THE BINDING OF MAGNESIUM IONS TO DNA

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Various studies have demonstrated that divalent cations exert different effects when they interact with DNA. Eichhorn (1962) and Eichhorn and Clark (1965) reported that both Ca^{++} and Mg^{++} enhanced the stability of DNA and increased the Tm whereas Cu^{++} decreased the Tm.

Thomas (1954) and Shack (1958) observed that the UV spectrum of native DNA was unchanged in the presence of Mg^{++} and a similar observation was made by Eichhorn et al (1966), when Cu^{++} was used. It was suggested that, under these conditions, divalent ions are bound to the phosphate groups in DNA. In further studies by Katz (1952), Thomas (1954), Yamane and Davidson (1961), Hiai (1965), and Eichhorn et al (1966), it was shown that the combination of either Hg^{++} or Cu^{++} with denatured DNA led to a spectral shift of the OD_{max} to longer wave lengths. These results were interpreted as a binding of the cations to the nitrogen bases once hydrogen bonds were disrupted. In this paper, we wish to report that magnesium ions can lead to a similar effect.

In our experiments, we studied the effect of increasing concentrations of Mg^{++} on the thermal denaturation of DNA and these were compared with the effect of Cu^{++}. In agreement with Hiai (1965) and Eichhorn et al (1966), it was found that the addition of Cu^{++}, either before or after denaturation, caused an increase in OD, higher than that of denatured DNA, together with a shift in the OD_{max}. The results with Mg^{++}, which are given in Fig. 1 and Table 1, were somewhat different from those for Cu^{++}.

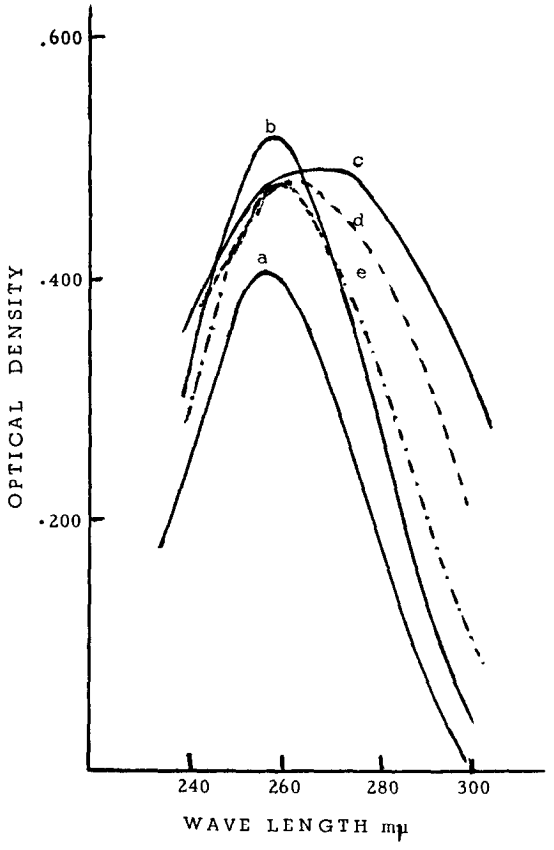

Fig. 1. Absorption spectra of DNA in the presence of Mg^{++}. a. Native DNA in 5 mM NaCl. b. DNA denatured in 5 mM NaCl. c. DNA denatured in 5 mM NaCl and .04 M $MgCl_2$. d. DNA denatured in 5 mM NaCl and .018 M $MgCl_2$. e. DNA denatured in 5 mM NaCl and .01 M $MgCl_2$.

When Mg^{++} was added, at room temperature, to calf-thymus DNA which had already been heat-denatured, the spectral curve was similar to that of the denatured DNA alone, except for a reduced absorbance. However, when Mg^{++} was added to native DNA and this was followed by denaturation, there was a spectral shift to longer wave lengths as well as a reduced absorbance. It would thus appear that Mg^{++} which is added to heat-denatured DNA binds only to the phosphate groups. However, the

Table 1. Absorption Maxima of Denatured DNA in 5 mM NaCl and with Varying Concentrations of Mg^{++}.

Conc. of Mg^{++}	Added Before Denaturation		Added After Denaturation	
	λ	OD_{max}	λ	OD_{max}
.010 M	258	.490	258	.460
.018 M	260	.489	258	.458
.030 M	263	.479	258	.457
.040 M	265	.500	258	.451

(OD of native DNA = .411 (258 mμ); OD of denatured DNA = .530 (258 mu))

combination of Mg^{++} with nitrogen bases is facilitated when DNA is denatured in the presence of Mg^{++} and hydrogen bonds between the bases are broken.

This apparent difference in the nature of the complex has also been observed with regard to sedimentation constants. In a preliminary experiment which was carried out on a sample of phage α C_3 DNA obtained from Dr. S. Aurisicchio of the International Laboratory of Genetics and Biophysics, it was observed that there was a 3-fold increase in the S_{20} values when the DNA was denatured in the presence of Mg^{++} as compared with either denatured DNA alone or DNA with Mg^{++} added after denaturation.

EXPERIMENTAL

Calf-thymus DNA was purchased from the Worthington Biochemical Corp., Freehold, New Jersey and was dissolved in 5 mM NaCl. Thermal denaturation was carried out in a boiling water bath for 10 minutes, followed by quick cooling in an ice bath. Absorbance measurements were made with a Beckman DU spectrophotometer.

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REFERENCES

Eichhorn, G.L., Nature, 194, 474 (1962).
Eichhorn, G.L. and Clark, P., Proc. Natl. Acad. Sci., 53, 586 (1965).
Eichhorn, G.L., Clark, P. and Becker, E.D., Biochemistry, 5, 245 (1966).
Hiai, S., J. Mol. Biol. 11, 672 (1965).
Katz, S., J. Amer. Chem. Soc., 73, 2238 (1952).
Shack, J., J. Biol. Chem. 233, 677 (1958).
Thomas, C. A., J. Amer. Chem. Soc., 76, 6032 (1954).
Thomas, R., Trans. Faraday Soc., 50, 304 (1954).
Venner, H. and Zimmer, C., Biopolymers, 4, 321 (1966).
Yamane, T. and Davidson, N., J. Amer. Chem. Soc., 83, 2599 (1961).