SOME ASPECTS OF STRUCTURAL DISTURBANCES IN THE DNP COMPLEX WHEN DAMAGED BY N-NITROSO-N-METHYLUREA

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The effect of solubilization of deoxyribonucleoproteins (DNP) in a medium of near-physiological ionic strength after treatment with the mutagen N-nitroso-N-methylurea (NMU) is highly dependent on the NMU concentration. To convert DNP into a soluble state, the critical number of groups in the DNA and protein must evidently be modified. On the basis of data obtained by the circular dichroism method and by viscosimetry it is concluded that after treatment with NMU the DNP complex becomes soluble in solvents with near-physiological ionic strength largely as a result of labilization and dissociation of the DNA–protein bonds.

KEY WORDS: deoxyribonucleoproteins (DNP); DNA; solubility of DNP; single breaks in DNA.

The writers showed previously that the classical supermutagen N-nitroso-N-methylurea (NMU) cannot only damage free DNA but can also substantially modify the structural integrity of the deoxyribonucleoprotein complex (DNP), altering its solubility in a medium of near-physiological ionic strength [4].

To study the nature of this phenomenon, the dependence of the solubility of DNP on the NMU concentration, the degradation of DNA in the DNP complex, and changes in the optical properties of DNP during the reaction with NMU were investigated.

EXPERIMENTAL METHOD

Preparations of DNP were isolated from calf thymus and their solubilities were studied by methods described previously [4]. Circular dichroism spectra were measured on the Roussel Joan CD-185 dichrograph at 20°C, with an optical path of 0.1 dm in the solution. All preparations had a concentration as DNA of 60 μg/ml. Single breaks in the polynucleotide strands of DNA in the composition of the DNP were determined viscosimetrically in 0.1 N NaOH [3]. The relationship between the reduced viscosity of the soluble fraction of DNP and the ionic strength of the solvent were studied with a single-ball capillary viscosimeter of the Ostwald type (diameter of the capillary tube 1 mm, volume of the ball 1 mm^3, gradient relative to H_2O under standard conditions 117.95 sec^{-1}).

EXPERIMENTAL RESULTS AND DISCUSSION

It was shown previously that in the presence of a comparatively high concentration of NMU (100 molecules per nucleotide) incubation for 24 h at 37°C leads to injury to the DNP complex, as a result of which, in a medium with near-physiological ionic strength (0.11 M

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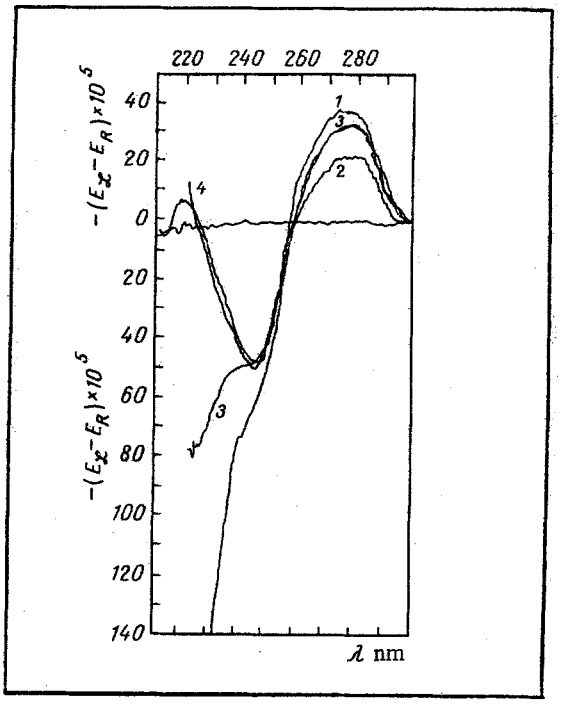

Fig. 1. Circular dichroism spectra: 1) DNA, solvent 0.14 M NaCl, concentration 60 μg/ml; 2) DNP, solvent 0.6 M NaCl ± 0.1 M phosphate buffer, pH 7.0, concentration as DNA 60 μg/ml, as protein 96.3 μg/ml; 3) soluble fraction of DNP (in 0.11 M NaCl + 0.01 M phosphate buffer, pH 7.0) after incubation with NMU for 24 h at 37°C, concentration as DNA 60 μg/ml, as protein 57.3 μg/ml. NMU concentration 0.2 M; 4) DNA incubated with NMU for 24 h at 37°C in 0.6 M NaCl + 0.1 M phosphate buffer, pH 7.0, DNA concentration 700 μg/ml, NMU concentration 0.2 M. After incubation, solution diluted fivefold with water and DNA concentration in 0.12 M NaCl + 0.02 M phosphate buffer adjusted to 60 μg/ml.

TABLE 1. Effect of Ionic Strength of Solvent on Reduced Viscosity of Control DNP Preparations and of DNP Fraction Soluble in 0.11 M NaCl + 0.01 M Phosphate Buffer, pH 7.0, after Incubation with NMU

Preparation	NaCl concentration (M)				
	0.11	0.2	0.7	2.6	0.001
	η_{sp}/c [dl/g]				
DNP^{cont}_{exp}	—	—	49,4	98,1	—
$DNP^{cont}_{37°}$	—	—	44,7	83,7	—
Soluble DNP	32,6	29,4	30,3	30,6	62,8

NaCl, 0.01 M phosphate buffer, pH 7.0), all the DNA of the complex and about 60% of the protein pass into solution [4]. Ultracentrifugation showed that half of the dissolved protein was not bound with DNA. Evidence of structural changes in the nucleic acid component of the DNP complex and a decrease in the protein content in the soluble fraction of DNP after its treatment with NMU was given by experiments in which the circular dichroism of the solutions was measured (Fig. 1). Circular dichroism spectra of DNP in solvents with both low and high (0.5-0.7) ionic strength had a characteristic maximum of ellipticity at 275 nm and a minimum at 245 nm. For DNA in the composition of DNP, the maximum of ellipticity was substantially lower in value, and in the spectral region 220-245 nm a negative band due to the protein com-

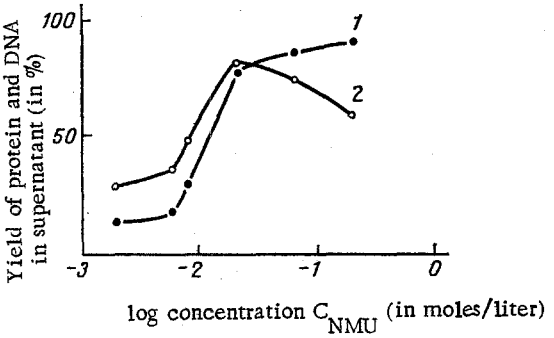

Fig. 2. Solubility of DNP in 0.11 M NaCl + 0.01 M phosphate buffer as a function of NMU concentration. Incubation for 24 h at 37°C. 1) DNA; 2) protein.

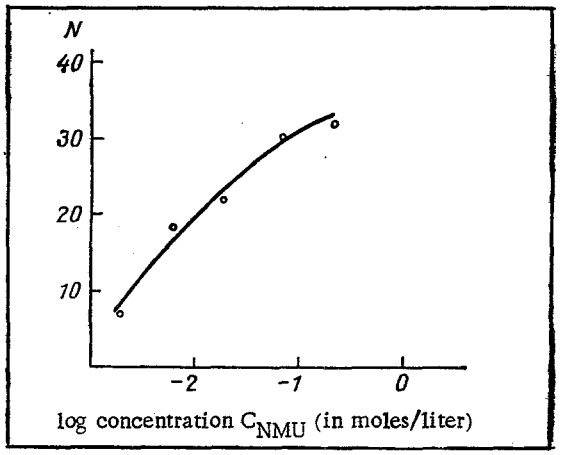

Fig. 3. Accumulation of single breaks in polynucleotide strains of DNP as a function of NMU concentration. Conditions of incubation: DNP concentration (as DNA) 600 μg/ml, 37°C, 24 h in 0.55 M NaCl + 0.05 M phosphate buffer.

ponent of the complex was observed. A shoulder characteristic of DNA was observed on the negative band at 245 nm. The difference between the circular dichroism spectra of DNA and DNP in the 275-nm region was due to different conformational states of DNA in the free form and in the complex with protein. It has been suggested that the DNA in such complexes has a more tightly coiled helix than the canonical B form [2].

After treatment with NMU the spectrum of the soluble fraction of DNP in the 275-nm region came to resemble that of double-stranded DNA free from protein, but the amplitude of the negative band caused by protein fell sharply. The circular dichroism spectra of DNA treated with NMU and of the control preparations were almost indistinguishable.

Substantial changes were observed in the viscosimetric characteristics of the soluble fraction of DNP. The viscosity of DNP solutions is known to depend on the conformational state of the DNA and, as a rule, the viscosity of salt solutions of DNP is two to three times less than the viscosity of equimolar solutions of DNA isolated from the corresponding solutions of the DNP preparations [1, 5]. In particular, if DNP preparations were transferred to a solvent with high ionic strength (2.6 M NaCl), in which electrostatic interaction between DNA and proteins was disturbed and the complex was in a dissociated state, the viscosity of the solutions increased. This phenomenon was observed both for freshly isolated preparations (DNP^{cont}_{exp}) and also for preparations kept at 37°C for 24 h ($DNP^{cont}_{37°}$) (Table 1).

Unlike the viscosity of the control preparations, the viscosity of the soluble fraction of DNP fell after NMU treatment by about one-third, but in the NaCl concentration range from 0.11 to 2.6 M it was virtually unchanged. The observed decrease in viscosity may be evidence that after NMU treatment the DNA changed into a more compact state, independent of electrostatic interaction between DNA and histones. By interacting with DNP, NMU evidently caused the formation of single breaks in the polynucleotide strands of the DNA, and at the sites opposite to the breaks the DNA molecule became more flexible, so that it could be twisted into a more compact coil. This hypothesis is confirmed by the change in viscosity of the soluble fraction of DNP after its transfer by dialysis into a solvent with low ionic strength. With a decrease in the NaCl concentration in the solvent to 0.001 M, electrostatic screening by counterions of the phosphate groups of DNA was reduced. In that case, interaction between the similarly negatively charged phosphate groups of the polynucleotide strands would begin and the DNA molecule ought to uncoil, and this would naturally lead to an increase in the degree of its asymmetry and, consequently, to an increase in its viscosity.

The effect of solubilization of DNP in a medium of near-physiological ionic strength after treatment with the mutagen depended strongly on the NMU concentration (Fig. 2). After treatment with low concentrations (1-3 molecules NMU per nucleotide) the DNP remained virtually insoluble, or at least it was indistinguishable from the control preparations in this test. However, with a further increase in the concentration of mutagen (5-10 molecules per nucleo-

tide, the solubility increased sharply, and at 100 molecules per nucleotide all the DNA went into solution. This relationship may indicate that the conversion to the soluble state requires modification of the critical number of groups in the DNA and protein.

In connection with this hypothesis of the formation of single breaks in the DNA in the complex and the absence of any significant degradation of the DNA, the results of quantitative determination of the accumulation of single breaks in the polynucleotide strands in relation to NMU concentration are of fundamental importance (Fig. 3). Incubation of DNP for 24 h with NMU in the maximal concentration (100 molecules per nucleotide) led to the appearance of about 30 single breaks. As was shown previously [4], during the action of NMU on DNA, one paired break occurs during the formation of 30-40 single breaks. Considering that the results obtained by the viscosimetric method are possibly a little on the high side [7], it can be concluded that under the conditions used there was no significant degradation of DNA.

During the action of NMU on DNP the secondary structure of the DNA remained stable, no ruptures evidently occurred in the double-stranded structure of the DNA, but as a result of the single breaks the DNA molecule became more flexible. This conclusion agrees well with the results of investigation of the chemical properties of DNA after methylation by dimethyl sulfate [6]. The increase in the solubility of DNP in solvents with near-physiological ionic strength was evidently due mainly to labilization or dissociation of the DNA–protein bonds.

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EFFECT OF THE PROTEASE INHIBITOR CONTRYCAL ON GRANULATION TISSUE METABOLISM

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Investigation of the effect of contrycal on granulation tissue metabolism in rats showed that the inhibitor stimulated incorporation of glycine-C^{14} into collagen proteins and modified the lactate dehydrogenase isozyme spectrum; meanwhile maturation of the granulation tissue was delayed despite its well-marked growth.

KEY WORDS: protein synthesis; granulation tissue; protease inhibitor, contrycal; lactate dehydrogenase and its isozymes.

Inhibitors of proteolytic enzymes are extensively used in surgical practice [5]. However, the desirability of using inhibitors to accelerate the healing of clean and infected wounds is still under discussion in the literature. For instance, besides papers giving evidence of the beneficial effect of inhibitors on wound healing [2, 8], there are others describing the absence of any marked therapeutic effect [10]. The contradictory nature of the

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