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Native 및 Denatured Calf Thymus DNA의 DNase 1에 대한 Susceptibility에 미치는 Spermine의 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. Spermine은 DNase 1에 대한 native 및 denatured calf thymus DNA의 susceptibility에 상반된 효과를 갖는다. spermine은 그 native DNA의 그 susceptibility를 증가시키지만 그 denatured DNA의 것은 감소 시킨다. spermine이 존재하지 않을 경우에는 그 deoxyribonuclease의 반응성은 native 및 denatured DNA의 conformation 차이에 외견상 무감각한 것으로 나타난다.

ABSTRACT. Spermine has opposite effect on the susceptibility of native and denatured calf thymus DNA toward deoxyribonuclease I (DNase I; deoxyribonucleate 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 mucleic acids. For example, conformational transitons of the promoter regions of DNA might be involved in the recognition of DNA by such regulatory proteins as RNA polymerase[1~3]. Importance of possible physicochemical structural factors of a nucleic acid in the elucidation of its metabolic or expression process has been emphasized[4]. 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 acids[5~7].

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 used[8]. The DNase solution was prepared by dissolving 5mg of the DNase just before use, in 3.5m*l* of cold 0.15*M* NaCl solution. Stock solution of the DNA substrate was prepared by dissolving 10mg of the DNA in 30m*l* 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 2.9m*l* of the substrate solution and 0.1m*l* of enzyme solution were mixed together. The final composition of the buffer components in the reaction mixture of 3m*l* was: 0.03*M* $MgCl_2$, 0.005*M* NaCl, and 0.008*M* acetate, pH 5.0. The native calf thymus DNA concentrations were determined by assuming a molar extinction coefficient at 260nm of $6600M^{-}$ cm^{-} per nucleotide. The enzyme reaction was initiated by adding and mixing preincubated (at 20°C) 0.1m*l* of the DNase solution and 2.9m*l* 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 1, 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 dimersional 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 $3\times10^{-4}M$, 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

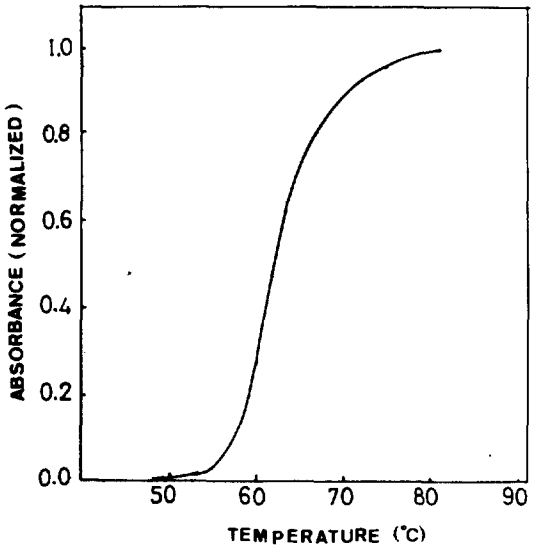

Fig. 1. Absorbance-temperature profiles of native ——and denatured (- - - -) calf thymus DNA in the phosphate buffer, pH 7, composed of 0.006*M* sodium phosphate and 0.001*M* EDTA. For further experimental details, see text and the Ref. 16.

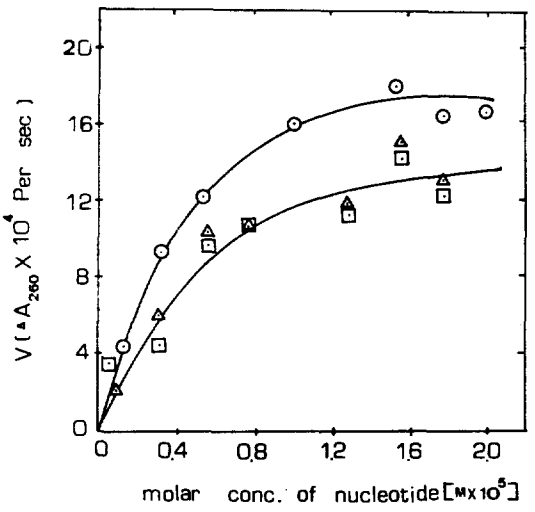

Fig. 2. Susceptibility of native and denatured DNA to DNase *vs.* DNA concentration in the absence and presence of spermine. The reactions were carried out at 20°C, pH 5.0 in a buffer of 0.03*M* $MgCl_2$, 0.005 *M* NaCl, and 0.008*M* acetate. The absorbance increase at 260nm ($\triangle A_{260}$) was 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.

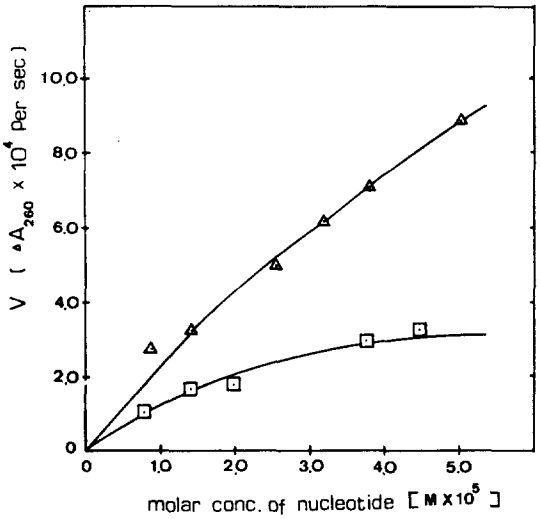

Fig. 3. Enzymatic susceptibility of denatured DNA *vs.* DNA concentration in the absence and presence of spermine. All experimental conditions were as in *Fig.* 2. △ : Denatured DNA, minus spermine. □ : Denatured DNA, plus spermine.

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 structure[9~11]. 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 molecule[12]. 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

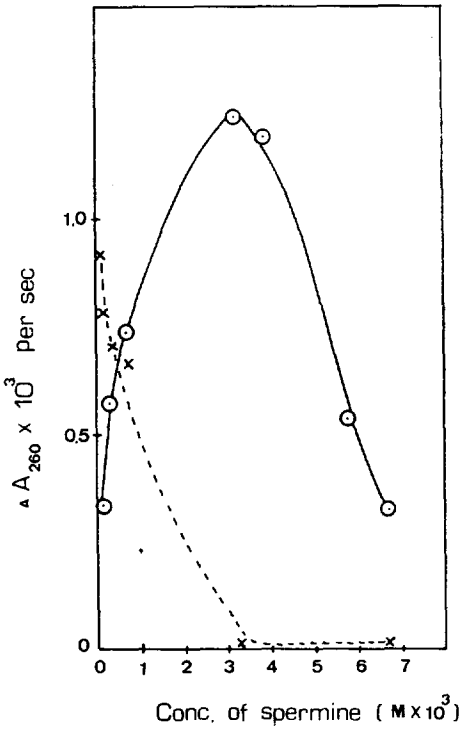

Fig. 4. Effect of spermine on the susceptibility of native and denatured DNA for DNase. The concentration of DNA was $1.2\times10^{-4}M$ of nucleotide phosphorus in each case. Experimental conditions were as in *Fig.* 2. ○ : Native DNA. : Denatured DNA.

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 pancretic ribonuclease (RNase) is depressed by spermine[13~15].

Since it is expected that spermine, which is bound with DNA, is involved in the coopera tive 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

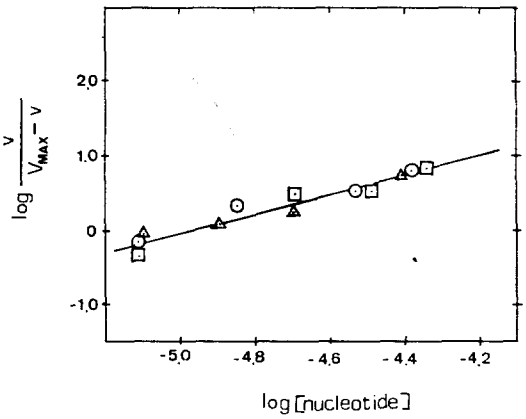

Fig. 5. Hill plots of DNA susceptibilities to DNase in the absence and presence of spermine. The plots were obtained from the experiments of *Fig.* 2 and *Fig.* 3. ○ : Native DNA, +spermine. □ : Native DNA, −spermine. △ : Denatured DNA, −spermine.

coefficient of unity. Apparently the differential effect of shermine 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 matabolism *in vivo*.

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