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PROPERTIES OF AN ACID PHOSPHATASE IN *ESCHERICHIA COLI*

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SUMMARY

1. Some properties of an acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) in *Escherichia coli*, strain K-12, are described.

2. The pH optimum of the acid phosphatase lies at pH 2.5 in an assay medium without added KCl. Addition of 140 mM KCl to the medium changes the shape of the pH–activity curve without shifting the pH optimum. The activity is increased between pH 3.1 and 6.2, maximally at pH 3.6.

3. Addition of chlorides of a variety of monovalent cations increases the phosphatase activity at pH 3.6 by the same amount, indicating that the increased activity is due to Cl^-.

4. Cl^-, Br^-, I^- and NO_3^-, whether added as potassium or sodium salts in 140 mM final concentration, have about the same stimulating effect on the phosphatase activity at pH 3.6.

5. Of twelve phosphate esters tested, fructose 1,6-diphosphate and 2,3-diphosphoglyceric acid, next to *p*-nitrophenylphosphate, give the highest activities at pH 2.5. Enhancement of the activity at pH 3.6 by addition of KCl was only observed with *p*-nitrophenylphosphate and acetylphosphate as substrates.

6. Several differences between this acid phosphatase and the (Na^+–K^+)-ATPase (ATP phosphohydrolase, EC 3.6.1.3) in *Escherichia coli* are described and lead to the conclusion that the acid phosphatase has no relation with the (Na^+–K^+)-ATPase.

INTRODUCTION

It is now generally assumed that the active transport of Na^+ and K^+ across the cell membrane is closely related to a (Na^+–K^+)-activated ATPase (ATP phosphohydrolase, EC 3.6.1.3). The mechanism of this enzymic reaction has been studied in several laboratories and is thought to involve a Mg^{2+}- and Na^+-stimulated phosphorylation, followed by a K^+-dependent dephosphorylation, which can be inhibited by ouabain[1–6].

In many animal tissues the presence of a K^+-dependent phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) with optimal activity at neutral

pH, which can also be inhibited by ouabain, has been demonstrated. Several investigators[7–11] have concluded that this phosphatase activity appears to be related to the K^+-dependent dephosphorylation step of the (Na^+–K^+)-ATPase system.

Recently HAFKENSCHEID AND BONTING[12] demonstrated the occurrence of a (Na^+–K^+)-ATPase in *Escherichia coli*. Therefore an effort was made to detect in *E. coli* a K^+-dependent phosphatase activity at neutral pH, but without success. Instead, however, an acid phosphatase activity was observed, which has a pH optimum 2.5 and is stimulated by KCl between pH 3.1 and 6.2, maximally at pH 3.6.

The present paper describes various properties of this acid phosphatase activity in *E. coli* in comparison to those of the (Na^+–K^+)-ATPase and the accompanying Mg^{2+}-ATPase in this organism, as well as to those of the neutral K^+-dependent phosphatase of many animal tissues.

MATERIALS AND METHODS

E. coli strain K-12, kindly supplied by Professor T. O. WIKÉN, Laboratory of Microbiology, Institute of Technology at Delft, The Netherlands, was cultivated and harvested as described previously[12]. All homogenates were prepared by homogenization of the freeze-dried bacteria in twice-distilled water. The final homogenate concentration was 0.025 mg dry weight per ml incubation medium.

The acid phosphatase assay was carried out in 1.0 ml of a medium containing 7.5 mM *p*-nitrophenylphosphate and 85 mM glycine–HCl at pH 2.5. After incubation at 37° for 1 h the reaction was stopped by adding 2 ml of 0.1 M NaOH, the precipitate removed by centrifugation and the absorbance of the supernatant measured at 410 mμ.

The pH–activity curve over the range 1.5–6.5 was obtained by using glycine–HCl (1.5–3.0), succinic acid–Tris (3.5–5.5) and histidine–HCl (6.0–6.5). The influence of different ions on the phosphatase activity at pH 3.6 was tested by adding various salts in 140 mM final concentration to the incubation medium. The substrate specificity was measured by replacing *p*-nitrophenylphosphate by each of various phosphate esters. After the incubation, the reaction was stopped by adding trichloroacetic acid and the released P_i was measured according to the method of BONTING, SIMON AND HAWKINS[13]. For acetylphosphate hydrolysis the hydroxamate ester method of LIPMANN AND TUTTLE[14] was applied. (Na^+–K^+)-ATPase and Mg^{2+}-ATPase activities were measured according to the method by HAFKENSCHEID AND BONTING[12]. Localization of the acid phosphatase, (Na^+–K^+)-ATPase and Mg^{2+}-ATPase activities was achieved by employing the osmotic shock procedure described by DVORAK, BROCKMAN AND HEPPEL[15]. The 'shock fluid' was concentrated by lyophilization. The different enzyme activities were determined in untreated bacteria, in the shock fluid and in the residue obtained after osmotic shock. The protein content was measured according to the method of LOWRY *et al.*[16].

RESULTS

Properties of the acid phosphatase activity

The enzymic reaction was linearly dependent on the amount of freeze-dried bacteria added. There was also a linear relationship between substrate hydrolyzed and time of incubation for 60 min. Equimolar amounts of *p*-nitrophenol and P_i were

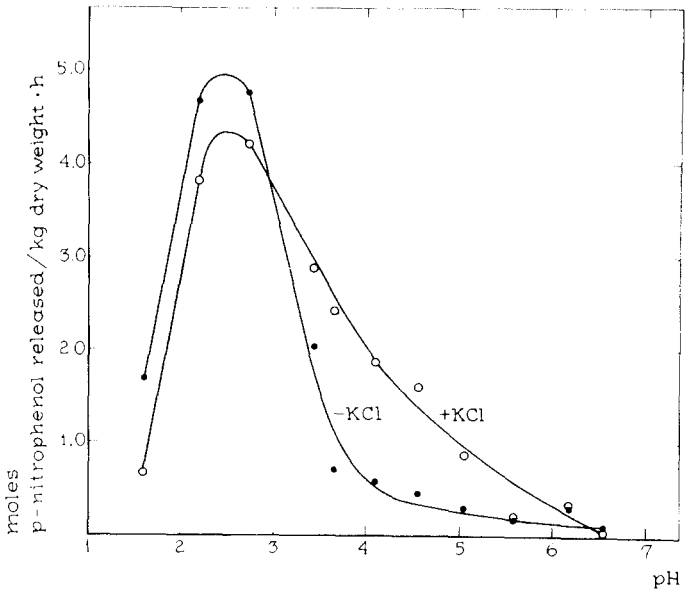


Fig. 1. Effect of pH on acid phosphatase activity of *E. coli* in the absence or presence of KCl (140 mM final concn.).

released by the enzyme system. Mg^{2+} had no effect on this phosphatase activity, as was previously demonstrated in *E. coli* by VON HOFSTEN AND PORATH[17].

The pH–activity curve is reproduced in Fig. 1. In the absence of added salts the phosphatase activity had a maximum at pH 2.5. ROGERS AND REITHEL[18] also observed an acid phosphatase with the same low pH optimum in *E. coli*. Beside acid phosphatases with a pH optimum between 4.3 and 6.0, they described an acid phosphatase with a pH optimum at 2.6–2.8 with fructose 1,6-diphosphate as substrate. The acid phosphatase here described gave with fructose 1,6-diphosphate the same low pH optimum.

When *E. coli* strain K-12 used in all our experiments was grown on the media described by VON HOFSTEN AND PORATH[17], by DVORAK, BROCKMAN AND HEPPEL[15] and by ROGERS AND REITHEL[18], acid phosphatases could be demonstrated with the pH optima of respectively 6.0, 3.0 and 5.5 and 3.5 and 6.0. Only with the growth medium described in this paper, the acid phosphatase with a pH optimum at pH 2.5 could be obtained.

When KCl was added to the incubation medium in 140 mM final concentration the pH optimum remained at pH 2.5, but the phosphatase activity decreased by about 12% at this pH, while the activity between pH 3.1 and 6.2 increased (Fig. 1). Because the increase of activity upon addition of KCl was greatest at pH 3.6, it was thought that a K^+-dependent phosphatase was present at this pH and should be studied for a possible relationship to the (Na^+–K^+)-ATPase system present in *E. coli*.

Table I shows the effect of different monovalent cations on the enzymic activity at this pH. It is clear that all chlorides, including choline chloride, gave about the same increase of the phosphatase activity. Replacement of KCl by NaCl, in such a manner that the total concentration of the ions added is constant, did not change

TABLE I

EFFECT OF DIFFERENT MONOVALENT CATIONS ON ACID PHOSPHATASE ACTIVITY OF *E. coli* AT pH 3.6

The cations were added as chlorides, final concn. 140 mM.

Cation added	*Activity (moles p-nitrophenylphosphate hydrolyzed per kg dry wt. per h)*
—	0.63
Li^+	2.60
Na^+	2.70
K^+	2.67
Rb^+	2.43
Cs^+	2.70
NH_4^+	2.54
Choline	2.40

the phosphatase activity. Therefore, we concluded that this increase is caused by the added anion.

Table II shows that the potassium and sodium salts for each anion gave about the same effect, while the effects of different anions differed markedly. In both series I^- causes the highest increase, while SO_4^{2-} inhibited 70% of the normal phosphatase activity at pH 3.6. For the acid phosphatase activity of *Tetrahymena pyriformis*, LAZARUS AND SCHERBAUM[19] reported that all ions except cysteine, citrate and EDTA brought about varying degrees of inhibition, with SO_4^{2-} (50 mM) giving an inhibition of 50%.

Ouabain in 10^{-4} M final concentration had no influence on the increase of the phosphatase activity by the addition of KCl: 1.91 ± 0.12 moles *p*-nitrophenylphosphate hydrolyzed per kg dry wt. per h with ouabain and 1.87 ± 0.07 moles *p*-nitrophenylphosphate hydrolyzed per kg dry wt. per h without ouabain ($P > 0.1$).

The substrate specificity of the acid phosphatase measured at pH 2.5 is shown in Table III. Among the various phosphate esters, fructose 1,6-diphosphate and 2,3-

TABLE II

EFFECT OF VARIOUS ANIONS ON ACID PHOSPHATASE ACTIVITY OF *E. coli* AT pH 3.6

Anion added (140 mM final concn.)	*Activity (moles p-nitrophenylphosphate hydrolyzed per kg dry wt. per h)*	
	Sodium salt	*Potassium salt*
No salt added	0.63	0.63
Cl^-	2.76	2.70
Br^-	3.15	3.66
I^-	4.59	5.25
NO_3^-	4.05	3.90
SO_4^{2-}	0.19	0.18

TABLE III

SUBSTRATE SPECIFICITY OF THE ACID PHOSPHATASE ACTIVITY OF *E. coli* AT pH 2.5 AND OF THE ACTIVATION BY KCl AT pH 3.6

Activity is expressed in moles of substrate hydrolyzed per kg dry wt. per h.

Substrate	*Activity at pH 2.5*	*Increase of activity at pH 3.6 by addition of 140 mM KCl*
p-Nitrophenylphosphate	3.92	1.97
Glucose 1-phosphate	0.33	0.01
Glucose 6-phosphate	0.16	0
Fructose 1-phosphate	0.09	—
Fructose 1,6-diphosphate	1.84	0
3-Phosphoglyceric acid	0.17	—
2,3-Diphosphoglyceric acid	3.79	0*
Phosphoenolpyruvate	0.32	—
Adenosine triphosphate	0.39	0.01
Adenosine diphosphate	0.20	0.05
Adenosine monophosphate	0.02	0.01
Acetylphosphate	1.14	0.87

* A decrease of 63% was observed.

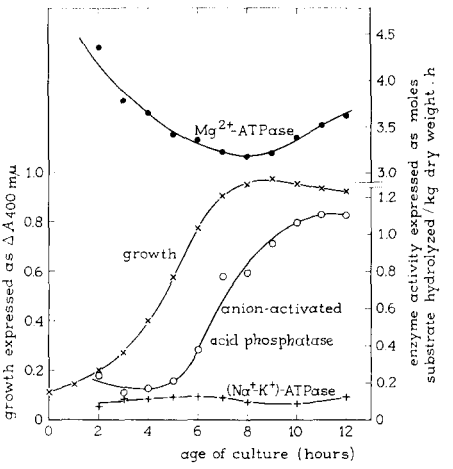
diphosphoglyceric acid were the best substrates. Glucose 1-phosphate and glucose 6-phosphate were rather slowly hydrolyzed by this acid phosphatase, in contrast to the findings of ROGERS AND REITHEL[18]. At pH 3.6 only the phosphatase activity with *p*-nitrophenylphosphate and acetylphosphate as substrates could be increased by the addition of 140 mM KCl. The other substrates tested were ineffective.

Differences in properties of the acid phosphatase and $(Na^+–K^+)$-ATPase and Mg^{2+}-ATPase

Because in many tissues there is a close relationship between a K^+-dependent phosphatase activity at neutral pH and the $(Na^+–K^+)$-ATPase, it was important to compare the properties of the acid phosphatase with those of a $(Na^+–K^+)$-ATPase and of a Mg^{2+}-ATPase present in *E. coli*.

Fig. 2 demonstrates a marked difference between the three enzyme activities. Both the $(Na^+–K^+)$-ATPase and Mg^{2+}-ATPase were nearly constant during the entire growth period from lag phase to stationary phase, while the enhancement of the acid phosphatase activity by addition of KCl increased, reaching its highest value in the stationary phase. The same applied for the acid phosphatase activity at pH 2.5. Therefore, the latter enzyme appears to be an induced enzyme, while the $(Na^+–K^+)$-ATPase and Mg^{2+}-ATPase are constitutive ones. This is in contrast with the results obtained by VON HOFSTEN[20], who found a decreased acid phosphatase activity during the exponential phase in sonicated cells of *E. coli* grown on a medium containing glucose.

The acid phosphatase at pH 2.5 has a K_m value of $1.8 \cdot 10^{-3}$ M for *p*-nitrophenylphosphate as substrate, which agrees with the value of $1.3 \cdot 10^{-3}$ M obtained by ROGERS AND REITHEL[18]. ATP was a competitive inhibitor for the acid phosphatase



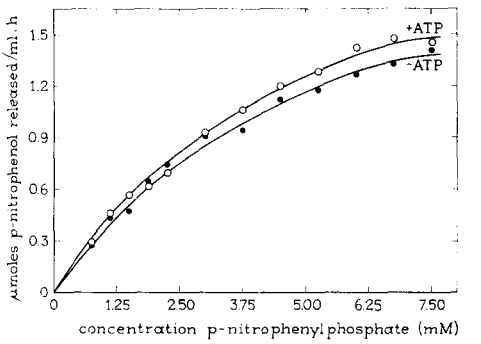


Fig. 2. $(Na^+–K^+)$-ATPase, Mg^{2+}-ATPase and enhancement of the acid phosphatase activity at pH 3.6 by the addition of KCl during growth of *E. coli*.

Fig. 3. Rate of *p*-nitrophenylphosphate hydrolysis in the presence of 140 mM KCl and the concn. of *p*-nitrophenylphosphate at pH 3.6 in the absence or presence of 0.3 mM ATP.

at pH 2.5. In contrast ATP had no influence on the enhancement of the acid phosphatase activity at pH 3.6 by the addition of KCl, as is shown in Fig. 3. Also *p*-nitrophenylphosphate had no influence on the $(Na^+–K^+)$-ATPase activity at pH 7.5 and on the Mg^{2+}-ATPase activity at pH 8.7.

By employing the osmotic shock method of DVORAK, BROCKMAN AND HEPPEL[15] for a selective release of enzymes from *E. coli*, it was possible to obtain some information about the localization of the different enzymes. The results in Table IV show that treatment of *E. coli* with a hypertonic solution of 20% sucrose, containing 0.033 M Tris–HCl (pH 7.3) and $2 \cdot 10^{-3}$ M EDTA, released the acid phosphatase, while the $(Na^+–K^+)$-ATPase and Mg^{2+}-ATPase were retained by the cells. By this treatment the acid phosphatase is enriched about 6 times. Also the increase of the acid phosphatase at pH 3.6 by the addition of KCl is released. NEU AND HEPPEL[21] had evidence that an ATPase was set free by osmotic shock, but this was not apparent in our experiments.

TABLE IV

ENZYME DISTRIBUTION AFTER OSMOTIC SHOCK TREATMENT

Activities are expressed in moles of substrate hydrolyzed per kg protein per h.

	Untreated bacteria	*Treated bacteria*	*Shock fluid*
Acid phosphatase activity at pH 2.5	7.4	2.5	40.9
Increase of acid phosphatase activity at pH 3.6 by addition of 140 mM KCl	2.7	1.0	36.0
Mg^{2+}-ATPase activity	3.4	3.9	1.1
$(Na^+–K^+)$-ATPase activity	0.2	0.1	0.1

DISCUSSION

In *E. coli* two phosphatases with different pH optima have been demonstrated by various investigators. Alkaline phosphatase with a pH optimum between 9.3 and 9.5 has only been observed in cells, which were growing in a medium where P_i becomes limiting [22,23].In our experiments with *E. coli* growing on a medium containing 64 mM P_i, no alkaline phosphatase could be demonstrated. A phosphatase activity at neutral pH, activated by K^+ and inhibited by ouabain, could not be detected either; only an acid phosphatase has been observed.

A number of acid phosphatases in *E. coli* with pH optima ranging from 2.6 to 6.0 has been described[15,17,18,20,21]. In our experiments an acid phosphatase with pH optimum 2.5 was demonstrated. Only with the growth conditions used in this paper, could this acid phosphatase in *E. coli* be demonstrated. Apparently the growth conditions determine the type of acid phosphatase in *E. coli*.

The acid phosphatase at pH 2.5 resembles in pH optimum and K_m value one of the acid phosphatases obtained by ROGERS AND REITHEL[18], although there are some differences in substrate specificity. Similarly to the hexose phosphatase and the non-specific acid phosphatase[15], this acid phosphatase could be released by osmotic shock from cells in the late stationary phase.

While VON HOFSTEN[20] observed a decrease in acid phosphatase activity of cells grown in a glucose-containing medium during the exponential growth phase, we observed a steady increase in this activity. VON HOFSTEN[20] has pointed out that glucose has a repressive effect on the synthesis of acid phosphatase in *E. coli*, while succinate or glycerol supported the synthesis of high levels of acid phosphatase. During the growth of the bacteria, the pH of our growth medium fell from 7.0 to 5.2. Therefore, it is possible that during the exponential phase, the synthesis of the acid phosphatase increased as an adaptation of the cells to the increase of the level of organic acids in the growth medium.

This acid phosphatase is unlike the neutral K^+-dependent phosphatase, which is a part of the (Na^+–K^+)-ATPase in many animal tissues[7–11], in the following respects: (1) The pH optima of the acid phosphatase and the (Na^+–K^+)-ATPase differ greatly. (2) Mg^{2+} is not essential for the acid phosphatase, while the (Na^+–K^+)-ATPase requires Mg^{2+} for activity. (3) The activating effect of KCl on the acid phosphatase is due to the anion rather than to the cation. (4) Replacement of K^+ by Na^+ does not inhibit the acid phosphatase activity. (5) The enhancement of the acid phosphatase activity by addition of KCl is not inhibited by ouabain. (6) The subcellular distribution of the K^+-dependent phosphatase and the (Na^+–K^+)-ATPase are quite similar[8]. However in *E. coli* the acid phosphatase can be released by osmotic shock, while the (Na^+–K^+)-ATPase is retained by the cells. (7) ATP does not inhibit the enhancement of the acid phosphatase by the addition of KCl nor does *p*-nitrophenylphosphate have any influence on the (Na^+–K^+)-ATPase of *E. coli*.

It is concluded therefore, that the acid phosphatase in *E. coli* cannot be related to the (Na^+–K^+)-ATPase system of this microorganism.

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