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Some Features of the Salts of Deoxyribonucleic Acid with Bivalent Metals in Relation to Deoxyribonuclease Activity

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Mg++, Mn++, and Zn++ salts of deoxyribonucleic acid (DNA) were prepared from the sodium salt of calf thymus DNA. The products were characterized by ultraviolet absorption spectra, viscosity measurements and titration curves, in addition to nitrogen, phosphorus and metal analyses. From the pH titration curves it is apparent that bivalent metals may interact with the titratable groups of DNA to produce structures similar to the chelated complexes. Pancreas deoxyribonuclease I (DNase I) showed a pH optimum at 6.0 for ZnDNA and at 6.5 for NaDNA, MgDNA and MnDNA when tested without addition of bivalent ions. Mg++ at 0.003 M concentration (a considerable excess over the amount of stoichiometrically bound metal) increased the rate of hydrolysis of MgDNA and MnDNA but was without appreciable effect on the action of DNase I when ZnDNA was used as substrate. It can be assumed that complex formation between the metal and the enzyme may also be essential for the activity of DNase I.

Previous studies on the activation of pancreas deoxyribonuclease I (DNase I) indicate a correlation between metal-binding of deoxyribonucleic acid (DNA) and its rate of hydrolysis [1–4]. It has been suggested that magnesium and similar ions may form chelate bridges between an amino group and a phosphate group of DNA [5].

Jungner [6] has obtained large molecular aggregates on precipitation of sodium thymonucleate from aqueous solution with ethanol in the presence of some bivalent ions. It has been found in this laboratory that the content of sodium in these aggregates is rather low (< 0.1 %) when the precipitation procedure has been repeated several times. Thus, the aggregates, albeit artificial products, may also be considered as salts of DNA with bivalent metals.

In order to gain some qualitative information on the degree of chelation of the metals, potentiometric titration of the aggregate compounds was undertaken. In addition, a study was made of the action of DNase I on these "metallosubstrates".

MATERIALS AND METHODS

Salts of DNA. DNA was isolated from calf thymus as the sodium salt by the method of Kay, Simmons and Dounce [7]. The product was stored as a solution in the frozen state. Analysis of two different preparations: I, N 14.0; P 8.0; N/P 3.87; η_{rel} (0.1 %) 4.4; ε(P) 7 500. II, N 12.0; P 7.0; N/P 3.79; η_{rel} (0.1 %) 4.7; ε(P) 8 350.

Mg^{++}, Mn^{++}, and Zn^{++} salts of DNA were prepared mainly by Jungner's method [6]. To 300 ml of sodium deoxyribonucleate solution containing 0.50 g of NaDNA, NaCl was added to bring the NaCl concentration up to 1 M. The nucleate was precipitated with ethanol and dissolved with stirring in 50 ml of distilled water in the refrigerator. The solution was dialyzed for 9 h against ion exchange water and 18 h against re-distilled water. The chloride of the bivalent metal in 10 ml of water was added to the concentration of approximately 0.05 M and the mixture was shaken for several minutes and placed in the refrigerator. The nucleate was precipitated with 5 volumes of ethanol, washed with ethanol and dissolved in water. The precipitation process was repeated twice.

DNase I was the once crystallized preparation D537 from the Worthington Biochemical Corp. It was found that in the cold a stock solution, containing 0.14 mg of enzyme per ml, remained stable for several days.

Spectral measurements were carried out with a Unicam spectrophotometer, model S.P. 500, 1 cm silica cells being employed. The nucleate concentration was 0.03 %.

Viscosity measurements were made at 25 ± 0.05° in an Ubbelohde viscometer with distilled water as solvent. The data were plotted as relative viscosity.

Potentiometric titration. A sample containing 20 mg of nucleate in 12 ml of water was used in the titration experiments, carried out mainly by following the directions of Gulland, Jordan and Taylor [8]. No attempt was made to maintain the ionic strength constant during the titrations. All pH measurements were made with a Beckman Glass Electrode pH Meter.

Measurements of DNase I activity. The enzyme activity was measured by Kunitz's spectrophotometric procedure [9]. One ml of enzyme solution, containing 5 μg of DNase I, and 3 ml of substrate solution (2 mg of nucleate and 5 ml of phosphate buffer in 50 ml of H_2O or 0.005 M $MgSO_4$) was kept 10—15 min at 25°, then mixed and placed in a 1-cm quartz cell with mixing. The linear change in the optical density at 260 mμ was followed during 5—10 min against a substrate-water control. The DNase activity was expressed as the increase in absorption per min.

Table 1. Composition and properties of Mg, Mn, and Zn salts of deoxyribonucleic acid.

		MgDNA	MnDNA	ZnDNA
N, %	found	13.7	12.4	12.5
	calc. *	16.3	15.6	15.4
P, %	found	8.0	7.0	7.3
	calc.	9.7	9.3	9.2
N/P,	found	3.80	3.93	3.79
	calc.	3.72	3.71	3.70
Mg, %	found	4.0		
	calc.	3.80		
Mn, %	found		7.54	
	calc.		8.19	
Zn, %	found			10.7
	calc.			9.6
Metal/2 P		1.28	1.22	1.38
$\eta_{rel.}$(0.1 % solution)		4.7	3.6	3.9
ε(P)		7 350	7 100	7 350

* Calculated for $(C_{39}H_{45}O_{24}N_{15}P_4Me_2)_x$.

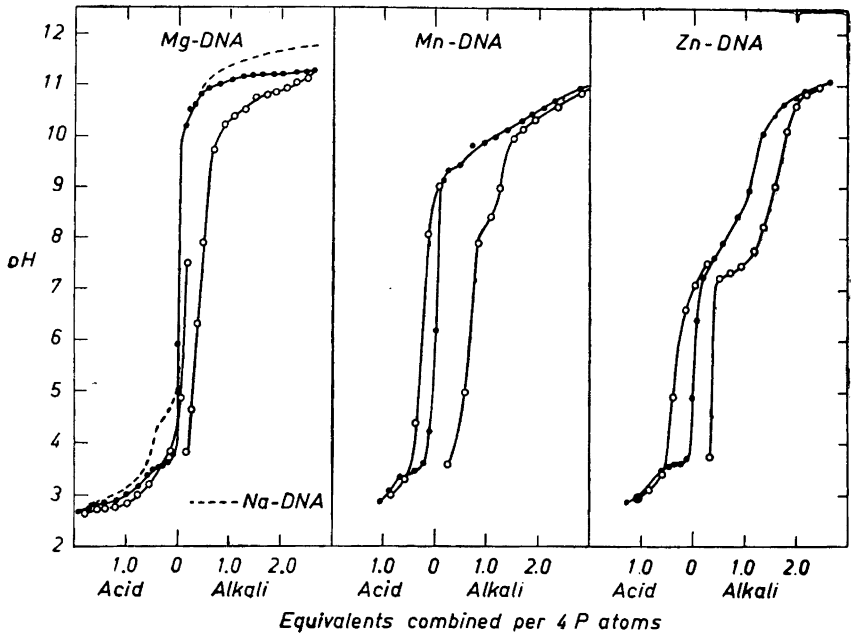

Fig. 1. Titration curves of the salts of DNA with bivalent metals. ● Forward-titration; O Back-titration.

RESULTS AND DISCUSSION

General properties of the preparations. The low nitrogen values of the preparations (Table 1) are probably due to the Kjeldahl procedure having been used. The atomic N/P ratio is in good agreement with the calculated figures. The protein content of the aggregates was not estimated, but in an earlier preparation of MgDNA, precipitated in a similar way, a protein content of about 3 % was found.

It can be seen from the analytical data that the molar ratio of metal to phosphorus is considerably greater than the theoretical value. One explanation, proposed by Jungner, is the possibility that the metal can also enter less acid radicals and not only the phosphoric acid groups. However, Shack, Jenkins and Thompsett [10] have reported data which suggest that there is no binding of metal on DNA with groups other than phosphorus. Cavalieri, Angelos and Balis [11] suggest that at least two types of sites, one with monovalent and one with divalent phosphate, may be involved. In our view, the excess of metal can also be interpreted as due to the retention of chloride ions.

A partial denaturation of the nucleate in dilute solutions during preservation and prolonged dialysis [12] may contribute to the rather high values of ε(P).

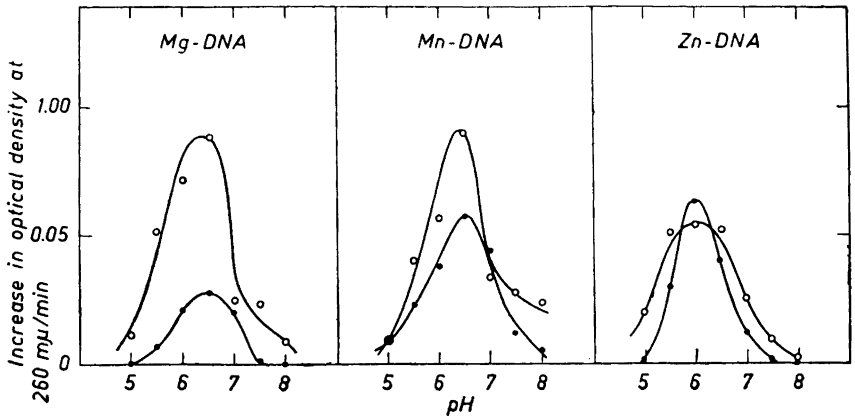

Fig. 2. Effect of pH on the rate of enzymatic digestion of salts of DNA. ● Without added Mg^{++}; ○ With 3.75×10^{-3} mmoles of Mg^{++}. Test solution: 0.03 mg DNA/ml; 5 μg DNase I; phosphate buffer; total volume 4 ml; 25°C.

Titration curves. Fig. 1 summarizes the changes in pH on forward- and back-titration of preparations. The titration curve for NaDNA is also included. All the preparations show a clear-cut pH drop in the acid range as compared with the titration curve of NaDNA. It can be concluded that some acidic protons of DNA, titrating between pH 5 and 3.5, have been displaced by the chelating bivalent metals, and that the amino groups of DNA are involved. This conforms with the views of Brown and Watson [5] who suggest the possibility that magnesium and similar ions may form chelate bridges between the amino group of guanine and a neighbouring phosphate group.

Neither in the sodium nucleate nor in the magnesium nucleate are any groups titrated in the alkaline region below pH 10. On the other hand, the titration curves of MnDNA and ZnDNA indicate that there is a very distinct tendency for Mn^{++} ions and especially for Zn^{++} ions to form chelates with the groups dissociating in the alkaline range. The different titration steps with distinct inflection points are most clearly separated in the titration curve of ZnDNA. The liberation of the titrating groups in the alkaline region begins at pH 7, and at approximately pH 7.3 the back-titration curve of ZnDNA shows a very steep bend. All this evidence indicates linkages of a different kind in ZnDNA as compared with the other preparations investigated.

Experiments with DNase I. Parallel studies (Fig. 2) were made without added Mg^{++} and with addition of 0.003 M Mg^{++}. The concentration of metal in the substrate solution without added Mg^{++} was 4.95×10^{-5} M for MgDNA, 4.12×10^{-5} M for MnDNA and 4.93×10^{-5} M for ZnDNA.

It will be noted that the optimal pH for the digestion of MgDNA and MnDNA is 6.5, as shown by Kunitz [9]. The same optimal pH was also obtained with NaDNA. With ZnDNA, however, the optimal pH for the action of DNase I is about 6.0. When no Mg^{++} is added to the digestion mixture, the reaction at the optimal pH range is most rapid with ZnDNA as substrate.

On the other hand, addition of Mg^{++} at 0.003 M concentration (a considerable excess over the amount of stoichiometrically bound metal in the substrate) increases the rate of hydrolysis of MgDNA and MnDNA but has no activating effect on the action of DNase I with ZnDNA at pH 6.0.

The activating effect of added Zn^{++} was not studied at the pH optimum of the DNase because free zinc ions cause precipitation of phosphate above pH 5.5. In an experiment made at pH 5.0 and with NaDNA as substrate, 0.003 M $ZnSO_4$ accelerated the reaction rate to approximately 200 % of that with 0.003 M $MgSO_4$ activation.

Our results are not in agreement with the observation of Gilbert, Overland, and Webb [13] that 0.01 M of zinc sulphate completely inhibited the action of deoxyribonuclease. These workers followed the course of the hydrolysis by measuring the increase in acid-soluble phosphorus. It seems possible that the increase in ultraviolet absorption, catalyzed by DNase I during the initial stage of the degradation, and the cleavage of the phosphate ester bonds are not simultaneous changes.

The formation of complexes of DNA with Mg^{++}, Ca^{++}, and Mn^{++} and the respective dissociation constants have been reported in the work of Wiberg and Neuman [3]. In a recent paper [4], Wiberg has studied the mechanism of metal activation of DNase I. He found that Ca^{++} is a potent synergist in the Mg-activated reaction.

The data reported here and the evidence presented by Wiberg indicate that the mechanism of the metal activation of DNase I cannot be explained satisfactorily by the concept of metallosubstrate alone.

DNase I is found to excercise its catalytic power within the pH range where no substrate groups are dissociated and where the hydrogen bonds of the Watson-Crick double-helical model are intact. The pH of optimum stability of the enzyme — from pH 5 to 6 — lies within the same pH range. Since the isoelectric point of DNase is in the region of pH 4.7 to 5.0 [9], the active form of the enzyme has a negative charge. It can be assumed that complex formation between the metal and the enzyme may also be essential for the activity of DNase I.

The chelation of the substrate with the bivalent metals may alter the substrate either in such a way that the enzyme system can function, as postulated by Weissman and Fisher [1], or also, we would add, in such a way that the combination of the metallosubstrate with the metalloenzyme is no longer possible. In this way the observations, to some extent contradictory, regarding the effect of the metals and the observed synergistic or antagonistic behaviour of the pairs of metals could be satisfactorily explained.

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