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Arsanilic acid–Sepharose chromatography of pyruvate kinase from KB cells

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Abstract

In the present study, arsanical-based affinity chromatography for pyruvate kinase (PK) isolation was explored. *p*-Arsanilic acid (4-aminophenyl arsonic acid), which contains an arsonic acid moiety structurally similar to inorganic pentavalent arsenate, was conjugated to Sepharose 4B via its *para*-amino group to form an As(V)–Sepharose matrix. The cellular proteins from KB cells bound to arsonic acid moieties were eluted by 50 m*M* sodium arsenate in Tris–HCl buffer (50 m*M*, pH 7.6). A single protein band with a molecular mass of 58 kDa was shown on a sodium dodecyl sulfate–polyacrylamide gel. By immunoblotting, amino acid sequencing and enzymatic analysis, the sodium arsenate-eluted 58-kDa protein was demonstrated to be a human PK (type M2). By using this one-step As(V)–Sepharose chromatography, PK from KB cells was purified 35.4-fold with a specific activity of 153.15 U/mg protein in the presence of 6 m*M* fructose-1,6-biphosphate. Although PK was eluted from an As(V)–Sepharose column with sodium arsenate, PK activity was apparently inhibited by the used eluent system, but not by *p*-arsanilic acid, indicating a specific interaction of As(V) to PK. In summary, our results indicate that As(V)–Sepharose can serve as a simple and efficient chromatographic support for PK purification from KB cells.

Keywords: Arsanilic acid; Pyruvate kinase; Enzymes

1. Introduction

Pentavalent arsenate, an environmental carcinogen structurally similar to phosphates, replaces phosphate and results in uncoupled oxidative phosphorylation and inhibition of adenosine triphosphate (ATP) formation [1,2]. Pentavalent arsenate is incorporated by cells through the phosphate transport system in both bacteria and mammalian cells [3,4]. Compounds in which the $-O-PO_3H_2$ group has been substituted by the $-O-AsO_3H_2$ moiety have been used for a long time in biochemical studies of analogues of natural substrates [5].

The genetic materials DNA and RNA are phos-

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phodiesters, hence phosphates are ubiquitous in the living organism. The principal reservoirs of biochemical energy, such as ATP, creatine phosphate and phosphoenolpyruvate, are also inorganic phosphate. Phosphorylation of a protein can modify its biological activity and influence the binding of another protein [6–8]. Enzymes having phosphorus compounds as their targets often exert their actions on arsenic analogues, e.g., adenylate kinase [9,10], and RNA polymerase [11].

p-Arsanilic acid (4-aminophenyl arsonic acid), a growth promotant used in veterinary medicine, contains a pentavalent arsonic acid moiety and an amino group that can be easily coupled on Sepharose 4B particles [As(V)–Sepharose] (Fig. 1). Since arsenate is a good structural analogue of phosphate in enzyme reactions [5], in the present study we explored the applicability of As(V)–Sepharose affinity chromatography in phosphoenzyme isolation. Our previous study showed that pentavalent arsenate and phosphate share a common transport system in human oral epidermal carcinoma KB cells [3] and therefore this cell was adopted in these experiments. Experimental results indicated that an As(V)–Sepharose affinity matrix was an effective chromatographic support for the single-step purification of pyruvate kinase (PK) from KB cells.

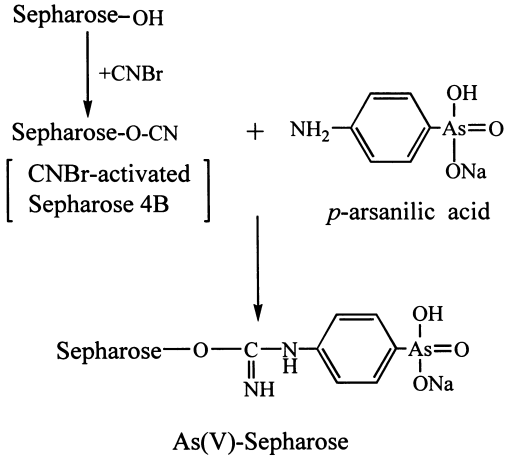


Fig. 1. Chemical reactions involved in the preparation of As(V)–Sepharose.

2. Experimental

2.1. Chemicals and cell culture

Inorganic pentavalent arsenate (Na_2HAsO_4) was purchased from Merck (Darmstadt, Germany). *p*-Arsanilic acid (4-aminophenyl arsonic acid) is a commercial product of Tokyo Chem. (TCI, Kasei, Tokyo, Japan). CNBr-activated Sepharose 4B was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Rabbit muscle pyruvate kinase (RMPK) was purchased from Boehringer Mannheim (Mannheim, Germany). All reagents used for cell culture were from Gibco (Life Technologies, Grand Island, NY, USA). KB cells (oral epidermoid carcinoma cells) were obtained from American Type Culture Collection (Rockville, MD, USA), cultured in RPMI medium and harvested by trypsinization as previously described [3].

2.2. As(V)–Sepharose column preparation and affinity chromatography for pyruvate kinase

The conjugation of *p*-arsanilic acid to Sepharose 4B gel was essentially performed according to the manufacturer's instruction [12]. In brief, after sequentially swelling and washing the CNBr-activated Sepharose 4B (3.4 g) with 1 m*M* HCl and coupling buffer (0.1 *M* $NaHCO_3$), the gel was reacted with *p*-arsanilic acid (1 mg/ml) dissolved in coupling buffer for 2 h with gentle agitation. The reaction was terminated by adding ethanolamine for 2 h. Afterward, the As(V)–Sepharose gel (ca. 12 ml) was placed into a 10×2.5 cm I.D. column and extensively washed with 50 m*M* Tris–buffer, pH 7.6 prior to use.

All chromatographic steps were carried out at 4°C. KB cells were homogenized by sonication in buffer A [50 m*M* Tris–HCl, pH 7.6 containing 0.2 m*M* phenyl methanesulfonyl fluoride (PMSF) and 1 m*M* dithiothreitol (DTT)]. The cell homogenates were centrifuged at 15 800 *g* for 30 min at 4°C and the clear supernatants (12 mg protein in 1 ml buffer A) were directly applied onto an As(V)–Sepharose column pre-equilibrated with buffer A. The column was rinsed at hydrostatic pressure at a flow-rate of 40 ml/h with buffer A containing 0.5 *M* NaCl for

removal of the non-specific binding proteins. When the baseline, recorded at 280 nm, reached a stable value, the specifically bound protein material was eluted with 50 m*M* sodium arsenate or 100 m*M* sodium phosphate in buffer A. The eluted peaks were pooled, concentrated and desalted with Amicon ultrafiltration cells using a 3-kDa cutoff membrane (Amicon, Beverly, MA, USA). The concentrated proteins were stored at 4°C.

2.3. Electrophoresis and Western blotting analysis

Protein content in the eluted fraction was determined using a Bio-Rad protein reagent kit [13], and the protein composition was investigated on an 8% sodium dodecyl sulfate (SDS)–polyacrylamide gel [14]. The gels were either visualized by silver staining [15] or transferred to a nitrocellulose membrane for immunoblotting analysis. In brief, the nitrocellulose membrane was air dried for 5 min, shaken for 1 h in phosphate-buffered saline (PBS, KCl 0.2 g/l; KH_2PO_4 0.2 g/l; NaCl 8 g/l; Na_2HPO_4 1.13 g/l) containing 5% non-fat milk, and incubated overnight at 4°C with the antibody against rabbit PK-M1 (Rockland, Gilbertsville, PA, USA) diluted with PBS containing 1% non-fat dry milk. After repeated washings with PBS containing 0.1% Tween 20 (PBST) (Sigma, St. Louis, MO, USA), the nitrocellulose membrane was incubated with an alkaline phosphatase conjugated secondary antibody [anti-goat IgG (H+L), Kirkegaard & Perry Labs., Gaithersburg, MD, USA) for 1 h at room temperature (RT). The nitrocellulose membrane was extensively washed with PBST and the immunoreactive PK was detected using a NBT/BCIP kit (nitroblue tetrazolium/5-bromo-4-chloro-3-indolylphosphate, Zymed, San Francisco, CA, USA).

2.4. Partial amino acid sequence analysis

For analysis of the internal sequence of the purified protein, the purified protein was subjected to either acidic or cyanogen bromide (CNBr) treatment. The purified protein was dissolved in 70% formic acid at RT for 24 h (acid cleavage) or in 70% (v/v) formic acid with a 100-fold molar excess of CNBr with respect to the methionine (i.e., 0.18 μg/μg protein) content for 48 h (CNBr cleavage). The resulting peptide mixture was separated by 12.5% SDS–polyacrylamide gel electrophoresis (PAGE) and electroblotted onto polyvinylidene difluoride (PVDF) membranes (Pall Ultrafine Filtration, Glen Cove, NY, USA) with a semi-dry blotting system (Nihon-Eido, Tokyo, Japan) at 1 mA/cm^2 for 4 h at room temperature. The membranes were then stained with Coomassie Blue and the stained peptide bands were cut down and analyzed automatically using a microsequencing sequencer (Model 476A, Perkin-Elmer, USA).

2.5. Assay of PK

The enzymatic activity of PK was determined by lactate dehydrogenase-coupled enzyme assay [16]. The reaction mixture in a total volume of 1 ml consisted of 50 m*M* Tris–HCl (pH 7.5), 72 m*M* $MgCl_2$, 0.3 m*M* NADH, 10 μg/ml lactate dehydrogenase, 43 m*M* PEP, 6 m*M* ADP. The reaction was initiated by adding 0.1 ml PK (0.1–0.2 U/ml), which had been preincubated with various concentrations of fructose-1,6-bisphosphate at 25°C for 1 h. The rate of decrease in the absorbance at 340 mm, resulting from the oxidation of NADH, was taken as a measure of PK activity.

3. Results

Atomic absorption spectrophotometry revealed that about 3.5–5 μ*M* *p*-arsanilic acid had been covalently bound per gram (related to dry mass) of Sepharose gel. The adsorbent could be stored in the refrigerator without measurable dissociation of ligands. Fig. 2 shows the elution profile of a protein from an As(V)–Sepharose column. After an extensive wash with buffer A containing 0.5 *M* NaCl, a small peak of proteins bound to As(V)–Sepharose was eluted from the column with 50 m*M* sodium arsenate. Measurement of enzymatic activity showed that PK activity was preponderantly located in the peak eluted with 50 m*M* sodium arsenate. No PK activity was detectable in the flow-through volume or in fractions eluted with regeneration buffer. SDS–PAGE of fractions eluted with sodium arsenate

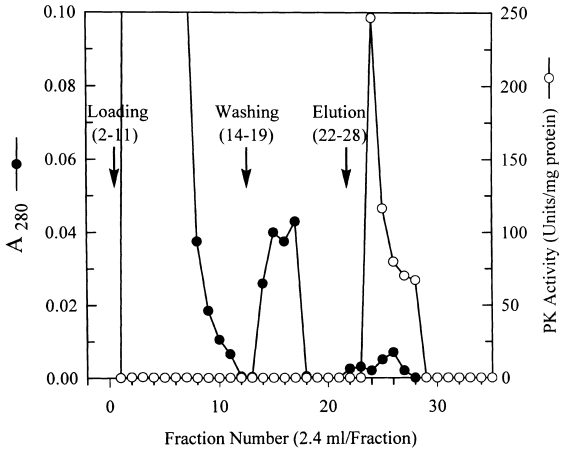


Fig. 2. Affinity chromatographic separation of As(V)-binding proteins of KB cells. The clear extract from KB cells was applied to the As(V)–Sepharose affinity column as described in Experimental. After extensive washing, the specific binding protein was eluted with 50 m*M* sodium arsenate in buffer A. Fraction proteins were individually concentrated and desalted by ultrafiltration, and aliquots analyzed for PK activity as described in Experimental. The fraction numbers collected are indicated in parentheses.

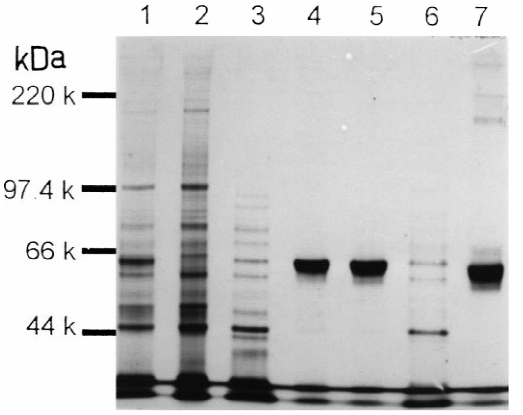
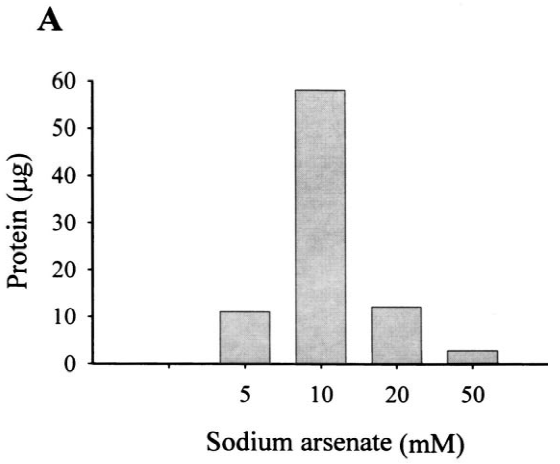


Fig. 3. SDS–PAGE analysis of protein fractions obtained by As(V)–Sepharose affinity chromatography. Each protein fraction (Fig. 2) was pooled and concentrated by Amicon ultrafiltration. An aliquot containing 2.5 μg of protein was separated on an 8% SDS–polyacrylamide gel and visualized by silver staining. Lane 1, clear cell lysate; lane 2, flow-through fraction; lane 3, fraction rinsed with buffer A; lane 4, sodium arsenate-eluted protein; lane 5, binding proteins eluted with 100 m*M* sodium phosphate; lane 6, proteins eluted with column regenerating buffer (2 *M* NaCl in loading buffer); lane 7, rabbit muscle pyruvate kinase (RMPK).

revealed a single protein band with a molecular mass of 58 kDa (Fig. 3, lane 4). This 58-kDa protein was apparently specifically bound to As(V)–Sepharose, since no corresponding protein band could be found in the flow-through volume or in fractions eluted with either 0.5 *M* NaCl or 2 *M* NaCl (Fig. 3, lanes 2, 3 and 6). Similar electrophotographs were obtained when the As(V)–Sepharose binding proteins were eluted with 100 m*M* sodium phosphate (Fig. 3, lane 5). These results further confirmed that pentavalent arsenate may be involved in phosphate-mediated biochemical processes.

The PK of KB cells could be eluted from As(V)–Sepharose column by a stepwise increase of sodium arsenate concentration starting from 5 m*M*, and most of the PK protein (96.5%) was eluted at sodium arsenate concentrations between 5 and 20 m*M* (Fig. 4A). When the sodium arsenate concentration was further increased to 50 m*M*, a homogeneous band attributable to a PK protein was still obtained by SDS–PAGE (Figs. 2 and 4B). However, a further increase in sodium arsenate concentration (e.g., 100 m*M*) resulted in the appearance of other minor bands in the SDS–polyacrylamide gel (data not shown).

Amino acid sequencing provided evidence that the 58 kDa protein, eluted with sodium arsenate, contains a N-blocked terminus. Therefore, we decided to determine the partial sequence of some fragments generated by acid digestion and CNBr cleavage. Two peptides, one attributable to CNBr cleavage and the other to partial acid digestion, were isolated and subjected to amino acid sequencing. Their N-terminal amino acid sequences were determined to be VFASFIRKASDVHEVR and PTEATAVGAVE. A homology search in the protein data bank (Swiss-Port protein sequence database release 34.0) showed that the two peptide sequences are completely identical to amino acid residues 239–254 and 407–417 of human PK-M1 and PK-M2. Western immunoblotting confirmed that this 58-kDa protein was PK. As shown in Fig. 5, the sodium arsenate- and sodium phosphate-eluted proteins were recognized by the PK antibody. Immunoblotting of commercial PK showed one major band attributable to proteins with a molecular mass of 58 kDa. As reported by others, the molecular mass of PK ranges from 57 to 60 kDa [17,18]. PK-M1 and -M2 are encoded by the same gene with differential splicing and share similar



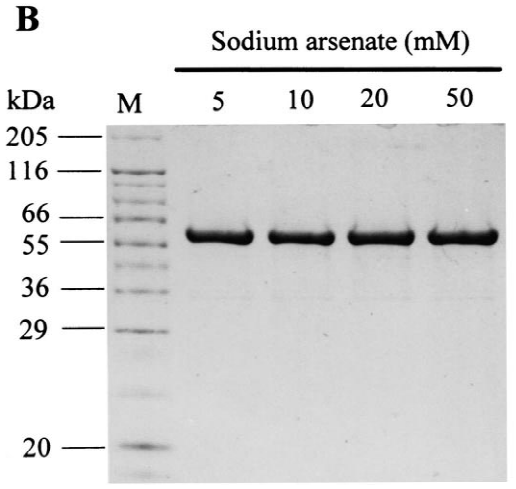


Fig. 4. Stepwise elution of PK from the As(V)–Sepharose affinity column with sodium arsenate. The KB cell extract was prepared and loaded onto the As(V)–Sepharose affinity column as depicted in Fig. 2. After extensive washing, the bound proteins were stepwise eluted with increasing concentrations of sodium arsenate in buffer A. (A) Proteins in each fraction were concentrated and quantified as described in Experimental. (B) SDS–PAGE (12.5%) of an aliquot of 3 μg proteins from each fraction. The proteins were visualized by Coomassie Blue staining. M indicates protein marker.

immunological properties (the amino acid sequence of PK-M1 from rabbit show 93% identity to that of PK-M2 from human) [19], hence we concluded that this 58-kDa protein was a human M type PK.

Table 1 summarizes the results from the PK purification procedure. As(V)–Sepharose affinity

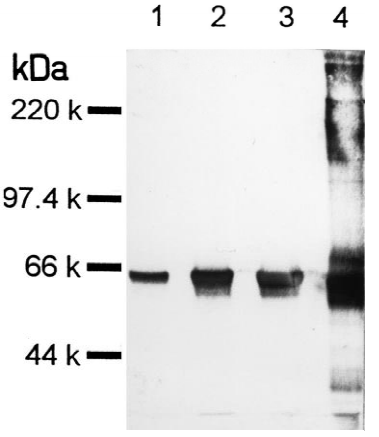


Fig. 5. Immunoblot analysis of As(V) binding proteins with pyruvate kinase antibody. The sodium arsenate- and sodium phosphate-eluted proteins were electrophoretically separated (8% SDS–PAGE) and transferred to a nitrocellulose membrane. The membrane was immunoblotted with rabbit PK-M1 antibody. Lane 1, cell lysate (5 μg); lanes 2 and 3, 50 m*M* sodium arsenate- and 100 m*M* sodium phosphate-eluted proteins (1.5 μg), respectively; lane 4, RMPK (1.5 μg).

chromatography showed that the yield of PK was 33.7% and the specific activity was 153.15 U/mg protein in the presence of 6 m*M* fructose-1,6-biphosphate as an enzyme activator. Without fructose-1,6-biphosphate, sodium arsenate-eluted PK showed a very low specific activity of 1.13 U/mg protein (data not shown). Fructose-1,6-biphosphate was previously shown to activate PK activity by induction of tetramer formation [17,20]. The activation of PK by fructose-1,6-biphosphate is a common characteristic also shared by PK-L, R and M2 type isozymes [17], but not by PK-M1 [21]. Therefore, PK isolated from KB cells by As(V)–Sepharose chromatography could be further characterized as a M2 type PK monomer that is known to be present in most adult tissues. It should be emphasized that PK could also be eluted with sodium phosphate. However, even in the presence of fructose-1,6-biphosphate (6 m*M*), the yield was 0.74% and the specific activity 6.67 U/mg protein, i.e., 45.5- and 22.96-fold less effective compared to elution with sodium arsenate.

Fig. 6A shows the Lineweaver–Burk plots of purified PK. The K_m value toward PEP is 0.12 m*M* which is much smaller than the K_m values toward PEP in other reports [22–25] which ranged from 0.2 to 0.8 m*M*. Furthermore, PK activity was dose-

Table 1
Purification of pyruvate kinase from KB cells by As(V)–Sepharose chromatography

Step	Protein (mg)	Total activity (U)	Specific activity[a] (U/mg)	Yield (%)	Degree of purification (fold)
Crude extract	12	52	4.33	100	1
Flow-through	10.12	0	0	0	
Wash	0.36	1.36	3.77	2.6	
Arsenate eluent	0.1144	17.52	153.15	33.7	35.37
Regeneration	0.0612	0	0	0	

[a] One unit U is defined as that PK activity responsible for the release of one micromole of pyruvate from phospho(enol)pyruvate (0.625 m*M*) in the presence of ADP per minute at pH 7.5 and 25°C.

dependently inhibited by sodium arsenate (K_i=28.3 m*M*), but not by *p*-arsanilic acid (Fig. 6B).

4. Discussion

Our present study showed that a single protein band with a molecular mass of 58 kDa eluted from an As(V)–Sepharose affinity column with 50 m*M* sodium arsenate was a PK monomer. PK activity was only detected in the protein fraction eluted with sodium arsenate, but not in the flow-through volume or washing and regeneration buffers (Fig. 2), indicating that the As(V)–Sepharose column is highly efficient for purification of PK.

PK from a variety of sources [21,26–29] has been purified to homogeneity. In general, a series of chromatographic procedures has been used for purification of PK, including phosphocellulose, Sephadex G200, blue-dextran agarose, and ADP-agarose column chromatography [21,27,30,31]. Compared with other supports for purification, As(V)–Sepharose column chromatography is an alternative and simple method for PK enrichment. The specific activity of purified PK was 153.15 U/mg protein (Table 1), which is substantially higher than the specific activity measured in commercially available PK ranging from 108 to 125 U/mg protein [23]. However, the specific activity of our preparation was slightly lower than the reported values for other sources of PK [17,27,30]. This difference is likely due to the different sources of PK used in the various studies.

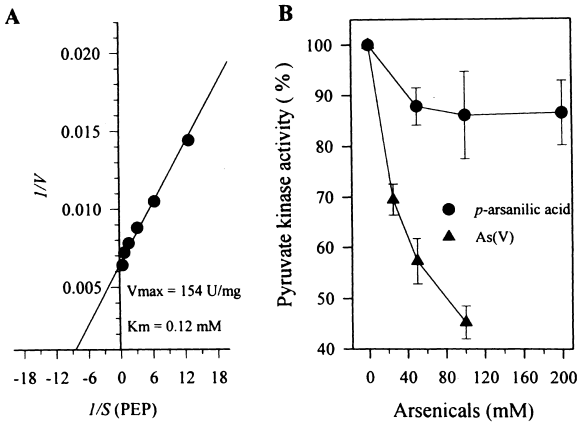


Fig. 6. Lineweaver–Burk plot of purified PK and inhibitory effects of arsenical compounds on PK activity. (A) Kinetics of PK purified by affinity chromatography were performed as described in Experimental. (B) PK activity assayed in the presence of various concentrations of sodium arsenate and *p*-arsanilic acid.

The specific activity of PK obtained from KB cells has not been previously reported. The KB cells used in the present study were derived from a malignant transformed oral tumor and require a high level of intrinsic PK activity for growth. Numerous reports have shown that the specific activity of PK in rapidly growing cells is greater than in normal cells [32–34]. Due to the high abundance of PK in KB cells (specific activity: 4.33 U/mg), we were able to purify PK to 35.4-fold using one-step As(V)–Sepharose affinity chromatography.

PK consists of a family of four isozymes, designated as L, M1, M2 (or A) and R [18,21]. Although the amino acid sequences of two peptides after acid digestion and CNBr degradation clearly indicated that the isolated 58-kDa protein is a M-type PK, it was impossible to differentiate between M1 or M2 types (the amino acid sequence of PK-M1 from rabbit show 93% identity to that of PK-M2 from human). Without external addition of 6 m*M* fructose-

1,6-biphosphate, the specific activity of sodium arsenate-eluted PK was extremely low. These results are consistent with other reports that showed that the catalytically inactive monomeric PK-M2 enzyme is presumably converted into the active tetrameric form in the presence of fructose-1,6-biphosphate [17–19]. From a kinetic point of view, PK-M1 activity is independent of the presence of fructose-1,6-biphosphate [21], indicating that PK isolated from KB cells is a PK-M2 isozyme.

In contrast to sodium arsenate, the specific activity of phosphate-eluted PK was only 4% of sodium arsenate-eluted PK. Since phosphate is the putative substrate for PK, it may bind more strongly than arsenate to the enzyme and is not readily removed from the enzyme through ultrafiltration. Due to the structural similarity between phosphate and arsenate, the As(V) moiety of *p*-arsanilic acid may serve as a ligand for protein binding, similar to the binding via a phosphate moiety located on the enzymes' active site. This view is further supported by the fact that phosphate can be substituted by arsenate in ATP synthesis [1,2]. Enzymes that act on phosphorus compounds often act similarly on arsenic analogues [9–11]. For example, arsonopyruvate, an analogue of phosphonopyruvate with an As(V) moiety, is also a competitive inhibitor for phosphoenolpyruvate mutase [35,36]. It is worthy to note that the radius of arsenate and phosphate differ significantly and thus there are differences in catalytic actions; in this respect, arsenate can replace phosphate in an enzyme reaction (namely arsenylation), however, the resultant product is unstable and hydrolyzes spontaneously [9,37]. Therefore, arsenate offers an advantage over sodium phosphate as an eluent for PK in view of its ability to remove the ligand from enzymes by dialysis.

PK is an enzyme responsible for catalyzing an irreversible transfer of a phosphate moiety to an ADP molecule. The inhibitory effects of sodium arsenate on PK activity confirm the specific interaction between PK and As(V) moiety. However, it still remains unclear why PK activity is not inhibited by *p*-arsanilic acid. Further investigations are required to clarify whether the relatively large phenyl substituent in free *p*-arsanilic acid affects the interaction of the bound As(V) moiety with the enzyme. We also used As(V)–Sepharose chromatography for purifying of PK from human placenta and pig liver, and our preliminary investigations suggest that As(V)–Sepharose can also be effective for these purposes. Since pentavalent arsenate is a good analog of phosphate, studies targeting on the feasibility of using As(V)–Sepharose for its binding ability for other phosphoproteins are currently underway.

5. Abbreviations

As(V)	Pentavalent arsenate
As(V)–Sepharose	Arsanilic acid–Sepharose
CNBr	Cyanogen bromide
PEP	Phospho(enol)pyruvate
PK	Pyruvate kinase
Pi	Phosphate group
RMPK	Rabbit muscle pyruvate kinase
PBS	Phosphate-buffered saline

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References

[1] R.A. Mitchell, B.F. Chang, C.H. Huang, E.G. DeMaster, Biochemistry 10 (1971) 2049.
[2] M.J. Gresser, J. Biol. Chem. 256 (1981) 5981.
[3] R.N. Huang, T.C. Lee, Toxicol. Appl. Pharmacol. 136 (1996) 243.
[4] T. Thiel, J. Bacteriol. 170 (1988) 1143.
[5] K. Kamiya, W.B.T. Cruse, O. Kennard, Biochem. J. 213 (1983) 217.
[6] T. Pawson, Nature 373 (1995) 573.
[7] H.K. Kim, J.W. Kim, A. Zilberstein, B. Margolis, J.G. Kim, J. Schlessinger, S. Rhee, Cell 65 (1991) 435.
[8] S. Nishibe, M.I. Wahl, S.M.T. Hernandez-Stotomayer, N. Tonks, S.G. Rhee, G. Carpenter, Science 250 (1990) 1253.
[9] S.R. Adams, M.J. Sparkes, H.B.F. Dixon, Biochem. J. 221 (1984) 829.

[10] R. Lagunas, D. Pestana, J.C. Diez-Masa, Biochemistry 23 (1984) 955.
[11] T.A. Rozovskaya, V.O. Rechinsky, R.S. Bibilashvili, Biochem. J. 224 (1984) 645.
[12] A.B. Ljungforetagen, Affinity Chromatography – Principle and Methods, Pharmacia Fine Chemicals, Uppsala, 1979.
[13] M.A. Bradford, Anal. Biochem. 72 (1976) 248.
[14] U.K. Laemmli, Nature 227 (1970) 680.
[15] A.S. Dion, A.A. Pomenti, Anal. Biochem. 129 (1983) 490.
[16] V.S. Tanphaichitr, V. Eys, J. Clin. Chim. Acta 41 (1972) 41.
[17] K. Ashizawa, M.C. Willingham, C.M. Liang, S.Y. Cheng, J. Biol. Chem. 266 (1991) 16842.
[18] K. Imamura, T. Tanaka, in: W.A. Wood (Ed.), Methods in Enzymology, Vol. 90, Academic Press, New York, 1982, p. 150.
[19] H. Kato, T. Fukuda, C. Parkison, P. McPhie, S.Y. Cheng, Proc. Natl. Acad. Sci. USA 86 (1989) 7861.
[20] K. Ashizawa, P. McPhie, K.H. Lin, S.Y. Cheng, Biochemistry 30 (1991) 7105.
[21] G. Farrar, W.W. Farrar, Int. J. Biochem. Cell Biol. 22 (1995) 1145.
[22] M. Gosalvez, L. Lopez-Alarcon, S. Garcia-Suarez, A. Montalvo, S. Weinhouse, Eur. J. Biochem. 55 (1975) 315.
[23] J.E. Feliu, J. Diaz-Gil, R. Garcia-Canero, M. Gosalvez, FEBS Lett. 50 (1975) 334.
[24] D. Balinsky, E. Caynis, L. Bresohn, Biochemistry 12 (1972) 863.
[25] K. Imamura, K. Taniuchi, T. Tanak, J. Biochem. 72 (1972) 1001.
[26] W.R. Kiffmeyer, W.W. Farrar, J. Prot. Chem. 10 (1991) 585.
[27] Z. Hu, W.C. Plaxton, Arch. Biochem. Biophys. 333 (1996) 298.
[28] T. Kedryna, M. Guminska, E. Marchut, Biochim. Biophys. Acta 1039 (1990) 130.
[29] A. Kahn, J. Marie, in: W.A. Wood (Ed.), Methods in Enzymology, Vol. 90, Academic Press, New York, 1982, p. 131.
[30] X. Cheng, R.H. Friesen, J.C. Lee, J. Biol. Chem. 271 (1996) 6313.
[31] J. Parkinson, J.S. Easterby, Biochim. Biophys. Acta 481 (1977) 471.
[32] M. Guminska, M. Stachurska, B. Christiensen, V. Tromholt, J. Kieler, C. Radzikowski, D. Dus, Experientia 45 (1989) 571.
[33] E. Marchut, M. Guminska, T. Kedryna, C. Radzikowski, H. Kusmierczyk, Experientia 44 (1988) 25.
[34] D. Schafer, B. Hammkunzelmann, K. Brand, FEBS Lett. 417 (1997) 325.
[35] S. Chawla, E.K. Mutenda, H.B.F. Dixon, S. Freeman, A.W. Smith, Biochem. J. 308 (1995) 931.
[36] H.B.F. Dixon, E.K. Mutenda, M.J. Sparkes, Appl. Organomet. Chem. 11 (1997) 251.
[37] L. Slooten, A. Nuyten, Biochem. Biophys. Acta 725 (1983) 49.