

Native 및 Denatured Calf Thymus DNA 의 DNase 1 에 대한 Susceptibility 에 미치는 Spermine 의 영향

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Opposite Effect of Spermine on the Susceptibility of Native and Denatured Calf Thymus DNA to DNase 1.

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요 약, Spermine 은 DNase 1 에 대한 native 및 denatured calf thymus DNA 의 susceptibility 에 상반된 효과를 갖는다. spermine 은 그 native DNA 의 그 susceptibility 를 증가시키지만 그 denatured DNA 의 것은 감소 시킨다. spermine 이 존재하지 않을 경우에는 그 deoxyribonuclease 의 반응성은 native 및 denatured DNA 의 conformation 차이에 의견상 무감각한 것으로 나타난다.

ABSTRACT. Spermine has opposite effect on the susceptibility of native and denatured calf thymus DNA toward deoxyribonuclease I (DNase I; deoxyribonuclease 5'-oligonucleotidohydrolase; EC 3.1.4.5; from bovine pancreas). It enhances the susceptibility of the native DNA, whereas depresses that of the denatured DNA. In the absence of spermine, the reactivity of the deoxyribonuclease is apparently insensitive to the conformational difference of the native and denatured DNA.

INTRODUCTION

Functional properties of enzymes or some other agents acting on nucleic acids would often be dependent upon the conformation of the nucleic acids. For example, conformational transitions of the promoter regions of DNA might be involved in the recognition of DNA by such regulatory proteins as RNA polymerase¹⁻³. Importance of possible physicochemical structural factors of a nucleic acid in the elucidation of its metabolic or expression process has been emphasized⁴. On the other hand,

numerous studies have shown that polyamines interact with nucleic acids and have a variety of effects on three dimensional structural and functional properties of nucleic acids⁵⁻⁷.

We have been interested in the effect of conformational change of a nucleic acid as a substrate in some enzymatic reactions. Our purpose in the present work is to show that the conformational difference of the native and denatured DNA, which is not reflected in their susceptibility for the DNase, can become manifested through the intermediation of spermine.

MATERIALS AND METHODS

Calf thymus DNA (highly polymerized sodium salt, type I), DNase I (EC 3.1.4.5), and spermine tetrahydrochloride were purchased from Sigma Chemical Company. In the DNase reactions, the procedure of Kunitz with some modifications was used⁸. The DNase solution was prepared by dissolving 5mg of the DNase just before use, in 3.5ml of cold 0.15M NaCl solution. Stock solution of the DNA substrate was prepared by dissolving 10mg of the DNA in 30ml of cold water and allowing to stand overnight in the cold. To the DNA solution was added appropriate volume of distilled water, 10X enzymatic reaction buffer, and spermine, if needed, to make final concentration of 1X buffer components in the enzyme reaction mixture, when 2.9ml of the substrate solution and 0.1ml of enzyme solution were mixed together. The final composition of the buffer components in the reaction mixture of 3ml was: 0.03M MgCl₂, 0.005M NaCl, and 0.008M acetate, pH 5.0. The native calf thymus DNA concentrations were determined by assuming a molar extinction coefficient at 260nm of 6600M⁻¹cm⁻¹ per nucleotide. The enzyme reaction was initiated by adding and mixing preincubated (at 20°C) 0.1ml of the DNase solution and 2.9ml of the substrate solution in a cell at 20°C. The absorbance increase at 260nm was scanned with Pye Unicam Spectrophotometer, SP 1800, and the reaction velocity was calculated from the slope of the progress curve. The heat-denatured DNA was prepared by heating the aqueous solution at 95°C for 20min and then cooling rapidly to 0°C in ice-water.

RESULTS AND DISCUSSION

Spectrophotometric tests with respect to the structural integrity of the native and denatured

DNA species used in the experiments were carried out. When the enzyme, DNase I, was omitted in the complete reaction mixtures containing either the native DNA or denatured DNA, any appreciable absorbance increase at the wavelength of 260nm could not be observed during the reaction period, thus eliminating the possibility of any self-denaturation of the native DNA and self-degradation of the denatured DNA. The integrity of the preparation of the heat-denatured DNA and the native DNA was confirmed respectively by the absorbance-temperature profile at 260nm as shown in Fig. 1.

At first, we were interested to see whether or not the three dimensional structural differences of native and denatured DNA could be recognized by the DNase and the differences would be reflected in the enzyme kinetics observed. One can see in Fig. 2 that there is no any significant differences in the susceptibility of the native and denatured DNA for the DNase function. However, as shown in Fig. 2 and Fig. 3, in the presence of spermine at the concentration of $3 \times 10^{-4}M$, the susceptibility of the native DNA for the DNase is enhanced, while that of the denatured DNA is lowered. Apparently, in the absence of spermine, the DNase is insensitive for the structural difference of the two conformational species of the DNA, whereas in the presence of spermine, the enzyme would be able to recognize the structural differences and the spermine has opposite effects on the susceptibility of the native and denatured DNA. We further tested the susceptibility of the native and denatured DNA for the DNase versus a variety of concentrations of spermine. The result, agreeable with the results of Fig. 2 and Fig. 3, is shown in Fig. 4. The figure shows that spermine enhances the susceptibility of the native

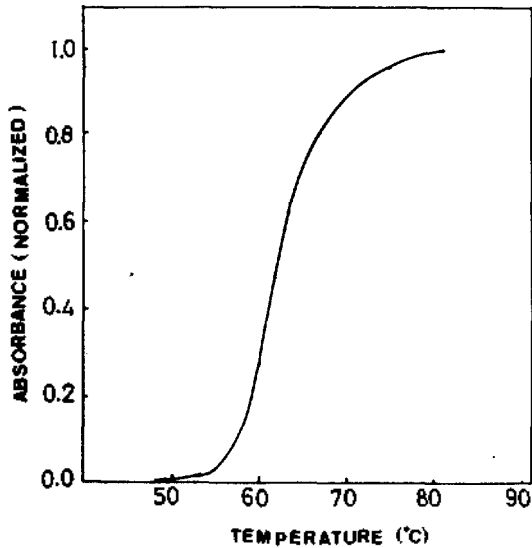


Fig. 1. Absorbance-temperature profiles of native — and denatured (---) calf thymus DNA in the phosphate buffer, pH 7, composed of 0.006M sodium phosphate and 0.001M EDTA. For further experimental details, see text and the Ref. 16.

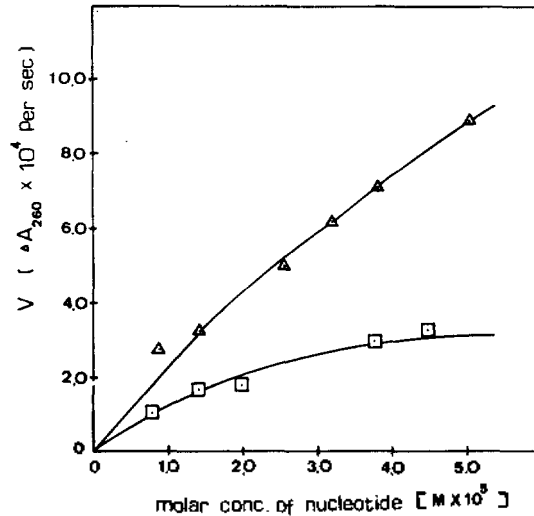


Fig. 3. Enzymatic susceptibility of denatured DNA vs. DNA concentration in the absence and presence of spermine. All experimental conditions were as in Fig. 2. Δ : Denatured DNA, minus spermine. \square : Denatured DNA, plus spermine.

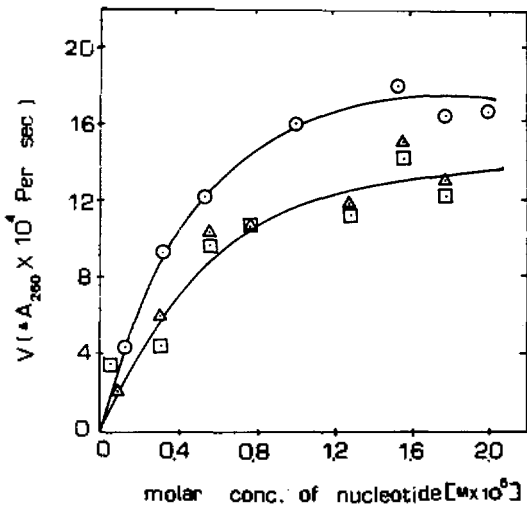


Fig. 2. Susceptibility of native and denatured DNA to DNase vs. DNA concentration in the absence and presence of spermine. The reactions were carried out at 20°C, pH 5.0 in a buffer of 0.03M MgCl₂, 0.005M NaCl, and 0.008M acetate. The absorbance increase at 260nm (ΔA_{260}) was scanned with the spectrophotometer, and the reaction velocity (v) was obtained from the slope of the progresscurve. —○: Native DNA, +spermine. —□: Native DNA, -spermine. —△: Denatured DNA, +spermine. —▽: Denatured DNA, -spermine.

DNA for the DNase, having an optimum concentration for the susceptibility, whereas the susceptibility of the denatured DNA is decreased rapidly as the concentration of the spermine is increased.

It has been shown that polyamines have strong affinity for binding with multiple-stranded helical structure of polynucleotides, giving rise to the stabilization effect of the structure⁹⁻¹¹. The binding of spermine with DNA may take place *via* both the formation of intramolecular cross-linking bridge between different DNA molecules and the formation of intramolecular tilted bridges across shallow grooves between separate polynucleotide chains of a DNA molecule¹². In contrast with the case of native DNA, in the case of the denatured DNA molecules which might have lost much of its double-stranded helical structures, the former type of spermine effect may become increasingly important, resulting in the decrease

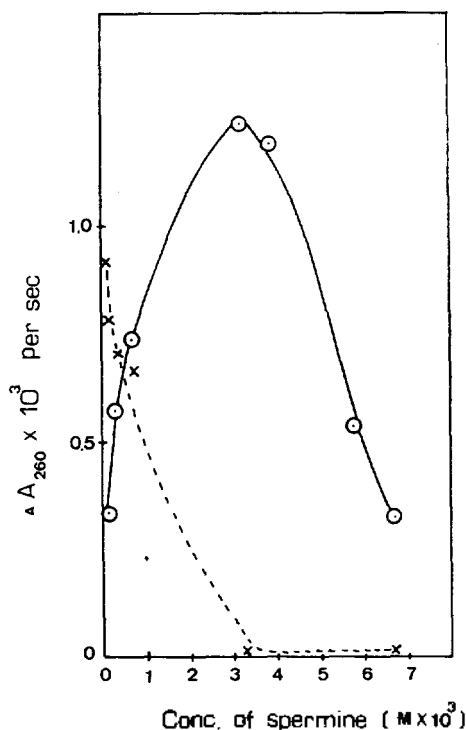


Fig. 4. Effect of spermine on the susceptibility of native and denatured DNA for DNase. The concentration of DNA was $1.2 \times 10^{-4} M$ of nucleotide phosphorus in each case. Experimental conditions were as in Fig. 2. ○: Native DNA. ×: Denatured DNA.

of the susceptibility of the DNA for the DNase as shown in Fig. 4. This assumptive explanation can be supported by previous reports that the susceptibility of polyribonucleotides for pancreatic ribonuclease (RNase) is depressed by spermine¹³⁻¹⁵.

Since it is expected that spermine, which is bound with DNA, is involved in the cooperative transition of DNA conformation (Huh *et al.*, unpublished data), we tested the Hill plot of the experimental data of the DNase reactivity on the native DNA in the presence and absence of spermine, and the denatured DNA in the absence of spermine. We do not see any significant difference in their Hill plots, as shown in Fig. 5. They do not show cooperativity, having approximately the same Hill

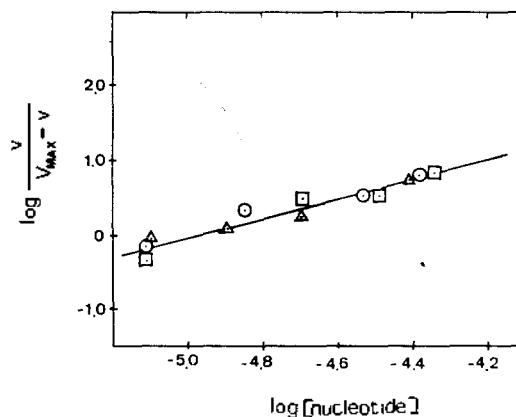


Fig. 5. Hill plots of DNA susceptibilities to DNase in the absence and presence of spermine. The plots were obtained from the experiments of Fig. 2 and Fig. 3. ○: Native DNA, +spermine. □: Native DNA, -spermine. △: Denatured DNA, -spermine.

coefficient of unity. Apparently the differential effect of spermine on the susceptibility of the native and denatured DNA for the DNase may not be due to any difference in their cooperativity characteristics in the enzymatic reaction.

While the mechanism of the opposite effect of spermine on the native and denatured DNA in their susceptibility for the DNase remains to be elucidated, the present result emphasizes the possibility of altered mode of reactivity of nucleic acid dependent upon its structural changes, which being reacted upon by some agent such as spermine. This kind of consideration would be especially important in studies of nucleic acid metabolism *in vivo*.

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