Purification and Properties of Yeast Histidinol Phosphate Phosphatase†

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ABSTRACT: Histidinol phosphate phosphatase from baker's yeast has been purified and two forms of the enzyme isolated. The two forms have identical K_m values for histidinol phosphate (0.25 mM) and pH profiles. Separation by preparative polyacrylamide gel electrophoresis could only be accomplished after some impurities were removed by preparative gel electrophoresis in 8 M urea. The enzyme activity could be completely renatured from 8 M urea solutions although a temperature-dependent irreversible loss of activity was observed with prolonged treatment. This loss of activity may be associated with the modification of the enzymes by cyanate ion. Neither the kinetic and electrophoretic properties nor the heat stability of the two forms were changed by urea treatment. Both enzymes have a molecular weight of 38,000 and consist of a single polypeptide chain as shown by sodium dodecyl sulfate gel electrophoresis. The enzyme was not inhibited by low concentrations of histidinol in contrast to the bifunctional phosphatase from *Salmonella typhimurium*, nor was it affected by manganese chloride as is the *Salmonella* enzyme.

The genetic relationship of the enzymes that carry out the reactions of histidine biosynthesis have been extensively analyzed in *Saccharomyces cerevisiae* (Fink, 1964), *Neurospora crassa* (Ahmed *et al.*, 1964) and *Salmonella typhimurium* (Hartman *et al.*, 1960, 1971; Loper *et al.*, 1964). Fink has shown that the *his2* locus of yeast specifies an enzyme which hydrolyzes histidinol phosphate and in *Neurospora crassa*, a single locus has also been identified by Ahmed *et al.* that codes for histidinol phosphate phosphatase. The enzyme performing the same catalytic event in *Salmonella typhimurium* exhibits markedly different characteristics than the yeast and *Neurospora* enzymes, and is, in fact, specified by a gene which also codes for an additional nonconsecutive catalytic activity—the removal of water from imidazoleglycerol phosphate to produce imidazoleacteol phosphate (Loper *et al.*, 1964; Hartman *et al.*, 1971; Brenner and Ames, 1971). In addition to this difference in the nature of the enzymes that hydrolyze histidinol phosphate, the genes that code for the histidine biosynthetic enzymes are clustered together into an operon in *Salmonella* (Ames and Martin, 1964), but are distributed throughout several linkage groups in *Saccharomyces cerevisiae* and *Neurospora crassa* with only a residual clustering of three enzyme activities surviving the evolutionary process. The comparison of these enzymes would, therefore, be of some interest with respect to both the basic enzymology of the separate monofunctional and bifunctional enzymes and the evolution of enzyme systems.

Some information on histidinol phosphate phosphatase from yeast was reported by Gorman and Hu (1969). The phosphatase, a heat-stable enzyme with an alkaline pH optimum, was physically separated from nonspecific alkaline phosphatases on the basis of chromatographic behavior on DEAE-Sephadex and was functionally distinguished from alkaline phosphatases by its insensitivity to berylium ion and by its strict substrate specificity. The *Salmonella* bifunctional enzyme has been partially purified by Brady and Houston (1973) and mutant enzymes produced by strains having nonsense lesions have been studied and purified (Houston, 1973a,b). The purification of the yeast monofunctional enzyme and the description of its properties is reported here.

Methods and Materials

Histidinol phosphate was purchased from Cyclo Chemical Co. and histidinol dihydrochloride was obtained from Sigma. All other chemicals were reagent grade. Red Star compressed baker's yeast was purchased from a local bakery and stored at −20° until needed.

Enzyme Assay. Histidinol phosphate phosphatase was assayed at 37° by a modification of the procedure of Ames *et al.* (1960). The substrate was dissolved in water and was neutralized to prevent pH changes in the assay if substrate concentrations above 5 mM were employed. The final volume of the assay was 60 μl containing 100 mM triethanolamine hydrochloride (pH 8.5), 5 mM histidinol phosphate, and a volume of enzyme less than 10 μl. When needed suitable corrections were made for inorganic phosphate present in the substrate. The reaction was usually stopped in 5 min or less by the addition of 140 μl of ascorbate–molybdate reagent and the phosphate–molybdate complex formed at 45° for 20 min. An extinction coefficient at 820 nm of 2.60×10^4 l. mol^{-1} cm^{-1} was used to calculate the amount of phosphate released. One unit of activity is defined as the amount of enzyme releasing 1 μmol of phosphate/min under the above conditions.

Crude Extract. Following the procedure of Fink,[1] 1 lb of yeast was suspended in 225 ml of 0.6 M Tris-chloride (pH 8.0), containing 0.01 M mercaptoethanol. The mixture was agitated with a wrist-action shaker at 37° with 700 ml of toluene for 2 hr. All other operations were carried out at 4° unless otherwise specified. The crude extract was collected by removal of the aqueous layer after centrifugation at 10,800*g*. The cell debris was washed with 100 ml of standard buffer (0.05 M

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[1] Gerald Fink, personal communication.

Tris-chloride–0.01 M mercaptoethanol, pH 8.5) and the extracts were combined. The extract was placed in a large beaker and incubated in a 60° water bath for about 6–10 min until an obvious precipitate had formed. It was then chilled to 4° and centrifuged for 30 min to remove precipitated protein. The supernatant was made to 45% saturation with ammonium sulfate by slow addition of the salt over 20 min and centrifuged after being stirred for an additional 40 min in an ice bath. The precipitate was discarded and the supernatent was made to 60% saturation with ammonium sulfate, stirred for 60 min in ice, and centrifuged. The precipitate was recovered and dissolved in a minimal volume of pH 8.5 buffer.

Column Chromatography. The above solution was immediately applied to a 5 × 45 cm column of Sephadex G-100, which had been previously equilibrated with standard buffer. Upward flow elution was by standard buffer and 12-ml fractions were collected. The fractions (600–900 ml) containing the activity were pooled and concentrated by an Amicon Diaflo ultrafiltration apparatus under 50-psi nitrogen pressure using a PM-10 membrane. Approximately 20 ml of concentrated solution was obtained, which was applied to a DEAE-Sephadex A-50 column (4 × 30 cm) and washed on with about 250 ml of buffer containing 0.1 M NaCl. This column had been previously equilibrated with standard buffer containing 0.1 M NaCl. Elution was by a concave gradient made by feeding 1 l. of 1.0 M NaCl in buffer in a 1-l. erlenmeyer flask into 1 l. of standard buffer with 0.1 M NaCl contained in a stirred 1-l. cylindrical reagent bottle. Fractions of 12 ml were collected. Fractions containing activity (1400–1560 ml of the gradient) were pooled and concentrated as before by ultrafiltration using a UM-2 membrane. The concentrate was then dialyzed against standard buffer.

Polyacrylamide Gel Electrophoresis. Preparative polyacrylamide gel electrophoresis using a Canalco apparatus was employed to obtain a preparation with a high degree of purity. The system developed by Ornstein and Davis (1962) was used for this purpose. A 10-cm long gel was used with a PD/320 column at 10°. The separating gel concentration is described as $T = 12.54°$, $c = 0.30$ (Hjerten, 1962) and the 2-cm stacking gel was $T = 4.13°$, $c = 15.15$. The slit elution buffer was standard buffer and 5–10-ml fractions were normally collected at a flow rate of about 2 ml/min. The eluate was passed through a LKB ultraviolet monitor using Bromophenol Blue dye to detect the front.

Polyacrylamide gel electrophoresis using the preparative apparatus was also carried out in 8 M urea. The smaller preparative column PD/150 was employed and a 10-cm long gel was used. Fresh reagents were made, adding solid recrystallized urea to make a final concentration of 8 M urea. The separating gel was $T = 7.7°$, $c = 2.6$ and urea was not added to the electrode reservoir buffer (buffer F) or to the slit elution buffer. The enzyme was made 8 M in urea less than 1 hr before the separation was carried out. When needed, the enzyme obtained from this procedure was run on a third gel with a composition of $T = 10.27°$, $c = 2.6$ and of similar dimensions as the urea gel. All electrophoretic separations were carried out at 10° using a constant voltage of 700 V.

The Ornstein and Davis system was used to electrophoretically determine the purity of the enzyme preparations on 7.5 and 10% analytical polyacrylamide gels.

Specific Staining of Phosphatase Activity on Gels. Histidinol dehydrogenase was purified by the procedure described by Martin *et al.* (1971) and added at a concentration of 0.5 mg/ml with 0.4 mM NAD^+, 0.3 mM Nitroblue Tetrazolium, and 0.2 mM phenazine methosulfate at room temperature. Histidinol phosphate was added at a final concentration of about 5 mM and the release of histidinol by the action of the phosphatase was indicated by the presence of precipitated blue formazan at the surface of the gel.

Protein Determination. The concentration of protein was determined by the ratio of absorbances at 280 and 260 nm as proposed by Layne (1957). The concentration of protein in the final steps of purification are reported with respect to the absorbance at 280 nm.

Molecular Weight Determination. A 1.5 × 90 cm column of Sephadex G-100 was equilibrated with standard buffer at pH 7.5 containing 1 mM $MnCl_2$ and was carefully standardized by passing 0.5-ml volumes of individual proteins solutions through the column at 4°. Bovine serum albumin, ovalbumin, chymotrypsinogen A, and cytochrome *c* were used as standards. Duplicate determinations were made of the elution volumes and the void volume was determined with Blue Dextran. After standardization, the phosphatase was filtered through the column, the elution volume was monitored by assaying for activity and the molecular weight was estimated by the usual plot (Whitaker, 1963).

The subunit molecular weight of the enzyme was determined by the method of Weber and Osborn (1969). The enzyme was dissolved in sodium dodecyl sulfate–mercaptoethanol buffer and incubated overnight at 37° or 45° to effect complete denaturation. Electrophoresis in gels containing sodium dodecyl sulfate was then carried out for about 5 hr at 8–10 mA/tube and stained with Coomassie Blue. Rabbit γ-globulin, ovalbumin, aldolase, trypsinogen, ribonunclease, and cytochrome *c* were used as standard proteins to construct a plot of molecular weight *vs.* mobility.

Results

Purification of Histidinol Phosphate Phosphatase. Table I shows the relative purification in each step of the procedure. The ammonium sulfate precipitation allowed the reduction of the material to manageable amounts for application to the Sephadex G-100 column. The small percentage of nonspecific phosphatase mentioned by Gorman and Hu which survived the heat treatment was found to be separated by the G-100 column. Previous experiments had indicated a molecular weight for this enzyme of about 90,000. DEAE-Sephadex chromatography of the fraction from Sephadex gel filtration provided about 80- to 125-fold purification with the recovery of about 55–70% initial activity.

A single peak of activity was observed to elute from the preparative gel approximately 3 hr after the Bromophenol Blue dye. The total run required approximately 9 hr. No increase in the absorbance at 280 nm was detected in these fractions, but analytical gels from several of the fractions across the peak of activity showed at least five closely migrating bands. Repeated preparative gel electrophoresis on the pooled fractions using various acrylamide to bisacrylamide ratios showed additional improvement in purity, but never succeeded in reducing the number of bands seen on analytical gels to fewer than two or three. No run was made in which a separation of two active fractions was observed without further treatment. The same band pattern was also found after a single preparative run if strain S288C of *Saccharomyces cerevisiae* was used for the purification. At this point, the specific activity had increased over 500 times over that in the crude extract with the recovery of about 20–50% of the initial activity.

TABLE I: Purification of Histidinol Phosphate Phosphatase from Baker's Yeast.

Step	Protein (mg)	Sp Act. (μmol of P_i/min per mg)	Total Act. (μmol of P_i/min)	% Yield	-Fold Purificn
Crude extract	26,200	0.073	1955	100	1.0
Heat treated	10,980	0.183	1925	98.4	2.3
$(NH_4)_2SO_4$ fraction	4,750	0.317	1497	76.6	4.2
Sephadex G-100	1,750	1.03	1797	91.9	13.8
DEAE-Sephadex	119	9.33	1107	56.6	125
First preparative gel	27	45.7	1240	63.4	610
Urea preparative gel	1	426.7	440	22.5	5800

The presence of two forms of histidinol phosphate phosphatase that also migrate very closely on polyacrylamide gel electrophoresis has been demonstrated from a nonsense mutant of *Salmonella typhimurium* (Houston, 1973b). If analytical polyacrylamide gels were examined for yeast phosphatase activity *in situ*, as described in Methods, a broad band of activity in the region of two minor bands was observed even though the coupled staining procedure was not very satisfactory. Resolution was poor because the formazan did not adhere to the surface sufficiently well and the band formed only transiently.

Since information was needed to determine whether or not two proteins in the preparation were active phosphatases, other preparative methods were tried. Isoelectric focusing (Haglund, 1968) was not successful and the enzyme lost activity rapidly due to the acidic nature of the region in which it focused, about pH 5. Precipitation also occurred at the isoelectric point. Chromatography on hydroxylapatite or sulfoethyl-Sephadex were similarly incapable of resolving the components seen after preparative gel electrophoresis.

Stability to Urea Treatment. The protein bands could be shown to separate reasonably well when 8 M urea was included in the analytical polyacrylamide gel electrophoretic system. In contrast to their similar mobilities under native condition, the bands appeared to be well separated in 8 M urea gels. Therefore, experiments were initiated to explore the effect of urea on the phosphatase activity. Various concentrations of urea were made in standard assay buffer (0.1 M triethanolamine hydrochloride, pH 8.5) and histidinol phosphate added at 1.67 mM concentration. The assay (60 μl) was initiated by the addition of about 0.016 unit of enzyme (obtained after the first preparative gel electrophoresis separation) and terminated after 30-sec incubation at 37° by adding 140 μl of ascorbate–molybdate reagent. The enzyme was progressively less active as the urea concentration was increased to 8 M where the activity was about 25% of the control activity assayed without urea. The enzyme retained 50% of its activity in 4 M urea; however, a further 10–15% decrease in activity was observed if the enzyme was first incubated in urea (above 2 M urea) for 10 min and then the assay was initiated by the addition of substrate. With a 10-min preincubation, no activity was observed in 8 M urea.

Enzyme incubated in 8 M urea did undergo irreversible loss of activity with extended periods of treatment. Complete recovery of activity was observed if the enzyme was incubated in 8 M urea and immediately diluted into histidinol phosphate in standard assay buffer so that the concentration of urea was minimal (0.3 M). This concentration of urea caused a loss of less than 10% activity compared to the enzyme assayed without urea. Fifty per cent of the activity was lost if the enzyme was kept in 8 M urea for 20 hr at 25° before dilution and assay. Loss of activity followed first-order kinetics for 50 hr and showed a dependence on temperature. The inhibition could not be reversed by dialysis against buffer. At 10°, 30-hr incubation in 8 M urea was required for the loss of 50% of the activity.

The enzyme must be renatured almost instantaneously after dilution in substrate since only 30 sec was allowed for hydrolysis of histidinol phosphate to proceed before the reaction was terminated. If the enzyme was diluted into assay buffer without substrate, incubated for 10 min at 37°, and then histidinol phosphate added, no further increase in the amount of activity recovered was observed.

Effect of Cyanate in 8 M Urea on Phosphatase Activity. The spontaneous formation of ammonium cyanate in concentrated urea solutions is particularly troublesome at the higher pH values used during electrophoresis. The possibility of the production of electrophoretic artifacts is always present during these types of experiments even when precautions are taken to minimize the effects. Gerding *et al.* (1971) has shown that high concentrations of cyanate in 6 M urea are needed to modify the electrophoretic mobility of α-crystallin. Several days of incubation at 20° in 6 M urea were required for substantial modification of the electrophoretic pattern while short periods of incubation had little effect on the mobility of the protein. The data of Hagel *et al.* (1971) would suggest that under the conditions used for our electrophoresis (assuming the initial absence of cyanate), the concentration of ammonium cyanate would reach concentrations only on the order of 10^{-5} M after 10-hr incubation at a pH greater than 8.

The effect of potassium cyanate was investigated by adding the enzyme to solutions of 8 M urea containing cyanate. A rapid loss of activity was observed by treatment with 0.5 M KCNO, but the rate of loss was reduced considerably by using lower concentrations of potassium cyanate. Only about 15–20% of the initial activity was lost in 0.05 and 0.20 M KCNO under these conditions after 60-min incubation. It cannot be determined whether the inactivation was due to modification of a crucial catalytic residue necessary for activity or to the modification of groups which are necessary to proper three-dimensional folding. The loss of activity observed during long periods of incubation in 8 M urea was probably due to the presence of low concentrations of cyanate ion either present initially or formed during the course of the incubation in urea. The enzyme was also shown to be more slowly inactivated by similar concentrations of KCNO in the absence of urea.

Preparative Gel Electrophoresis in 8 M Urea. Since a better separation of at least the minor bands could be obtained in analytical gel electrophoresis in 8 M urea and the enzyme was shown to withstand urea treatment for reasonably long

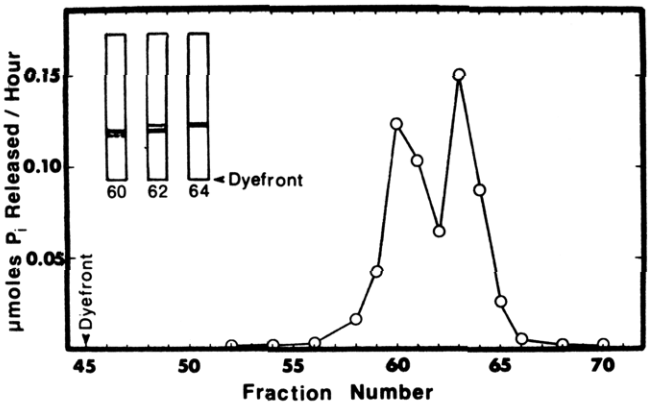

FIGURE 1: Polyacrylamide preparative gel electrophoresis. The enzyme after two prior separations on preparative gel electrophoresis, one in 8 M urea, was run in 10° as described in the text and assayed for activity. The inset shows schematically the results of analytical gel electrophoresis on fractions 60, 62, and 64. The fractions in the peak on the left are referred to as fraction 1 and those of the right-hand peak as fraction 2.

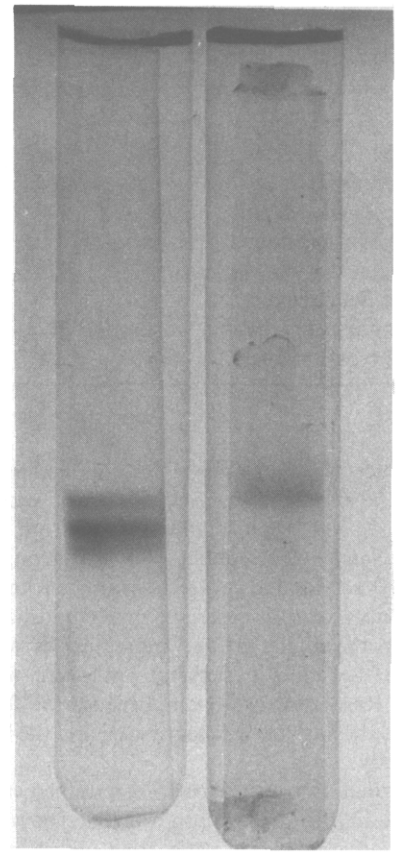

FIGURE 2: Analytical gel electrophoresis of the preparation after urea gel electrophoresis. A typical gel is on the left with a fast-moving minor band. Some preparations have been obtained with only two bands indicated on the right-hand gel.

periods of time at 10° with good recovery of activity, preparative polyacrylamide gel electrophoresis was carried out in 8 M urea. A single peak of phosphatase activity was again found to elute approximately 4 hr after the dye band. The recovery of activity from this procedure was about 50% of the applied activity. No separations of two peaks of activity were seen with this procedure. However, if this material were placed on a preparative gel electrophoresis column (T = 7.7°, c = 2.6), separation into two peaks (fraction 1 and 2) could then be demonstrated (Figure 1). Less than 200 µg of protein was recovered and due to the small amount available, the specific activity was not measured. Figure 2 shows the analytical gels of the preparation after the urea preparative gel electrophoresis runs; fraction 1 has an identical mobility on analytical gels to the fast-moving major band observed after the first preparative electrophoresis run and fraction 2 corresponds to the slower moving band. A minor band is the fastest moving component.

Molecular Weight of Native Enzyme. A Sephadex G-100 column was calibrated with proteins of known molecular weight and a standard plot was constructed. The molecular weight of the enzyme after three different stages of purification was estimated to be 38,000. The presence of 1 mM $MnCl_2$ in the elution buffer has no effect on the molecular weight of the yeast enzyme in contrast to its effect on the *Salmonella* bifunctional enzyme (Brady and Houston, 1973).

Subunit Composition. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was used to determine the subunit composition of the enzyme. A molecular weight of 38,000 was estimated from a standard plot. Therefore, histidinol phosphate phosphatase from yeast is a monomeric enzyme of 38,000 molecular weight.

Effect of pH. The pH profile for fraction 1 and 2 enzyme are not significantly different as shown in Figure 3. The optimum pH for activity is 8.8 and drops sharply on either side of this value. The curve is not affected by the presence of 1 mM manganese chloride. Houston (1973b) has shown that the pH profile of histidinol phosphate phosphatase from strain TR691 of *Salmonella typhimurium* is significantly affected by Mn^{2+}, although the native enzyme from strain *his01242* is not (Brady and Houston, 1973).

Effect of Heating. The enzyme after the first preparative gel electrophoresis was incubated at the desired temperature for 10 min and immediately put into an ice bath. After all samples were collected, they were assayed at 37° by the standard procedure. The enzyme is quite stable up to about 60° (Figure 4), but complete loss of activity occurs at 80°. The inclusion of 17 mM histidinol seemed to increase the rate of inactivation, whereas 17 mM aminotriazole had little effect.

Kinetic Properties. The K_m of histidinol phosphate for fractions 1 and 2 were evaluated using a double-reciprocal plot. The plots were linear and showed no differences in the K_m value (0.25 mM) between the two fractions. A similar value (0.38 mM) was obtained with the preparation after the first preparative gel electrophoresis. The effect of pH over a wide range on the K_m value was evaluated as shown in Figure 5. A titratable group of approximately pK_a = 9.3, either on the substrate or the enzyme, can be implicated by this data. It is possible that this pK_a is reflecting the ionization state of the amino group of histidinol phosphate.

Histidinol phosphate phosphatase from *S. typhimurium* is very sensitive to histidinol and has a competitive inhibition constant of 30–50 µM (Brady and Houston, 1973; Houston, 1973b). In contrast, a competitive inhibition contrast for histidinol was found to be 5–10 mM for various preparations of purified yeast phosphatase using both a Dixon plot and a double reciprocal plot. Preparations at different stages of purification showed similar results. Histidine is a competitive inhibitor of the *Salmonella* enzyme (Brady and Houston, 1973); no inhibition with 7 mM histidine could be demonstrated

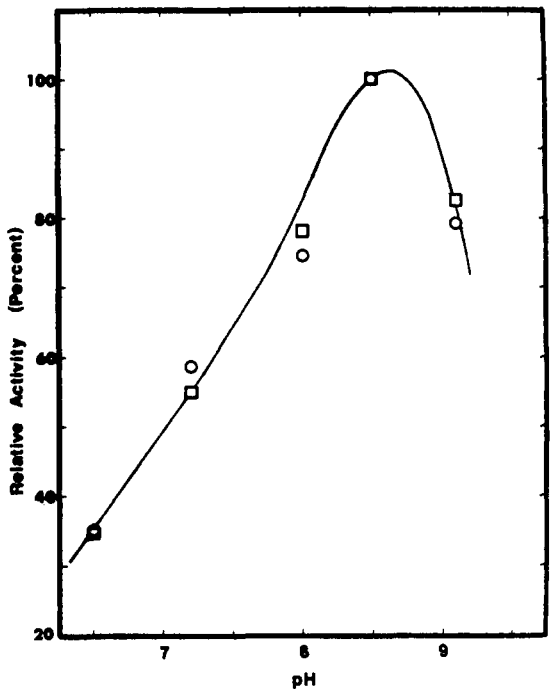

FIGURE 3: pH–activity relationship for fractions 1 and 2. Assays were carried out at 37° in 0.1 M triethanolamine (titrated to pH with HCl) containing 1.67 mM histidinol phosphate. Both fraction 1 (○) and fraction 2 (□) were assayed.

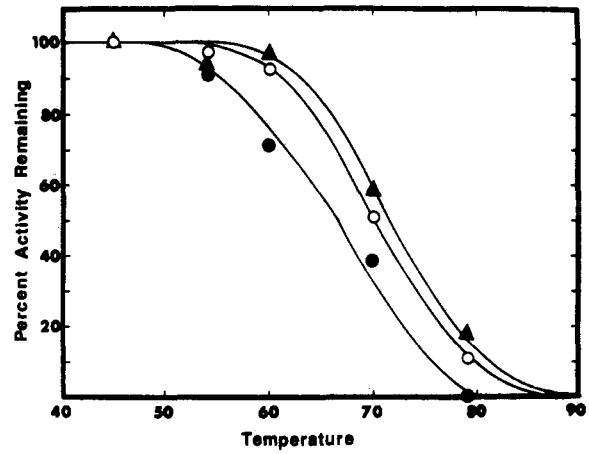

FIGURE 4: Effect of heating on phosphatase activity. The enzyme in standard buffer (○), 17 mM histidinol in buffer (●), or 17 mM aminotriazole in buffer (▲) was heated for 10 min at the desired temperature, cooled in ice for 1 min, and then assayed for phosphatase activity. The activity for an unheated control was determined for each point.

with the yeast enzyme. Imidazoleglycerol phosphate had no effect at a concentration of 4.3 mM, nor did 1.7 mM aminotriazole or 15 mM phosphate (determined using the coupled assay procedure of Brady and Houston, 1972).

Discussion

Through the use of a rather unusual purification procedure employing polyacrylamide gel electrophoresis in 8 M urea, two forms of histidinol phosphate phosphatase have been isolated from baker's yeast. The purification depends upon the fact that the enzyme is unchanged by the urea treatment as detected by both kinetic and electrophoretic analysis. Carbamylation by cyanate during urea treatment is a possibility even though fresh reagents were used together with a low temperature during the work-up. Nevertheless, neither of the electrophoretic or kinetic parameters are demonstrably different before and after the separation in 8 M urea. We have no explanation as to why separation of two forms of the enzyme can be attained after urea gel electrophoresis, but not before. The removal of some of the impurities may be important. Only one gene has been identified in yeast (Fink, 1964), although it is possible that two alleles are present. However, the same electrophoretic patterns are seen throughout the purification if a pure strain of yeast is used for purification. Artifacts that occur in the work-up before electrophoretic separation are always a possibility, such as deamination, which might possibly give rise to the electrophoretic variant we observe.

Proteolysis may serve as an explanation for the two forms of the histidinol phosphate phosphatase isolated from a nonsense mutant of the *hisB* gene of *S. typhimurium* (Houston, 1973b), but this would not serve to explain the findings in this work since both enzymes appear to have identical molecular weights in the native and denatured state. Proteolysis near the end of the polypeptide chain might go unnoticed upon molecular weight analysis by Sephadex chromatography, but would be apparent upon electrophoresis. The two forms seem to be present in roughly equal amounts as judged by the intensity of staining on analytical gels. Isolation using proteolytic inhibitors has not been attempted. In the absence of any other more definitive information, we cannot draw any conclusions about the relationship between the two phosphatases.

It is puzzling that the enzyme is not appreciably inhibited by histidinol since the *Salmonella* enzyme exhibits tight binding for histidinol (Brady and Houston, 1973; Houston, 1973b). This may reflect important changes in the catalytic and binding residues participating in the active site of the enzymes. The specific activity of the mutant enzymes isolated from *S. typhimurium* differ substantially from the yeast enzymes. In yeast, the specific activity shown here is more than 100-fold greater than that found in the purified *Salmonella* mutant enzymes (Houston, 1973b), indicative of the occurrence of some modification in the catalytic site during the process of evolution. These changes are not reflected in the K_m values, both yeast and *Salmonella* enzymes having similar values. The higher specific activity of the yeast enzyme may account for

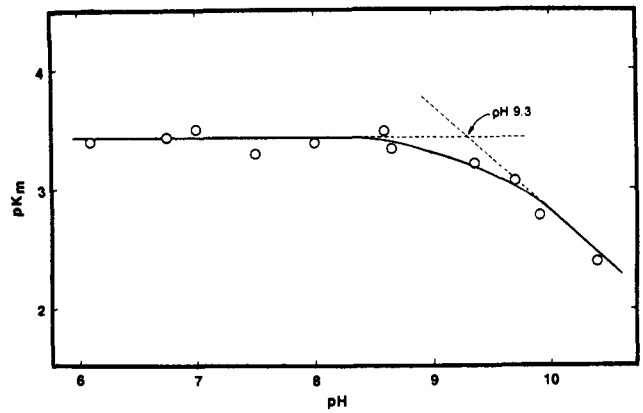

FIGURE 5: Effect of pH on K_m of histidinol phosphate. The buffer was 0.1 M triethanolamine titrated to pH with HCl at pH 7.0–8.0 and 0.1 M Tris titrated with succinic acid from pH 6.1 to 6.8 and with HCl from pH 8.6 to 10.5.

the fact that apparently so little of the enzyme is present in the cell. The *Salmonella* and yeast enzymes also differ markedly in their sensitivity to heat. In contrast to the heat stable yeast enzymes, the *Salmonella* enzyme is extremely heat sensitive (Brady and Houston, 1973). The *Salmonella* enzyme is very sensitive to even low concentrations of urea[2] in contrast to the relative stability of the yeast enzyme.

An interesting relationship would seem to exist between the yeast phosphatase and the *hisB* bifunctional enzyme of *S. typhimurium* first described by Hartman *et al.* (1960). A subregion of *hisB* has been identified on the amino terminal end of the gene (operator-proximal) which can alone specify a polypeptide having phosphatase activity and having roughly one-half the length of the entire gene product (Houston, 1973a). Although the smaller of the two forms isolated from *Salmonella* has two subunits of identical size, the native enzyme has a molecular weight of 38,000 (Houston, 1973b); exactly the same as shown there for the yeast enzyme. It would appear that the "splitting" of the bifunctional *Salmonella* gene into two genes in yeast controlling single activities results in a monofunctional phosphatase and represents nearly all of the genetic region specified by the amino-terminal subregion of *hisB* in *S. typhimurium*.

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