THERMAL DENATURATION OF DNP

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Thermal denaturation of DNP was studied by spectrophotometry, spectropolarimetry, viscosimetry, and gel-filtration on Sephadex G-200. The results showed that changes in the specific rotation at 290 nm and optical absorption at 260 nm take place in DNP in a low ionic strength ($0.7 \cdot 10^{-3}$) without dissociation of the DNA-protein bonds, by contrast with DNP in a high ionic strength (0.7), in which thermal denaturation produces changes in viscosity, specific rotation, and optical absorption which correlate to a definite degree with dissociation of the DNP complex.

Denaturation is a term applied to any modification of the secondary, tertiary, or quaternary structure of the protein molecule with the exception of rupture of its covalent bonds [7]. If this definition is applied to the DNP molecule it is clear that the concept includes not only changes in the components of the complex, but also changes in relations between the protein and nucleic acid components linked together by noncovalent bonds. As a rule in the many papers published on DNP denaturation attention is concentrated on individual aspects of this complex phenomenon.

EXPERIMENTAL METHOD

DNP was isolated from calf thymus by the method of Zubay and Doty [9] in an ionic strength of $0.7 \cdot 10^{-3}$ (phosphate buffer), pH 6.9 ("aqueous" DNP) and by a modified method of Mirsky and Pollister [8] in an ionic strength of 0.7 (NaCl) ("saline" DNP). The method of isolation and the characteristics of the total histone preparations are described in the literature [6]. The study of viscosity, UV-absorption, and rotation of the plane of polarization of the DNP preparations as functions of temperature was carried out as described previously [2, 3]. The degree of integrity of the DNP complex at different temperatures was studied by gel filtration on columns with Sephadex G-200. The columns were fitted with a thermostatically controlled jacket.

EXPERIMENTAL RESULTS

Thermal denaturation was studied in specimens of "aqueous" and "saline" DNP. The chemical composition of the isolated specimens was virtually identical, but the difference in ionic strengths at which the tissue DNP was soluble, namely three orders of magnitude, significantly affected the physicochemical properties of the object studied. During dispersion of the nucleoprotein gel in a low ionic strength a predominantly strong electrostatic interaction was established between DNA and protein, and this probably prevented the formation of specific hydrogen and hydrophobic bonds [5]. In "saline" DNP the electrostatic interaction was weakened by the presence of gegenions in the solution. This led to more complete nonionic interactions between the protein and DNA in the nucleoprotein. In view of these arguments, it was expected that there would be significant differences between the thermal denaturation of the "aqueous" and "saline" DNPs. As Fig. 1 shows, raising the temperature from 25 to 80°C did not lead to dissociation of the "aque-

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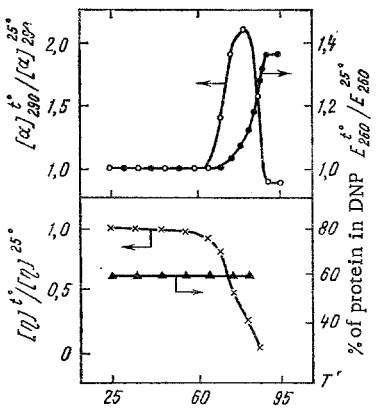

Fig. 1

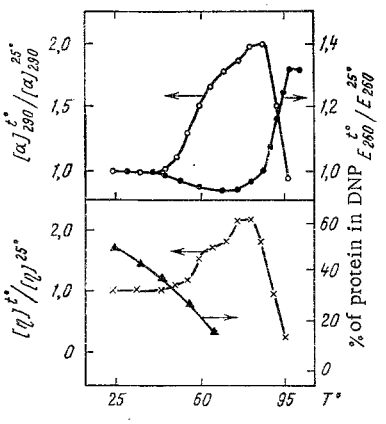

Fig. 2

Fig. 1. Thermal denaturation of "aqueous" DNP ($\mu = 0.7 \cdot 10^{-3}$).

Fig. 2. Thermal denaturation of "saline" DNP ($\mu = 0.7$).

ous" nucleoprotein. However, changes in $[\alpha]_{290}$*, E_{260}, and viscosity began in the region of 65°C. At 80°C $[\alpha]_{290}$ reached a maximum, the melting temperature as given by the test of the change in E_{260} was 82°C, and the viscosity of "aqueous" DNP was reduced by 50-60% at 80°C. Significant changes in the secondary structure of DNA and in the degree of asymmetry of DNP were thus observed although the integrity of the DNP as a DNA-protein complex was preserved. It is also known from the literature that changes in the secondary structure of the proteins present in the "aqueous" DNP are virtually complete at 80°C [4]. These results show that the distinguishing feature of thermal denaturation of "aqueous" DNP is a change in the physicochemical properties of the components of the complex while its integrity is preserved. In other words, strong electrostatic interaction between DNA and protein does not prevent changes in the conformation of the components and in turn, a change in the conformation of DNA and protein has no significant effect on the strength of the DNA-protein bond. In the course of thermal denaturation of "aqueous" DNP, the DNA and protein probably assume conformations in which sufficiently strong electrostatic interaction is preserved between a large number of the protein amino groups and phosphatic groups of the DNA, which is not overcome as the result of increased Brownian movement of the molecules at temperatures below 80°C.

Denaturation of "saline" DNP showed certain fundamental differences from denaturation of the "aqueous" DNP (Fig. 2). Heating significantly increased the degree of dissociation of the protein. At 65°C the complex still retained 12-20% of protein. Although removal of the first 20% of more easily separated proteins did not lead to changes in the parameters of the DNP (in agreement with results obtained with "aqueous" DNP [4]), the beginning of the change in viscosity, $[\alpha]_{290}$, and E_{260} for "saline" DNP was shifted to a temperature 20° lower, and the viscosity of "saline" DNP rose parallel to the increase in $[\alpha]_{290}$. The values of E_{260} in the region of temperatures just below the melting temperature were reduced and the hypochromic effect was 4-5%. The parallel nature of the changes in $[\eta]$ and $[\alpha]_{290}$ indicates that the more compact arrangement of the DNA in the DNP molecule is connected with certain changes in the secondary structure of the DNA during complex formation with the protein. Thermal dissociation of the protein leads to restoration of the secondary and tertiary structures of the DNA.

It must be pointed out that the profile of the curves $[\eta] = f(t°)$ and $[\alpha]_{290} = f(t°)$ observed in these experiments was not an artifact due to aggregation of the DNP. This is confirmed by the results obtained for denaturation of DNP in different concentrations. The profile of the curves of viscosity and optical rotation at 290 nm as functions of temperature is independent of the DNA concentration within the range from 20 to 100 μg/ml as DNA. Moreover, as Fig. 3 shows, urea in a concentration of 1 M virtually completely suppressed the increase in nonspecific absorption of DNP arising through the protein dissociated and aggregated during the rise of temperature in an ionic strength of 2.6, in which the effect of dissociation and aggregation of the DNP proteins is maximal. The profile of the melting curve of DNP in the presence of 1 M urea solution was similar to that of the melting curve taking account of the scattered light, although the

*Changes in $[\alpha]_{290}$ of DNP are determined purely by the conformational state of the DNA. Results obtained in the writers' laboratory show that changes in the conformation of total histone have no effect on the value of this index.

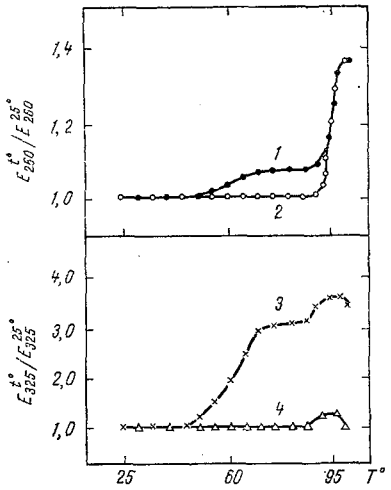

Fig. 3. Curves showing optical absorption of DNP in an ionic strength of 2.6 at 260 nm as a function of temperature disregarding the contribution of nonspecific absorption (1), in the presence of 1 M urea solution (2), and of nonspecific absorption at 325 nm without (3) and in the presence of urea (4).

melting temperature was reduced by 1°C. Urea thus prevents aggregation of DNP on account of the dissociated protein. Investigation of the curve of viscosity of the "saline" DNP as a function of temperature in the presence of urea revealed no change in its profile.

Unfortunately, the results relating to thermal denaturation of DNP described above do not allow a final decision to be made regarding the level of organization of DNA at which the changes arising in its structure during complex formation with protein are primary. Is the more compact arrangement of DNA in the DNP molecule a function of disturbances in the secondary structure, or vice versa? Most probably the changes in the secondary and tertiary structures of the DNA during complex formation with protein take place simultaneously, at least in the macromolecules of "saline" DNP. Before this question can be answered unequivocably for "aqueous" DNP more thorough investigations of the character of the change in tertiary structure of the complex with temperature are required. It can be categorically stated that the changes in the conformation of the protein moiety of "aqueous" DNP within the temperature range preceding a change in the secondary and tertiary structures of the DNA [4] do not affect the viscosity of the nucleoprotein. This, in the writers' opinion, indicates that the tertiary structure of the DNA is the factor chiefly responsible for the hydrodynamic behavior of the molecules of "aqueous" DNP in solution and it throws doubt on the view that the DNP solution is a system in which the molecular chain of DNP is folded into a more compact structure than in DNA as the result of protein crosslinkages [1].

The foregoing account can be summed up in the statement that denaturation of the nucleoprotein macromolecules may be of two types: with rupture of the complex and with preservation of its integrity but modification of its components. Denaturation of "aqueous" DNP is of the second type. Significant changes in the conformation of DNA and protein are observed in the temperature region within which the protein-nucleic acid complex behaves as a single entity. In "saline" DNP, dissociation of the protein is a component of the denaturation process. The parallel course of the changes in viscosity and in $[\alpha]_{290}$ observed in these experiments is evidence of correlation between the changes in the secondary and tertiary structures of DNA and of the complex as a whole. By the investigation of thermal denaturation of "saline" DNP it is thus possible to detect more precisely the effect of interaction between DNA and protein on changes in the physicochemical properties of DNA during the formation of specific nucleoprotein complex.

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