-Lys-Ala-... . For comparison, we investigated in parallel the interaction with DNA of the synthetic polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$* modeling the composition of the

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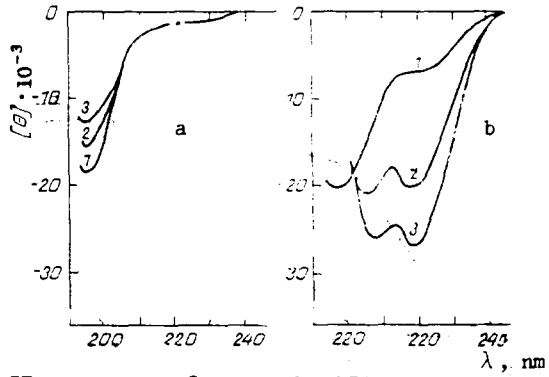

Fig. 1. CD spectra of peptide 152–184 of the C-end of histone H1 (a) and of the polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ (b) in aqueous solutions under various conditions: 1) H_2O, pH 4-6; 2) 1 M NaCl; 3) H_2O, pH 10.5.

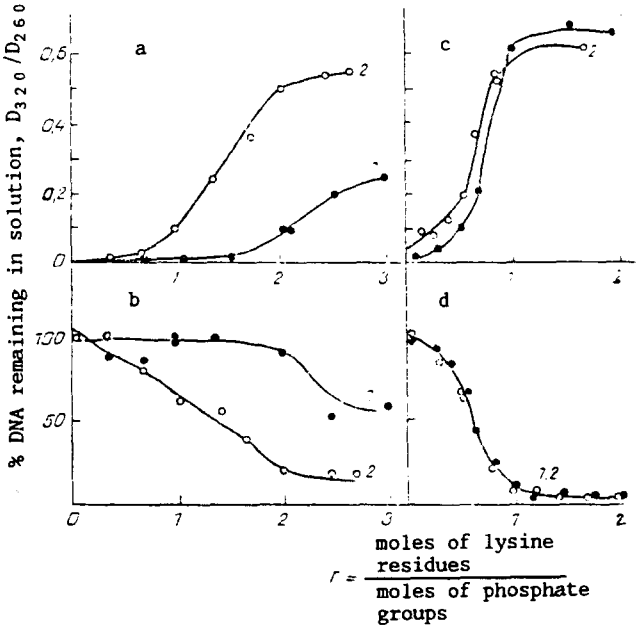

Fig. 2. Curves of the titration of DNA by peptide 152-184 of histone H1 (a, b) and by the polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ (c, d) in 0.015 and 0.15 M NaCl solutions [(1) and (2), respectively]; a, c) the change in the turbidity of the solutions of the complexes as a function of the amount of peptide component in them (r); b, d) percentage of DNA remaining in a solution of the complex after its centrifugation for 20 min at 12,000 rpm as a function of the amount of peptide component incorporated in the complex on its preparation (r).

C-terminal fragment of histone H1. The nature of the interaction with DNA under various ionic conditions was studied by titrating it with these polypeptides and recording the turbidity of the solutions (from the ratio of absorptions D_{320}/D_{260}). The capacity of the polypeptides for precipitating DNA from solution was evaluated in percentages of the DNA remaining in the solution after centrifugation of the complex that had formed with a given value of r (r being the ratio of the number moles of lysine in the polypeptide to the number of moles of DNA phosphate groups) in the course of 20 min at 12,000 rpm in comparison with the initial amount of DNA incorporated in the complex.

The circular dichroism (CD) method enabled us to characterize the difference in the structural possibilities of the polypeptides in solution and also of their complexes with DNA and the conditions and nature of the association and the conformational changes of the components of the complex under various ionic conditions. Figure 1 shows the CD spectrum of

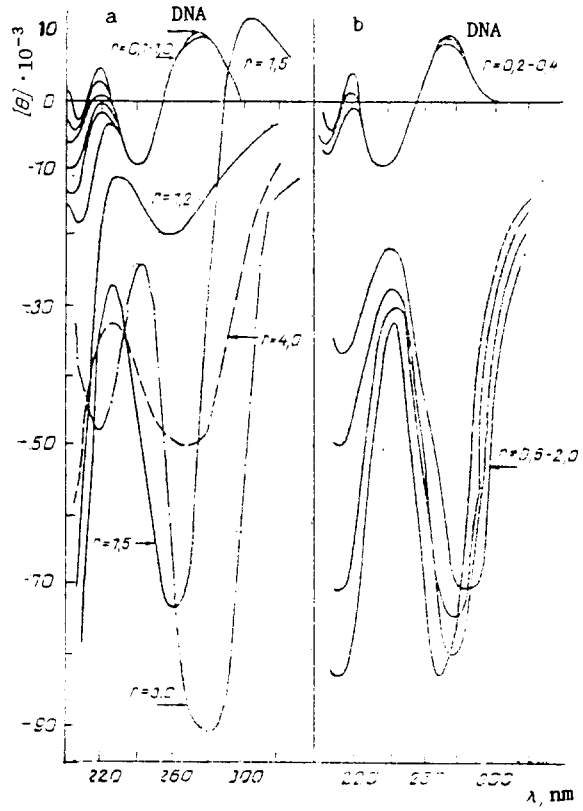

Fig. 3. CD spectra of DNA and its complexes with the 152-184 peptide from the C-end of histone H1 with different amounts of the peptide component (r) in 0.015 and 0.15 M NaCl solutions [(a) and (b), respectively].

both polypeptides in water under various conditions. In spite of some analogy in the composition of the amino acids (about 30% of lysine residues and a large number of alanine residues), the structural possibilities of these polypeptides differ strongly. While the Pro-II conformation of the peptide fragment 152-184 of histone H1 was extremely stable and no formation of other types of secondary structure was observed in aqueous solution (Fig. 1a), the CD spectra of the polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ indicated that, in spite of the high density of positive charge, in water at pH 4-6 its polypeptide chain contained a certain amount of α-helical or β-folded structures (Fig. 1b, curve 1, and [12]), the amount of which increased substantially when the charges were screened in 1 M NaCl solution (curve 2) or when they were neutralized in the region of alkaline pH values (curve 3). On the one hand, these differences may be due to the higher molecular mass of the polypeptide $(Lys\text{-}ala\text{-}Ala)_n$ (by a factor of 2-4 in comparison with peptide 152–184 of histone H1 [12]) – a factor promoting the stabilization of α-helical and β-folded structures. On the other hand, the absence of proline residues, which prevent the formation of α-helical and β-structural sections and stabilize the left-handed helical conformation in histones, also explains the observed structural differences of the polypeptides investigated. It is natural to assume that the differences detected should have an effect on the nature of the interaction of these polypeptides with DNA and on the structure of the complexes obtained.

To prepare the complexes we used calf thymus DNA that had been treated with ultrasound (5' at 22,000 kHz) to M_W 300,000 Da. The complexes were prepared by the method of slow mixing at two ionic strengths: 0.015 and 0.15 M NaCl.

Figure 2 shows curves of the titration of DNA by the peptide 152–184 (Fig. 2a, b) and by the polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ (Fig. 2c, d) in solution with the two ionic strengths. In the solution with the low ionic strength (0.015 M NaCl), the peptide fragment 152–184 bound with the DNA noncooperatively – the peptide molecule was distributed uniformly between the DNA molecules, and only at large values of $r > 1.5$, did the addition of new portions of the peptide lead to a rise in the turbidity of the solution and to the precipitation of the complexes obtained. However, complete precipitation did not take place, and even at $r = 3.0$ half of all the DNA molecules remained in solution.

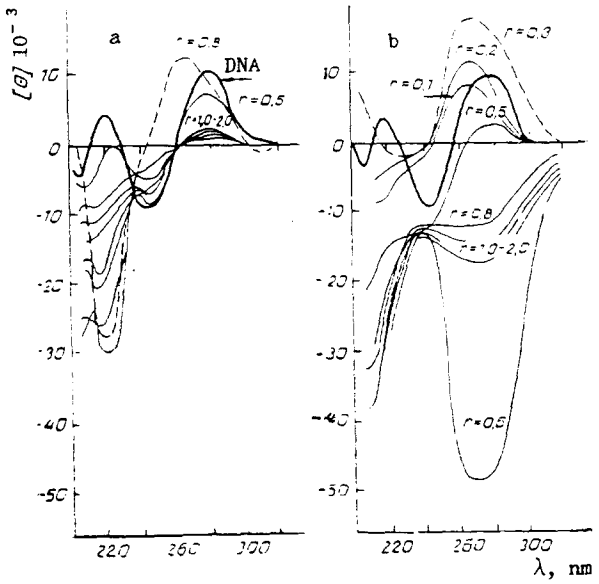

Fig. 4. CD spectra of DNA and its complexes with the polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ with different contents of the peptide component (r) in 0.015 and 0.15 M NaCl solutions [(a) and (b), respectively].

The interaction of this peptide with DNA at physiological ionic strengths apparently takes place differently. An increase in the turbidity of the solution and in the precipitation of the complexes was observed even at low values of r, which indicates the cooperative nature of this binding with DNA under these conditions: on the addition of the peptide to the solution, its molecules preferably add to DNA already bound with the peptide and not on the free DNA molecules. At r = 2.0, almost complete precipitation of the complexes from the solution took place (Fig. 2a, b).

The longer molecules of the polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$, which is capable of helicalization on the neutralization of the charges, bound cooperatively with the DNA at both ionic strengths (Fig. 2b, d) and precipitated it from solution more effectively than the 152–184 peptide (Fig. 2d).

The CD spectra of the complexes of DNA with the peptide fragment 152–184 in a solution of low ionic strength (0.015 M NaCl) that are given in Fig. 3a, show that up to r = 1.0 the CD of DNA as a component of complexes does not differ from the CD of free DNA in solution in the 240-300 nm region. An increase in the negative contribution of the CD of the complexes in the 200-240 nm region with a rise in r is due to the contribution of the peptide component. The form of the CD spectra of the complexes with a high content of the peptide component ($r \geq 1.5$) indicates the formation under these conditions of highly ordered asymmetric associates which give an intense negative CD band in the 260-280 nm region. This type of CD spectra (spectra of the ψ-type) has been observed for complexes of DNA with polylysine [13, 14], with histone H1 and its C-terminal domain [15, 16], and with polypeptide models of histones [17, 18].

They reflect the formation of a highly ordered asymmetric supermolecular packing of associates of the nucleoproteins. The ψ-type of CD spectra is not the result of an influence of light scattering, since in this case such spectra are also observed in the complete absence of turbidity (compare Figs. 2a and 3a). At the physiological ionic strength (Fig. 3b), the formation of highly assymetric associates takes place at lower values of $r \geq 0.6$ [sic] and the condensed structure of DNA has more rigid, stable, parameters that do not change with an increase in r to 2.0.

Thus, the peptide fragment 152–184 of the C-end of histone H1, just like the whole C-terminal domain of this histone, possesses a highly pronounced capacity for condensing DNA, i.e., converting it into a more compact state on interacting with it and forming highly ordered asymmetric structures on the association of the complexes. In this process the conformation of the DNA double helix is apparently not distorted – the transition from the CD spectrum typical for the B form of DNA to the ψ-type takes place without intermediate stages (Fig. 3).

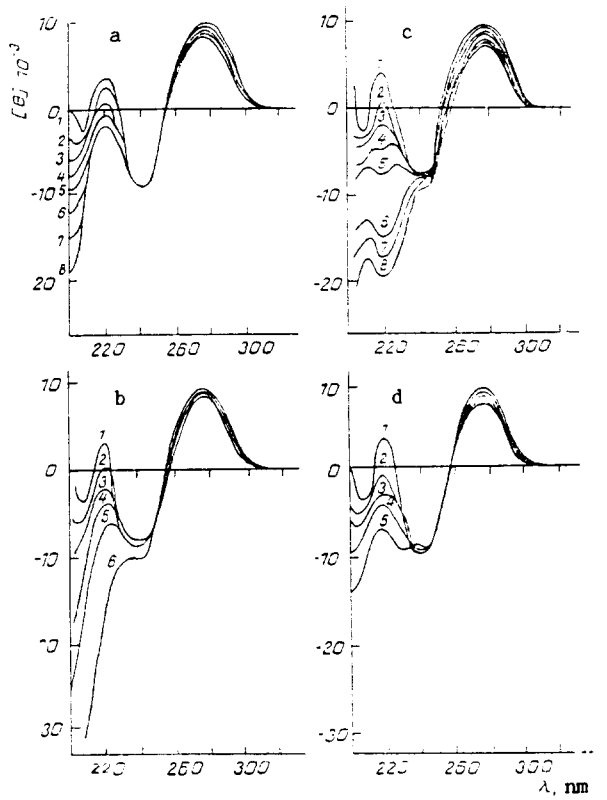

Fig. 5. CD spectra of DNA and its complexes with peptide 152–184 of histone H1 (a, b) and of the polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ (c, d) with various amounts of the peptide component (r) incorporated in the complex in 0.015 M NaCl solution (a, c) and 0.15 M NaCl solution (b, d) taken after the clarification of the solutions by centrifuging at 12,000 rpm for 20 min: a, b, d: 1) r = 0; 2) r = 0.2; 3) r = 0.4; 4) r = 0.6; 5) r = 0.8; 6) r = 1.0; 7) r = 1.2; 8) r = 1.5; c: 1) r = 0; 2) r = 0.1; 3) r = 0.2; 4) r = 0.3; 5) r = 0.4; 6) r = 0.6; 7) r = 0.7; 8) r = 0.8.

On the interaction of DNA with the polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ in a solution of low ionic strength (0.015 M NaCl) (Fig. 4a), the changes in the positive CD band began even at low values of r, leading to a fall in its intensity by 25% at r = 0.5. This indicates that the interaction of DNA with a polypeptide capable of changing its conformation on the neutralization of the charges with the formation of regular structures of the α-helical or β-folded type takes place differently from its interaction with the extended structure of a left-handed helix of a polypeptide chain, which leads to an appreciable deformation of the parameters of the double helix. The substantial changes in the CD spectra observed at r > 0.8 can be considered as an artefact caused by light scattering (compare Fig. 2c and Fig. 4a). In contrast to the 152–184 peptide of histone H1, this polypeptide, under conditions of low ionic strength, causes no condensation of the DNA with the formation of regular associates whatever, although the scattering of light (Fig. 2c) indicates that it has a more pronounced associating capacity. It is possible that the nature of the association in this case is different and the associates themselves introduce no additional asymmetry into the system leading to the ψ-type of CD spectra.

In spite of the fact that the change in the degree of association of the complexes of DNA with polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ with a rise in r and the capacity of this polypeptide for precipitating DNA from solutions differ little with a change in the ionic strength of the solution from 0.015 to 0.15 NaCl (Fig. 2c), the changes taking place in the CD spectra in 0.15 M NaCl differ substantially from those at a low ionic strength (Fig. 4a and b). At r = 0.1-0.2 (where the scattering of light is negligibly small), substantial changes take place in the structure of the DNA double helix corresponding to its passage from the B to the A form. At r = 0.5, the CD of DNA as a component of complexes with the polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ corresponds to its transition into the C form (turbidity low: $D_{320}/D_{260} \leq 0.1$). How-

ever, at r = 0.6, as in the case of the peptide 152–184, the DNA in the complex gives a ψ-type of CD spectrum which in this case is unstable and is destroyed with an increase in the proportion of the peptide component in the complex (r > 0.8).

Thus, the capacity for condensing DNA with the formation of highly ordered asymmetric associates is expressed more feebly for the polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ than for the peptide fragment 152–184, and these associates are less stable. Clarification of the solutions by centrifugation at 12,000 rpm for 20 min led to the precipitation of these associates and, apparently, changed the peptide/DNA ratio, particularly in the case of the cooperative addition of the peptides.

The CD spectra given in Fig. 5 show that in the case of both polypeptides and both ionic solutions the DNA double helix as a component of the complexes that have remained in solution after centrifugation does not change and retains the spectral parameters of the canonical B form. It is possible that the changes in the CD of DNA in complexes with the polypeptide $(Lys\text{-}Ala\text{-}Ala)_n$ at small values of r, in spite of the absence of light scattering recorded by the D_{320}/D_{260} ratio, are the result of association not recorded by this method. It is not excluded that the initial relatively low-molecular-mass associates can introduce asymmetry into the system and distort its spectral parameters. All the more does the structuring of this polypeptide observed on its interaction with DNA affect the parameters of its double helix. At the same time, the conformationally flexible and labile structure of a left-handed helix of the Pro-II type does not affect these parameters. It is possible that the left-handed helical conformation of the C-terminal fragment of histone H1 and its stability because of the possibility of the formation of other types of secondary structure in it form two of the determining factors of the condensing action of this fragment on DNA.

EXPERIMENTAL

The synthesis of the peptide fragment of the C-end of histone H1 was carried out by the classical methods of peptide synthesis, as described in [19]. After the removal of the protective groupings, the peptide was completely soluble in water. Its amino acid composition was confirmed by amino acid analysis. The concentrations of the peptide solutions were determined by weight. The isolation of the calf thymus DNA, its sonication to obtain low-molecular-mass DNA, and the parameters of the DNAs obtained were determined as described in [10]. The weight-average value of the molecular mass of the DNA was 6×10^6 Da. The molecular mass of the sonicated DNA was in the region of 3×10^5 Da.

The complexes of the DNA with peptides were obtained by the method of the slow direct mixing of solutions of the peptide and the DNA with continuous shaking. Equal volumes of the solutions of the peptide and the DNA were used for mixing. The initial concentration of the sonicated DNA was 0.06 mg/ml, and the concentration of the peptide depended on the required amount of it in the complex. The amount of peptide in the complex was characterized by the value of the ratio r of moles of lysine residues in the peptide to moles of phosphate groups in the DNA. The mixtures obtained were characterized by the initial r values predetermined in the preparation of the complexes and by the values of the optical densities of the solutions at 260 and 320 nm. The turbidities of the solutions, expressed by the ratio D_{320}/D_{260}, characterized the degree of aggregation of the complexes obtained at the given ionic strength of the solution and the given values of r. To determine the percentage amount of DNA remaining in solution and, consequently, the degree of precipitation of the DNA from the solution by the corresponding peptide, the mixtures obtained were centrifuged to precipitate the associates at 12,000 rpm for 20 min, and then the optical densities of the supernatants at 260 and 320 nm were measured again.

Features of the secondary structure of the complexes were studied by the CD method. The CD measurements were performed on a Cary-60 CD combined spectropolarimeter-dichrograph in the 200-320 nm region in quartz cells with a path length of 1 cm. The absorption of a cell with a solution in no case exceeded one optical density unit. The values of the molar ellipticities in $deg \cdot cm^2/dmole$ were calculated for the DNA–peptide complexes per 1 mole of DNA nucleotides ($M_0 = 330$), and, for the peptides, on the basis of the average weight of an amino acid residue in the corresponding peptide. The accuracy of the measurements amounted to ±0.002°. The instrument was calibrated with a solution of d-camphor-10-sulfonic acid.

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SYNTHESIS OF A NEW CYCLIC ANALOGUE OF LULIBERIN

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A new cyclic analogue of luliberin possessing the capacity for stimulating ovulation in sexually mature and infantile rats and also exhibiting a pronounced prolongation of its influence on a number of behavioral reactions of animals has been synthesized.

Among the numerous directions of the synthesis of active analogues of luliberin, considerable interest is caused by the preparation of cyclic peptides. As early as 1977, a hypothesis was expressed on the existence of a hydrogen bond between the pyroglutamic acid and glycinamide residues [1]. Conformational investigations of the luliberin molecule have confirmed the energetic advantageousness of a quasi-cyclic structure – for example, [2]. The first cyclic analogues of luliberin, cyclo(βAla^1, D-Ala^6)LH-RH and cyclo(6-aminohexanoyl^1, D-Ala^6) LH-RH had 1.2% and 0.65% of its releasing activity, respectively [3]. The low activity of these peptides was apparently due to the considerable modification of the first position. It is known that this usually leads to a pronounced fall in activity while at the same time weakly influencing the capacity of the compound for binding with the receptor. The linear precursors of these peptides were inactive.

The analogue cyclo(4-7)(Glu^4,D-Ala^6,Orn^7)LH-RH also proved to be inactive [4]. According to spectroscopic results, such cyclization leads to pronounced conformational changes in the molecule.

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