DEREPRESSION OF POLYPHOSPHATASE IN *ESCHERICHIA COLI* BY STARVATION FOR INORGANIC PHOSPHATE

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1. Introduction

Several micro-organisms are known to accumulate polyphosphate (polyP) when subjected to unbalanced growth conditions such as low pH, sulphur starvation or following a period of phosphate-starvation. The accumulated polyP, which can be degraded, is thought to regulate the level of inorganic phosphorous (P_i) of the cells or, alternatively, to serve as a microbial phosphagen for the control of the cellular level of ATP [1]. The enzyme polyP kinase catalyzes the biosynthesis of polyP by transfering the terminal phosphoryl group of ATP to a primer polyP and in *Escherichia coli* it is a reversible enzyme [2,3]. The enzyme polyphosphatase catalyzes the hydrolysis of polyP to P_i [4]. It has been reported that in *Aerobacter aerogenes* P_i-starvation derepresses these two enzymes by a factor of 4–8 [5]. However, in many micro-organisms, P_i-starvation is also known to derepress strongly the synthesis of alkaline phosphatase, an unspecific phosphoesterase [5–7] and in this communication we show that in *E. coli* polyphosphatase is induced only two-fold and that this induction is obscured by the derepression of alkaline phosphatase.

2. Materials and methods

2.1. *Chemical synthesis of polyphosphate*

^{32}P-labelled polyP was chemically synthesized by the method described by Muhammed, Rodgers and Hughes [8]. To 4 ml of 1M KH_2PO_4, 1.2 mCi of carrier $^{32}P_i$ free were added. The solution was brought to pH 4.5 with 0.2 M KOH; it was evaporated in a platinum crucible, heated at 770°C and processed as described in [8]. The yield of poly[^{32}P] on the basis of undialysable radioactivity retained was 48.4%.

2.2 *Bacterial strains*

Strain K10 is an HfrC wild type strain and E15 is a mutant strain derived from K10; it carries a detetion for the structural gene of alkaline phosphatase (*phoA*⁻ [6]).

2.3. *Media, growth conditions and preparation of enzyme source*

Medium A consists of minimal Tris-buffered medium lacking P_i but enriched with 0.5% bacto-peptone and 0.5% glucose [9]. The bacto-peptone contains traces of P_i sufficient for the growth of a cell inoculum to approximately 5×10^8 cells/ml; at this cell density growth is arrested, due to phosphate limitation. For cultures growing in excess P_i, KH_2PO_4 was added to the medium to a final concentration of 10^{-3} M.

Overnight cultures were washed once with 0.02 M Tris–HCl buffer pH 9.0 and resuspend in one fifth volume of the same buffer. This suspension was used to measure the activity of whole cells. Extracts were prepared by sonic oscillation.

2.4. *Enzyme assays and protein determination*

Polyphosphatase was assayed in Tris buffer pH 9.0 by the method described by Harold [5]. The release of labelled [$^{32}P_i$] from poly [^{32}P] was determined. The reaction mixture (0.30 ml) contained either 50 μl of the cells, cell extract or commercially purified *E. coli* alkaline phosphatase (Worthington, 320 EU/ml diluted ×20). The reaction was stopped with 1.0 ml 0.5 N perchloric acid and 0.1 ml of bovine serum albumin (10 mg/ml) was added. The precipitate

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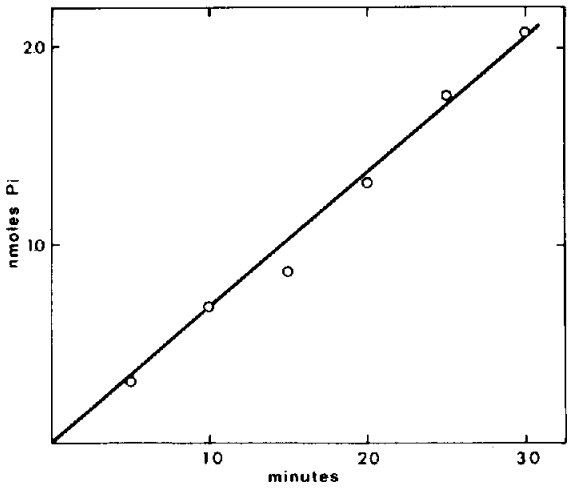


Fig.1. Polyphosphatase activity as a function of time. Cell extracts from strain K10 growth in excess P_i were used as a source of enzyme.

was centrifuged and P_i was extracted from the supernatant with isobutanol and benzene by the method described by Avron [10]. One ml of the isobutanol–benzene phase was mixed with 5 ml Tritol scintillation solution (Packard) and counted. The reaction proved to be linear with time for at least 30 min (fig.1). One Enzyme Unit (EU) was defined as nmoles P_i released per min at 37°C.

Alkaline phosphatase was assayed as described by Schlesinger [11] using p-nitrophenyl phosphate as substrate. One EU was defined as the amount of enzyme producing one absorbancy unit at 410 nm per minute at 37°C.

Protein was determined by Hartree's modification of the Lowry method [12], bovine serum albumin was used as standard.

3. Results and discussion

Cell were grown in media containing either excess or limited concentration of P_i, cell extracts were prepared and the specific activity of polyphosphatase and of alkaline phosphatase determined (table 1A). It is seen that phosphate-limitation causes a considerable increase (of approx. 4-fold) in polyphosphatase activity. Since, however, these conditions are also known strongly to derepress alkaline phosphatase activity (approx. 65-fold, table 1A) it is not clear whether the derepression of polyphosphatase is specific. We have therefore repeated the same assays in a mutant strain (E15) carrying a deletion in the structural gene for alkaline phosphatase (table 1B). The increase of polyphosphatase activity under P_i limitation is now 1.6-fold. To see whether polyphosphate can serve as a substrate of alkaline phosphatase we compared the rate of the reaction of commercially purchased alkaline phosphatase and cell extracts of strain E15 (fig.2). The data show that polyP is a substrate of purified alkaline phosphatase; both enzyme sources have similar K_M values (8.3×10^{-3} M) and alkaline phosphatase has a somewhat lower V_{max}. Muhammed et al. [8] obtained a K_M value of 7.7×10^{-4} M for polyphosphatase from *Corynobacterium xerosis*. Since the concentration of substrate is based on the P_i moieties in the polyP chain, variations in average chain length of the polymer lead to variations in its concentration.

To distinguish further between the activity of alkaline phosphatase and that of polyphosphatase, advantage was taken of the fact that alkaline phosphatase is located on the cell surface between the inner and outer membranes, in the so-called periplasmic space [13]. Therefore, the enzyme is active on external substrates when whole cells are used as the enzyme source [14]. Furthermore, alkaline phosphatase is

Table 1
Derepression of polyphosphatase and alkaline phosphatase activity in *phoA*$^+$ and *phoA*$^-$ cells

Specific activity	A Strain K10 (*phoA*$^+$)		B Strain E15 (*phoA*$^-$)	
	High P_i	Low P_i	High P_i	Low P_i
Polyphosphatase	1.53	6.48	1.96	3.20
Alkaline phosphatase	0.08	5.35	0	0.23

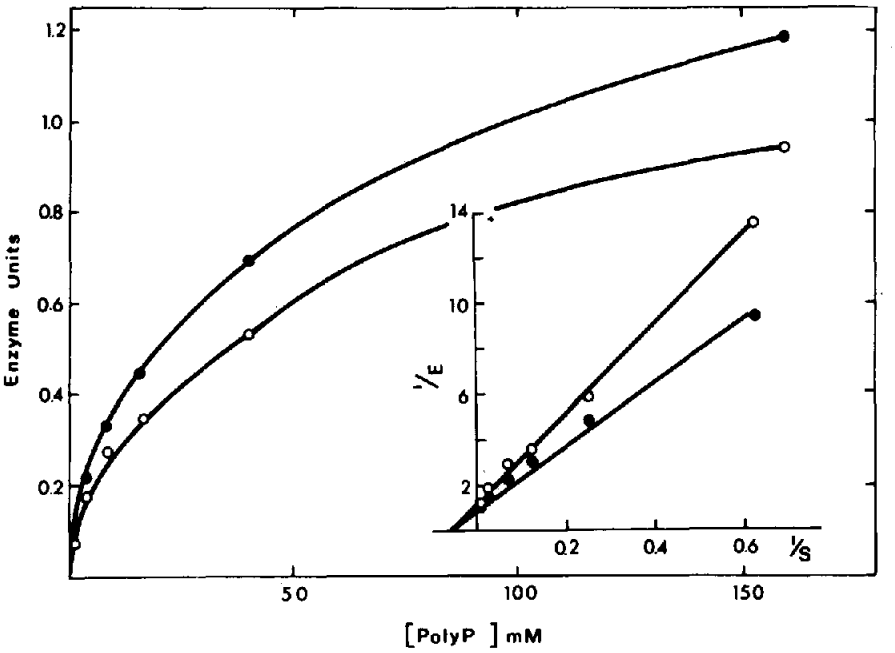
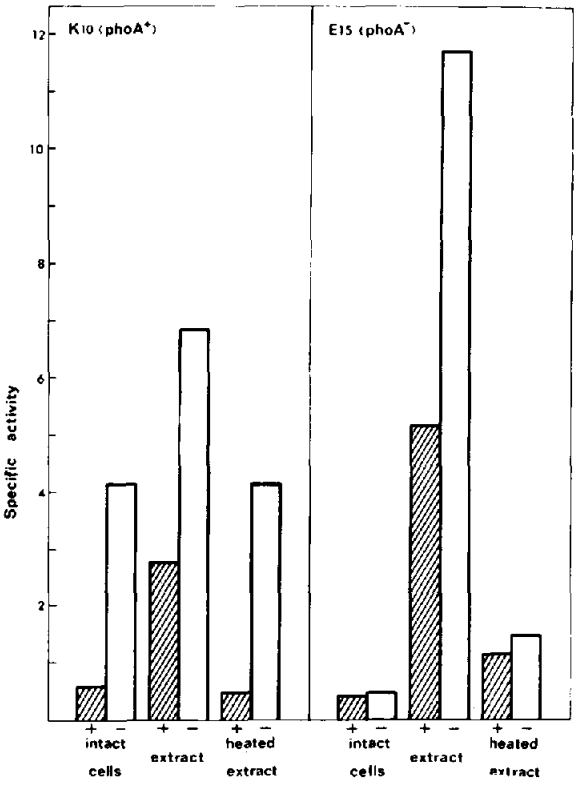


Fig.2. Polyphosphatase activity as a function of polyP concentration using E15 (*phoA*⁻) extracts (○) or commercial alkaline phosphatase (Worthington) (●) as sources of enzyme. The insert is a Lineweaver-Burk plot of the data. The concentration of polyP is based on that of its P_i moieties.



exceptionally stable to heat [15]. Polyphosphatase, on the other hand, is presumed to be an 'internal' enzyme (polyP accumulates within the cell) and is heat labile [8]. Cells were grown in limited and in excess P_i and of each culture three enzyme sources were prepared: intact cells, cell extracts and heated cell extracts. Polyphosphatase activity was then determined (fig.3). Intact cells of the *phoA*⁺ strain show activity (when starved for P_i and the *phoA*⁻ cells are only weakly active. This shows that the bulk activity obtained by intact cells is due to the periplasmic alkaline phosphatase. The extracts show increased activity both in excess and low P_i due to the exposure of the internal polyphosphatase. When these extracts were heated (10 min in 60°C) polyphosphatase became inactivated and only the heat-stable alkaline phosphatase activity was retained. It can be concluded that polyphosphatase is an internal enzyme and its

Fig.3. Polyphosphatase activity of intact cells, cell extracts (sonicated cells) and heated extracts (10 min in 60°C) of strain K10 (*phoA*⁺) and strain E15 (*phoA*⁻) grown either in excess P_i (striped bars) or in limited P_i (blank bars).

activity is derepressed by approx. 2-fold by P_i-starvation. In order to reveal this derepression the cell must be free of alkaline phosphatase, which is derepressed under the same conditions and can use polyP as a substrate. It should be pointed out that the activity of alkaline phosphatase was demonstrated on externally-supplied polyP. The question whether it can cleave in vivo internally-formed polyP remains open.

Acknowledgement

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