INVESTIGATION OF COMPLEXES OF DNA WITH SYNTHETIC PEPTIDE FRAGMENTS OF THE N-END OF HISTONE H2B

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The nature of the interaction of high- and low-molecular-weight DNAs (6×10^6 and 3×10^5 daltons) with synthetic oligopeptides of the N-end of histone H2B having the sequences 1-21, 1-10, 1-13, 11-21, and 14-21, differing in molecular weight and amino acid composition, as a function of the amount of peptide component in the complex and the ionic strength of the solution has been studied by the methods of UV and CD spectroscopy and the spectrophotometric analysis of melting curves. It has been shown that of all the peptides studied only the 1-21 peptide possesses the capacity of condensing DNA. This capacity depends on the amount of peptide component in the complex, the molecular weight of the DNA, and the ionic strength of the solution. The interaction with peptides under all the conditions studied, without changing the conformational parameters of the DNA, stabilizes its secondary structure in relation to the action of the temperature, which depends on the number of lysine residues in the peptides.

One of the approaches to elucidating the nature of DNA-protein interactions in chromatin is an investigation of artificial complexes of DNA with individual fractions of histones and their polypeptide models. The complex domain nature of the structure of histones presupposes different mechanisms of the interaction of individual sections of histones with DNA. The investigation of the interaction of DNA with individual fractions of histones, by considerably simplifying the model under investigation, will therefore permit the roles of the various sections of the histone molecule in the structural organization of chromatin to be revealed. While the central sections of histone H2A, H2B, H3, and H4 form globules constituting the protein core of nucleosomes, their terminal sections, containing a large amount of positively changed amino acid residues, are present outside the globules and, interacting with the phosphate groups of DNA, may promote internucleosomal interactions and, thus, the packing of the chromatin into structures of higher order.

In the present work we consider the nature of the interaction of DNA with the peptide fragment of the N-end of histone H2B with the sequence 1-21: Pro-Gly-Pro-Ala-Lys-Ser-Ala-Pro-Ala-Pro-Lys-Lys-Gly-Ser-Lys-Ala-Val-Thr-Lys-Lys-Ala and its 1-10, 1-13, 11-21, and 14-21 fragments, which differ both in their amino acid compositions and in their molecular weights. The capacity of these fragments for condensing DNA as a function of its molecular weight, the ionic strength of the solution, and the relative amount of the peptide in the complex, and also the capacity of these peptides for stabilizing the structure of DNA in relation to heat denaturation, were investigated.

We have shown previously that in aqueous solutions these peptides assume the conformation of an extended left-handed helix of the type of polyproline (II) (ppII), which is stable in a wide range of conditions of the medium [1]. Analysis of the circular dichroism (CD) spectra of artificial DNA-peptide complexes that we have performed have shown the influence of the interaction of the above-mentioned peptides with DNA on the conformational parameters of both components of each complex.

Since the peptides studied contained different numbers of lysine residues (from 10% in the 1-10 peptide to 50% in the 11-21 peptide), the amount of peptide included in the complex was determined by the value of the ratio of the moles of lysine residues of the given peptide to the moles of the phosphate groups of DNA (r), Figures 1A and 1B show the results of the

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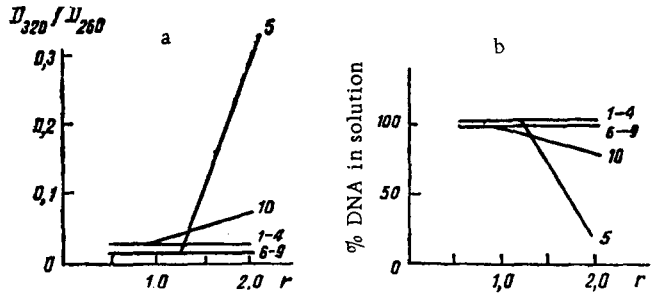

Fig. 1. Dependence of the turbidity of the solutions, D_{320}/D_{260} (a), and of the percentage of DNA precipitated in the solution (b), on the amount of histone H2B peptides in the complexes (r) in 0.015 M NaCl solution: peptide: 1) 1-10; 2) 11-21; 3) 1-13; 4) 14-21; 5) 1021; 6-10) complexes of peptides in the same sequence but in 0.15 M NaCl solution.

titration of DNA by the oligopeptide 1-21 and its 1-10, 1-13, 11-21, and 14-21 fragments under the conditions of low (0.015 M NaCl) and physiological (0.15 M NaCl) ionic strengths. Calf thymus DNA in the high-molecular-weight ($M_W = 6 \cdot 10^6$ daltons) from and in the sonicated (5 min at 20 kHz) low-molecular-weight form with $M_W = 3 \cdot 10^5$ daltons were used in the experiments. The study of the dependence of the scattering of light on the amount of peptides in the complex showed that both in the solutions with a low ionic strength (0.015 M NaCl) and under physiological conditions (0.15 M NaCl) no scattering of light was observed in the range of values of r from 0 to 2.0 for any of the peptides except the 1-21 peptide (Fig. 1A). Beginning from r = 1.2 in 0.015 M NaCl and r = 0.8 in 0.15 M NaCl, the complexes of DNA with the 1-21 peptide scattered light, which indicated the beginning of aggregation, and this increased with a rise in the amount of peptide in the complex; in the solution having a low ionic strength this process took place far more effectively than under physiological conditions and was accompanied by the precipitation of the DNA from the solution (Fig. 1B), while in 0.15 M NaCl the precipitation of the DNA took place feebly. None of the other peptides precipitated DNA from solution at all at either of the ionic strengths studied. Similar behavior was observed for complexes of the peptides with the high-molecular-weight DNA.

The curves of the titration of DNA by the 1-21 peptide in 0.015 M NaCl solution indicate the noncooperative attachment of this peptide to the DNA. This means that the peptide molecules are distributed uniformly between the DNA molecules and when they accumulate to a definite value r > 1.0 pronounced precipitation of the DNA from the solution begins. This process is apparently not determined solely by the neutralization of the phosphate groups of the DNA, the completeness of which we cannot judge from these results. It is possible that one of the necessary conditions for the precipitation of DNA is the length of the chain of the peptide, which must be sufficient to bind the DNA in the complexes into intermolecular aggregates. The process of intermolecular association of complexes of DNA with the 1-21 peptide and the process of the precipitation of DNA from the solution that is connected with it do not depend on the molecular weight of the DNA.

It can be seen from Fig. 2 that for complexes of the high-molecular-weight and the sonicated DNAs with the 1-10 and 11-21 peptides having different r values and at different ionic strengths of the solution on change in the turbidity of the solution was observed under the conditions studied. Only for the complexes of DNA with the 1-21 peptides was their precipitation from the solution at ionic strengths below 0.2 M NaCl observed. This process took place more effectively with a further lowering of the ionic strength of the solution from 0.2 M to 0.015 M NaCl and with an increase in the concentration of peptide in the complex (at r > 1.5). Thus, for all the peptides apart from the 1-21 peptide it is possible to state that on interaction with DNA they do not change its spectral parameters, do not scatter light, and do not precipitate DNA from solution.

It is possible that all the properties mentioned above are characteristic only of peptides with higher molecular weights and do not depend on their amino acid composition. In our case, the 1-21 peptide consisted of two peptides — 1-10 and 11-21 — and therefore it is

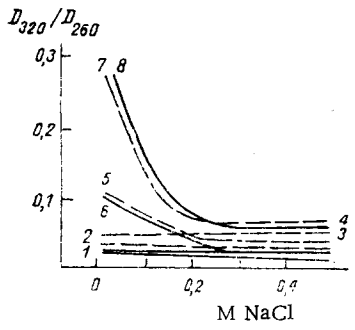

Fig. 2

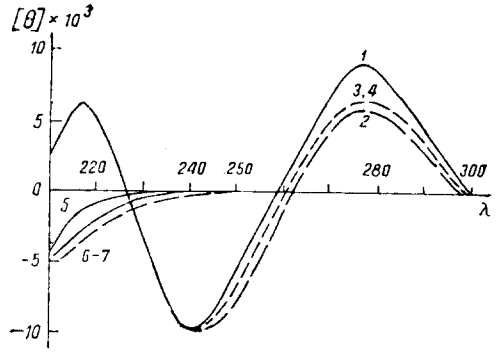

Fig. 3

Fig. 2. Dependence of the turbidity of solutions of complexes of high- and low-molecular-weight DNAs (—— and ---) with various peptides at different values of r on the ionic strength of the solution: peptides 1, 2) 1-10 at r = 2.0; 3, 4) 11-21 at r = 2.0; 5, 6) 1-21 at r = 1.5; 7, 8) 1-21 at r = 2.0.

Fig. 3. CD spectra in 0.15 M NaCl solutions: 1) low-molecular-weight DNA; 2) high-molecular-weight DNA; 3, 4) complexes of the 1-21 peptide with low- and high-molecular-weight DNAs at r = 2.0; 5) initial 1-21 peptide; 6, 7) CD spectra of the 1-21 peptide obtained by deducting the contribution of DNA to the spectrum of the complex.

difficult to speak of differences between the amino acid compositions of the short peptide and of the 1-21 peptide. One of the most obvious differences between this peptide and all the others is its greater length and, consequently, its higher molecular weight. The 1-21 peptide at small values of $r < 1.2$, behaved in the same way as its fragments — it did not scatter light and did not precipitate DNA from solution at any ionic strength.

Figure 3 gives the CD spectra of complexes of the high- and low-molecular-weight DNAs with the peptide having the highest molecular weight and with the highest value of r = 2.0 — the 1-21 peptide — in the 250-300 nm region, which is more sensitive to changes in the structural characteristics of DNA. At other values of r and with other peptides the nature of the CD curves remained the same.

An investigation of the CD spectra of the complexes obtained showed that in 0.15 M NaCl interaction with peptides did not change the spectral parameters of the DNAs in the complexes at any values of r, i.e., the configurational parameters of DNA within complexes with the peptide fragments of histone H2B remained unchanged from its structure in the free state over a wide range of values of r. The absence of the aggregation of these complexes in a solution with the physiological ionic strength (0.15 M NaCl) at r = 1.0 permitted the contribution of the DNA to be deducted and the structure of the peptide component within the complexes to be evaluated (Fig. 3). The absence of a positive contribution in the spectral region of 210-220 nm indicated some deformation of the left-handed helical structure of the peptides on their interaction with the DNA, but on the whole the main features of this conformation were retained and no formation of other ordered structures was observed. The same can be said about the conformational features of the complexes of DNA with the peptides in 0.015 M NaCl solution (with the exception of the complex of DNA with the 1-21 peptide). While in the case of the high-molecular-weight DNA in complexes with the 1-21 peptide its spectral parameters remained unchanged as far as r = 1.5, in spite of the fairly intense scattering of light (40% of the DNA was present in an aggregated state), in the case of the low-molecular-weight DNA at r = 1.5 substantial changes in the CD spectra were obtained that were connected with the beginning of specific association of the complexes, introducing additional asymmetry into the system (Fig. 4). The formation of highly asymmetric supermolecular associates at r = 2.0 made a 10 times more intensive contribution to the negative CD band. This type of CD spectrum (the ψ-type of spectrum) has been observed for complexes of DNA with polylysine [2, 3], with histone H1 [4, 5], and with peptides modeling histones [6, 7]. They are interpreted as a highly ordered asymmetric supermolecular packing of associates of the nucleoproteins in the presence of a salt. Of the five peptides that we have investigated, only the longest was capable, on its interacting with the low-molecular-weight DNA, of forming this type of

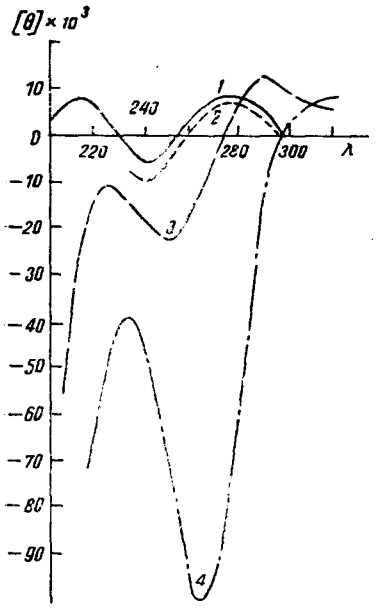

Fig. 4

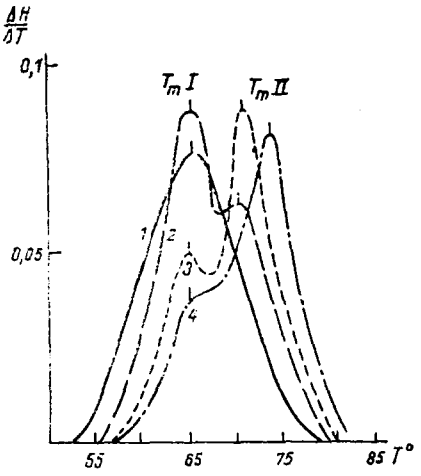

Fig. 5

Fig. 4. CD spectra of 0.015 M NaCl solutions of complexes of low-molecular-weight DNA with the 1-21 peptide at different amounts of the latter in the complexes: 1) r = 0; 2) r = 1.0; 3) r = 1.5; 4) r = 2.0.

Fig. 5. Differential melting curves of the low-molecular-weight DNA (1) and its complexes with peptides: 2) 1-10; 3) 1-21; 4) 11-21 in 0.015 M NaCl at r = 1.0.

structure at a fairly high content of the peptide in the complex. The associates that were formed with the low-molecular-weight DNA at high values of r may be an ordered aggregate not introducing any additional asymmetry, i.e., not making any contribution to the CD spectra, apart from the influence of the scattered light on the optical parameters of the spectra. The observed phenomenon disappeared at higher ionic strengths of the solution and, in particular, at physiological ionic strengths. Thus, the fundamental factors necessary for the formation of nucleoprotein complexes with highly ordered supermolecular structures are the length of the polypeptide chain, the molecular weight of the DNA, the amount of peptide component in the complex, and the ionic strength of the solution.

The differential curves of the spectrophotometric melting of the low-molecular-weight DNA and its complexes with the 1-10, 1-21, and 11-21 peptides in a solution of low ionic strength are shown in Fig. 5. These peptides contained different amounts of lysine residues (10, 30, 50%, respectively). In spite of the fact that the amount of each peptide included in the complex corresponded to r = 1.0, i.e., the number of lysine residues in the peptides was equal to the number of phosphate groups in the DNA, the curves given in Fig. 5 unambiguously show that for none of the three peptides did their complete binding with the DNA and, consequently, the complete neutralization of its phosphate group take place. All the complexes showed a band or shoulder corresponding to the melting of free DNA (T_mI = 65°C). The percentage of free DNA was a maximum in its complexes with the 1-10 peptide and a minimum with the 1-21 peptide. The interaction with DNA and, consequently, the degree of its stabilization differed somewhat for the different peptides. The greatest stabilizing effect on the structure of the DNA was possessed by the 11-21 peptide (T_mII = 73°C), which contains the largest number of lysine residues (Fig. 5), and the least effect by the 1-10 peptide (T_mII = 70°C). The 1-21 peptide, representing the sum of these two peptides, exhibited intermediate stabilizing properties (T_mII = 71°C). Thus, the stabilization of DNA was effected by all the peptides and was not directly connected with the conformational or aggregational properties of their complexes with the DNA but depended on the number of lysine residues in the peptides.

EXPERIMENTAL

The synthesis of the peptide fragments of the N-terminal sections of histone H2B was effected by the classical methods of peptide synthesis, as described in [8]. After the elimi-

nation of the protective grouping, the peptides were completely soluble in water. The concentrations of the peptides in solution were determined by weight. The isolation of DNA from calf thymus and its sonication to obtain low-molecular-weight DNA were performed and the parameters of the DNAs obtained were determined as described in [9]. The weight-average molecular weight of the initial DNA was $6 \cdot 10^6$ daltons. The molecular weight of the sonicated DNA was approximately $3 \cdot 10^5$ daltons.

The complexes of the DNA with the peptides were obtained by the direct mixing of solutions of the peptides and the DNA with continuous shaking. Equal volumes of solutions of the peptide concerned and the DNA were taken for mixing. The initial concentration of the high-molecular-weight DNA and the sonicated DNA was 0.04 mg/ml, while the concentration of the peptide depended on its required amount in the complex. The amount of a peptide in the complex was characterized by the value of the ratio r of the moles of lysine residues in the peptide to the moles of phosphate groups of the DNA. In our case, r was varied between 0 and 2.0. The mixtures obtained were characterized by the initial value of r, predetermined in the preparation of the complexes, and by the values of the optical densities of the solutions at 260 and 320 nm. The turbidity 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 value of r. To determine the percentage amount of DNA remaining in the solution and, consequently, the degree of precipitation of the DNA from the solution by the corresponding peptide, the mixtures obtained were centrifuged to precipitate associates at 15,000 for 15 min, and then the optical densities of the supernatants were measured again at 260 and 320 nm.

Features of the secondary structure and thermal stability of the DNA in the soluble part of the complexes were studied by the methods of CD and thermal melting. CD was measured on a Cary-60 CD combined spectropolarimeter-dichrograph in the 210-320 nm range in quartz cells with a path length of 1 cm. In no case did the absorption of the cell with the solution exceed an optical density unit. The molar ellipticities in degrees$\cdot$cm^2/dmole were calculated in the 250-320 nm region per mole of DNA nucleotides (M_0 = 330) and in the 210-245 nm region after the deduction of the contribution of the DNA per mean weight of an amino acid residue in the corresponding peptide. The accuracy of the measurements was ±0.0002°C. The instrument was calibrated with a solution of d-camphor-10-sulfonic acid.

The thermal melting of the low-molecular-weight DNA and its complexes with peptides was performed in 0.015 M NaCl solution on a Specord-40 M spectrophotometer in quartz cells with a pathlength of 1 cm thermostated with the aid of U-2 thermostat. The rate of heating was 0.5°C per minute. The temperatures were measured with the aid of a thermocouple directly in the cell with an accuracy of ±0.5°C. The change in optical density with a rise in the temperature was represented in the form of differential $\Delta H/\Delta T(T)$ curves of the temperature dependence of the temperature derivative of the hyperchromism. The smoothing of the curves was performed graphically. The curves were characterized by the value of the hyperchromism and by the temperatures corresponding to the peak maxima on the differential melting curves (T_mI and T_mII).

CONCLUSIONS

The nature of the interaction of high- and low-molecular-weight DNAs ($6 \cdot 10^6$ and $3 \cdot 10^5$ daltons) with synthetic oligopeptides corresponding to the N-end of histone H2B with the sequences 1-21, 1-10, 1-13, 11-21, and 14-21, differing in their molecular weights and amino acid compositions, has been studied as a fucntion of the amount of peptide component in the complex and the ionic strength of the solution by the methods of UV and CD spectroscopy and the spectrophotometric analysis of melting curves.

It has been shown that of all the peptides studied only the 1-21 peptide possessed the capacity for condensing DNA. This capacity depends on the amount of peptide component in the complex, the molecular weight of the DNA, and the ionic strength of the solution.

Interaction with the peptides under all the conditions studied, without changing the conformational parameters of the DNA, stabilized its secondary structure in relation to the action of the temperature, and this depends on the number of lysine residues in the peptides.

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