Purification of human adult and foetal intestinal alkaline phosphatases by monoclonal antibody immunoaffinity chromatography

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We have used the technique of monoclonal antibody immunoaffinity chromatography to purify adult and foetal intestinal alkaline phosphatases. Pure adult intestinal enzyme was obtained from a crude tissue extract with a single immunoaffinity chromatographic step in yields exceeding 95%. An additional ion-exchange chromatographic step was necessary for purification of the foetal enzyme, but yields still exceeded 70%. Experiments to optimize the efficiency of the monoclonal antibody immunoaffinity chromatography procedure suggest that the relative strength of binding of an antibody to its antigen is the most important factor to consider when constructing such columns. A column made from an antibody of too low an avidity will not retain the enzyme, while one of too high an avidity will make elution of enzyme in the active state difficult. A scheme is suggested for the application of this technique to a general approach to enzyme purification.

The human alkaline phosphatases [ALPs; ortho-phosphoric-monoester-phosphohydrolase (alkaline optimum), EC 3.1.3.1] are a closely related family of membrane-bound glycoproteins that hydrolyse a variety of monophosphate esters at high pH optima. They are produced in different forms and at different levels in a number of tissues. Genetic, biochemical and immunological evidence indicates that at least three gene loci code for the protein moieties of the various ALPs (reviewed in Harris, 1981; and see Mulivor *et al.*, 1978c; Sargent & Stinson, 1979; McKenna *et al.*, 1979; Stinson & Sargent, 1981). Liver, bone and kidney ALPs (L/B/K ALP) are encoded by at least one gene (Mulivor *et al.*, 1978b; Fishman & Sie, 1971). Placental ALP (ALP_p) is encoded by a separate locus and is produced in large quantities by placental trophoblastic cells under control of the foetal genome (Robson & Harris, 1965, 1967). In addition, it is expressed at low levels in several adult tissues (Goldstein *et al.*, 1980, 1982). Another form of ALP is found in intestinal mucosa and is the product of at least one further locus.

Two forms of intestinal ALPs have been recognized (Mulivor *et al.*, 1978a; Sugiura *et al.*, 1981). Previous work has shown that the ALPs isolated from adult and foetal intestinal mucosa

(ALP_{A1} and ALP_{F1}) have similar thermostabilities and identical sensitivities to a variety of enzymic inhibitors, and give precipitin lines of identity in Ouchterlony double diffusion tests against a cross-reacting polyclonal rabbit antiserum raised to ALP_p. However, distinct electrophoretic differences between the enzymes can be demonstrated. In part, these differences are due to the presence of sialic acid on ALP_{F1} but not on ALP_{A1}. However, the desialylated enzymes still do not co-migrate.

To characterize better these two isoenzymes, we undertook to develop a protocol to purify them in high yield. Human ALP_{F1} and ALP_{A1} have been purified by other laboratories. In general, however, the isolation procedures have involved multiple chromatographic steps (including gel filtration, ion-exchange, and affinity chromatography with a variety of ligands), preparative electrophoresis, and chemical fractionations (Stinson & Sargent, 1981; Sugiura *et al.*, 1981; Khattab & Pfeleiderer, 1976; Lehman, 1975; Williams *et al.*, 1982; Komoda *et al.*, 1981). Yields have been low, with one report of 25%, but usually less than 2%, unsatisfactory when limited amounts of starting material are available. Furthermore, the complicated organic syntheses necessary to produce many of the affinity ligands introduce a variable which is often difficult to control in different laboratories. Kirschberger & Kopperschlager (1982) and Bouriotis & Dean (1981) have reported the use of several

Abbreviations used: ALP, alkaline phosphatase; SDS, sodium dodecyl sulphate.

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organic dyes as ligands for purification of calf intestinal ALP; however, additional steps were also necessary. Overall yields of 33% and 66% respectively were obtained. The use of these dyes for human intestinal ALP has not been reported.

We report the use of monoclonal antibody immunoaffinity chromatography to purify ALP_{AI} in a single chromatographic step with greater than 95% yield, and to purify ALP_{FI} in two chromatographic steps with greater than 70% yield. A general scheme is presented to aid in maximizing the efficiency of monoclonal antibody columns.

Materials and methods

Enzyme and protein assays

ALP activity was assayed as previously described (Mulivor *et al.*, 1978). 1 unit of ALP is defined as the amount of enzyme necessary to release 1 μ mol of *p*-nitrophenol in 1 min. Protein was assayed according to the method of Lowry *et al.* (1951).

Monoclonal antibody production

ALP-D98/Sp2/21, a monoclonal antibody produced against an intestinal-type ALP found in D98/AH-2 HeLa tissue culture cells, has been described (Wray & Harris, 1983). Ascites fluid, rich in antibody, was made by intraperitoneal injection of hybridoma cells into pristane-primed mice (Potter *et al.*, 1972). Relative binding of antibodies to the ALPs was determined by an electrophoretic titration technique as described by Gogolin *et al.* (1982).

Production of monoclonal antibody immunoaffinity column

A quantity of CNBr-activated Sepharose-4B (Pharmacia), as specified in the text, was activated by swelling in 600 ml of 0.001 M-HCl for 15 min, and the beads were washed with several volumes of 0.1 M-NaHCO₃ (pH 8.4)/0.5 M-NaCl (bicarbonate buffer). Ascites fluid was added to the beads and allowed to incubate for 4 h at room temperature with gentle mixing. Remaining active sites on the beads were blocked by the addition of solid glycine (Sigma) to 0.2 M for 2 h at room temperature. Unbound protein was removed by washing with three alternating cycles of 0.1 M-sodium acetate (pH 4.0)/0.5 M-NaCl followed by bicarbonate buffer. The beads were packed into a Pharmacia column (1.6 cm $\times$ 40 cm) and equilibrated with 0.01 M-Tris/HCl (pH 7.4)/0.0005 M-MgCl₂ (Tris/MgCl₂ buffer) containing 0.5 M-NaCl.

Purification of ALP_{FI}

Meconium, the first stool of the newborn, has been shown to be a rich source of ALP_{FI} (Mulivor

et al., 1978), and was used as the starting material for its purification. Meconium was mixed with an equal volume/weight of distilled water and one half volume/weight of butan-1-ol, and homogenized with a Polytron unit (Brinkman Instruments) to separate the ALP from lipids. The homogenate was incubated for 30 min at 37°C, centrifuged at 20000g at 4°C for 15 min, and the aqueous phase was collected and dialysed overnight against Tris/MgCl₂ buffer. The dialysed material was mixed overnight at 4°C in a batch-wise fashion with Whatman DE-52 that had previously been prepared according to the manufacturer's specifications. The next morning the beads were washed extensively with Tris/MgCl₂ buffer, then packed into a Pharmacia column (2.6 cm $\times$ 70 cm). ALP was eluted with a 0–0.2 M-NaCl linear gradient in Tris/MgCl₂ buffer (1200 ml total volume) at 4°C. Peak ALP activity fractions were pooled and directly applied to the antibody column in appropriate aliquots.

Usually, approx. 3×10^3 units of ALP activity (approx. 2 mg of enzyme protein) at a time were loaded onto the antibody column. The DE-52 ALP fraction was applied to the antibody column with ascending flow by a peristaltic pump at a rate of 1.0 ml/min. The column was washed extensively with 0.5 M-NaCl in Tris/MgCl₂ buffer until the A_{280} returned to baseline. ALP activity was eluted by applying 4 M-KSCN in Tris/MgCl₂ buffer to the column with ascending flow at a rate of 1.5 ml/min. Peak ALP activity fractions were pooled and immediately desalted on a Sephadex G-25 medium column (Pharmacia). The preparation was checked for purity on 10% SDS/polyacrylamide gels, and on 9.5% non-denaturing polyacrylamide gels stained for protein and activity as described below. If impurities were seen, the entire preparation was passed through the antibody column again to remove them. This was generally necessary when purification of greater than $(1-2) \times 10^3$ units of ALP at one time was attempted. Washing the column with 1 M-KSCN in Tris/MgCl₂ prior to elution with 4 M-KSCN aided in removing non-specifically bound protein in these cases. The antibody column could be regenerated for multiple uses by washing with the starting buffer.

Purification of ALP_{AI}

This was essentially identical with the purification of ALP_{FI}. Adult intestinal samples obtained at autopsy and stored at –20°C until use were homogenized with butanol as above, and the dialysed aqueous phase was tested for ALP before and after heating at 56°C for 1 h to inactivate any L/B/K ALP present. Different intestinal samples varied in ALP_{AI} content.

Separate antibody columns were used in the

purification of ALP_{AI} and ALP_{FI} to eliminate the possibility of cross-mixing. The intestinal extracts, containing $(1-3) \times 10^3$ units of ALP (0.6–2.0 mg of enzyme protein) were applied directly to the antibody column and eluted with 4M-KSCN in Tris/MgCl₂ buffer as above. Generally, further purification was not necessary, but when contaminants were present, the sample was re-run on the antibody column.

Polyacrylamide-gel electrophoresis

Polyacrylamide-gel electrophoresis in 10% gels with SDS according to the method of Laemmli (1970) was used to check purity and estimate enzyme subunit sizes. Native molecular weight was determined by electrophoresis to equilibrium under non-denaturing conditions on pre-poured 4–30% gradient acrylamide gels (Pharmacia) in 0.09M-Tris/0.08M-boric acid, pH 8.4, buffer for 17 h at a constant 125 V at room temperature.

Results

Enzyme purification

Monoclonal antibodies to intestinal type ALPs have been produced in our laboratory, and represent a potentially highly specific ligand for affinity chromatography. Using monoclonal antibody immunoaffinity chromatography, ALP_{AI} and ALP_{FI} have been purified with high yields. The procedures were much less cumbersome than previously reported purifications of these enzymes, since fewer chromatographic steps were required.

For the purification of ALP_{FI} , the dialysed aqueous phase of meconium extracted with butanol was initially loaded directly onto an antibody-Sepharose 4B column. This was unsuccessful, as the extract was too viscous even when diluted to pass through the column. We therefore added a DE-52 ion-exchange chromatography step as described in the Materials and methods section. The DE-52 allowed us to bind the ALP while washing away much of the viscous material prior to packing the column. The eluent from this column, in turn, could be applied directly to the antibody column without problems. Fig. 1(a) shows that A_{280} tracing and ALP activity profile of the DE-52 column. The ALP peak was eluted at 0.06M-NaCl as determined by electrical conductivity. This step averaged a 72% yield in three separate experiments and was the most inefficient step of the purification. Fig. 1(b) shows the A_{280} tracing and ALP activity profile from an optimal antibody-Sepharose 4B column run, i.e. one in which all ALP_{FI} was bound (see below). Yields for this step were generally in excess of 95%.

For purification of ALP_{AI} , the dialysed aqueous phase of a butanol extract of total adult intestine

Fig. 1. A_{280} (○) and ALP activity (●) profiles in purification of ALP_{FI}

(a) Whatman DE-25 chromatography of butanol-extracted meconium, as described in the text. The peak ALP fractions were pooled and applied to the antibody column. (b) Immunoaffinity chromatography of peak DE-52 fraction on a column of the monoclonal antibody ALP-D98/Sp2/21 coupled to CNBr-activated Sepharose 4B, as described in the text.

could be applied directly to the antibody column without difficulty. The A_{280} and ALP activity profiles were essentially identical with those of Fig. 1(b). Yields were in excess of 95%.

Determination of molecular weight and specific activities

Fig. 2(a) shows a 10% SDS/polyacrylamide-gel electrophoresis gel of an ALP_{FI} and an ALP_{AI} preparation. Fig. 2(b) is a non-denaturing 4–30% gradient polyacrylamide gel of the two purified isoenzymes. Table 1 contains estimates of subunit size and native molecular weight based on these and similar gels, and the specific activity of the purified enzymes. It has previously been reported that the intestinal ALPs had specific activities several-fold higher than those of ALP_p or L/B/K ALP (Lehman, 1975; Sugiura *et al.*, 1975). We have not found this to be so, as the values determined in our laboratory for ALP_p and ALP_{FI} are similar to those reported here for ALP_{FI} and ALP_{AI} (Meyer *et al.*, 1982; H. Harris, unpublished work). Native molecular weight is approximately

Fig. 2. Polyacrylamide-gel electrophoresis of ALP_{FI} and ALP_{AI}

(a) 10% SDS/polyacrylamide-gel electrophoresis of ALP_{FI} and ALP_{AI} purifications stained with Coomassie Blue. Lane 1, aqueous phase of a butanol extraction of adult intestine; lane 2, ALP peak of the previous sample after elution from an ALP-D98/Sp2/21-Sepharose 4B column; lane 3, ALP peak of a Whatman DE-52 column of butanol-extracted meconium as seen in Fig. 1(a); lane 4, ALP peak after elution from an ALP-D98/Sp2/21-Sepharose 4B column as seen in Fig. 1(b). The migration of molecular weight markers (kDa) is indicated. (b) Non-denaturing polyacrylamide-gel electrophoresis of purified ALP_{FI} and ALP_{AI} stained with Coomassie Blue. Lane 5, ALP_{AI} ; lane 6, ALP_{FI} . The migration of molecular weight markers (kDa) is indicated.

Table 1. Physical properties of ALP_{FI} and ALP_{AI}

Subunit size was estimated by linear regression using molecular weight standards after SDS/polyacrylamide-gel electrophoresis. Native molecular weight was estimated by linear regression using molecular weight standards after non-denaturing polyacrylamide-gel electrophoresis as described in the Materials and Methods section.

Enzyme	Estimated subunit molecular weight	Estimated native molecular weight	$10^{-3} \times$ Specific activity (units/mg of protein)
ALP_{FI}	70900	140000	1.6
ALP_{AI}	77000	170000	1.4

double the apparent denatured size, indicating that both enzymes are dimers, as previously reported.

Optimizing construction and use of antibody columns

To characterize better the parameters that affect the efficiency of monoclonal antibody immunoaffinity columns and to provide a general scheme for the construction of immunoaffinity columns, we made antibody-Sepharose 4B columns using a variety of antibodies at various concentrations, and attempted elution with several different solutions. These experiments are summarized in Table 2. Initially, a column of antiserum made to placental ALP (which cross-reacts with ALP_{AI} and ALP_{FI}) bound to Sepharose 4B was constructed. This efficiently bound ALP_{FI} , but resisted elution

with 1M-NH₄OH, high and low pH buffers and high concentrations of NaCl (3M) in a neutral buffer (Table 2, line 1). We then constructed columns using three different monoclonal antibodies produced in our laboratory. $ALP_p/Sp2/5$ is an antibody raised against placental ALP which cross-reacts (although less strongly) with ALP_{FI} (Gogolin *et al.*, 1982). The antibody in 3.0 ml of this ascites fluid bound to 1.5 g of Sepharose 4B did not retain even a small amount of ALP_{FI} (Table 2, line 2). The antibody in 3.0 ml of $ALP_{FI}/Sp2/41$ ascites fluid, an antibody made against ALP_{FI} (J. Vockley & H. Harris, unpublished results), bound to 6.0 g of Sepharose 4B efficiently retained almost 2×10^3 units of ALP_{FI} , but it could not be eluted (Table 2, line 3).

Table 2. *Maximizing efficiency of monoclonal antibody immunoaffinity columns*

Details of these experiments can be found in the text. Column size is expressed as ml of ascites fluid or antiserum used per g (dry wt.) of CNBr-activated Sepharose 4B. pH 3.0 buffer is 0.1 M-sodium acetate, pH 3.0; pH 12.0 buffer is 0.1 M-diethanolamine, pH 12.0. Key to retention and elution efficiencies: (a), enzyme activity was retarded behind the general protein peak as sample was loaded onto the column, but trickled off upon continued washing with loading buffer; (b), enzyme could not be eluted with the stated buffers; (c), enzyme was eluted slowly over a large volume of buffer; (d), enzyme was eluted in a sharp peak. Abbreviation: N.D., not done.

Antibody used for column	Column size (ml of antibody/g of Sepharose)	Retention of ALP _{FI} by column	Eluent	Elution efficiency	Column capacity (units of ALP)
1. Anti-ALP _p serum	10/3	+	1M-NH ₄ OH pH 3.0 buffer pH 12.0 buffer 3M-NaCl, pH 7.4	(b) (b) (b) (b)	N.D.
2. ALP _p /Sp2/5	3/1.5	—	N.D.	N.D.	N.D.
3. ALP _{FI} /Sp2/41	3.6	+	1M-NH ₄ OH pH 3.0 buffer	(b) (b)	N.D.
4. ALP-D98/Sp2/21	2/6	± (a)	N.D.	N.D.	N.D.
5. ALP-D98/Sp2/21	3/6	+	3M-NaCl, pH 7.4	(c)	2 × 10 ³
6. ALP-D98/Sp2/21	4/6	+	4M-KSCN, pH 7.4	(d)	2 × 10 ³
7. ALP-D98/Sp2/21	6/6	+	4M-KSCN, pH 7.4	(d)	(3–5) × 10 ³
8. ALP _{FI} /Sp2/37	1/1	—	N.D.	N.D.	N.D.
9. ALP _{FI} /Sp2/36	7.5/6.0	+	4M-KSCN, pH 7.4	(d)	N.D.

A third antibody, ALP-D98/Sp2/21 (made to the intestinal-like ALP found in D98/AH-2 tissue culture cells), was tested next. The antibody in 2.0ml of ascites fluid bound to 6.0g of Sepharose 4B failed to retain the enzyme completely (Table 2, line 4). Although the enzyme activity was retarded behind the general protein peak, it continued to trickle off the column during washing with loading buffer. The antibody in 3.0ml of ascites fluid bound to 6.0g of Sepharose 4B was found to retain 2 × 10³ units of ALP_{FI} and release it upon elution with 3M-NaCl at neutral pH (Table 2, line 5). However, the elution was inefficient in that enzyme was released slowly over a large volume of buffer. A solution of 4M-KSCN, a stronger chaotropic agent, allowed the sharp elution of enzyme activity demonstrated in Fig. 1(b) from columns constructed with 4.0 or 6.0ml of ascites fluid (Table 2, lines 6 and 7). ALP_{FI} is stable in this reagent for at least 1 h (long enough to elute and desalt a sample).

ALP_{FI}/Sp2/37 and ALP_{FI}/Sp2/36, two additional monoclonal antibodies raised against ALP_{FI}, were tested as potential immunoaffinity chromatography reagents (see the Discussion) by constructing small test columns of 1.0ml of ascites fluid to 1.0g of Sepharose 4B. The ALP_{FI}/Sp2/37–Sepharose 4B column failed to retain even a small amount of ALP_{FI} (Table 2, line 8), while the ALP_{FI}/Sp2/36 column efficiently bound 1.2 × 10² units of ALP_{FI} and released it upon elution with 4M-KSCN. A larger ALP_{FI}/Sp2/36 column made with 7.5ml of ascites fluid bound to 6.0g of

Sepharose 4B was able to retain 3 × 10³ units of ALP_{FI} and efficiently release it upon elution with 4M-KSCN (Table 2, line 9). The protein eluted from this column migrated as a single band on an SDS/polyacrylamide gel.

ALP-D98/Sp2/21 binds more strongly with ALP_{AI} than does ALP_{FI} (Wray & Harris, 1983). As one might anticipate, ALP_{AI} also bound more efficiently to the ALP-D98/Sp2/21 Sepharose 4B columns. Thus, even the column made with 3.0ml of ALP-D98/Sp2/21 ascites fluid completely retained 5 × 10³ units of ALP_{AI}, and this could be recovered with elution by 4M-KSCN.

Discussion

Immunoaffinity chromatography using polyclonal antisera in enzyme purification has had limited success in the past. While a highly specific antiserum provides a theoretically ideal ligand for column chromatography, two major problems are present. Firstly, purified enzyme must initially be available to produce an antiserum. Secondly, when enzyme is bound to an antiserum immunoaffinity column, it is often quite difficult to elute in the native state.

Recently, growing attention has been paid to the use of monoclonal antibodies as an immunoaffinity chromatography reagent (Denny *et al.*, 1982; Liedgens *et al.*, 1980; Bailyes *et al.*, 1982; Choo *et al.*, 1980; Meyer *et al.*, 1982). These have several advantages over antisera. It is not neces-

sary to begin with pure protein as an immunogen in the production of the monoclonal antibodies. As long as a suitable screening method is available, the antibodies specific to a desired antigen can be identified. Recent advances in immunization techniques *in vitro* could make this step even more convenient as less immunogen is necessary to produce hybridomas in this manner. Since a variety of monoclonal antibodies recognizing many different antigenic determinants can be obtained, each with a single specificity, it becomes possible to test a number of antibodies with a wide range of antigen binding avidities for use in immunoaffinity chromatography. Finally, since a monoclonal antibody is specific for only a single antigenic determinant on the enzyme (or one per identical subunit), binding of an enzyme to such a column should in principle be weaker than to one made of a polyclonal antiserum which recognizes a larger number of different determinants. This weaker binding of the enzyme to the immunoaffinity column should make subsequent elution in the active state easier. In this report we describe simplified high yield purification schemes for ALP_{AI} and ALP_{FI} using monoclonal antibody immunoaffinity chromatography.

Two major parameters are likely to affect the success or failure of an immunoaffinity column: the avidity of an antibody towards its antigen, and the quantity of antibody present in a column able to bind antigen. A high avidity antibody is likely to present problems when elution of active enzyme is attempted, while an antibody with too low an avidity may not retain the enzyme. Similarly, too much antibody immobilized on a column may lead to difficulty in elution whereas too little may lead to a low capacity column. Examples of these phenomena can be seen in our experiments. The column made with an anti-ALP_p antiserum which cross-reacts with ALP_{AI} and ALP_{FI} proved to be too avid and resisted elution of ALP_{FI}. Similarly, a column made with ALP_{FI}/Sp2/41 monoclonal antibody bound the enzyme too tightly for recovery. ALP_p/Sp2/5, a monoclonal antibody made against placental ALP (which reacts with ALP_{FI} but less strongly than with placental ALP) bound to Sepharose 4B failed to retain ALP_{FI}. A fourth antibody column made with ALP-D98/Sp2/21 proved to efficiently bind both ALP_{AI} and ALP_{FI} and could subsequently be eluted with 4M-KSCN to give high yields of active enzyme.

Optimal conditions for the construction and operation of an ALPD98/Sp2/21 monoclonal antibody column were then explored. A decreasing amount of antibody used to construct the column resulted in lower capacity and eventually failure to retain ALP_{FI}. Even when the column capacity was exceeded, the ALP_{FI} was retarded significantly by

the column. Increasing the antibody/Sepharose 4B ratio led to increased column capacity, and efficient elution was maintained. ALP_{AI} was efficiently retained even at the lowest antibody/Sepharose ratio used here; this is in keeping with the fact that it reacts more strongly with the antibody in solution. Thus, it seems that the relative binding of an antibody to its antigen is the major parameter in achieving successful retention of enzyme, while the amount of antibody bound to the column is more important in determining column capacity. It must be remembered, however, that the measured binding of different ascites fluids to the same antigen is dependent on both the avidity of the antibody and its concentration in the ascites. The concentration of specific antibody is difficult to measure in ascites fluids since they contain other endogenous immunoglobins.

The choice of an eluent was critical to the success of the purification. Solutions such as high and low pH buffer, and NH₄OH were often able to elute the enzyme, but also at least partially denatured it. NaCl, a chaotropic agent which can disrupt antibody-antigen interactions, was inefficient in eluting enzyme, giving too broad an elution peak. KSCN, a stronger chaotropic agent than NaCl, was able to elute the ALP rapidly without affecting enzyme activity.

Considering these factors, we propose the following scheme as a general approach to the application of immunoaffinity chromatography in enzyme purifications. A limited number of steps consisting of conventional methods of chemical fractionation and column chromatography can be used to generate an enriched, but none the less impure, enzyme preparation in fairly high yield. This preparation can be used to produce monoclonal antibodies via the hybridoma method. Small scale columns of each potential antibody linked to Sepharose 4B can then be prepared (1 ml of ascites fluid rich in monoclonal antibody bound to 1 g dry wt. of Sepharose beads is a convenient starting point). Small quantities of crude enzyme preparation containing 100–200 µg of enzyme can be passed through each of the columns and tested for retention of enzyme by the column. Several eluents that do not destroy enzyme activity can then be tested. If enzyme cannot be eluted, the columns can be remade with less antibody. An antibody that allows successful retention on the column and efficient elution of the bound enzyme in an active state can then be used to construct large scale columns, and the capacity of the column increased to maximum by adjusting the ratio of antibody to Sepharose. The end result is a highly specific affinity column which can be reused many times.

To test the efficacy of this general scheme, ALP_{FI}/Sp2/36 and ALP_{FI}/Sp2/37, two additional

monoclonal antibodies raised against ALP_{FI}, were studied as potential immunoaffinity reagents. ALP_{FI}/Sp2/36 binds ALP_{FI} about 500 times more strongly than does ALP_{FI}/Sp2/37 (Vockley, J., Meyer, L. & Harris, H., unpublished work). Test columns constructed of 1.0 ml of ascites fluid bound to 1.0 g of Sepharose 4B showed that ALP_{FI}/Sp2/37 would not retain small amounts of enzyme, while ALP_{FI}/Sp2/36 could bind 75 µg of ALP_{FI} and release it upon elution with 4 M-KSCN. A larger scale column constructed using ALP_{FI}/Sp2/36 proved to be equally efficient as the ALP-D98/Sp2/21 columns and allowed purification of ALP_{FI} to homogeneity from the DE-52 fraction of meconium.

In summary, we have used the technique of monoclonal antibody immunoaffinity chromatography in a simple purification scheme to produce active ALP_{FI} and ALP_{AI} in high yield. Experiments to optimize performance of the antibody column suggest that the relative strength of binding of an antibody to its antigen is the single most important factor to consider when constructing such a column. A general approach is presented to integrate this technique into a general approach to enzyme purification.

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