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Specific Ion Effects in Affinity Chromatography. The Case of L-Histidinol-phosphate Aminotransferase†

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ABSTRACT: Specific ion effects provide an additional parameter which can be adjusted for optimizing the purification of a protein by affinity chromatography. This principle is illustrated in the case of L-histidinol-phosphate aminotransferase (EC 2.6.1.9) from *Salmonella typhimurium,* which was purified 15-fold in one step by chromatography on L-histidinol phosphate coated agarose. The choice of ions in the buffer and the adjustment of their concentration dramatically affect the elution pattern of the column. A 0.7 M Na_2HPO_4 buffer adjusted to pH 7 with citric acid promotes adsorption of the enzyme onto the column, while subsequent dilution of the same buffer results in selective desorption.

The unique affinity of an enzyme for its substrates, inhibitors, cofactors, or other physiological effectors provides a variety of specific interactions which can be used in the design of efficient columns for enzyme purification (Lerman, 1953; Arsenis and McCormick, 1964, 1966; McCormick, 1965; Cuatrecasas *et al.,* 1968; Wilchek and Gorecki, 1969). Initially applied to only a few enzymes, this approach is now extensively used due to the introduction of simple, widely applicable procedures for the activation of beaded agarose (Axen *et al.,* 1967; Porath *et al.,* 1967) and for binding to it a large variety of ligands (Cuatrecasas, 1970; Feinstein, 1971; Cuatrecasas and Anfinsen, 1972).

The choice of the solid matrix, the ligand, and the mode of binding of the ligand to the matrix were all shown to be highly important for achieving efficient and selective retention of a desired protein. Ideally, the desorption of bound protein would be mildest and most efficient if a high affinity substrate analog is used. Very often, however, this is not feasible, either because such a compound is not available, or because it is prohibitively expensive. In most cases, elution of the protein from the column is performed by altering the pH of the buffer, changing its ionic strength, or by using a protein denaturant such as guanidine hydrochloride or urea (Cuatrecases and Anfinsen, 1972).

Several years ago, while studying the process of resolution of glycogen phosphorylase, it was shown that specific ions may act as "deforming agents," *i.e.,* loosen or tighten the structure of presumably unique sites in proteins (Shaltiel *et al.,* 1966). In principle, such specific ion effects could be utilized for optimizing retention and elution of proteins during affinity chromatography. In fact, the possible use of such specific ion effects in protein chromatography was recently shown in the desorption of glycogen phosphorylase from a hydrophobic chromatography column (Er-el *et al.,* 1972; Shaltiel and Er-el, 1973).

This paper illustrates the use of specific ion effects both for achieving retention of an otherwise excluded enzyme and for its subsequent desorption from an affinity chromatography column. The enzyme used, L-histidinol-phosphate aminotransferase (EC 2.6.1.9), catalyzes an essential step (the conversion of imidazoleacetol phosphate to L-histidinol phosphate) in the biosynthesis of L-histidine (Ames *et al.,* 1967), and was recently purified from *Salmonella typhimurium* in a crystalline homogeneous form (Henderson and Snell, 1973).

Studies on the effect of various salts on the activity of the enzyme had shown (G. B. Henderson and E. E. Snell, manuscript in preparation) that it is inhibited by polyvalent ions, such as phosphate or sulfate, and that these ions affect the rate of removal of pyridoxal phosphate from the enzyme, suggesting that phosphate and sulfate have a pronounced effect on the structure of the enzyme.

Materials and Methods

Preparation of Cell Extracts. The histidine auxotroph *S. typhimurium hisE11* was used as a source of L-histidinephosphate aminotransferase. The cells were grown and disrupted as described previously (Henderson and Snell, 1973), and the resulting homogenate was dialyzed against a buffer composed of sodium acetate (25 mM) and 2-mercaptoethanol (25 mM), adjusted to pH 5.0 with HCl. The precipitate was removed by centrifugation for 60 min at 30,000*g* (4°). The clarified protein solution was adjusted to pH 7.5 with solid Tris and stored at 4°.

Crystalline L-histidinol-phosphate aminotransferase (for reference purposes) was prepared by the method of

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Henderson and Snell (1973). The preparation used had a specific activity of 53 units/mg and was homogeneous when subjected to gel electrophoresis.

Apoenzyme. Removal of pyridoxal 5'-phosphate from L-histidinol-phosphate aminotransferase was carried out at pH 10 using the phenylhydrazone method described previously (Henderson and Snell, 1973). The potential catalytic activity of the apoenzyme was measured after re-formation of the holoenzyme by incubation (10 min, 37°) with 20 mM pyridoxal 5'-phosphate in an assay mixture devoid of L-histidinol-phosphate.

Enzyme Assay. The procedure of Ames *et al.* (1960) as modified by Henderson and Snell (1973) was used for the assay of L-histidinol-phosphate aminotransferase. Enzyme units represent micromoles of imidazoleacetol phosphate formed per minute at 37°.

Protein concentrations in crude extracts were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. The concentration of pure or partially purified enzyme (specific activity >5 units/mg) was determined spectrophotometrically using a molar absorption $A_{280nm}^{1\%} = 10.6$ (Henderson and Snell, 1973).

Gel electrophoresis was carried out at pH 8.9 and 4° in 7.5% polyacrylamide as described by Davis (1964). Gels were stained for protein with 0.25% Coomassie Blue (20 hr, 23°) and destained electrophoretically. An activity stain was used for detection of L-histidinol-phosphate aminotransferase on the gels (Henderson and Snell, 1973).

L-Histidinol Phosphate Coated Agarose. Packed Sepharose 4B (20 ml) was suspended in 40 ml of water and activated at pH 11 by the addition of 2 g of CNBr dissolved in 3 ml of dioxane (Axen *et al.*, 1967). The reaction was allowed to proceed with gentle swirling for 8 min at 22°, maintaining the pH between 10.6 and 11 by the addition of 5 N NaOH. Activation was terminated by filtration and washing with 500 ml of ice-cold deionized water. The activated agarose was then suspended in 30 ml of cold 0.1 M $NaHCO_3$, pH 9.0 (4°). A sample of 250 mg of L-histidinol phosphate · $2H_2O$ (Cyclo) dissolved in 5 ml of the same bicarbonate buffer was added to the activated agarose and the reaction mixture was readjusted to pH 9.0. Coupling was allowed to proceed for 25 hr at 4°, with gentle swirling. Excess reagent was then removed by filtration and washing with (a) 500 ml of water, (b) 100 ml of 0.1 M $NaHCO_3$ (pH 9.0), (c) 200 ml of water, (d) 100 ml of 0.05 M CH_3COOH, and (e) 200 ml of water and finally with 300 ml of the buffer used for chromatography. Each of the washings was tested for residual unbound ligand (by ninhydrin) to ascertain that all excess of L-histidinol phosphate was removed.

The amount of ligand coupled to the agarose was determined after hydrolysis (6 N HCl, 110°, 22 hr) of a sample of the washed column material. The hydrolysate was filtered through a Millipore filter and the ninhydrin-positive material in it was determined by the chromatographic procedure described previously (Shaltiel and Patchornik, 1963). A reference sample containing agarose mixed with a known amount of L-histidinol phosphate was similarly treated to determine losses of ninhydrin-positive material during hydrolysis. Using this procedure it was established that the various batches of L-histidinol phosphate coated agarose used throughout this work contained between 17 and 34 μmol of ligand/ml of packed agarose.

Other Affinity Chromatography Columns. Four additional types of affinity chromatography columns were prepared by the foregoing procedure, replacing L-histidinol phosphate with one of the following ligands: (a) *O*-phosphoethanolamine (Sigma), 4 g dissolved in 40 ml of the bicarbonate buffer; (b) L-histidine (Nutritional Biochemical Co.), 6 g in 50 ml of buffer; (c) L-glutamic acid (Nutritional Biochemical Co.), 6 g in 100 ml of buffer; (d) carnosine (Aldrich), 2 g in 100 ml of buffer.

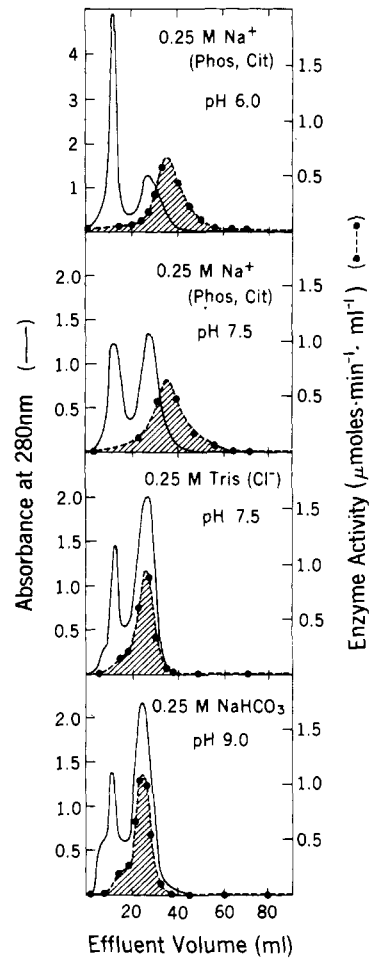

FIGURE 1: Effect of various buffers in the affinity chromatography of the enzyme on an L-histidinol phosphate column. Buffers used: (A) 0.25 M Na_2HPO_4 adjusted to pH 6.0 with citric acid: (B) 0.25 M Na_2HPO_4 adjusted to pH 7.5 with citric acid; (C) 0.25 M Tris adjusted to pH 7.5 with HCl; (D) 0.25 M $NaHCO_3$ adjusted to pH 9.0 with NaOH.

General Procedure for Column Chromatography. Unless indicated otherwise, the affinity columns used were 1.6 cm in diameter and 8–10 cm long. They were equilibrated in each case with the buffer indicated, which included also 10 mM 2-mercaptoethanol. Samples (1 ml) of the bacterial extract described above (protein concentration, 27 mg/ml; specific activity, ~0.4 unit/mg) were dialyzed against 100 ml of the appropriate buffer and applied on the column. Chromatography was carried out at 22–24°. Fractions of 1.5–2.0 ml were collected and their absorption at 280 nm, as well as their L-histidinol-phosphate aminotransferase activity, were monitored.

Between runs, the column was washed with high salt (1 M NaCl, ~100 ml) followed by water (~300 ml). After several runs, washing with 0.05 M $NaHCO_3$ (pH 9.5, 100 ml) or, if necessary, with 0.05 M NaOH (100 ml) was carried out, to restore the flow rate of the column.

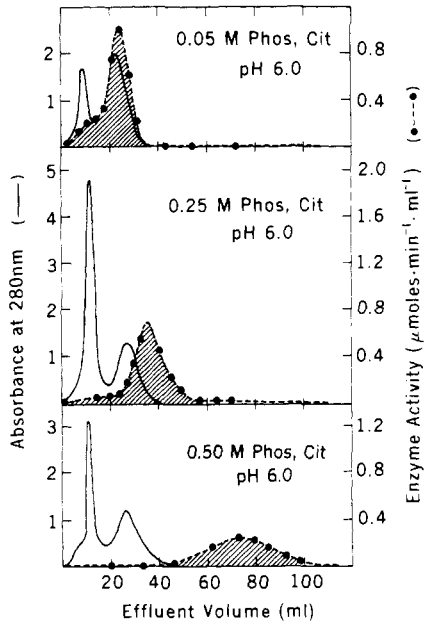

FIGURE 2: Increased retardation of L-histidinol-phosphate aminotransferase upon increasing the concentration of a phosphate-citrate buffer. Buffers used contained Na_2HPO_4 (at the indicated concentration) and were adjusted to pH 6.0 with citric acid.

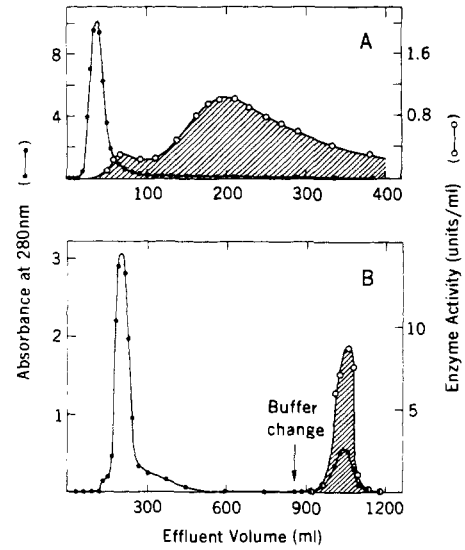

FIGURE 3: Preparative scale purification of the enzyme on L-histidinol phosphate coated agarose. (A) A sample of extract (12.4 ml; protein concentration, 33.2 mg/ml; specific activity, 0.49 unit/mg) was applied on a column (8 × 1.8 cm) equilibrated and run at 23° with a buffer composed of 0.5 M Na_2HPO_4 and 0.2 M citric acid, and adjusted to pH 7.5 with NaOH. (B) A sample of extract (66 ml; protein concentration, 5.7 mg/ml; specific activity, 0.97 unit/mg) was applied on a column (25 × 2.5 cm) equilibrated at 23° with a buffer composed of 0.7 M Na_2HPO_4 adjusted to pH 7.0 with citric acid. Unadsorbed protein was washed off and then the column was washed with a buffer composed of 0.05 M Na_2HPO_4 adjusted to pH 7.0 with citric acid.

Results

In an attempt to establish optimal conditions for purification of L-histidinol-phosphate aminotransferase by affinity chromatography, we applied samples of the crude extract obtained from *Salmonella typhimurium* on columns of L-histidinol phosphate coated agarose, and followed the protein elution pattern, as well as the aminotransferase activity.

Initially, the column was run with three different buffers: phosphate-citrate (pH 6.0), Tris (pH 7.5), and bicarbonate (pH 9.0). As seen in Figure 1, the enzyme was somewhat retarded when the column was run with the pH 6.0 buffer. However, this seemed to be associated at least in part with the buffer itself rather than its pH, since a similar retardation occurred also at pH 7.5, if the Tris buffer was replaced by a phosphate-citrate buffer of the same pH (Figure 1). Moreover, the retardation became more and more pro-

TABLE 1: Factors Affecting the Retardation of L-Histidinol-phosphate Aminotransferase by L-Histidinol Phosphate Coated Agarose.[a]

Factor Varied	Eluent Composition	pH	Position of Enzyme Peak[b] (ml)
Ionic strength and ion composition	(I) Na_2HPO_4 (0.05 M); citric acid[c]	6.0	23
	(II) Na_2HPO_4 (0.05 M); NaCl (0.9 M); citric acid[c]	6.0	24
	(III) Na_2HPO_4 (0.05 M); Na_2SO_4 (0.45 M); citric acid[c]	6.0	58
	(IV) Na_2HPO_4 (0.05 M); $(NH_4)_2SO_4$ (0.45 M); citric acid[c]	6.0	49
	(V) Na_2HPO_4 (0.5 M); citric acid[c]	6.0	72
	(VI) Na_2HPO_4 (0.5 M); succinic acid[c]	6.0	52
	(VII) Na_2HPO_4 (0.5 M); acetic acid[c]	6.0	41
pH	(VIII) Na_2HPO_4 (0.5 M); citric acid[c]	6.0	73
	(IX) Na_2HPO_4 (0.5 M); citric acid[d]	7.0	>100[e]
	(X) Na_2HPO_4 (0.5 M); citric acid[d]	8.0	>120[f]
Presence of a substrate analog	(XI) Na_2HPO_4 (0.5 M); citric acid[c]	6.0	72
	(XII) As XI + L-histidine (0.1 M)[g]	6.0	53
Presence of the coenzyme	(XIII) Na_2HPO_4 (0.5 M); citric acid[c]	6.0	73 (holoenzyme)
	(XIV) Na_2HPO_4 (0.5 M); citric acid[c]	6.0	61 (apoenzyme)

[a] For further details on the procedure see Materials and Methods. [b] Greater retardation of the enzyme was accompanied by a spread of enzyme peak (as exemplified in Figure 2). [c] At the concentration required for adjusting the pH to 6.0. [d] This eluent was prepared from VIII by adding solid NaOH, so that the final concentration of phosphate and citrate ions was identical in eluents VIII, IX, and X. [e] Some enzyme activity started to emerge after an effluent volume of 100 ml. [f] No enzyme activity emerged up to an effluent volume of 120 ml. [g] The pH was readjusted to 6.0 with solid NaOH.

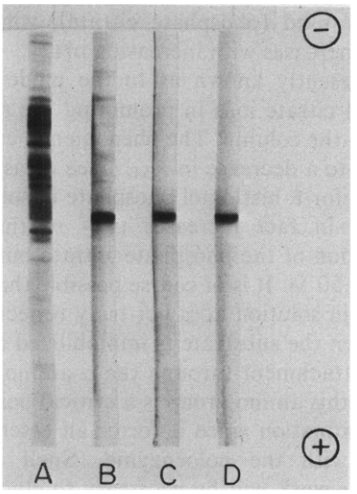

FIGURE 4: Purification of L-histidinol-phosphate aminotransferase as monitored by disc gel electrophoresis. Gel A contained 54 μg of crude extract; B contained 10 μg of enzyme purified on L-histidinol phosphate coated agarose; C and D contained 4.5 and 0.9 μg, respectively, of crystalline enzyme. Gels A–C were stained for protein with Coomassie Blue and gel D was stained for enzymatic activity.

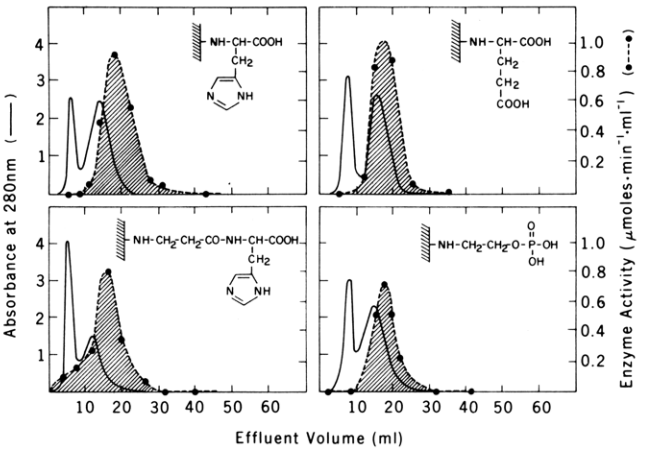

FIGURE 6: Comparison between various agarose derivatives in their ability to retard L-histidinol-phosphate aminotransferase; buffer used: 0.5 M Na_2HPO_4 adjusted to pH 6.0 with citric acid. Note that under the same conditions, the L-histidinol phosphate column exhibited a greater retardation (Figure 2, lowest panel).

nounced upon increasing the concentration of the phosphate–citrate buffer while keeping the pH constant (Figure 2). This increased retardation could not be attributed merely to the increase in ionic strength. As seen in Table I there is no change in the position of the enzyme peak when the ionic strength of the 0.05 M phosphate–citrate buffer (pH 6.0) was increased by adding NaCl up to 0.9 M. The capacity to retard the enzyme on the column was not restricted to the phosphate–citrate system. Sulfate or phosphate ions alone were also effective (Table I). Nevertheless, the citrate ions, being polyvalent, do contribute to the retardation; with a series of carboxylic acids used to adjust the pH of 0.5 M Na_2HPO_4 to 6.0 it was found that the retardation of the enzyme increased with increasing valency (acetate < succinate < citrate, Table I).

Once it was established that the original observations regarding the effect of pH on the elution pattern of the enzyme were associated with the nature of the buffer and its concentration, we reexamined the effect of pH with three buffers which contained identical concentrations of phosphoric and citric acid and were adjusted to three different pH values with NaOH. In contrast to the results obtained initially (decreased retardation with buffers of higher pH,

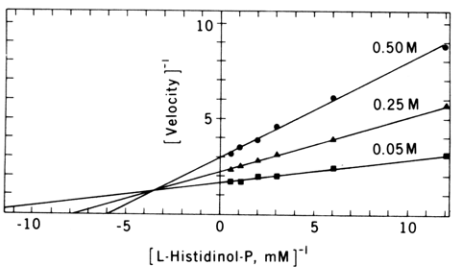

FIGURE 5: Effect of the phosphate–citrate buffer on the K_M and V_{max} of the enzyme. The activity of the enzyme in the presence of various concentrations of L-histidinol phosphate was determined in three different buffers containing the indicated concentrations of phosphate–citrate as well as α-ketoglutarate (6.7 mM), EDTA (6.7 mM), and 2-mercaptoethanol (10 mM, pH 7.5). The enzyme activity (V) is measured by the change in absorbance at 284 nm after 15 min (37°) following enolization of the imidazoleacetol phosphate (Henderson and Snell, 1973).

Figure 1) it was now possible to show that in a phosphate–citrate system, raising the pH favors retardation (Table I).

On the basis of these observations, it was attempted to purify the enzyme on a preparative scale using a buffer composed of 0.5 M Na_2HPO_4 and 0.2 M citric acid adjusted to pH 7.5 with NaOH. As seen in Figure 3A, purification was achieved, but the enzyme activity emerged in many fractions resulting in a large volume of very dilute enzyme. By increasing the buffer concentration (to 0.7 M Na_2HPO_4 adjusted to pH 7.0 with citric acid) it was possible to retain the enzyme on the column even at a lower pH. At this point, desorption of the enzyme was achieved by simply lowering the concentration of the eluting buffer to 0.05 M. The enzyme then emerged from the column in a relatively small volume (Figure 3B), and was considerably purified as judged by gel electrophoresis (Figure 4). On the basis of activity measurements, it was established that a 15.5-fold purification was obtained in one step, with a quantitative yield.[1]

The simplest way through which the polyvalent anions could promote retardation of the enzyme on the column would be to increase the affinity of the enzyme for its substrate. However, this need not be the case: when the K_M of the enzyme for L-histidinol phosphate was measured at various concentrations of the phosphate–citrate buffer, it was found (Figure 5) that the affinity of the enzyme for its substrate actually decreased with increasing concentration of the buffer: at 0.05 M K_M = 0.07 mM, while at 0.5 M K_M = 0.17 mM.

For affinity chromatography of this enzyme, L-histidinol phosphate seems to be a much better ligand than another substrate of the enzyme (L-glutamic acid) or other ligands which contained only part of the structural features of L-histidinol phosphate: *O*-phosphoethanolamine, L-histidine, or carnosine (*cf.* Figure 6 with Figure 2, lowest panel).

If 0.1 M L-histidine (which competes for the active site) is included in the buffer, the enzyme emerges from the column somewhat earlier than it would otherwise (Table I), as one would expect with a column that retards the enzyme by

[1] The yield found was 125%, based on activity measurements. This most probably reflects inaccuracies in measuring specific activities in crude preparations, which may contain inhibitory components.

specific interactions with its active site. However, this effect is not very pronounced, probably since the affinity of the enzyme for L-histidine (K_M = 7.5 mM) is considerably lower than its affinity for L-histidinol phosphate (K_M = 0.2 mM) (Albritton and Levin, 1970).

It is interesting to note that apo-L-histidinol-phosphate aminotransferase, which lacks an indispensable constituent of the active site, is still retarded by the column, although to a less extent than the enzyme (Table I). This result suggests that even after removal of the coenzyme, the inactive protein still recognizes L-histidinol phosphate. This is in agreement with the observation of Henderson and Snell (manuscript in preparation) that L-histidinol phosphate retards the reconstitution of the apoenzyme with pyridoxamine phosphate.

Discussion

This paper illustrates the dramatic effect of specific ions on the elution pattern of an affinity chromatography column. By taking advantage of such effects it was possible to optimize both the adsorption and desorption of L-histidinol-phosphate aminotransferase during affinity chromatography on L-histidinol phosphate coated agarose. The finding that specific ions which have a pronounced effect on the structure of glycogen phosphorylase *b* (Shaltiel *et al.*, 1966) are effective in the desorption of the enzyme during hydrophobic chromatography (Er-el *et al.*, 1972) indicates that this is probably not a unique case. There are numerous examples of enzymes which are sensitive to specific buffers (or ions) under certain conditions. These buffers affect the structure of the enzyme as reflected in its activity, aggregation state, or stability. Screening of such buffers seems to be a promising approach in the design of conditions for affinity chromatography.

The use of specific ion effects is not limited to elution, as in the case of glycogen phosphorylase *b*. In the present study it is shown that they can be utilized to cause retardation of an otherwise excluded enzyme, and, by further raising the concentration of these ions, to bring about selective adsorption. Optimal adsorption was achieved with a rather high buffer concentration (0.7 M Na_2HPO_4, adjusted to pH 7 with citric acid).

The possibility that the phosphate ions would be interacting directly with the L-histidinol phosphate binding site seems remote, as it was previously shown (G. B. Henderson and E. E. Snell, manuscript in preparation) that this polyvalent ion is a noncompetitive inhibitor of the enzyme. However, this does not exclude the possibility that the polyvalent ions act at a vicinal subsite, *e.g.*, that involved in binding the second substrate, α-ketoglutarate, which is also a polyvalent anion.

Since the substrate bound on the column contains ionized functional groups, it is reasonable to assume that ionic interactions could be involved in the recognition of the substrate by the enzyme. If so, then increasing the concentration of the buffer should decrease the affinity of the enzyme for the column, as it raises the ionic strength of the medium. This should promote desorption. The finding that adsorption is in fact promoted suggests that other effects of the phosphate–citrate buffer on the enzyme counteract the mere increase in ionic strength.

While choosing conditions for optimal purification it was observed that the retardation of the enzyme on the column would occur at low pH. This initial observation was found to be misleading, as the retardation was in fact due to the specific buffer used (phosphate–citrate), whose effectiveness actually increases with increasing pH.

Little is presently known as to the mode of action of phosphate and citrate ions in promoting the retardation of the enzyme on the column. The phenomenon cannot be simply attributed to a decrease in K_M, since measuring the K_M of the enzyme for L-histidinol phosphate in solution showed that the K_M in fact increases two- to threefold when the concentration of the phosphate–citrate buffer increases from 0.05 to 0.50 M. It is of course possible that this type of measurement in solution does not truly reflect the changes in affinity when the substrate is immobilized and sterically hindered by attachment through the α-amino group to the bulky matrix; this amino group is a critical point of binding during transamination since it forms an essential covalent intermediate with the holoenzyme (Snell and DiMari, 1970). Further work will be necessary to elucidate the detailed mechanism involved here.

The preparative procedure reported in this paper is quantitative and results in 15.5-fold purification in the affinity chromatography step (Figure 3B). By dialyzing the crude extract at pH 5.0 prior to chromatography, a 47-fold overall purification is achieved. However, the enzyme obtained has a specific activity of 15 units/mg compared with 53 units/mg for the crystalline enzyme. An additional purification step, using an ω-aminoalkylagarose column described in the following paper in this issue (Henderson *et al.*, 1974), results in an essentially pure enzyme.

Use of specific ion effects in affinity chromatography has the advantage of being much less drastic than extreme changes in pH or exposure to denaturing agents such as urea or guanidine. The effects of such ions are often fully reversible, increasing the chances of isolating an undamaged enzyme which retains its native structure.

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ω-Aminoalkylagaroses in the Purification of L-Histidinol-phosphate Aminotransferase†

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ABSTRACT: A homologous series of ω-aminoalkylagaroses was tested in the purification of L-histidinol-phosphate aminotransferase (EC 2.6.1.9) from *Salmonella typhimurium.* Out of this series, the hexyl derivative was found to be the most suitable for this purpose, providing a purification of 15-fold in one step. This purification factor is very similar to that obtained by affinity chromatography. Combining the biospecific step with chromatography on ω-aminohexylagarose results in an enzyme preparation which is homogeneous when subjected to gel electrophoresis and has a specific activity of 40 units/mg, compared with 53 units/mg for the crystalline enzyme.

Using classical methods of enzyme purification (Henderson and Snell, 1973), L-histidinol-phosphate aminotransferase (EC 2.6.1.9) from *Salmonella typhimurium* was recently purified to homogeneity. In the preceding paper (Shaltiel *et al.,* 1974) we have described a biospecific method for the purification of this enzyme, using L-histidinol phosphate coated agarose. By this procedure, a 15.5-fold purification was achieved in one step, but the enzyme had a specific activity of only 15 units/mg, compared with 53 units/mg reported for the crystalline enzyme (Henderson and Snell, 1973).

In view of the remarkable purifications achieved recently with homologous series of alkyl- or ω-aminoalkylagaroses (Er-el *et al.,* 1972; Shaltiel *et al.,* 1973; Shaltiel, 1974a,b), we attempted to apply these new columns to the purification of L-histidinol-phosphate aminotransferase and thus compare their effectiveness with that of affinity chromatography.

Materials and Methods

Preparation of crude extracts from *Salmonella typhimurium,* assay of L-histidinol-phosphate aminotransferase, determination of protein concentrations, and gel electrophoresis were carried out by the methods quoted in the preceding paper (Shaltiel *et al.,* 1974). ω-Aminoalkylagaroses were prepared as described previously (Shaltiel and Er-el, 1973) except for the following modifications: the α,ω-diaminoalkane was dissolved in each case in a solvent composed of equal volumes of *N,N*-dimethylformamide and 0.1 M $NaHCO_3$ (pH 9.0) and the pH of the solution was readjusted to that value, if necessary. If a precipitate formed (with the higher members in the series) the suspension was warmed to 60° and then cooled rapidly before mixing with the activated agarose. After coupling at 22° and washing, each column was equilibrated with the buffer chosen for chromatography. DEAE-cellulose (DE 52) was purchased from Whatman.

Results

In order to establish which member within the homologous series Seph-C_n-NH_2[1] is most suitable for the purification of L-histidinol-phosphate aminotransferase, we used an exploratory kit (Shaltiel, 1974a,b) containing eight different small columns of ω-aminoalkylagaroses. These agarose derivatives differed in the number of carbon atoms (n) in their hydrocarbon side chains. Aliquots of the crude extract (0.1 ml) were applied on each of these columns. The amount of excluded aminotransferase activity was measured and plotted as per cent activity *vs. n.* As seen in Figure 1A, Seph-C_6-NH_2 retained all the aminotransferase activity when the columns were run with buffer A described in the legend to the figure. However, if the same series of columns were run with a buffer which was identical with A but contained also 0.25 M NaCl (Figure 1B), part of the aminotransferase activity was excluded by Seph-C_6-NH_2, indicating that increasing the ionic strength decreases retention of the enzyme and thus promotes elution. Under the conditions where the enzyme is retained by Seph-C_6-NH_2, ~75%

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[1] Abbreviations used are: Seph-C_n-NH_2, Sepharose 4B activated with CNBr and reacted with an α,ω-diaminoalkane n-carbon atoms long.