IS THE ACID PHOSPHATASE OF *ESCHERICHIA COLI* WITH pH OPTIMUM OF 2.5 A POLYPHOSPHATE DEPOLYMERASE?

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1. Introduction

Inorganic polyphosphates are widely distributed among microorganisms, and their metabolism has been studied over the last 30 years (review [1]). Their physiological function, however, remains ill-defined. Besides the possibility that polyphosphates might represent a form of energy storage [2], they are more often believed to participate in the regulation of the intracellular [P_i] by constituting a phosphorus reserve [3]. Their metabolism appears rather complex and has been more extensively studied in bacterial species such as *Aerobacter aerogenes* [4,5] or *Corynebacterium xerosis* [6], in which these compounds substantially accumulate.

In *Escherichia coli* where only traces of polyphosphates are found [7], an enzymatic activity has been described, which reversibly transfers the γ-phosphate group of ATP to a growing chain of inorganic polyphosphates [8,9]. Which mechanism is actually responsible in vivo for the degradation of polyphosphates in this organism, is presently unknown. The presence at the periphery of the cell of a polyphosphatase operating near pH 7 has been reported [10,11], but little is known on this enzyme at the molecular level. Another periplasmic enzyme referred to as an 'acid phosphatase with a pH optimum of 2.5', has been shown to hydrolyze preferentially molecules possessing several phosphoanhydride bonds, and more particularly the guanine nucleoside polyphosphates [12]. This enzyme, first identified as a monomeric protein of M_r 45 000 [13] was purified to homogeneity [14]. Its synthesis was shown to take place exclusively in the stationary phase of the growth or to be induced by P_i starvation or by anoxia [14].

We show here that inorganic polyphosphates, as judged by their comparatively low K_m-values, are in fact the best substrates so far tested in vitro for the pH 2.5 acid phosphatase, leaving open the possibility that this enzyme might play a role in vivo in the catabolism of polyphosphates.

Abbreviations: PNPP, *para*-nitrophenylphosphate; P_i, inorganic phosphate; poly$(P)_3$, polyphosphate with 3 phosphoryl residues; poly$(P)_5$, polyphosphate with 5 phosphoryl residues; poly$(P)_{15}$, polyphosphate with 15 phosphoryl residues

2. Materials and methods

2.1. *Source of enzyme*

The pH 2.5 acid phosphatase was extracted from stationary-phase cultures of *E. coli* strain K-10, grown in LB medium [15] and purified to homogeneity by the method in [14].

2.2. *Enzyme assays*

All the enzymatic assays were performed in 250 mM glycine–HCl buffer, at pH 2.5 and at 37°C, with the same amount of purified enzyme. One unit of enzyme is defined as the amount of enzyme which catalyzes the hydrolysis of 1 nmol PNPP/min at 37°C in the above buffer. The spectrum of activity of the enzyme as a function of pH was established in 250 mM sodium phtalate buffer adjusted at the required pH-values. The hydrolysis of polyphosphates was measured after 15 min in the same buffers and P_i release was measured on aliquots as in [16]. All the results were corrected for the spontaneous hydrolysis of polyphosphates under acidic conditions.

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2.3. *Chemicals and reagents*

PNPP was from Fluka; sodium pyrophosphate, poly(P)$_3$, poly(P)$_5$, poly(P)$_{15}$, and cyclic poly(P)$_3$ were from Sigma.

3. Results and discussion

The hydrolysis of the phosphoanhydride bonds in different short-chain polyphosphates by the purified pH 2.5 acid phosphatase of *E. coli* has been compared with that of PNPP, usually employed as reference substrate for phosphatases (fig.1). The efficiency of the reaction clearly increased with the length of the polyphosphate chain up to 5 residues. Only 1 phosphate residue was liberated/molecule of linear poly(P)$_3$ at the plateau obtained after 20 min. In the case of poly(P)$_5$, ~3 residues were hydrolyzed, and the reaction proceeded linearly with poly(P)$_{15}$ during the time of the experiment. Pyrophosphoric acid was accordingly not hydrolyzed and cyclic poly(P)$_3$ was not a substrate for the enzyme. These observations and the linearity of P_i accumulation during the early stages of action of the enzyme on poly(P)$_{15}$ for

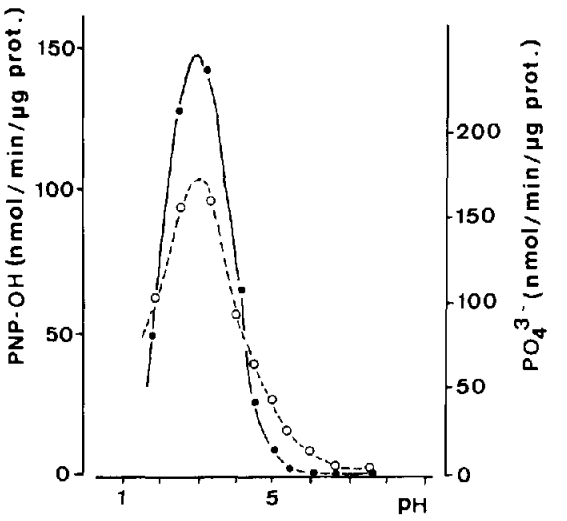

Fig.2. pH dependence of the poly(P)$_5$ depolymerase activity of the enzyme. The buffer system used consisted in 250 mM Na-phtalate/HCl adjusted at the required pH-values. poly(P)$_5$ (0.5 mM) was incubated at 37°C in presence of 7 units purified enzyme/ml and P_i release was measured after 15 min as in [16] at 820 nm. The hydrolysis of PNPP (25 mM) was measured comparatively by the absorbance at 410 nm of the *para*-nitrophenol formed (see section 2).

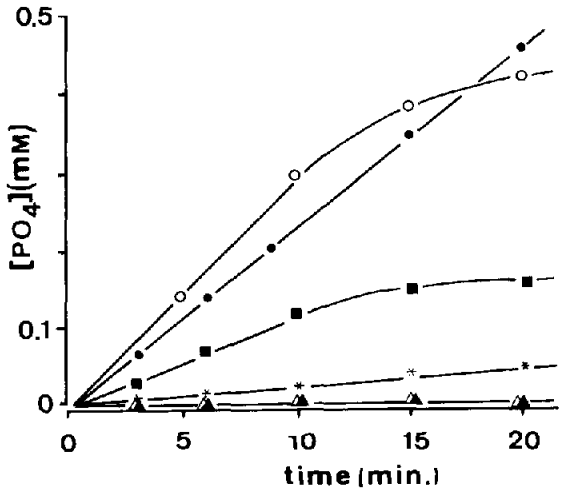

Fig.1. Comparison of the kinetics of hydrolysis of various short chain polyphosphates. The experiments were performed in 250 mM glycine/HCl buffer at pH 2.5 and at 37°C in presence of 7 enzyme units/ml. All substrates were at 0.2 mM final conc. The concentration of the P_i released was measured as in section 2. (–○–) poly(P)$_5$; (–●–) poly(P)$_{15}$; (–■–) poly(P)$_3$ (linear); (–▲–) trimetaphosphate (cyclic); (–△–) pyrophosphoric acid; (–*–) *para*-nitrophenylphosphate (PNPP).

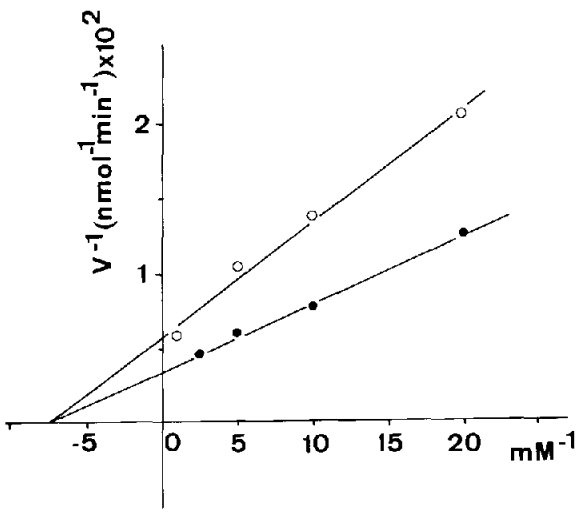

Fig.3. Lineweaver-Burk representation of the kinetics of hydrolysis of short chain polyphosphates. The experimental conditions are the same as those in fig.1, except for substrate concentrations: (–●–) poly(P)$_5$; (–○–) poly(P)$_{15}$. The kinetic parameters derived from these data are given, with others, in table 1.

Table 1
Kinetic constants for the hydrolysis of short chain polyphosphates by the acid phosphatase at pH 2.5

Substrate	K_m (mM)	V_{max} (nmol . min^{-1} . μg $protein^{-1}$)	K_{cat} (s^{-1})
Pyrophosphoric acid	Not degraded		
$Poly(P)_3$ (linear)	0.8	210	159
$Poly(P)_5$	0.13	293	221
$Poly(P)_{15}$	0.13	190	144
Cyclic $poly(P)_3$	Not degraded		
PNPP	2.8	207	157

instance, are consistent with an exopolyphosphate depolymerase activity and with observations on the hydrolysis of nucleoside polyphosphates [12–14].

Since another polyphosphatase working optimally near pH 7 has been described in *E. coli* [10,11], the spectrum of activity of the present enzyme as a function of pH has been explored (fig.2). The pH optimum for the polyphosphate depolymerase activity was exactly the same as for the hydrolysis of PNPP, i.e., 2.5, as reported [13,14]. The main kinetic constants of the enzyme for the hydrolysis of short chain polyphosphates, calculated from the data of Lineweaver-Burk representations (fig.3), are indicated in table 1. The app. K_m for $poly(P)_5$ was ~3-times below that reported for GTP and the V_{max} was high as compared with other substrates [14].

Polyphosphates apparently do accumulate in *E. coli* under conditions shown to reduce the expression of the pH 2.5 acid phosphatase, such as a starvation for sulphur [14]. Hence, a reverse relationship seems to exist between the abundance of this enzyme and the accumulation of polyphosphates, suggesting a polyphosphatase activity in vivo.

The exclusive presence of this enzyme in the periplasmic space [12,17] is at first glance difficult to reconcile with its possible role in polyphosphate catabolism, mainly in view of the cytoplasmic localization of such molecules in most bacteria studied [1]. The existence of polyphosphates in the periplasmic space of certain species, including *E. coli*, has nevertheless been reported [18]. The degradation of long chain-polyphosphates present in the cytoplasm of *A. niger* has been shown to proceed via a preliminary endolytic cleavage leading to molecules of $poly(P)_5$ [19]. If a similar mechanism also exists in *E. coli*, then the enzyme studied here is a good candidate for the in vivo catabolism of such small polyphosphate chains, which might well be excreted across the cytoplasmic membrane.

Alternatively, the pH 2.5 acid phosphatase might play a role in the degradation of short-chain polyphosphates of exogenous origin.

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