COMPARISON OF THE ALKALINE PHOSPHATASES OF E. COLI AND RENAL TISSUE

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The isolation of a phosphoesterase from a mutant of E. coli (Garen and Levinthal, 1960) has raised the question of a possible relationship of this enzyme to the alkaline phosphatases of mammalian tissue; the application of the term "alkaline phosphatase" to the bacterial phosphoesterase would give an impression that some relationship does exist. Accordingly a comparison of the bacterial enzyme with the purified renal alkaline phosphatase (Binkley, 1961) was undertaken.

Since the bacterial phosphoesterase as purchased (Worthington Biochemical Corporation) was supplied as a suspension in ammonium sulfate, the comparison with the renal enzyme was made on the basis of absorbancy at 280 millimicrons and the results are presented in terms of Roche units (Roche and Bouchilloux, 1950) per unit absorbancy. Since the renal enzyme has an unusually high absorbancy at 280 millimicrons, the comparison of activity on this basis should greatly favor the bacterial enzyme. From the results given in Table I, it is apparent that there are many differences.

Table I

Activity in relationship to pH and metal ion

Assay as described by Binkley (1961) with beta glycerophosphate and with appropriate Tris or ethanolamine buffers. Activity is expressed as Roche units per unit absorbancy at 280 millimicrons.

	Bacterial phosphatase		Renal phosphatase	
pH	No $Mg^{\#}$	0.001 M $Mg^{\#}$	No $Mg^{\#}$	0.001 $Mg^{\#}$
7	480	480	0	0
8	750	750	100	900
9	850	850	200	49,000
10	510	510	200	97,000*

* From the extinction of enolase, one may calculate a protein content of about 1.1 mg or a nitrogen content of about 0.2 mg thus indicating a specific activity of about 500,000 whereas, on a determined total nitrogen basis, the specific activity was about 200,000.

The bacterial enzyme was more than half maximally active at pH 7 whereas the renal enzyme was without activity until above pH 8. The addition of magnesium ion was without effect on the bacterial enzyme whereas the renal enzyme was entirely dependent upon magnesium ion for activity. Furthermore, the renal enzyme was at least two orders of magnitude more active than the bacterial enzyme. This particular preparation, when freshly prepared, had a specific activity of about 400,000 Roche units per mg total N but had lost about half activity during storage.

One of the characteristics of the renal enzyme is the complete resistance to proteolysis. As illustrated in Table II, the bacterial enzyme was destroyed by digestion with Panprotease (Worthington) whereas the renal enzyme was resistant

Table II

Resistance to proteolysis

An amount of each enzyme representing 0.01 absorbancy units per ml was digested with 1 mg Panprotease per ml at pH 8 for 3 and 24 hrs and the digests were assayed with beta glycerophosphate at pH 9; results are expressed as percentage of the activity of the unincubated enzymes.

	Bacterial		Renal	
Time	Digested	Control	Digested	Control
3 hr	21	100	103	100
24 hr	0	81	109	100

Several phosphoesterases have been suggested as reagents for the study of the structure of polynucleotides; the bacterial and renal enzymes were compared in the removal of phosphate from RNA (dialyzed sodium nucleate, Schwarz Bio-Research, Inc.) and RNA "cores" (Worthington). The results given in Table III are expressed in terms of indicated chain length. It was found that the bacterial enzyme was much less active in the removal of phosphate from polynucleotides; almost 1000-fold amounts of bacterial enzyme were required to achieve a rate of hydrolysis similar to that of the renal enzyme. However, with prolonged hydrolysis with the bacterial enzyme, similar chain lengths are indicated. It is possible that both preparations contain diesterase activity, but according to the assay of Hilmoe (1961), the purified renal enzyme was free of diesterase activ-

Table III

Phosphatase action on polynucleotides

The bacterial enzyme and the renal enzyme were at a level of 0.02 and 0.00002 absorbancy units per ml respectively with RNA and "core" representing 315 and 55 micrograms P respectively per ml digest. Results are expressed as apparent chainlength.

	Bacterial		Renal	
Time	RNA	"Core"	RNA	"Core"
Min	Chain length		Chain length	
30	156	11.4	48	10.2
60	132	10.6	41	9.1
120	87	10.1	39	8.9
360	47	8.9	39	8.8

ity. Crude preparations of the renal enzyme contain significant amounts of diesterase activity that may be removed by the procedure of Sevag et al (1938) and by filtration with Sephadex G-50; the diesterase activity passes through the Sephadex and the phosphatase is retarded. In any event, the two preparations appear to be of equal value in the study of polynucleotides; the much greater activity of the renal preparation may be of some advantage.

These results illustrate that there is little in common between the two preparations insofar as pH-activity relationships, metal activation, order of activity, and resistance to proteolysis are concerned. It is unfortunate that the term "alkaline phosphatase" has been applied to the bacterial preparation; it does appear possible, however, that the bacterial enzyme may operate as a phosphatase, but it is rather unlikely that the renal preparation will be found to operate as a phosphatase under in vivo conditions.

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