SOME ASPECTS OF THE SPECTROPOLARIMETRIC INVESTIGATION OF THE HISTONE COMPONENT OF DNP

I. I. Byal'skii, N. V. Chelyapov, A. L. Gorin, and P. I. Tseitlin

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The total histone was determined in the region 250-350 nm by the optical rotary dispersion method. Within the range 280-300 nm the optical rotary dispersion spectrum is insensitive to conformation changes in the histone protein molecule. It is postulated that the abnormal specific rotation in the region of the positive Cotton effect of DNP with a maximum at 290 nm is due to changes in the nuclear component of the complex.

The macromolecular organization of DNA in the composition of nuclear DNP of eukaryotes remains unsolved [2, 4], although its solution is of fundamental importance in the elucidation of transcription of the genome and also of the nature of the primary effects as a result of interaction between mutagens and genetic target molecules. An important argument in support of the view that during the formation of protein–DNA complexes certain changes take place in the DNA is the abnormal decrease in specific rotation $[\alpha]_{290}$ in the region of the positive Cotton effect of DNA, characteristic of the optical rotatory dispersion of DNP [1, 9]. It is impossible simply by examination of these anomalies to give an unequivocal answer to the question of whether they are connected with changes in DNA conformation or with changes in the conformation of the protein component, although individual proteins (of nonnuclear origin) and synthetic polypeptides do not change the specific rotation values in the region of 280-300 nm in solvents changing their conformation state [10].

The object of this investigation was to study, by the optical rotatory dispersion method, the principal protein component of DNP (total histone) in salt solutions and also in 6 M urea and 2-chloroethanol in the region of the positive Cotton effect of DNP (290 nm).

EXPERIMENTAL METHOD

Total histone was isolated from calf thymus by extraction with 0.2 N HCl and dried lyophilically. Amino-acid analysis, using a Hitachi analyzer, showed that the total histone thus isolated was virtually identical in its amino-acid composition with that described in the literature [6]. The absorption spectrum of an aqueous solution of histone (Unicam SP-8000 spectrophotometer) had a maximum at 193 nm, in agreement with data in the literature [3].

It was most important to free the histone from traces of DNA and from contamination with nonhistone proteins, and accordingly the protein preparation was tested for phosphorus and sulfur on a Varian-V-IEE-15 electronic emission spectrometer, with facilities for the fine analysis of the bond energy of electrons on the inner shells of the elements and compounds on the basis of x-ray irradiation of the specimen and measurement of the electrons emitted. The energy of the emitted electrons is a function of the elements and, consequently, the IEE spectrometer can be used for chemical analysis. As is clear from Fig. 1, neither

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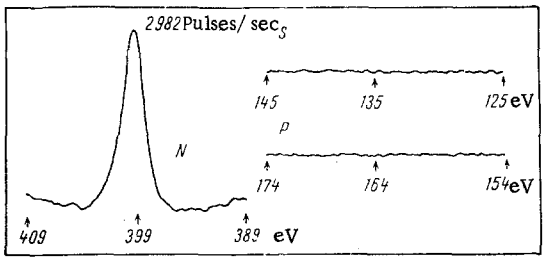

Fig. 1. Graph of electron emission for nitrogen, phosphorus, and sulfur of total histone preparations.

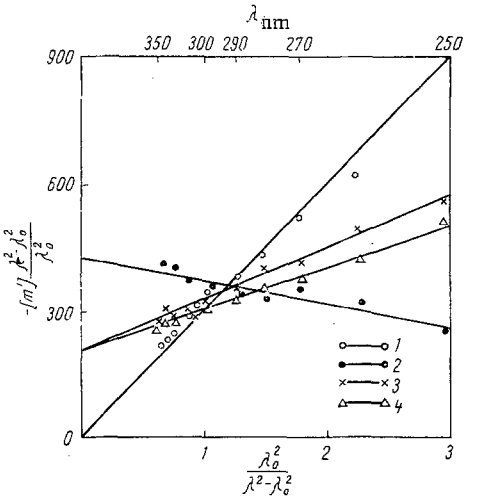

Fig. 2. Moffitt diagram for total histone in various solvents: 1) 2-chloroethanol; 2) 6 M urea; 3) 0.7 M NaCl; 4) 0.7 M NaCl after denaturation of protein for 15 min at 92°C.

phosphorus nor sulfur was found in the protein preparation, indicating absence of nonhistone proteins or nucleotide residues.

The optical rotary dispersion was photographed in the SPU-M spectropolarimeter in a 1-cm cell. The optical density of the sample throughout the spectral range 250-350 nm did not exceed 2 optical units. The histone was dissolved in the corresponding solvent immediately before measurement of the optical rotary dispersion. The 6 M urea solution was made up from weighed sample of dry urea. The solution of 2-chloroethanol was distilled before the measurements were made.

EXPERIMENTAL RESULTS

In the region 250-350 nm the optical rotatory dispersion curves of the total histone in all solvents tested were smooth in character, so that they could be analyzed by Moffitt's method [7]:

$$[m']_\lambda \frac{\lambda^2-\lambda_0^2}{\lambda_0^2} = a_0 + b_0 \frac{\lambda_0^2}{\lambda^2-\lambda_0^2},$$

where [m'] is the molar rotation; a_0 and b_0 are constants, b_0 being a linear function of the percentage of helicity in the protein. The value of λ_0 was chosen as 216 nm for this particular optical region [6].

The parameters of linear dependence of $[m']_\lambda \frac{\lambda^2-\lambda_0^2}{\lambda_0^2}$ on $\frac{\lambda_0^2}{\lambda^2-\lambda_0^2}$ were calculated by the method of least squares. These straight lines are shown in Fig. 2.

The values of b_0 in 2-chlorethanol, 0.7 M NaCl, and 0.7 M NaCl after denaturation of the protein for 15 min at 92°C, and in 6 M urea were −300, −120, −100, and +50, respectively. The percentage of helicity was calculated from the value of −567 for b_0 at $\lambda_0 = 216$ nm for 100% and +52 for the random conformation [5], giving 57% helicity in 2-chloroethanol, 28% in 0.7 M NaCl, and 25% in 0.7 M NaCl after denaturation of the protein for 15 min at 92°C, and complete despiralization of the protein in 6 M urea.

As is clear from the Moffitt's diagram (Fig. 2) the straight lines intersect in the region of 280-300 nm, from which it follows that the changes in the conformation of the protein molecules of the total histone from random (in 6 M urea) to maximum helicity (in 2-chloroethanol) do not lead to changes in rotation of the protein in the region of 290 nm, i.e., any changes in the conformation of the total histone molecules are reflected only in the region of the negative peak at 233 nm or in the visible region. This suggests that the region of 280-300 nm is not a characteristic region for total histone in optical rotatory dispersion.

Considering that the maximum of the positive Cotton effect for DNA and DNP is located in this region of the spectrum and that the positive peak of the optical rotary dispersion curve of DNA at 290 nm is sensitive to conformation changes in polynucleotide chains [8], it can be postulated that the abnormal decrease in $[\alpha]_{290}$ of DNP compared with $[\alpha]_{290}$ of DNA is connected with a change in the nuclear component of the complex. This hypothesis follows from the fact demonstrated above that total histone does not change the parameters of optical rotatory dispersion during conformation changes in this region of the spectrum.

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