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Single step purification of rat liver aldolase using immobilized artificial membrane chromatography

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Abstract

Aldolase B is a peripheral membrane protein. Immobilized artificial membrane (IAM) surfaces were used to purify rat liver aldolase B in a single chromatographic step. Selective elution required dipalmitoylphosphatidylcholine (DPPC) to be included in the mobile phase. Selective elution of aldolase from the IAM column when DPPC (0.2 m*M*) was added to the mobile phase indicates that DPPC was an affinity displacing ligand for this membrane associated protein. Since tissue preparation involved only homogenization and centrifugation, the single step purification of aldolase B using IAM chromatography is a very convenient method. The IAM stationary phase (1.5 g) has a loading capacity of at least 4.39 mg total protein from rat liver homogenates and typically ≈17.7 μg of pure aldolase in a single step from ≈60 mg wet weight rat liver cytosol can be obtained. © 1997 Elsevier Science B.V.

Keywords: Aldolase; Enzymes

1. Introduction

Immobilized artificial membranes (IAMs) are immobilized lipid monolayers which provide virtually identical physico-chemical properties at the polar interfacial region as endogenous cell membranes [1,2]. IAM chromatography was used to purify cytochromes P450 [3], phospholipase A_2 [4,5], cholesterol carrier protein [6] and N-acetylphosphatidylethanolamine synthase [7]. Although the protein–lipid interaction responsible for the purification of these proteins has not been characterized, several concepts [4,5,7] suggest that proteins exhibiting their functions when adsorbed to membrane interfaces are amenable for purification using IAM chromatographic surfaces. Peripheral membrane proteins rather than integral membrane proteins are thus more likely suited to exhibit selective adsorption/desorption on IAMs. This is because peripheral membrane proteins are expected to exhibit similar recognition properties on the IAM surface as found in endogenous membranes, whereas in contrast, transmembrane proteins can not.

Fructose-1,6-bisphosphate aldolase (EC 4.1.2.13) is a peripheral membrane protein that forms tetramers at the surface of biological membranes [8–12].

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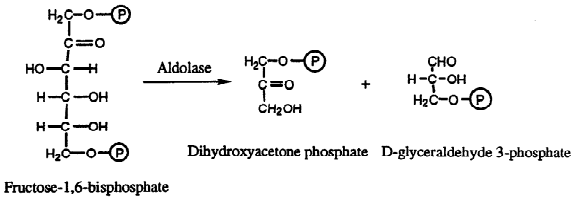
Aldolase is composed of three isoenzymes denoted as aldolases A, B and C. Aldolase B, used for this work, is found in the liver, kidney and small intestine [13,14]. Aldolase isoenzymes cleave both fructose 1-phosphate and D-fructose-1,6-bisphosphate



which demonstrates aldolase's biological role in both carbohydrate metabolism and de novo biosynthesis of carbohydrates [14]. Its properties as a peripheral membrane protein and its affinity for phosphatidylcholine (PC) fluid membrane surfaces [8,9] suggest that IAM chromatography stationary phases containing monolayers of PC analogues would function as an affinity matrix for the purification of aldolase.

2. Experimental

2.1. Chemicals

Disodium hydrogenphosphate, potassium dihydrogenphosphate, β-mecaptoethanol and methanol were obtained from Mallinkcrodt (Paris, NY, USA). Sodium azide, CHAPS and Tris base were ordered from Aldrich (Milwaukee, WI, USA). Glycine, trichloroacetic acid (TCA), D-fructose 1,6-bisphosphate, urea, L-lactic dehydrogenase, α-glycerolphosphate dehydrogenase and triosephosphate isomerase were purchased from Sigma (St. Louis, MO, USA). Dipalmitoylphosphatidyl choline (DPPC) was purchased from Avanti phospholipids (Alabaster, AL, USA). Micro BCA protein assay reagent kit was obtained from Pierce (Rockford, IL, USA). Hydrochloric acid (concentrated, 37.0%), silver nitrate, 37% formaldehyde solution, glacial acetic acid were ordered from Fisher Scientific (Fair Lawn, NJ, USA). Sodium chloride and sodium thiosulfate were from J.T. Baker (Philipsburg, NJ, USA). Acrylamide, ammonium persulfate (AP), N,N′-methylene-bisacrylamide (BIS), sodium dodecyl sulfate (SDS), N,N,N′,N′-tetra-methylethylenediamine (TEMED) and low-range molecular-mass markers for gel electrophoresis were purchased from Bio-Rad (Hercules, CA, USA). Lys-C protease was provided from the Purdue peptide synthesis and sequencing center. Absolute ethanol was obtained from McCormick Distilling (Pekin, IL, USA). Centricon devices were purchased from Amicon (Beverly, MA, USA). Fresh frozen rat tissues were obtained from Pel Freeze Biologicals (Rogers, AR, USA).

2.2. Rat liver cytosol preparation

The buffer used to prepare rat liver cytosols for injection on IAM columns contained 0.25 *M* sucrose, 1.0 m*M* EDTA and 10 m*M* Tris–HCl (pH 7.4) (abbreviated as SET buffer). Rat liver cytosol was prepared as described [15] with minor modifications. Rat livers were thawed and rinsed with SET buffer. Then SET rat liver homogenate (30%, v/v) was prepared and centrifuged for 20 min at 14 000 *g*. The supernatant was adjusted to pH 5.1 with 3.0 *M* HCl and stirred for 30 min at 4°C. The pH of the supernatant obtained from a second centrifugation (14 000 *g*, 20 min) was readjusted to pH 7.2 using 1.0 *M* Tris base. The supernatant was filtered through a 0.2 μm filter (nylon-66) before injection onto an IAM HPLC column.

2.3. IAM chromatography

IAM HPLC columns were packed at Regis Technologies (Morton Grove, IL, USA). Analytical size ^{ether}IAM.PC$^{C10/C3}$ and $^{\delta G}$IAM.PC$^{C10/C3}$ columns (150×4.6 mm) containing 12 μm particles with 300 Å pore diameter were used. The structure and synthesis of these surfaces have been described elsewhere [16,17]. Mobile phase compositions, A, B, C and D are given in the legend to Fig. 1. Prior to sample injection, the IAM column was equilibrated with at least 60 column volumes (i.e., ~60 ml) of mobile phase A. The mobile phase flow-rate was adjusted to 1.0 ml/min and detection was performed at 254 nm. To preserve aldolase activity, 10 m*M* β-mecaptoethanol was included in all mobile phases except mobile phase D. Fractions (1.0 ml/fraction) were collected throughout IAM chromatography. Chromatograms were obtained using a Rainin HPLC pumping system equipped with a Knauer Model 87

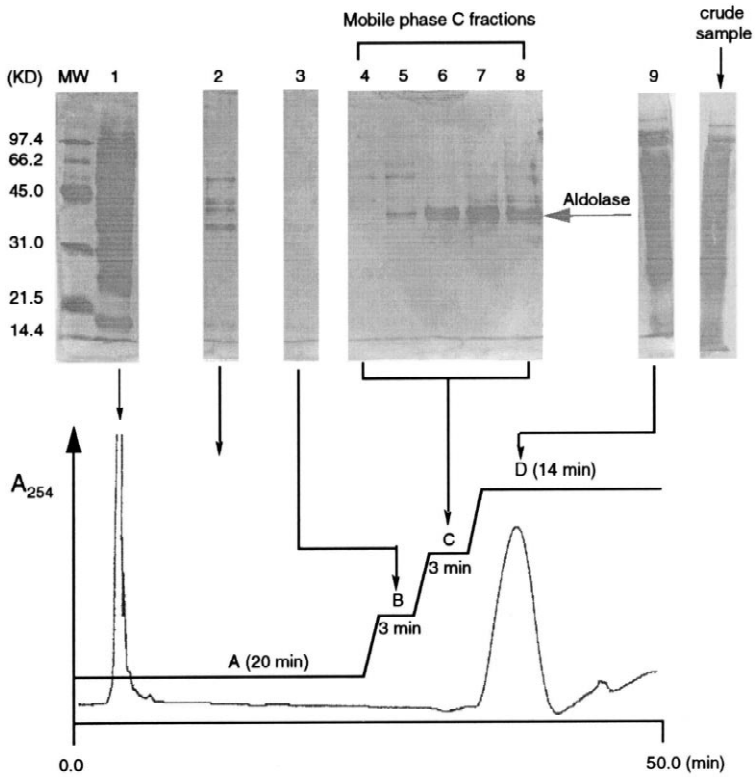


Fig. 1. Aldolase purification using an 150×4.6 mm ^{ether}IAM.PC$^{C10/C3}$ column. A rat liver cytosol preparation (60 μl, corresponding to ≈1.32 mg of total protein) was injected into the IAM column. Mobile phase A contained 50 m*M* Tris–HCl (pH 7.2), 10 m*M* β-mecaptoethanol and 0.02% sodium azide. Mobile phase B was prepared by addition of 150 m*M* sodium chloride to mobile phase A. Mobile phase C was prepared by the addition of 1% of CHAPS and 0.2 m*M* DPPC to mobile phase A. Mobile phase D was prepared as a 10% (v/v) acetonitrile solution in water. The change from one mobile phase to another was accomplised within 2 min. The SDS–PAGE patterns of several chromatographic fractions (1.0 ml/fraction) are depicted above the chromatogram. Gel lanes 1 and 2 show proteins that eluted using mobile phase A. Lane 3 shows proteins eluting during a brief 3 min elution period of mobile phase B. Aldolase eluted using mobile phase C. The intense single band in fractions 6–8 is attributable to aldolase B. Lane 9 shows that several high affinity proteins non-selectively eluted by mobile phase D (10% acetonitrile).

detector (Rainin Instrument, Emeryville, CA, USA) and interfaced with a Macintosh computer (Apple Computer, USA). Rainin Dynamax software (Rainin Instrument) was used to record chromatograms. All chromatography experiments were performed at room temperature.

2.4. SDS–polyacrylamide gel electrophoresis (PAGE)

SDS–PAGE analysis were performed according to Cai et al. [7] with the minor modification that 12% polyacrylamide gels were used.

2.5. Aldolase activity assay

Aldolase activity was measured using a coupled enzymatic reaction [18]. Basically, D-glyceraldehyde 3-phosphate is generated from the aldolase reaction. Then triosephosphate isomerase converts D-glyceraldehyde 3-phosphate to dihydroxyacetone phosphate. This latter compound is then reduced to glycerol 3-phosphate catalyzed by α-glycerolphosphate dehydrogenase in the presence of a reducing reagent, i.e., NADH. The decrease in UV absorbance at 334 nm due to the consumption of NADH is directly proportional to the aldolase activity in the sample. In brief, 2.5 ml of 5 m*M* D-fructose-1,6-bisphosphate in 0.1 *M*

Tris–HCl (pH 8.0) buffer, 50 μl of 8.5 m*M* NADH, 10 μl of α-glycerolphosphate dehydrogenase/triosephosphate isomerase/L-lactic dehydrogenase (30 IU/ml, 500 IU/ml, 425 IU/ml) in 3.5 *M* ammonium sulfate, and 100 μl of the sample were mixed together. One unit of α-glycerolphosphate dehydrogenase is defined as converting 1.0 μ*M* dihydroxyacetone phosphate to α-glycerophosphate per min at pH 7.4 at 25°C. One unit of triosephosphate isomerase is defined as converting 1.0 μ*M* D-glyceraldehyde 3-phosphate to dihydroxyacetone phosphate per min at pH 7.6 at 25°C. One unit of L-lactic dehydrogenase is defined as reducing 1.0 μ*M* of pyruvate to L-lactate per min at pH 7.5 at 37°C. The assay solution was vortexed (~2–3 s) and then incubated for 3 min prior to measuring enzyme activity by UV spectroscopy. Interference by endogenous substrates in the sample (e.g., pyruvate) was removed by the presence of L-lactic dehydrogenase in the sample during the pre-incubation period. Mobile phase C was used as the background absorbance. A UV-7 Beckman spectrophotometer (Beckman Instruments, Irvine, CA, USA) was used to record the absorbance at 334 nm for 5 min. UV spectroscopic data were converted into aldolase activities as described [18].

2.6. Scanning densitometry

Gels were scanned using a Shimadzu CS9000U dual-wavelength scanner in the transmission mode. Silver-stained gels were scanned at 400 nm using a 0.05×2.0 nm beam size and 1 mm width for peak integration.

2.7. Protein content measurements

The protein contents in both rat liver cytosol and chromatography fractions were determined by MicroBCA assay. Since detergents and β-mercaptoethanol interfere with the assay, TCA protein precipitation was performed prior to the MicroBCA assay. This protein assay procedure has been previously described [5].

2.8. Aldolase protein digestion and amino acid sequencing

Following IAM chromatography, eight fractions (1.0 ml/fraction) containing aldolase were pooled and concentrated to about 230 μl using a Centricon-10 apparatus. The concentrated sample was then injected onto a 250 mm×2.0 mm C_{18} microbore column (Supelco, Bellefonte, PA, USA) for removal of salts and detergents prior to protein digestion. Aldolase was eluted by gradient HPLC at a flow-rate of 150 μl/min with 0% to 70% acetonitrile in water–0.1% TFA (v/v) in 60 min and the column effluent monitored at 215 nm. The chromatogram showed a single major peak at 48.3 min attributable to aldolase and a few low intensity UV absorbing peaks attributable to contaminating proteins.

The aldolase fraction (≈200 μl) was collected, lyophilized and the residue subjected to Lys-C protease digestion overnight at 37°C. Protein digestion required a completely deionized 6.0 *M* urea solution. The deionized 6.0 *M* urea solution was prepared by suspending mixed ion resin (≈2 g) in 10 ml of 6.0 *M* urea at room temperature and handshaking the suspension several times. The protein digestion solution contained 10 μl Tris–HCl buffer (50 m*M*, pH 9.0), aldolase (≈10 μg-protein/20 μl), 20 μl urea (6.0 *M*), 9.0 μl of water and 1.0 μl of Lys-C (1.63 μg/μl). After digestion, the peptides were fractionated by C_{18} microbore chromatography running a gradient from 0% to 56% acetonitrile in water–0.1% TFA (v/v) for 60 min. Peptide sequencing was performed by Purdue's Cancer center in the peptide synthesis and sequencing core facilities using Edman degradation chemistry.

3. Results

Fig. 1 shows aldolase purification on an analytical ^{ether}IAM.PC$^{C10/C3}$ column. A rat liver cytosol preparation (60 μl, ≈1.32 mg total protein) was injected into the column. Similar to other protein purifications using IAM chromatography, a large pass through peak near the void volume containing low affinity proteins eluted when mobile phase A was used (gel lane 1, Fig. 1). Mobile phase B (containing 150 m*M* sodium chloride) did not elute proteins from the IAM column (gel lane 3, Fig. 1). However, mobile phase C (containing CHAPS and DPPC) selectively eluted aldolase (~40 000) which is the darkest stained spot on the gels in lanes 6, 7 and 8 in Fig. 1. No contaminating proteins were found in fraction 6, but, a few spots of low intensity attributable to high-

molecular-mass proteins were present in fraction 7 and fraction 8. Based on scanning densitometry, aldolase purities in fractions 6, 7 and 8 were 98.7%, 96.7% and 95.7% respectively.

For aldolase purification (Fig. 1), mobile phase D containing 10% acetonitrile was used to remove DPPC and CHAPS from the column prior to injection of the next sample. In addition to column-cleaning by removal of DPPC and CHAPS, a vast array of high affinity proteins adhering to the IAM surface were removed by the acetonitrile (Fig. 1 gel lane 9). Previous studies [3–5,7] have shown that preparations derived from other biological systems contain proteins with both high and low affinity for IAMs. IAM column regeneration between experiments requires desorption of high affinity proteins after the target protein has been eluted. In our studies, the mobile phase used to regenerate the IAM therefore included 100 ml of 1.0% SDS detergent, followed by 100 ml of acetonitrile in addition to mobile phase D.

Reproducible chromatograms, as shown in Fig. 1, were obtained on the same column after eight sample injections. Fig. 2 compares the SDS–PAGE results of the first to the eighth injection. Virtually no difference in the SDS–PAGE was observed after eight injections and aldolase purity decreased less than 1% based on scanning densitometry. However, column reproducibility as shown in Fig. 2 could not be

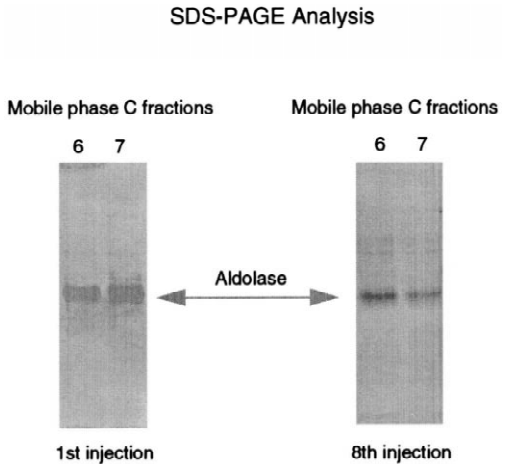


Fig. 2. Reproducibility of aldolase purification on IAM columns. The conditions shown in Fig. 1 were reproduced eight times and the SDS–PAGE analysis of fractions 6 and 7 eluting when mobile phase C was used are shown for the first and eighth injections.

achieved without the column washing step between injections as described above.

Previous work from our laboratory has demonstrated the importance of adding a phospholipid to the mobile phase during the purification of NAPES which is an interfacial binding enzyme. Virtually no enzyme activity was recovered during NAPES IAM purification unless DPPE was included in the mobile phase [7]. For this reason, the potential requirement for mobile phase DPPC in selectively eluting the aldolase from IAM columns was explored. Fig. 3 shows experimental results using the exact chromatographic condition shown in Fig. 1 except DPPC was removed from mobile phase C. Comparing Fig. 1 to Fig. 3 shows that aldolase did not elute when DPPC was omitted from mobile phase C. Fig. 3 thus demonstrates that DPPC is critical for the purification of aldolase using IAM chromatography and therefore DPPC was included in all further purifications.

After the requirement for the presence of DPPC in mobile phase C was confirmed (Fig. 3) and the elution conditions for the selective purification aldolase in a single chromatographic step were established, the conditions of protein loading on the analytical IAM column were investigated. The main objective of the protein loading scale-up experiments was to determine the total amount of protein which could be loaded on analytical IAM columns without impairment of aldolase binding which would result in substantial losses. Compared to the conditions used to obtain the data shown in Fig. 1, protein loadings as well as injection volumes were varied either 3.2- or 32-fold. The experimental results are shown in Figs. 4 and 5, respectively. Column overload decreased retention times and peak resolution, and as a consequence, yielded a concomitant reduction in the number of theoretical plates. However, high protein load facilitated the purification. In order for optimizing yield and purity of aldolase, the eluting time using mobile phase B and C was increased from 3 min to 13 min during scale-up of aldolase purification.

Fig. 4 shows the chromatogram for the 3.2-fold scale-up experiment and Table 1 summarizes aldolase activity recovered for each of the different mobile phases. Similar to Fig. 1, aldolase immediately eluted when mobile phase C was initiated (Fig. 4, gel lane 1). Unlike the chromatogram shown in

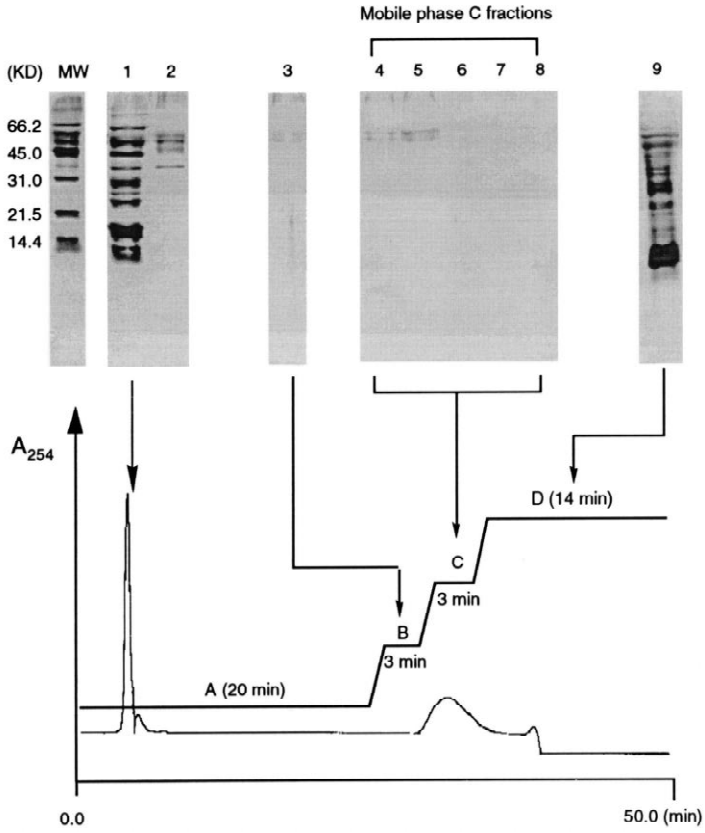


Fig. 3. Dependence of aldolase B purification using IAM chromatography on mobile phase DPPC. The experimental conditions were the same as described in Fig. 1 except that DPPC was omitted from mobile phase C.

Fig. 1, the chromatogram in Fig. 4 (gel lane 2) shows numerous proteins eluting at the end of mobile phase C. No aldolase activity was found in the unretained material indicating that the IAM column was not overloaded, i.e., all of the injected aldolase adsorbed to the column and did not elute until mobile phase C was applied (Table 1).

Fig. 5 shows the chromatogram and SDS–PAGE gel analysis of several fractions after a 32-fold scale-up (i.e., 43.94 mg total protein/injection). In contrast to the 3.2-fold scale-up procedure (i.e., 4.394 mg total protein/injection), which did not reveal contaminating proteins (Fig. 4, gel lane 1), the 32-fold scale-up corresponding to 29.3 mg protein per g of IAM stationary phase yielded aldolase fractions contaminated with several co-eluting proteins (Fig. 5, gel lanes 2–5).

The specific activity of rat liver aldolase B after IAM chromatography shown in Fig. 4 was 0.512 IU/mg protein (Table 1) which is similar to the specific activity of rabbit liver aldolase (1.46 IU/mg) purified by Rutter et al. in 5 steps [19]. The small differences may be due to the species (rabbit vs. rat), the temperature used to assay aldolase B activity, or partial enzyme inactivation during IAM chromatography. Rat liver aldolase activity was measured at ≈23°C, whereas the rabbit liver aldolase was determined at 28°C. Aldolase activity from human serum samples is temperature sensitive as evidenced by a 2.5-fold increase in activity at 37°C compared with 25°C [18]. Since the primary objective of this work was aldolase purification, the small observed difference in specific activity between rat liver aldolase (this work) and rabbit liver aldolase [19] was not pursued further.

Protein sequencing was performed in order to

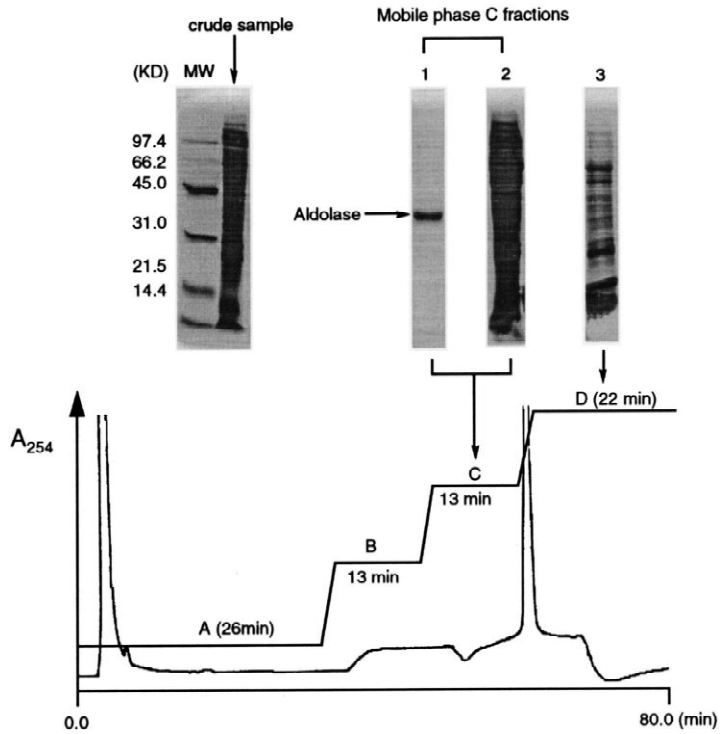


Fig. 4. Maximum protein loading for aldolase purification on analytical size IAM columns. Mobile phase compositions were as described in Fig. 1. However, mobile phase B and C elution times were increased to optimize purification and recovery of aldolase B during increasing column loading. Rat liver cytosol (200 μl, corresponding to 4.94 mg protein) was injected onto the analytical ^{ether}IAM.PC$^{C10/C3}$ column. SDS–PAGE analysis above the chromatogram (lane 1) shows that aldolase eluted in fraction 1.

eliminate the possibility that other proteins co-migrated with aldolase during SDS–PAGE analysis. After protein digestion, a key fragment of aldolase was sequenced and compared to the protein database. Table 2 shows that there was a 85% to 90% homology between rat liver aldolase purified in this work and rat liver aldolase B purified by conventional procedures [20]. For comparison, sequence information for human liver aldolase B [21] is also given in Table 2.

As shown in Figs. 1–4 as well as Tables 1 and 2, purification of aldolase to homogeneity using IAM chromatography was achieved yielding high specific activities. Previous work from our laboratory revealed that the interfacial binding enzyme PLA_2 did not require glycerol backbone or its esters for affinity purification using IAM surfaces [4]. In other words, selectivity of IAM for the recognition of PLA_2 did not significant depend on glycerol backbone of immobilized phospholipid. PLA_2 utilizes membrane phospholipids as substrates, and one of the primary goals of the present study was to determine if other interfacial enzymes that do not use phospholipids as substrates also do not require the glycerol backbone region for affinity purification using IAMs. For this reason, aldolase serves as a good model protein to elucidate affinity purification of interfacially bound enzymes that do not use membrane lipids as substrates. Thus, the purification strategies shown in Figs. 1 and 4 were tested on $^{\delta G}$IAM.PC$^{C10/C3}$ columns in which the IAM packing material lacks glycerol backbone, but, the phosphocholine headgroup still remains. Under identical conditions, the $^{\delta G}$IAM.PC$^{C10/C3}$ and ^{ether}IAM.PC$^{C10/C3}$ surfaces provide very similar results (data not shown). The primary difference was that a contaminating protein was found during the use of a $^{\delta G}$IAM.PC$^{C10/C3}$ column with mobile phase C. The capability to

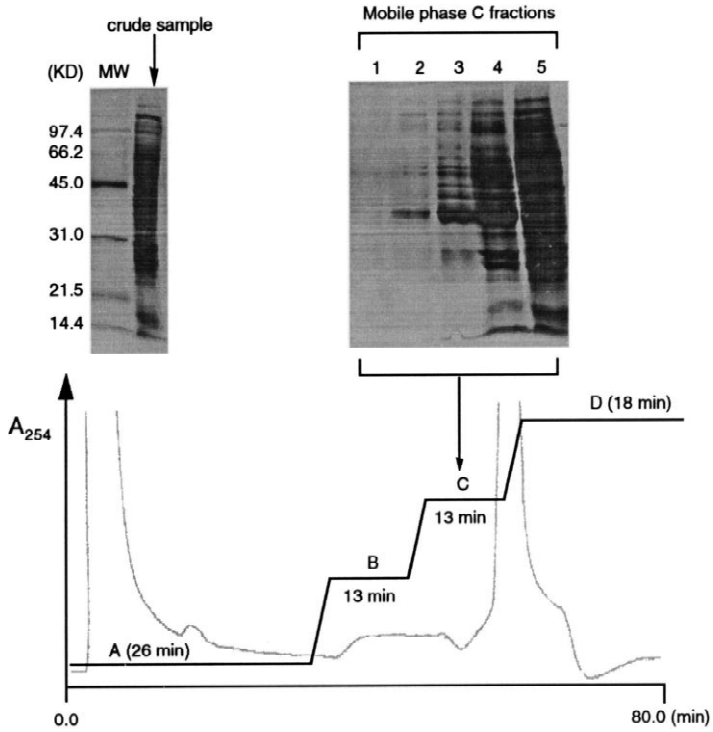


Fig. 5. Aldolase elution during column overloading conditions. The same conditions used to generate the elution pattern depicted in Fig. 3 were used to obtain the above data except that the scale-up was 32-fold instead of 3.2-fold.

purify aldolase on both $^{\delta G}$IAM.PC$^{C10/C3}$ and ^{ether}IAM.PC$^{C10/C3}$ surfaces suggests that phosphocholine headgroups are the primary binding sites for aldolase.

4. Discussion

The efficient purification of aldolase on IAM columns can be explained on the basis of affinity of

Table 1
Summary of aldolase activity eluting throughout the mobile phase gradient shown in Fig. 4[a]

Mobile phase condition	Sample	Volume (μl)	Protein (μg)[b]	Total activity (IU)[c]	Specific activity (IU/mg protein)	Recovery (%)	Purification factor
	Rat liver homogenate	200	$4.39 \cdot 10^{3}$	$2.66 \cdot 10^{-2}$	$6.05 \cdot 10^{-3}$	100	1
Mobile phase A	Unretained material	6000	nd[d]	no activity	0	–	–
Mobile phase B	Salt washings	6000	nd[d]	no activity	0	–	–
Mobile phase C	Fraction 1 in Fig. 4	6000	17.7	$9.10 \cdot 10^{-3}$	0.514	34.2	248[e]

[a]Fractions (1.0 ml/fraction) were collected and analyzed for aldolase activity during the mobile phases step gradient conditions shown in Fig. 4.
[b]The protein content was determined by MicroBCA assay.
[c]Activity was measured as described [18].
[d]Not determined because there was no activity.
[e]The purification factor is based on the amount of protein injected vs. the amount of protein recovered in the aldolase fraction collected from the IAM column. The purification factor may also be expressed by the ratio of enzymatica activity (IU/mg protein) in the crude rat liver cytosol preparation to the enzymatic activity (IU/mg protein) in the final product obtained by IAM HPLC, i.e., 85.0.

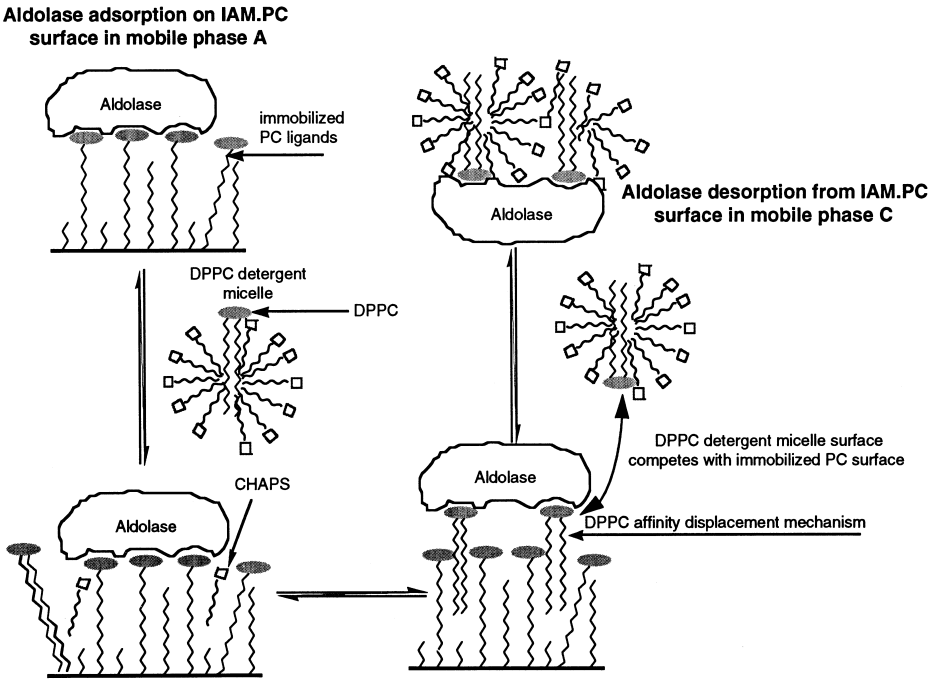
Table 2
Sequence homology among aldolases

Source aldolase B	Sequencing data[c]
Rat liver[a]	–Thr–Val–Pro–Ala–Ala–Val–Pro–Ser–Ilr–Cys–Phe–Leu–Ser–Gly–Gly–Met–Ser–Glu–Glu–
Human liver[b]	–Thr–Val–Pro–Ala–Ala–Val–Pro–Gly–Ilr–Cys–Phe–Leu–Ser–Gly–Gly–Met–Ser–Glu–Glu–
Rat liver (this work)	–Thr–Val–Pro–Ala–Ala–Val–Pro–Gly–Ilr–Thr–Phe–Leu–Ser–Gly–Gly–Met–Met–Glu–Glu–

[a]The sequence data was from [20].
[b]The sequence data was from [21].
[c]Amino acid differences are underlined.

aldolase for phosphatidylcholine membranes. Gutowicz and Modrzycka [9] found the rabbit muscle aldolase A isoenzyme binds to PC liposomes mainly by electrostatic interactions and can be desorbed by high salt from the surface. It is noteworthy that in our work high mobile phase salt concentrations, i.e., 150 m*M* NaCl, included in mobile phase B could not effect elution of aldolase from the IAM surface. This results indicate that electrostatic interactions do not contribute significantly to the binding of the aldolase B isoenzyme to the IAM surface.

Because detergent solubilized DPPC was also needed in the mobile phase during purification of aldolase on analytical IAM columns, phosphocholine headgroups of DPPC in the mobile phase micelles probably provide binding sites for aldolase that compete with the immobilized phosphocholine binding sites (Scheme 1). DPPC may effect aldolase purification by two mechanisms: (1) as an affinity displacing ligand whereby DPPC binds to the IAM surface and weakens aldolase affinity for the immobilized phospholipids, and (2) DPPC detergent micelles compete with the IAM surface for aldolase adsorption. It is likely that both mechanisms contribute as shown in Scheme 1. Furthermore, multivalent



Scheme 1. Rat liver aldolase purification by IAM HPLC using CHAPS–DPPC detergent mixtures.

binding of aldolase to immobilized phosphate groups has been demonstrated by several groups [13,22]. Thus, as depicted in Scheme 1, both the monolayer of phosphatidylcholine headgroups on the IAM surface and mixed micelles in the mobile phase containing DPPC–CHAPS provide multivalent binding sites for aldolase.

The high binding affinity of rat liver aldolase B to IAM surfaces suggests that IAMs can also function as a matrix for the non-covalent immobilization of this enzyme. Enzyme immobilization on IAM surfaces have been used for quantitating ligand–enzyme binding constants by HPLC. In this regard, Zhang and Wainer [23] have utilized IAM as a solid support for immobilization of PLA_2 to characterize PLA_2–substrate reactions. In addition Chui and Wainer [24] immobilized trypsin on IAMs and evaluated ligand–enzyme binding constants of the immobilized enzyme. The combination of purification of enzymes using IAMs followed by enzyme immobilization on IAMs may be a useful strategy for establishing drug screening methods. For an example, trypanosomal aldolase is a potential drug target site [25] and purification and immobilization of aldolase using IAM surfaces may evolve as a method for drug screening against this pathogen.

5. Conclusions

Rat liver aldolase was purified in a single chromatographic step using IAM HPLC. The glycerol backbone of the immobilized phospholipid does not significantly influence the affinity of aldolase B to immobilized phosphatidylcholine on IAM surfaces. Analytical IAM HPLC columns (150 mm×4.6 mm) can be used to purify ~17.7 μg of pure aldolase B in a single step from ~60 mg wet rat liver cytosol.

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