ACTION OF SARCOLYSIN ON THE MACROMOLECULAR STRUCTURE OF DNA

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High sensitivity of the temperature dependence of the characteristic viscosity of DNA to the brief action of small doses of sarcolysin has been demonstrated. It is postulated on the basis of these results that the primary action of sarcolysin on the molecular structure of DNA may be expressed by weakening of the phosphoester bonds with the subsequent formation of single breaks in the polynucleotide chains of DNA.

Sarcolysin [2] has been shown to produce depolymerization and cross linking of the DNA molecule [5, 6]. Kushner and Khodosova [1] found that the secondary structure of DNA is weakened and cross-linkages are formed between the complementary strands of the macromolecule. However, in the investigations cited above, concentrations of the agent used to demonstrate disturbance in the DNA structure were much higher than those permissible biologically, and incubation took place for a long period of time (measured in tens of hours).

The object of the present investigation was to study the action of sarcolysin in vitro on double-stranded and single-stranded DNA in near-biological doses (2 or 3 orders of magnitude smaller than in the investigations cited above), during the first few hours of interaction.

EXPERIMENTAL METHOD*

Calf thymus DNA was isolated by the detergent method of Kay et al. [10]. The molar coefficient of extinction of the preparations in a standard salt–citrate buffer (SSC 0.15 M NaCl + 0.015 M sodium citrate) was 6600-6800, the hyperchromic effect was 35-38%, T_m = 87°, and the characteristic viscosity in SSC varied from 85 to 160 dl/g, corresponding to molecular weights of 1.74×10^7-4.4×10^7 daltons [8]. Single-stranded DNA (sDNA) was obtained by heating 0.008% solutions of native DNA (nDNA) in a medium with low ionic strength (0.01 as Na^+) for 16 min at 95°C followed by rapid cooling in ice-cold water. Completeness of separation of the DNA strands was determined by investigation of the flexibility factor (FF) and the temperature of elevation of viscosity (TEV) [3]. DNA was incubated with a commercial preparation of sarcolysin in different concentrations at 37° without mixing, and also at 25° with slow mixing of the solution. In the first case the reaction was stopped and the agent removed by reprecipitation of DNA with two volumes of 96% ethanol, and in the second by dilution and dialysis at 4°. Dialysis of nDNA was carried out against

*The following abbreviations and symbols are used in this paper: T_m) melting temperature (mean temperature of the helix-coil transformation observed from measurements of optical density); ΔT_m) difference between melting temperature of DNA denatured by Geiduschek's method [9] and T_m; $\Delta E\%$) hyperchromism of DNA; $\Delta Ei\%$) hyperchromism of DNA denatured by Geiduschek's method [9]; $[\eta]$) characteristic viscosity of DNA in a zero shear gradient; $T_{[\eta]1/2}$) the temperature at which the characteristic viscosity of DNA is equal to half its viscosity at 25°

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TABLE 1. Spectrophotometric and Viscosimetric Characteristics of nDNA Incubated with Sarcolysin in Different Concentrations for 5 h in Medium with Ionic Strength 0.2

Sarcolysin concentration	T_M	ΔT_M	$\Delta E\%$	r%	[η]	$T_{[\eta]/2}$
—	68	7	36	28	155	85
10^{-4} M	68	7	36	28	140	81
$5 \cdot 10^{-4}$ M	68	6	35	26	145	78
10^{-2} M	69	5	34	24	140	70

TABLE 2. Relationship between Spectrophotometric and Viscosimetric Parameters of nDNA and Duration of Incubation with 10^{-4} M Sarcolysin Solution in Medium with Ionic Strength 0.01

Time of incubation with sarcolysin	T_M	ΔT_M	$\Delta E\%$	r%	[η]	$T_{[\eta]/2}$
Control	68	5	37	32	158	83
1/2 h	68	4	36	33	160	76
1 h	68	4	35	30	158	69
2 h	69	2	28	35	100	62
4	69	1	26	27	86	59

1/20 SSC ($\mu \simeq 0.01$), and of sDNA against phosphate buffer (pH 6.9, $\mu = 0.01$). Aqueous or saline solutions of sarcolysin neutralized by an equal volume of 0.04 M $NaHCO_3$ solution, followed by dilution to the working concentration with phosphate buffer ($\mu = 0.01$), 1/20 SSC, or NaCl of the required concentration, were used for incubation. The ionic strength of the incubation mixture (as Na^+) was either 0.01 (low) or 0.2 (high). The pH of the test solutions ranged from 7.2 to 7.6. The final concentration of double-stranded DNA in all the experiments was 0.01%, and that of single-stranded DNA 0.004%. DNA solution of the corresponding ionic strength, but not containing sarcolysin, was used as the control. The relationship between the optical density of the preparations at 260 mμ and temperature was measured on the SF-II spectrophotometer in 1-cm cuvettes with constant heating. Reversibility of DNA denaturation was studied by Geiduschek's method [9]. To estimate the ability of DNA to restore hydrogen bonds during rapid cooling, the value $r = (1 - \Delta Ei\%/\Delta E\%) \cdot 100\%$ was calculated. This value of r (renaturability) characterizes the highest percentage of DNA renaturation after rapid cooling of the specimen. The viscosity of DNA in a concentration of 0.002% was measured on a three-ball glass viscosimeter of the Ostwald type with water gradients under standard conditions of 45, 28.5, and 18 sec^{-1}. Values of the reduced viscosity were extrapolated to zero shear gradient. The maximum error of the method was ±7%. To measure the temperature dependence of viscosity, i.e., $[\eta] = f(T)$, the viscosimeter was placed in a water bath and heated for 5-10 min at the corresponding temperature, after which the time taken for the solution to flow out was measured at that temperature.

EXPERIMENTAL RESULTS

One method of qualitative determination of disturbances in the primary structure of DNA is the method of temperature dependence of the characteristic viscosity [4]. To determine the applicability of this method to the phenomena examined in the present investigation, spectrophotometric and viscosity parameters of DNA were compared during incubation in a solvent with ionic strength 0.2 and with different concentrations of sarcolysin (10^{-3}, $5 \cdot 10^{-4}$, 10^{-4} M) for 5 h at 37°. No significant disturbances in the secondary structure of DNA, as revealed by optical density and formation of cross-linkages, tested by Geiduschek's method, were observed (Table 1). Meanwhile, the value of $T_{[\eta]/2}$ fell appreciably with an increase in dose of the agent. Consequently, disturbances in the structure of DNA induced by low sarcolysin concentrations must logically be sought at the level of disturbances of the primary structure. One possible approach to the solution of this problem is to investigate disturbances in single-stranded DNA present in a solvent with ionic strength 0.01, at which the relative proportion of helical segments is extremely low. The duration of incubation of sDNA with the agent corresponded to the time during which nDNA underwent

TABLE 3. Effect of Sarcolysin on Viscosity of sRNA in Medium with Ionic Strength 0.01

Time of incubation with sarcolysin	$[\eta]_{25°}$	$[\eta]_{70°}$	$[\eta]_{70°\rightarrow 25°}$	Number of single breaks
Control	97	92	93	—
1/2 h	95	28	15	5.3
1 h	94	20	11	7.5

structural changes permitting a significant difference to be determined between the values of $T_{[\eta]/2}$ of the experimental and control specimens. The sDNA and nDNA were incubated at 25° (the decrease of temperature was due to the marked temperature dependence of aggregation of sDNA [7] in a medium with ionic strength 0.01), with mixing. The molecular ratio between sarcolysin and nucleotide molecules for nDNA and sDNA was identical (1:3).

The results given in Table 2 show that incubation for 0.5 h and 1 h produce no significant changes in the spectrophotometric characteristics of nDNA. The viscosity of the specimens was unchanged, although $T_{[\eta]/2}$ was appreciably reduced. Comparison of the results given in Tables 1 and 2 shows that with respect to the $T_{[\eta]/2}$ parameter DNA in a medium with low ionic strength was substantially more sensitive to the action of sarcolysin than DNA in a medium of high ionic strength. The possibility is not ruled out that one of the factors determining the sensitivity of DNA to the action of this agent is the degree of screening of the phosphate groups of the polymer, which is proportional to the ionic strength of the surrounding medium, and another possibility is that in a medium with low ionic strength, contact between sarcolysin and guanine residues is facilitated because of the relaxation of the secondary structure of the DNA. After incubation with sarcolysin for 30 min and 1 h the viscosity of the experimental sDNA at 25° was indistinguishable from the control. Measurement of TEV (70°) of the experimental sDNA showed a steady fall in the viscosity of the specimens over a period of 1 h. The viscosity of the control sDNA did not change with time. After the process of decrease in viscosity of sDNA at 70° had reached saturation point, the specimens were cooled and viscosity measured at 25°. The viscosity of the experimental specimens of sDNA was not restored, indicating that irreversible changes had occurred in its structure (Table 3). The most probable explanation is that the observed decrease in viscosity of sDNA during heating in a medium with an ionic strength of 0.01 at 70° takes place through the depolymerization of the macromolecule because of cleavages induced by sarcolysin. Another possibility is that this depolarization also occurs at 25°, although much more slowly than at 70°. The results obtained for sDNA suggest that changes in the $[\eta] = f(T)$ curves for nDNA incubated with 10^{-4} M sarcolysin for 1 h take place because of disturbances of the primary structure of the nDNA.

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