ANALYSIS OF DISTURBANCES IN MACROMOLECULAR ORGANIZATION OF DNP INDUCED BY NITROGEN MUSTARD

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As a result of the action of nitrogen mustard on deoxyribonucleoprotein (DNP), changes are observed in the DNA-protein bonds. These changes may take the form either of labilization or of strengthening, and this affects the state of the DNA in the DNP-complex.

Previous experiments [5] showed that the phenylalanine analog of nitrogen mustard (sarcolysin) modifies the hydrodynamic properties of deoxyribonucleoprotein (DNP), and this is considered to be the result of changes in the interaction between DNA and the protein component of the DNP complex.

In the investigation described below, the effect of nitrogen mustard itself on the physicochemical state of DNP was studied. The action of this compound can be explained only by alkylation and there is no possibility of additional interaction between DNP and the phenylalanine radical. In addition, the fact that nitrogen mustard does not absorb in the UV region essentially facilitates the determination of the character of the changes of DNA-protein interaction under the influence of the bifunctional alkylating mutagen.

EXPERIMENTAL METHOD

Samples of DNP were isolated from calf thymus by a modified method of Mirsky and Pollister [2]. DNA was isolated from the DNP by Kay's method [8]. The DNP (DNA) was incubated with nitrogen mustard at 25°C. The working solution (pH 7.0) contained 0.1 mg/ml DNP (as DNA), 10^3 M nitrogen mustard, 0.7 M NaCl, and 0.02 M $NaHCO_3$. In the deproteinization experiments, to stop the reaction and remove the unreacted agent, the DNP solutions were dialyzed at 4°C against 2M NaCl solution. The dialyzed specimens were deproteinized as described in [3]. Protein was determined by Lowry's method [11], and DNA by Spirin's method [4]. The viscosimetric spectrophotometric measurements were made as described earlier [5]. The method of determining the true absorption of the solutions at 260 nm, with allowance for "turbidity," is described in [1].

EXPERIMENTAL RESULTS

Just as when DNP was treated with the phenylalanine analog of nitrogen mustard [5], nitrogen mustard itself led to a sudden increase in the characteristic viscosity (Fig. 1:2). However, this increase averaged 24±4% for nitrogen mustard compared with 43±4% for the phenylalanine analog. The difference is statistically significant ($P < 0.01$). The second stage of the reaction took place more slowly and was marked by a steady decrease in viscosity which, by contrast with the action of phenylalanine mustard, reached much lower values than the corresponding control.

The initial increase in viscosity could be connected with partial deproteinization of the specimen through competitive interaction between the nitrogen mustard and the phosphate groups of the DNA previously occupied by protein molecules. Partial deproteinization of DNP is known to lead to an increase in the degree of asymmetry of the macromolecule [6, 7]. If this is true, partial deproteinization of the original DNP sample ought to prevent the initial increase in viscosity of DNP. In fact, nitrogen mustard, like its

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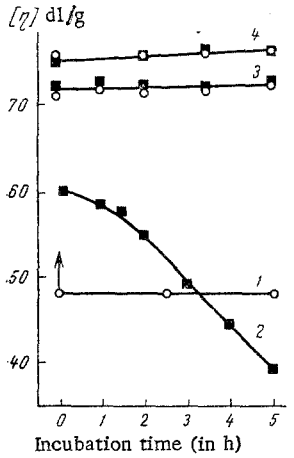

Fig. 1

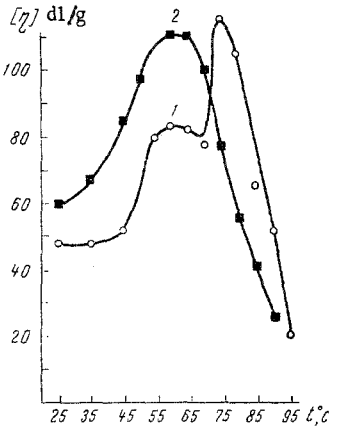

Fig. 2

Fig. 1. Effect of nitrogen mustard on characteristic viscosity of DNP in solvents of different ionic strength and with different protein content in the complex. Circles denote control, squares DNP + nitrogen mustard. 1, 2) $\mu = 0.7$, 60% protein; 3) $\mu = 0.7$, 42% protein; 4) $\mu = 1.0$, 60% protein. Ordinate, characteristic viscosity (dg/g); abscissa, incubation time (1 h).

Fig. 2. Effect of nitrogen mustard on relationship between characteristic viscosity of DNP and temperature: 1) control; 2) DNP + nitrogen mustard, incubation for 1 h. Ordinate, characteristic viscosity (in dl/g); abscissa, temperature.

phenylalanine analog, had no effect on the viscosity of DNP preparations in which the protein content in the complex had first been reduced either by partial deproteinization or by an increase in the ionic strength of the solution [Fig. 1 (3, 4)]. The descending part of the DNP viscosity curve [Fig. 1 (2)] can be attributed to at least two processes: reassociation of the dissociated protein with DNA and aggregation of the DNP molecules. Both these processes are accompanied by a decrease in the degree of asymmetry of the DNP, leading to a decrease in the characteristic viscosity. The aggregation process evidently progresses in time, as is shown by the increase in absorption due to scattering of light on the aggregates in the region in which DNP is optically empty. Aggregation of DNP after prolonged incubation with nitrogen mustard (5 h) is also demonstrated by the fact that centrifugation of the solutions for 1 h at 20,000 g precipitated up to 70% of the DNP, by contrast with the control. This is evidently the significant difference between the action of nitrogen mustard and its phenylalanine analog on DNP.

The molar extinction coefficient, an index of the degree of orderliness of the secondary structure of the DNP, fell during incubation of DNP with nitrogen mustard. This fact cannot be explained by aggregation of the DNP, for $E(p)_{260}$ was measured with allowance for the scattering of the light. This index for native DNP was rather higher than for DNA [10], as the result of conformational changes in the DNA during complex formation with protein; the decrease in optical absorption of DNP and, consequently, restoration of the normal DNA structure can be explained by weakening of the DNA–protein bond in the reassociated complex.

Melting curves were plotted from the results of tests of the optical density of the control DNP and of DNP after incubation with nitrogen mustard (DNP aggregates were removed by centrifugation, see above). The melting curve for the control DNP is typical of the native nucleoprotein: in the zone of raised temperatures preceding melting, hypochromism was observed, while the maximal increase in optical density was 30% of its initial value. The experimental samples melted like pure DNA and the hyperchromic effect of the samples was 37%. The view that the DNA–protein bonds were weakened is supported by the character of the characteristic viscosity versus temperature curve for the experimental and control samples (Fig. 2). It can be postulated that the displacement of the ascending limb of the viscosity curve in the region of lower temperatures [Fig. 2 (2)], just as during the action of phenylalanine mustard on DNP, is evidence of label-

TABLE 1. Effect of Nitrogen Mustard on Deproteinization of DNP by Chloroform and Sodium Dodecylsulfate

Type of treatment	Duration of interaction with agent (in h)	Percentage of protein in composition of DNP (aqueous phase)	Percentage of DNA passing into interphase
Chloroform	Control	28±3,6	12,5±2,9
	1	45,7±4,1	39,8±8,2
	5	63,8±9,2	77,5±8,9
Chloroform + SDS	Control	11±4,9	14,6±2,7
	1	36±4,6	57,6±8,1
	5	71±3,5	89,6±1,0

ization of the bond between DNA and the protein [6, 7] which, in the control DNA samples, starts to dissociate at 45°C.

Besides weakening of DNA–protein bond, the appearance of cross-linkages of the DNA–protein type would be expected, for nitrogen mustard is a bifunctional agent. To verify this hypothesis, experiments were carried out in which DNP was deproteinized once with a mixture of chloroform and isoamyl alcohol (24:1) and with the same mixture after treatment of the DNP with 0.5% sodium dodecylsulfate (SDS). Whereas chloroform induces denaturation of the free protein removed as a result of an increase in the ionic strength, and precipitates this protein at the chloroform–water boundary, SDS itself is a deproteinizing agent and is capable of rupturing both hydrophotic and electrostatic bonds. The results of these experiments are given in Table 1

As Table 1 shows, during interaction between DNP and nitrogen mustard the percentage of protein bound with DNA in the aqueous phase is increased and the effect of precipitation of DNA into the interphase, which also is the result of strengthening of the DNA–protein bond, is enhanced. The effects observed in the experimental DNP samples were significantly ($P < 0.02$) higher than the corresponding control values. SDS does not induce any additional deproteinizing effect on the experimental DNP samples. The results thus suggest that the DNA-protein bond is neither electrostatic nor hydrophobic in character during the action of nitrogen mustard on DNP, but is most probably covalent. The effect of precipitation of DNA into the interphase may occur during partial denaturation of the DNA, which may happen as a result of the action of alkylating compounds [9]. However, as control experiments showed, incubation of native DNA with nitrogen mustard does not lead to transfer of the experimental DNA samples into the interphase.

The kinetics of interaction between nitrogen mustard and DNP is thus a complex process involving the DNA–protein bond and the leading to both labilization and strengthening of these bonds.

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