LXXIII. STUDIES ON BACTERIAL PHOSPHATASES

III. THE PHOSPHATASES OF *AEROBACTER AEROGENES*, *ALCALIGENES FAECALIS* AND *BACILLUS SUBTILIS*

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PHOSPHATASES from different tissues show marked differences in their *p*H-activity relationships, and smaller differences in other properties. In some respects such as the effects of various ions, especially magnesium, all phosphatase preparations act similarly, though to a varying extent. While it would be convenient to believe that the mode of action of all orthophosphatases is the same, yet such a hypothesis must await a large accumulation of data. The interest attaching to specificity prompted an extension of the work on bacterial phosphatases already reported [Pett & Wynne, 1933].

This paper indicates the results obtained with three other bacterial species, *Aerobacter aerogenes*, *Alcaligenes faecalis* and *Bacillus subtilis*, in none of which has this enzyme been reported. These species have a special interest. The previous paper reported phosphatases in a facultative anaerobic, spore-forming, acid- and gas-producing rod (*Clostridium acetobutylicum*), and a non-spore-forming organism (*Propionibacterium jensenii*). This paper uses *Aerobacter aerogenes* as an acid- and gas-producing, spore-forming rod of aerobic type, in contrast to *Clostridium*. *Alcaligenes faecalis* was included because it does not produce acid or gas, and it is doubtful if it attacks carbohydrates. The *B. subtilis* was used because two distinctly different strains were available and it is valuable to see how the phosphatases differ. The *B. subtilis* A is the Marburg strain, and *B. subtilis* B the Michigan strain [Soule, 1932], which have been discussed by the American Society of Bacteriologists in the definition of a type-species of aerobic spore-formers (Genus *Bacillus*).

Materials and methods

Conditions were the same as those described by Pett & Wynne [1933] with the following variations. The reaction volume was only 5 ml. instead of 10, and the dried organisms were used at the rate of 0·03 g. per 5 ml. instead of 0·1 g. per 10 ml. The results have been shown to be comparable, however. In the acid range 0·025 *M* phthalate buffers were used; in the alkaline range either borate or

glycine buffers were used, no difference being observed in the amount of hydrolysis at a given *p*H. In the previous work 1 hr. hydrolysis, which is still on the linear part of the curve, was taken for comparison, but in the present work the activity was such that the curve is falling off slightly, so comparison is made on ½ hr. readings. No toluene of chloroform was used for this short period of digestion.

Aerobacter aerogenes was grown at 37° in 3 % glucose and 1 % peptone for 2 days. Longer times yielded no more organisms.

Alcaligenes faecalis was grown at 37° for 4 days in 3 % peptone. No increase in yield was obtained by using glucose, or by longer periods.

B. subtilis A (the Marburg strain) was grown at 37° for 4–5 days in 4 % glucose and 1 % peptone. It formed a thick wrinkly pellicle, and the liquid is not turbid if this is left undisturbed. A better yield is obtained if this is shaken down daily.

B. subtilis B (the Michigan strain) was grown at 37° for 6–7 days in a medium containing 1 % beef extract and 4 % glucose. The use of beef extract greatly increased the yield over bactopeptone.

Some properties of the enzymes

(1) p*H-activity.*

Table I shows the *p*H optima of the phosphatases studied in this paper, together with those for most other reported phosphatases, to facilitate comparison. These *p*H optima have been first reported or confirmed by the authors [*v.* Pett & Wynne, 1934].

Table I. *The optimum* p*H for phosphatases from different sources acting on three substrates*

Source of enzyme	Optimum *p*H on substrate indicated		
	Glycerophosphate	Hexosephosphate	Pyrophosphate
Aerobacter aerogenes	5·6	5·6	6·2
B. subtilis A	10·7	6·6	6·6
B. subtilis B	6·5	6·0	6·2
Alcaligenes faecalis	8·7	8·5	7·6
Cl. acetobutylicum	5·1	6·0	7·0
Prop. jensenii	7·0	6·0	7·0
Sacc. cerevisiae	4·0	6·5	—
Takadiastase	4·0	4·2	—
Mammalian tissues	8·9	8·9	—
Erythrocytes	6·0	6·6	—
Soya bean	5·3	—	—
White bean	5·8	—	—

It must be remembered for any enzyme that small differences in the optimum *p*H are observed from one preparation to another. These are usually of the order of 0·2 *p*H. Furthermore the relation of activity to *p*H is best expressed by a curve with a more or less broad peak. Curves have been established for every enzyme reported in Table I, and the actual peaks must be considered as spread around the figures given by about ±0·2 *p*H, followed by a rapid falling away on each side.

(2) *Effects of various ions.*

Previous work of the authors showed that phosphatases of two species of bacteria, in common with mammalian phosphatases, are stimulated by magnesium, inhibited by free phosphate, and respond to other ions in various ways. Similar studies have been carried out in the present case. Several factors must be

considered in such experiments where substances are added to enzyme digests—and much work on enzymes has not recognized these factors. It must be emphasized that the addition of any substance to an enzyme digest is liable to alter the *p*H, even in the presence of buffers. In the present work all solutions were adjusted to the desired *p*H before mixing and the *p*H after hydrolysis was determined. A variation of 0·3 unit discredited the experiment. The phosphate estimations were made with Fiske & Subbarow's method [1925] which is less sensitive to salts in solution than methods employing different reducers. In the present experiment it must also be admitted that in the alkaline range magnesium tends to precipitate and its exact concentration is doubtful.

The results with various ions may be summarized as follows. Magnesium, at some concentration, accelerated the rate of hydrolysis in every instance except with *B. subtilis* B. A very broad curve is the usual sign of this effect. As with the organisms previously studied, zinc, in low concentration, stimulated activity on pyrophosphate, except with *aerogenes*. Fluoride, in some concentration, was strongly inhibitory to glycerophosphatase, fairly inhibitory to pryophosphatase, and slightly so to hexosephosphatase, except with *aerogenes*. In this respect *aerogenes* resembles the organisms previously studied. Cyanide and calcium were without effect except in high concentrations ($M/10$). Phosphate was, as theory demands, inhibitory.

Discussion

This work is of interest in three ways: (1) the specificity of phosphatases, (2) the presence of a phosphatase in an organism (*Alcaligenes faecalis*) containing no glycolytic mechanism and (3) the marked differences between the two strains of *B. subtilis*.

Regarding specificity no conclusions can be drawn. Consistent differences in *p*H-optima both among bacterial phosphatases and these compared with other sources of enzyme form a striking argument for some kind of substrate and source specificity. Broadly speaking there is a similarity in the effects of various ions, especially magnesium, among all bacterial phosphatases. The differences observed may be due to the crudity of the preparations, though this is not necessarily the case.

Significant differences were found between the phosphatases of the two strains of *B. subtilis* (Marburg & Michigan). Not only are reactions to magnesium and the *p*H-optima distinctly different, but also the degree of inhibition by fluoride is much greater with the B (Michigan) strain, a fact possibly related to their different fermentation reactions. Undoubtedly biochemical differences of this sort will prove more and more valuable in differentiating bacterial species as they become worked out.

Summary

1. As an extension of previous work the phosphatase systems of *Aerobacter aerogenes*, *Alcaligenes faecalis*, and two strains of *Bacillus subtilis* (Marburg & Michigan) have been studied. Activity-*p*H curves on various substrates and the effects of magnesium, phosphate and other ions on the rate of hydrolysis were determined.

2. The significance of the observations has been discussed in relation to (1) the specificity of phosphatases, and (2) the difference between the two strains of *B. subtilis*.

3. It is noteworthy that *Alcaligenes faecalis* contains an active phosphatase since it lacks a glycolytic system.

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