[2] L. J. REED, *Advances in Enzymol.*, 18 (1957) 319.
[3] D. R. SANADI, M. LANGLEY AND F. WHITE, *J. Biol. Chem.*, 234 (1959) 183.
[4] D. R. SANADI, J. W. LITTLEFIELD AND R. M. BOCK, *J. Biol. Chem.*, 197 (1952) 851.
[5] S. KORKES, A. DEL CAMPILLO, I. C. GUNSALUS AND S. OCHOA, *J. Biol. Chem.*, 193 (1951) 721.
[6] L. P. HAGER AND I. C. GUNSALUS, *J. Am. Chem. Soc.*, 75 (1953) 5767.
[7] M KOIKE AND L. J. REED, *J. Am. Chem. Soc.*, 81 (1959) 505.
[8] D. R. SANADI, M. LANGLEY AND R. L. SEARLS, *J. Biol. Chem.*, 234 (1959) 178.
[9] V. MASSEY, *Biochim. Biophys. Acta*, 30 (1958) 205.
[10] V. MASSEY, *Biochim. Biophys. Acta*, 37 (1960) 314.
[11] C. A. PRICE, *Arch. Biochem. Biophys.*, 47 (1953) 314.
[12] I. C. GUNSALUS AND W. E. RAZZELL, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. III, Academic Press, 1957, p. 941.
[13] H. B. BURCH, O. A. BESSEY, R. H. LOVE AND O. H. LOWRY, *J. Biol. Chem.*, 198 (1952) 477.
[14] G. WEBER AND F. W. J. TEALE, *Trans. Faraday Soc.*, 54 (1958) 640.
[15] N. SAVAGE, *Biochem. J.*, 67 (1957) 146.
[16] V. MASSEY, *Biochim. Biophys. Acta*, 32 (1959) 286.
[17] F. B. STRAUB, *Biochem. J.*, 33 (1939) 787.
[18] H. S. CORRAN, D. E. GREEN AND F. B. STRAUB, *Biochem. J.*, 33 (1939) 793.
[19] F. LIPMANN, *Cold Spring Harbor Symposia Quant. Biol.*, 7 (1939) 248.
[20] V. MASSEY, *Biochim. Biophys. Acta*, 34 (1959) 255.

Biochim. Biophys. Acta, 38 (1960) 447–460

INFLUENCE OF INORGANIC PHOSPHATE IN THE FORMATION OF PHOSPHATASES BY *ESCHERICHIA COLI*

ANNAMARIA TORRIANI*

Biological Laboratories, Harvard University, Cambridge, Mass. (U.S.A.)

(Received June 11th, 1959)

SUMMARY

Both acid and alkaline phosphatase have been demonstrated in strains of *E. coli*. Bacterial acid phosphatase (pH optimum 4–5) displays a fairly high specificity for the hexosephosphates, while the alkaline enzyme (pH optimum 8.5–9.5) hydrolyzes all the phosphomonoesters tested.

The kinetics of formation of both enzymes have been studied and it has been shown that alkaline phosphatase in measurable amount is only formed when Pi becomes limiting in the medium, at which point the enzyme is formed in substantial amount at a maximum rate.

The implications of the findings with respect to mechanisms of controlling enzyme formation are discussed.

The following abbreviations are used: Pi (inorganic phosphate); Tris (trishydroxymethylaminomethane); NPP (*p*-nitrophenylphosphate); NP (*p*-nitrophenol); bis-NPP (bis-*p*-nitrophenylphosphate).

* Present adress: Dept. of Biology, Massachusetts Inst. of Technology, Cambridge, Mass. (U.S.A.).

INTRODUCTION

During the course of investigations on the effect of depletion of various nutrients on the level of certain enzymes in cultures of *E. coli**, high alkaline phosphatase activities were observed in cells harvested from media in which the inorganic phosphate concentration had become limiting. Since significant amounts of this enzyme are formed only under the unusual condition of limiting inorganic phosphate, perhaps it is not surprising that a search of the literature revealed but one short reference to the occurrence of alkaline phosphatase in *E. coli*[1].

In the present paper we are reporting on certain properties of two phosphomonoesterases, acid and alkaline phosphatase, and on the influence of inorganic phosphate on their formation by certain strains of *E. coli*.

METHODS

Bacterial strains: Most of the experiments to be reported were carried out using *E. coli* strain W although other strains (ML and K12) were shown to behave similarly.

Cultural conditions: Organisms were cultivated on minimal media of the following basal composition per l: (a) Basal Medium A; K_2HPO_4, 7 g; KH_2PO_4, 3 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $(NH_4)_2SO_4$, 1 g and sodium citrate, 0.5 g. The pH was adjusted to 7.0 with NaOH. (b) Basal Medium A-P: This medium was identical with medium A except that it contained 0.1 *M* Tris instead of phosphate. The pH was adjusted to 7.4 with HCl.

The sterile basal medium was distributed in Erlenmeyer flasks and glucose or sodium lactate, as carbon source, was added before inoculation. Care was taken to avoid adding Pi with the inoculum in experiments involving medium A-P, either by washing with Pi-free medium or by use of very small inocula. Cultures were incubated at 37°, with mechanical shaking to insure adequate aeration.

Bacterial growth: Growth was followed at 490 mμ in the Beckman spectrophotometer. It was found that an O.D. of 1 was equivalent to about 0.7 mg bacteria (dry wt.)/ml and approximately $5 \cdot 10^9$ cells/ml in cultures during exponential growth.

*Chemostat experiments***: Under conditions where Pi was limiting medium A-P contained either 0.1 % glucose or 0.2 % sodium lactate as carbon source and 50 μmoles of K_2HPO_4/l. When the carbon source was limiting, the Pi concentration was increased to 170 μmoles and the lactate concentration dropped to 0.02 %.

In these experiments the inoculum was grown overnight in minimal medium A-P containing 50 mμmoles/ml of K_2HPO_4 and 0.01 % sodium lactate; under these conditions the growth was limited by the energy source at O.D. 0.050. The following day enough lactate was added to bring its concentration to 0.2 %, growth resumed and when the O.D. reached 0.090 (see Fig. 1, curve A), the culture was transferred to the growth tube of the chemostat and feeding was started immediately. Flow rates were established for each experiment.

Determination of enzyme activity: Acid and alkaline phosphatase activities were determined in cell suspensions or cell-free extracts. 2-ml samples of culture were placed

* Preliminary experiments carried out in the Dept. of Cellular Physiology, Pasteur Institute, Paris.

** Chemostat experiments have been carried out in the Department of Bacteriology, Harvard Medical School. I thank Dr. B. D. DAVIS for permitting me to use this facility.

in centrifuge tubes containing 0.2 ml 1 : 1000 merthiolate in order to block instantly further enzyme formation. The killed cells were centrifuged at 2° and washed with chilled 0.1 *M* Tris buffer at pH 7.4. The washed cells were resuspended in exactly 2 ml of buffer. This suspension was either used directly or was first treated by shaking with 0.02 ml toluene/ml at 37° for 30 min. As will be shown below, treatment with merthiolate or with toluene or even complete disruption had no effect on enzyme activity.

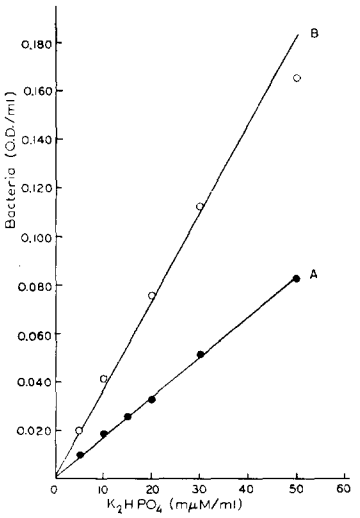

Fig. 1. Yield of bacteria as a function of Pi concentration in the medium. The cultures were grown in medium A-P with K_2HPO_4 added in increasing concentrations. Curve A shows the O.D. at cessation of the initial rapid logarithmic growth. Curve B shows the O.D. at the stationary phase (18–24 h).

Both acid and alkaline phosphatases were measured by a modification of the methods of OHMORI[2] and of BESSEY *et al.*[3]. The *p*-nitrophenol (NP) liberated from *p*-nitrophenylphosphate (Sigma 104) (NPP) at 37° was measured at 420 mμ and an alkaline pH. The following procedures were adopted.

Acid phosphatase: To 3 ml of 0.2 *M* acetate buffer at pH 4.0 and 37° are added 1 ml 0.04 *M* NPP and 1 ml of bacterial suspension (intact, toluenized or sonicated). The reaction mixture is maintained at 37° in a water bath. 1-ml samples are withdrawn at intervals and immediately added to 1 ml of 1 *M* Tris buffer, pH 8.5, containing 0.4 *M* phosphate. A high Pi concentration is essential at this point to inhibit any alkaline phosphatase activity which may be present. The amount of NP liberated is then measured at 420 mμ in the Beckman spectrophotometer. The amount of NP liberated after at least 4 successive intervals is plotted against time and the activity calculated from the slope of the resulting linear curve.

Alkaline phosphatase: 1 ml 1 *M* Tris, pH 8.8 and 0.5 ml of bacterial suspension are placed in a Beckman cuvette and brought to 37° in the chamber of the spectrophotometer. After the correct temperature is reached, 0.5 ml of 0.04 *M* NPP at 37° is added with rapid mixing. The appearance of free NP is followed directly at 420 mμ. The unit of enzyme activity is defined as that amount of enzyme which liberates 1 mμmole of Pi (or NP) per h under the prescribed conditions.

Pi determination: In determining the activity of the phosphatases on substrates other than NPP, it was necessary to follow liberation of Pi directly. Pi was determined using the method of FISKE AND SUBBAROW[4] as modified by DRYER *et al.*[5]. At low Pi concentrations (10 mμmoles/ml or less) it was necessary to use a bioassay method. The solution to be tested was used as a phosphate source for growth of coli W wild type. After 24 h the O.D. was measured and Pi concentration read on the standard calibration curve shown in Fig. 1B.

RESULTS

Effect of Pi on growth of E. coli

Preliminary experiments were performed to test the behavior of cultures growing in media containing limited amounts of Pi. Increasing amounts of K_2HPO_4 (5–50 mμmoles/ml) were added to medium A-P using 0.3 % lactate as energy source. The initial period of exponential growth was followed by a phase of decreasing growth rate beginning abruptly when Pi became limiting. An increase in turbidity per unit bacterial dry wt. from an O.D. of 1.54/mg during exponential growth to 2.28/mg when growth finally ceased after Pi became limiting was found. In curve A of Fig. 1 is plotted the O.D. of the culture at the time Pi became limiting as a function of Pi concentration. Curve B shows the O.D. 18–24 h later at the same Pi concentration. It is of interest to note that although the slope of curve B is 2.24 times that of curve A, the bacterial yield in terms of dry wt. increased by only 50 % over the 18–24-h period.

The effect of Pi on the rate of exponential growth was also studied but no significant change in rate of growth was observed even with Pi concentrations as low as 5 mμmoles/ml.

Demonstration of acid and alkaline phosphatase activity

Most living cells are known to contain at least two types of phosphatases, acid and alkaline. *E. coli* suspensions were therefore tested over a wide pH range using, as a buffer, mixtures of 0.1 *M* sodium acetate and 0.1 *M* Tris adjusted with 0.2 *N* HCl to pH values between 3 and 10.

Fig. 2 shows the effect of pH on phosphatase activity in two bacterial suspensions, each of which was washed twice with water and resuspended in water to approximately the same O.D. Suspension A was prepared from cells grown on excess glucose but limiting Pi. Suspension B was prepared from a culture in which glucose was the limiting factor and Pi was in excess. As seen from Fig. 2, suspension A showed two regions of phosphatase activity, a minor zone which was maximal near pH 4 and a

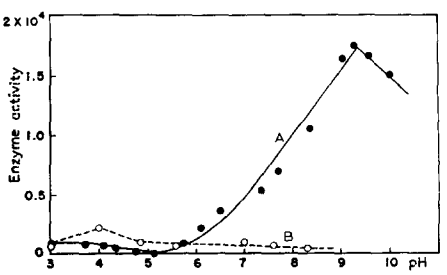

Fig. 2. Phosphatase activity as a function of pH. Enzyme activity: mμmoles of NP liberated/h/ml of cell suspension. Curve A: culture limited on Pi. Curve B: culture limited on glucose.

major region with peak activity at pH 9.3. Suspension B showed appreciable phosphatase activity only in the neighborhood of pH 4. Thus *E. coli* suspensions may show both acid and alkaline phosphatase but only cultures limited on Pi will show high activity at alkaline pH's.

Some properties of the two phosphatases

Both acid and alkaline phosphatase activities of *E. coli* are closely associated with the cells. After centrifugation only 10–15 % of the activity remains in the culture supernatant. Most of the activity is sedimented with the cells whether living or killed by merthiolate. The activity was the same whether or not the cells were intact and living or completely disrupted by sonication. The properties to be discussed below were all determined using sonic extracts from washed suspensions of cells harvested from an overnight culture grown on media containing excess carbon source and an initial concentration of 300 mμmoles/ml Pi so as to limit growth to about 1.2 O.D.

Stability

Toluenized suspensions of *E. coli* show no loss of either acid or alkaline phosphatase activity even after many days storage in the refrigerator. Even after 5 min at 100°, only 50 % of the alkaline phosphatase activity is destroyed.

pH optimum

The optimal pH found using NPP as substrate varied somewhat depending upon the nature of the buffer used. The optimum found for acid phosphatase ranged between pH 3 and pH 5; for alkaline phosphatase between 8.8 and 9.8. The same pH optima were found with sonic extracts as with whole bacterial suspensions.

Inhibitors

The effect of Pi on the activity of both acid and alkaline phosphatases has been tested at their respective pH optima. Fig. 3 shows that alkaline phosphatase is strongly inhibited by Pi and that inhibition is nearly complete in the presence of 0.1 *M* phosphate. Acid phosphatase is less affected by Pi. On the other hand the activity

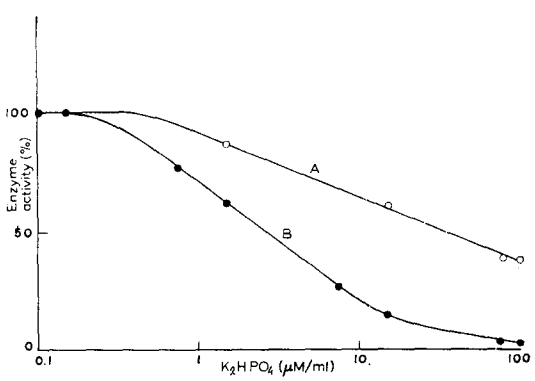

Fig. 3. Inhibition of phosphatase activity by Pi. The activities measured in presence of Pi are expressed as percentage of the activity measured in absence of Pi. For convenience the Pi concentrations have been plotted on a logarithmic scale. Curve A: activity measured at pH 4.0. Curve B: activity measured at pH 8.8.

of acid phosphatase is inhibited 85 % in the presence of 0.01 *M* sodium fluoride; this concentration is without effect on the activity of the alkaline enzyme. The behavior of the two phosphatases from *E. coli* with respect to Pi and fluoride inhibition is similar to that of the analogous enzymes from other microorganisms[6], and from animal sources[7–10].

Specificity

The activity of sonically disrupted bacterial suspensions was tested on several organic phosphate esters at pH 4 and at pH 8.8. The results summarized in Table I show that in both the acid and the alkaline range the enzyme preparation does not hydrolyze ATP and hydrolyzes bis-NPP to only slight extent (2 % or less of the NPP hydrolysis). Identical results were obtained using the cell suspensions before sonic treatment. It can be concluded that both acid and alkaline enzymes are phosphomonoesterases deprived of pyro- and di-esterase activity. While, as shown in Table I, the alkaline enzyme hydrolyzed all of the phosphomonoesters tested, the acid enzyme displayed a fairly high specificity for the hexosephosphates. Both enzymes hydrolyzed NPP readily.

TABLE I

SPECIFICITY OF ALKALINE AND ACID PHOSPHATASES

Substrate	*pH 8.8* *mμmoles Pi/h/ml*	*pH 4* *mμmoles Pi/h/ml*
NPP	27,000	690
AMP	19,300	0
β-glycerophosphate	16,100	43
Glucose 6-phosphate	18,000	560
Fructose-1,6-phosphate	17,350	645
Bis-NPP	trace	trace
ATP	0	0

Influence of Pi in the formation of alkaline phosphatase

In the experiment illustrated by Fig. 4, the kinetics of growth and alkaline phosphatase formation were followed in a culture limited on Pi. The bacteria were grown in duplicate flasks of medium A-P containing 0.2 % glucose and 200 mμmoles/ml K_2HPO_4. Fig. 4a shows the growth curves obtained for the two cultures. The growth rate remained constant at $\mu = 1.0$ (*i.e.* one division/h) until the bacterial density reached O.D. = 0.750/ml and then rapidly fell to a new rate $\mu = 0.2$ which was maintained for the next 3–4 h. As will be discussed below, the change in growth rate took place at almost the same time that the Pi concentration reaches its lowest value in the culture supernate. 1 h after the change in growth rate, 1 μmole/ml K_2HPO_4 was added to one of the flasks and, as seen from the figure, the original growth rate ($\mu = 1.0$) was resumed immediately. Fig. 4, in which alkaline phosphatase activity as ordinate is plotted against bacterial O.D., shows that enzyme formation began at the time when the change in growth rate occurred. Enzyme formation quickly ceased in the flask to which 1 μmole/ml Pi had been added and the enzyme activity in this culture remained constant thereafter. The differential rate of appearance of enzyme was high (= 106,000 units/O.D.) and remained constant for about 3 h after

the change in growth rate. It may be noted at this point that the amount of acid phosphatase formed per cell is always the same and independent of Pi concentration.

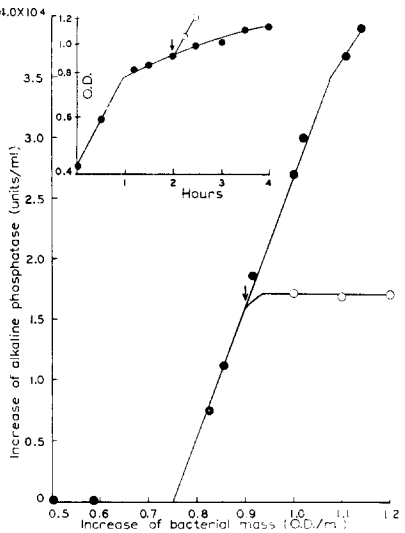

Fig. 4. Kinetics of formation of alkaline phosphatase. The main figure shows the increase of enzyme activity per ml as a function of the growth of the cultures. The insert shows the growth curve of the cultures. See text for details. ●—● culture in medium with limiting Pi. O—O culture after the addition of Pi. The arrow indicates the addition of 1 μmole of K_2HPO_4/ml.

Behavior of cultures grown in mixtures of organic and inorganic phosphate

In order to obtain more precise information regarding the relationship of Pi concentration to alkaline phosphatase formation, growth and enzyme synthesis were studied in cultures containing both Pi, in limited amount, and organic phosphate in excess. Curves 1 of Fig. 5 show the results of an experiment using *E. coli* W grown in medium A-P to which 50 mμmoles/ml K_2HPO_4 and 400 mμmoles/ml β-glycerophosphate had been added. Sodium lactate served as carbon source*. Curves 2 in Fig. 5 give the results in the control culture which contained only the inorganic source of phosphate. Inspection of Fig. 5 shows that in the control culture, change in growth rate, disappearance of detectable extracellular Pi and onset of a maximum rate of enzyme formation all coincided at a time indicated by the arrows. The behavior of the culture containing organic phosphate was different. A short, but reproducible diauxic lag lasting about 12–15 min is shown in curve 1 of Fig. 5A. The inorganic phosphate in the medium continued to fall during the diauxic lag. Enzyme formation began at about the time that growth was resumed and the Pi concentration had fallen to a minimum. The presence of enzyme caused a rapid release of Pi from glycerophosphate and further enzyme synthesis was suppressed, as soon as the Pi level reached 20–25 mμmoles/ml and the specific phosphatase activity had risen to ca. 5000 units/O.D. After the Pi concentration had increased to 75 mμmoles/ml, growth had reached a point where the rate of utilization of Pi exceeded the rate of its release by enzymic hydrolysis. Phosphatase synthesis was resumed a second time during

* Glucose was not used as the source of carbon in this experiment since the acid produced from glycolysis might favor activity of acid phosphatase.

the 4th hour of the experiment when the Pi concentration had again fallen to a minimum.

This experiment shows that when both organic and inorganic phosphate are available it is the inorganic Pi that is used preferentially.

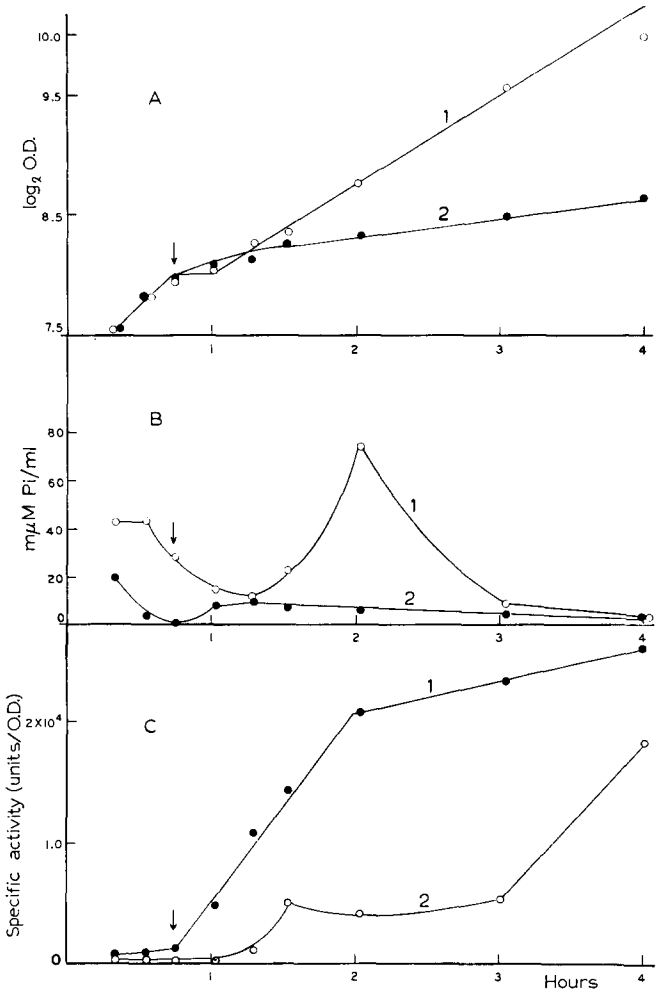

Fig. 5. Bacterial growth, extracellular Pi and alkaline phosphatase activity. The results with two cultures are shown one growing in a medium containing both inorganic and organic phosphate, and the other containing only inorganic phosphate. A, curve of growth; B, variation of Pi concentration in the cell free medium; C, alkaline phosphatase formation. Curves 1: medium containing K_2HPO_4 (50 mμmoles/ml) + β-glycerophosphate (400 mμmoles/ml). Curves 2: medium containing K_2HPO_4 (50 mμmoles/ml). The arrows indicate the onset of the diauxic lag.

Steady state levels of alkaline phosphatase in continuous cultures grown on limiting Pi

After the Pi becomes exhausted by a culture grown on limiting Pi, there is a prolonged period during which O.D. of the culture slowly increases and phosphatase is formed (see Fig. 4). During this period the ratio of bacterial mass to O.D. decreases and it is probable that the bacterial phosphorus content is also decreasing. The fact

that alkaline phosphatase is formed at a constant rate during this stage implies that its differential rate of synthesis is dependent of changes in growth rate of the cells and is independent of changes in their composition. This conclusion has been verified by experiments in the chemostat. Table II shows the steady state values attained for alkaline phosphatase activity in bacteria grown in continuous culture in which the

TABLE II

STEADY STATE LEVELS OF ALKALINE PHOSPHATASE

The inoculum for the chemostat was grown overnight in medium A-P as described. Time was allowed for at least 5 generations to occur before samples were withdrawn. The figures represent the mean of at least two determinations.

Doubling time (min)	*Alkaline phosphatase (Units/O.D.)*
62	0
68	37,500
84	74,000
90	65,000
114	75,000
180	71,200
240	67,500
360	66,000
540	65,400

rate of growth was limited by the Pi concentration. When the growth rate was maintained as close as possible to its maximal value, no enzyme was formed and analysis of the culture filtrate revealed the presence of 7–10 mμmoles Pi/ml*. Table II shows that when the doubling time was increased from 62 to 68 min, the steady state level of enzyme had already attained more than 50 % of its maximal value and the Pi remaining in the supernatant had fallen to 2–4 mμmoles/ml. At all lower growth rates enzyme activity remained constant at its maximum of 69,000 units/O.D. Similar experiments using glucose as carbon source gave identical results.

To demonstrate the specificity of Pi as a repressor of enzyme synthesis, Na_2HAsO_4 was tested in a chemostat experiment in which Pi was the limiting factor and increasing amounts of arsenate were added. No measurable inhibition of synthesis was noted until arsenate reached a concentration ($2 \cdot 10^{-4}$ *M*) that affected growth rate.

DISCUSSION

We have shown that *E. coli* is able to produce an acid and an alkaline phosphomonoesterase. The experiments reported concern only strain W, but both ML and K12 strains were also tested with similar results. The two phosphatases resemble analogous enzymes that have been studied in other microorganisms and in animal tissues in their pH optima as well as in their behavior toward inhibitors such as inorganic phosphate and fluoride. Neither enzyme is excreted into the culture medium; only after mechanical disruption of the cells are they obtained in the soluble fraction. All the substrates tested however, are equally well hydrolyzed by both disrupted and intact cells. Consequently these enzymes appear to be freely accessible *in vivo* to the substrates present in the surrounding medium.

* For Pi analysis 5-ml samples were withdrawn from the chemostat, rapidly chilled to 0° and filtered through a millipore filter. The whole operation required no more than 2–3 min.

The two enzymes differ in their substrate specificity. The alkaline phosphatase appears to hydrolyze to the same extent monohexosephosphates, mononucleotides and synthetic phosphomonoesters. The specificity of the acid phosphatase seems to be confined mostly to the hexosephosphates.

The conditions for formation of the two bacterial phosphatases differ strikingly from one another. Whereas the acid phosphatase is always present, the alkaline enzyme is only formed when the inorganic phosphate concentration becomes limiting (10 mμmoles Pi/ml or less). Thus the bacteria are provided with a means of obtaining Pi from organic phosphate when this becomes necessary. When Pi is present in excess and growth occurs at a pH of 7 or above, no alkaline phosphatase is formed and the acid enzyme is inactive presumably because of the unfavorable pH. Under such conditions there will be no hydrolysis of phosphomonoesters when present and Pi will be used preferentially until its concentration becomes limiting. Under these circumstances a diauxic growth curve[11] is obtained, as was shown in Fig. 5.

The inhibition of alkaline phosphatase formation appears to be specific for Pi. Glucose, a powerful repressor of formation of many bacterial enzymes[12] is without effect on alkaline phosphatase synthesis. Even the closely related arsenate ion does not repress formation of the enzyme.

Inhibition of phosphatase formation by Pi adds one more to the ever-increasing number of examples in bacteria where it is the product of an enzyme reaction or series of reactions which controls the synthesis of the enzyme or enzymes concerned (see[12–14]).

There is an additional reason why repression of enzyme formation may be of importance in the case of alkaline phosphatase. The presence of this enzyme may actually constitute a threat to the life of the cell which is dependent on a variety of phosphate esters for its vital processes.

ACKNOWLEDGEMENTS

I am indebted to A. M. PAPPENHEIMER, Jr. for his invaluable criticisms and suggestions in the preparation of this paper. This work was supported by Grant No. G 5467 from the National Science Foundation.

REFERENCES

1 A. BOIVIN AND L. MESROBEAU, *Compt. rend. soc. biol.*, 112 (1933) 611.
2 Y. OHMORI, *Enzymologia*, 4 (1937) 217.
3 O. A. BESSEY, O. L. LOWRY AND M. J. BROCK, *J. Biol. Chem.*, 164 (1946) 321.
4 C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
5 R. L. DRYER, A. R. TAMMES AND G. J. ROUTH, *J. Biol. Chem.*, 225 (1957) 177.
6 L. B. PETT AND A. M. WYNNE, *Biochem. J.*, 27 (1933) 1661; *Biochem. J.*, 32 (1938) 563.
7 J. ROCHE AND NGUYEN VAN THOAI, *Advances in Enzymol.*, 10 (1950) 83.
8 G. SCHMIDT in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. II, Academic Press, New York, 1955, p. 523.
9 R. K. MORTON in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. II, Academic Press, New York, 1955, p. 533.
10 L. A. HEPPEL in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. II, Academic Press, New York, 1955, p. 530.
11 J. MONOD, *Recherches sur la croissance des cultures bactériennes* IIème partie, Hermann and Co., Paris, 1942.
12 B. MAGASANIK, *Annual Rev. Microbiol.*, 11 (1957) 221.
13 H. J. VOGEL in *Symposium on the Chemical Basis of Development*, Johns Hopkins Press, 1958, p. 479.
14 L. GORINI AND W. K. MAAS, in *Symposium on the Chemical Basis of Development*, Johns Hopkins Press, 1958, p. 469.