# EFFECT OF SARCOLYSIN ON THE STRUCTURAL ORGANIZATION OF DEOXYRIBONUCLEOPROTEIN

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Anomalous changes in the viscosimetric characteristics of DNP when incubated in the presence of sarcolysin were discovered. It is postulated that sarcolysin affects the salt-like bonds between DNA and protein.

The writers showed previously that during the first few hours of interaction between sarcolysin and the double-stranded DNA molecule in vitro small doses of the alkylating agent (concentration $10^{-3}$, $5 \cdot 10^{-4}$, and $10^{-4}$) induce latent injuries in the macromolecular organization of the DNA. However, DNA in the cell nucleus is not in the free state, but in the form of a complex with histones and acid proteins. It can accordingly be expected that the appearance of primary molecular injuries in the chromosome in vivo is the result of interaction between the mutagen and the molecule of the deoxyribonucleoprotein complex (DNP). Virtually no investigations into the action of alkylating agents on the structural properties of isolated DNP macromolecules can be found in the modern literature. An object of particular interest in this field of research is the action of bifunctional alkylating agents, because this class of compound not only affects the structure of DNA, but can also modify the DNA–protein bond with all the consequences which stem therefrom.

The action of sarcolysin on the viscosimetric characteristics of DNP from the calf thymus were studied in the investigation described below.

## EXPERIMENTAL METHOD

DNP was isolated from the calf thymus by the writers' modification of Mirsky and Pollister's method in 0.7 M NaCl [4]. DNA was isolated from the test DNP by the detergent method of Kay et al. [3]. DNA and DNP were incubated with a commercial preparation of sarcolysin without mixing at 25°C in a water thermostat. A sample of sarcolysin hydrochloride, weighing 20.4 mg, with a molecular weight of 341, was dissolved at 60°C in 15 ml of 1.4 M NaCl, the solution was cooled and to neutralize the acid medium, 15 ml of 0.04 M $NaHCO_3$ was added, causing the pH of the solution to rise to 7.0. The resulting sarcolysin solution was mixed with an equal volume of DNP solution in 0.7 M NaCl to give a DNA concentration of 0.04 mg/ml. The solution, when ready for measurement, contained 0.02 mg/ml of DNP (as DNA) and $10^{-3}$ M sarcolysin. A DNP solution with corresponding ionic strength, but not containing sarcolysin, was used as the control. DNA was treated with sarcolysin in the same way. The viscosity of the DNA and DNP solutions was measured on a glass three-ball viscosimeter of the Ostwald type with gradients of 45, 28.5, and 18 $sec^{-1}$ relative to water under standard conditions. The values of the reduced viscosity were extrapolated to zero gradient shift. The maximum error of the method was ±7%. To measure the relationship between temperature and viscosity, the viscosimeter was placed in a water bath and heated for 10-15 min at a particular temperature, after which the time taken for the solution to flow out at that temperature was measured.

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TABLE 1. Effect of Sarcolysin on Viscosity of DNP Solutions ($[\eta]\beta-0$)

| Expt. No. | Control | Expt. | Age (in %) |
|---|---|---|---|
| 1 | 42 | 65 | 54 |
| 2 | 57 | 89 | 56 |
| 3 | 50 | 67 | 34 |
| 4 | 39 | 46 | 39 |
| 5 | 51 | 65 | 27 |

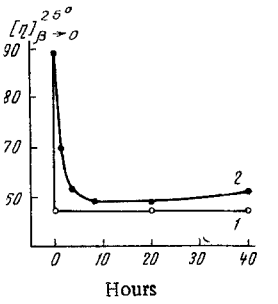


Fig. 1. Kinetics of change in characteristic viscosity of DNP when incubated with sarcolysin: 1) DNP in 0.7 M NaCl; 2) DNP + sarcolysin. Concentration rations are given in the section "Experimental Method."

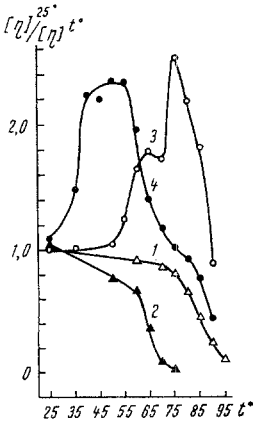


Fig. 2. Effect of sarcolysin on temperature dependence of characteristic viscosity of DNA and DNP: 1) DNA in 0.7 M NaCl; 2) DNA + sarcolysin, incubation for 35 h; 3) DNP in 0.7 M NaCl; 4) DNP + sarcolysin, incubation 35 h.

## EXPERIMENTAL RESULTS

Several stages can be clearly distinguished in the results obtained. By contrast with DNA solutions, whose viscosity either rises only very slightly or remains unchanged after treatment with sarcolysin, the viscosity of the DNP solution immediately rose in a stepwise manner after the addition of sarcolysin. It is clear from Table 1 that the mean viscosity increased by about 50% compared with the control. During the first 5 h of incubation after the stepwise increase, the viscosity of the DNP fell to values only slightly higher than their initial level. Later, for a considerable period of time, about 55-60 h of incubation, the viscosity of all the tested solutions without exception remained constant (Fig. 1).

The viscosimetric melting curves, which differed significantly from the corresponding curves for natural DNP preparations, are of considerable interest. Investigations have recently shown that the viscosimetric melting curve of native DNP preparations, by contrast with the analogous curve for DNA, consists of 2 sharply different segments [2]. During heating at certain temperatures the characteristic viscosity of DNP becomes approximately doubled, and in the region of the melting temperature of the DNA hydrogen bonds the viscosity falls sharply. The beginning of the rise and the width of the temperature range within which the viscosity increases depend on the ionic strength of the solvent, while the kinetics of the increase in viscosity evidently depend on the strength of interaction between DNA and histone [1]. Unlike the control solutions, the viscosity of DNP preparations incubated with mutagen begins to rise immediately on heating, reaches a maximum at lower temperatures, and begins to fall much sooner. The falling branch of the curve was more sloping in the experimental tests (Fig. 2).

It can be postulated that the reason for the observed anomalies in the change in viscosity and displacement of the melting temperature may be either aggregation or modification of the DNA–protein bonds. If aggregation takes place, the observed shift of viscosity (if this process initially begins with predominance of terminal intermolecular interactions, followed by lateral interactions) will be such that ultimately it leads to a decrease in the degree of asymmetry of the aggregate and, correspondingly, to a decrease in viscosity. With elevation of the temperature, under the conditions of measurement of the melting curves the opposite process may take place, and during destruction of the lateral aggregates the viscosity will increase. Meanwhile, the segment of decreasing viscosity (Fig. 2) indicates substantial weakening of the secondary structure of the DNA, which cannot be explained on the basis purely of aggregation processes.

Most probably the interaction between sarcolysin and DNA begins wherever the protein is located. As a result, temporary dissociation of the complex, characterized by an increase in viscosity, may be ob-

served. Since this process is based on salt-like interactions with zero activation energy and since it takes place very rapidly in time, the viscosity may increase stepwise. After the introduction of sarcolysin between the DNA and protein, transalkylation of the mutagen may take place on the basis of the DNA, and this may be followed by nonspecific interaction between the histone and DNA. This process will be characterized by a decrease in viscosity, and the nonspecific complex will dissociate at lower temperatures.

The possibility is not ruled out that both these processes actually take place together. In any case, the results of the investigations described above indicate a fundamental difference between the sensitivities of DNA and DNP to the action of sarcolysin, a typical member of the class of bifunctional alkylating agents and one which is widely used in medical practice. Although the results of these experiments do not yet provide a clear picture of the interaction between sarcolysin and DNP, they nevertheless indicate that this mutagen has a complex action on the structural organization of DNP and, in all probability, as well as modifying the structure of DNA, it also influences the DNA–protein bond. Further experiments will show which of these processes is dominant in the development of injury to DNP.

## LITERATURE CITED


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