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A STUDY OF THE BEHAVIOR OF HISTONE H1 AND ITS COMPLEX WITH DNA BY THE SPIN-LABEL METHOD

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The behavior of the tyrosine-72 residue of histone H1 has been studied by the spin-label method as a function of the ionic strength of the solution and of the temperature and on its interaction with DNA. It has been shown that in the formation of complexes of histone H1 with DNA the globular part of the protein not directly interacting with the nucleic acid retains a definite conformation enabling it to participate in various processes taking place in the chromatin.

In order to study the microstructure and the conformational properties of the globular part of the histone H1, we have developed a method for the selective attachment of a spin label to the single tyrosine-72 residue of the protein molecule [1].

In the present paper we give the results of an investigation of the behavior of the spin-labeled histone H1 as a function of the ionic strength of the solution and of the temperature and on its interaction with DNA. The interest in questions of the structural behavior of the globular section of histone H1 is due to the fact that, according to modern ideas, this region of the protein molecule is considered to be involved in the process of the specific recognition of twisted DNA [2]. The ESR spectrum of histone H1 of calf thymus labeled at the tyrosine-72 residue (Fig. 1a) indicates a weak immobilization of the spin label in the protein and shows that the tyrosine residue is most probably located on the surface of the globular segment of the histone.

To investigate the microenvironment and to determine the "accessibility" of the radical attached to the tyrosine-72 residue, the spin-labeled histone H1 was titrated with a 0.1 M solution of potassium ferricyanide. The change in the ESR spectra after the addition of each aliquot of the 0.1 M solution of potassium ferricyanide with a volume of 0.05–0.1 ml was followed from the broadening of the lines. The addition of potassium ferricyanide up to a concentration of 10^{-2} M did not lead to their broadening. According to the literature [3], such a phenomenon can be explained by the presence in the immediate microenvironment of the nitroxyl radical of a considerable accumulation of positively charged amino acid residues.

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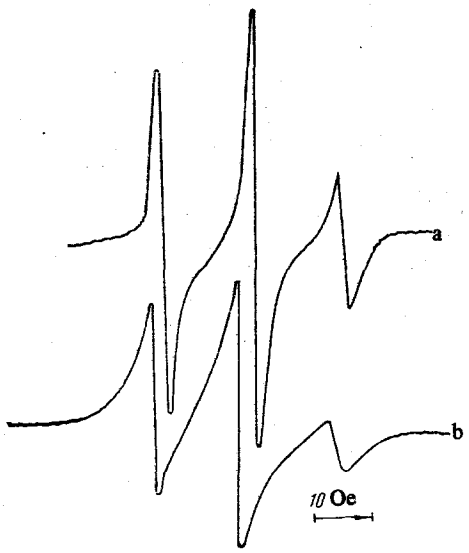

Fig. 1

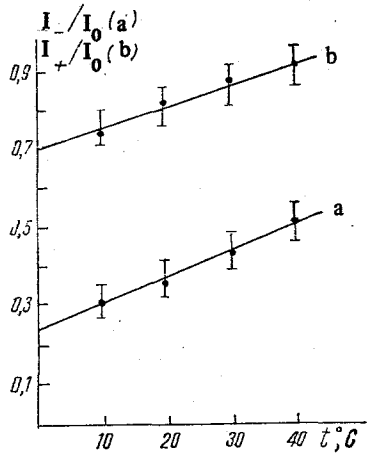

Fig. 2

Fig. 1. ESR spectrum of histone H1 labeled at the tyrosine-72 residue in 0.1 M NaCl at 20°C (a), and the same spectrum in a complex with DNA (b).

Fig. 2. Temperature dependence of the parameters of the ESR spectra of histone H1 labeled at the tyrosine-72 residue.

In order to study the structural behavior of the protein in the region of addition of the radical, we recorded the temperature dependences of the parameter I_-/I_0 and I_+/I_0 of the ESR spectra of the spin-labeled histone H1 (Fig. 2). Analysis of the curves of the temperature dependence shows the absence of conformational transitions of the section of the protein matrix in the region adjacent to the tyrosine residue. At the same time, an increase in the ionic strength of the solution as the result of the addition of increasing amounts of sodium chloride led to an appreciable change in the conformational state of the labeled histone, which was reflected in its ESR spectra. At salt concentrations from 0.5 to 2 M addition lines appeared (Fig. 3) apparently due to a change in the microenvironment of the radical through a conformational rearrangement of the globule section of histone H1.

A comparison of the ESR spectra of the spin-labeled histone in the free state and in the complex with DNA (see Fig. 1b) permits the conclusion that the change in the degree of immobilization of the label as the result of complex-formation was insignificant, probably because of the considerable conformational stability of the globular section in this process. At the same time, as we have shown previously [4], the formation of a complex with DNA by histone H1 spin-labelled at the N- and C-terminal sections led to considerable changes in the ESR spectra indicating a direct participation of these sections in the process of binding with DNA.

EXPERIMENTAL

The spin label used was 3-dichlorotriazinylamino-2,2,5,5-tetramethylpyrrolidone-1-oxyl, synthesized by the method of Shapiro et al. [5].

Histone H1 was isolated from calf thymus by Johns' method [6]. The calf thymus DNA was kindly supplied by B. A. Korol' of the Institute of Biophysics of the Academy of Sciences of the USSR; it had a molecular mass of $\sim 5 \cdot 10^7$, a melting point of 86°C, and hyperchromic effect of 34%.

The DNA–spin-labeled histone H1 complexes were obtained as described by Kirpichnikov et al. [7] by mixing 1 ml of DNA solution (c $\sim$ 9 mg/ml) and 0.5 ml of protein in 2 M NaCl (c $\sim$ 9 mg/ml) (weight ratio of DNA to histone, 2:1), followed by gradient dialysis with gradually decreasing concentrations of salt from 2 to 0.1 M.

The ESR spectra were recorded in Varian E-104 and RE-1406 (USSR) radiospectrometers.

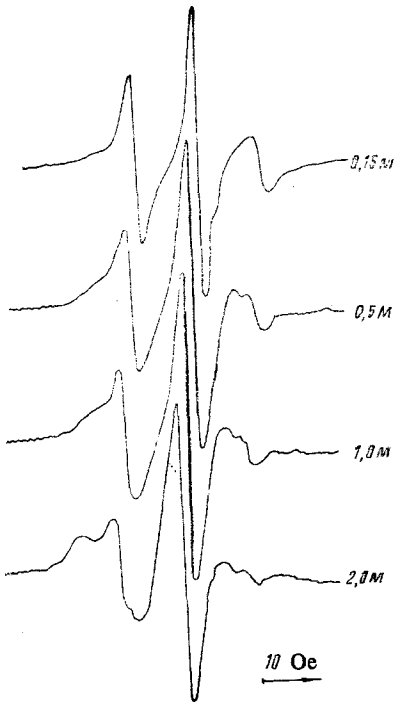

Fig. 3. Dependence of the nature of the ESR spectra of histone H1 labeled at the tyrosine-72 residue on the ionic strength of the solution.

SUMMARY

It has been shown that in the formation of complexes of Histone H1 with DNA its globular section not directly interacting with the DNA retains a definite conformation, which makes it possible for this protein to participate in various processes proceeding in the chromatin such as, in particular, enzymatic phosphorylation and protein–protein and protein–nucleic acid interactions.

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