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Enolase from *Klebsiella pneumoniae* and Human Muscle Cells. I. Purification and Comparative Molecular Studies

Enolaza z komórek *Klebsiella pneumoniae* i mięśniowa enolaza ludzka. I. Oczyszczanie i porównawcze badania właściwości molekularnych

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Abstract

Background. This report concerns the glycolytic enzyme enolase from the cytoplasm of *Klebsiella pneumoniae* bacterial cells and its similarity with human muscle-specific enzyme.
Material and Methods. Human muscle-specific enolase was purified from crude extract using standard chromatographic techniques, but this procedure was unsuccessful for isolation of bacterial enzyme. Gel filtration on a Sephadex G-100, anion-exchange chromatography on a DEAE-Sephadex A-50 column, and preparative electrophoresis were applied to obtain an electrophoretically homogenous cytosolic protein from *K. pneumoniae* cells.
Results. Human muscle-specific enolase was purified 83-fold with a specific activity of 75 U/mg. The new procedure resulted in a 76-fold purification of bacterial enolase, with a recovery rate of 14% and specific activity of 31 U/mg. The purified protein analyzed in SDS-PAGE appeared as a single band with a molecular mass of 47 kDa. A similar molecular weight (45 kDa) for the human enolase monomer was obtained. The molecular mass of the native *K. pneumoniae* enolase was estimated to be 94 kDa.
Conclusions. Although the specific activity of purified *K. pneumoniae* enolase is half that observed for human muscle-specific enzyme, the application of preparative gel electrophoresis for the purification of the bacterial enolase permits obtaining a homogenous enzyme with relative good recovery. The results presented in this report indicate that both enzymes have a dimeric native structure, comparable to enolases from other sources. In the second part of these investigations the kinetic differences between the bacterial and human muscle-specific enolase will be presented (**Adv Clin Exp Med 2009, 18, 1, 71–78**).

Key words: enolase, enzyme purification, preparative gel electrophoresis, molecular properties, *Klebsiella pneumoniae*.

Streszczenie

Wprowadzenie. Doniesienie dotyczy glikolitycznego enzymu enolazy z cytoplazmy komórek bakteryjnych *K. pneumoniae* i jego podobieństwa z ludzką enolazą mięśniowoswoistą.
Materiał i metody. Enolazę mięśniowoswoistą człowieka otrzymywano, stosując standardowe techniki chromatograficzne, ale były one nieskuteczne w wydzielaniu enzymu bakteryjnego. W celu uzyskania homogennego białka cytozolowego z komórek *K. pneumoniae* wykorzystano filtrację żelową na Sephadex G-100, chromatografię jonowymienną w żelu DEAE Sephadex A-50 i elektroforezę preparatywną
Wyniki. W procedurze wydzielania enolazy mięśniowoswoistej człowieka otrzymano enzym jednorodny, o aktywności specyficznej 75 U/mg i 83-krotnym stopniu oczyszczenia. W opracowanej nowej metodzie izolowania enzymu bakteryjnego uzyskano 76-krotne oczyszczenie, z wydajnością 14% i aktywnością specyficzną 31 U/mg. Analizowane metodą SDS-PAGE oczyszczone białko ujawniło się jako pojedynczy prążek o ciężarze cząsteczkowym 47 kDa. Dla monomeru enolazy ludzkiej określono niższy ciężar cząsteczkowy – 45 kDa. Masę cząsteczkową natywnej enolazy *K. pneumoniae* określono w warunkach elektroforezy niedenaturującej jako 94 kDa, co wskazuje na dimeryczną strukturę cząsteczki.

Wnioski. Mimo że specyficzna aktywność oczyszczonej enolazy *K. pneumoniae* jest dwukrotnie niższa od wartości otrzymanej dla enolazy mięśniowej człowieka, zastosowanie preparatywnej elektroforezy żelowej w oczyszczaniu enzymu bakteryjnego pozwoliło uzyskać homogenny enzym ze względnie dobrą wydajnością. Zaprezentowane w tym doniesieniu wyniki wskazują, że oba enzymy mają w formie natywnej dimeryczną strukturę, podobnie jak enolazy z innych źródeł. W drugiej części niniejszych badań zostaną przedstawione różnice kinetyczne między bakteryjną i mięśniowoswoistą enolazą człowieka (**Adv Clin Exp Med 2009, 18, 1, 71–78**).

Słowa kluczowe: enolaza, oczyszczanie enzymów, elektroforeza preparatywna, właściwości cząsteczkowe, *Klebsiella pneumoniae.*

Enolase (2-phosphopyruvate hydrolyase – EC 4.2.1.11) catalyses the Mg^{2+}-dependent conversion of 2-PGA (2-phospho-D-glycerate) to PEP (phosphoenolpyruvate) (Fig. 1). It is essential for the degradation of carbohydrates along the glycolysis pathway as well as for glucose synthesis via gluconeogenesis. As this reaction occupies a key position in the metabolic pathway of fermentation, enolase is ubiquitously present in abundance in the biological world [1]. Enolase has been found in almost all organisms in several isoforms. In vertebrates, among them mammalian and human cells, it is active as a homo- or heterodimer. Tissue-specific isoforms of the enzyme are formed by two of the three types of subunits, α, β, and γ. Each subunit is encoded by a distinct gene whose expression is regulated in a tissue-specific and development-specific manner. The αα embryonic form is widely distributed in most adult tissues. During development, the accumulation of specific isoforms accompanies the differentiation of two tissues with high energy demands: αβ and ββ in striated muscles and αγ and γγ in the brain [1, 2]. Alpha-enolase is present in most vertebrate tissues, including liver, kidney, lung, spleen, and adipose tissue, whereas β is located in the heart and skeletal muscles and the γ form is found only in neurons and neuroendocrine cells [3]. An approximately 82% amino-acid sequence identity between the three types of subunits in mammalian and human enolases was observed [3, 4].

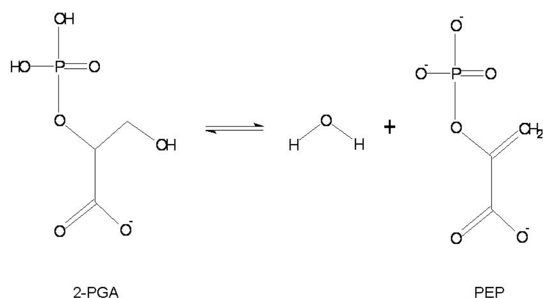

Fig. 1. The reversible dehydration of 2-phospho-D-glycerate (2-PGA) to phoshoenolpyruvate (PEP) catalyzed by enolase

Ryc. 1. Odwracalna reakcja dehydratacji 2-fosfo-D-glicerynianu (2-PGA) do fosfoenolopirogronianu (PEP) katalizowana przez enolazę

Enolase has been isolated and characterized from a broad spectrum of sources. The enzyme has been found in organisms from eubacteria to mammals and has maintained a highly conservative primary and tertiary structure throughout evolution [5, 6]. Comparison of the amino-acid sequences determined for about 80 enolases from different species demonstrated a high degree of identity in evolutionarily distant species. Between primates and lower organisms an about 50% sequence homology of this protein was observed [3, 6].

Although enolase from all eukaryotes and many prokaryotic species appears as a dimer, an octameric enzyme has been reported in some bacterial strains, for example the hyperthermophilic *Thermotoga maritima* and the Gram-positive pathogen *Streptococcus pneumoniae* [7, 8]. The subunit molecular weight of the most isolated enolases is in the range of 40–50 kDa, but for the monomer from *Streptococcus rattus* it is 22 kDa [9].

Enolase has been isolated from many different organisms in this department [10–12]. The subject of this investigation is a bacterial enolase from cytoplasm from *Klebsiella pneumoniae* cells. The cell surface enolase like protein of *K. pneumoniae* has been described in prerias report. This protein showed some epitope similarity with human muscle-specific enolase and maintained residual enzymatic activity in the inner-membrane fraction [13]. In the present study a simple method for the purification of enolase from the cytosolic fraction of *K. pneumoniae* cells is presented. The essential molecular properties of this enzyme are described and compared with those of human muscle enolase for a better understanding of some differences between the bacterial and human enzymes.

Material and Methods

All chemicals used were of analytical grade. 2-Phospho-D-glycerate was purchased from Fluka. The kit of molecular mass protein markers for SDS-PAGE (sodium dodecyl sulfate) was from Bio-Rad. Native molecular mass protein standards and other

reagents were purchased from Sigma-Aldrich. *K. pneumoniae* strain 21 was obtained from the Department of Microbiology of Wroclaw Medical University. Bacterial cells were cultivated in TSB (Tripticase Soy Broth) (BIOCORP). Tissue samples of human *tibialis anterior* muscle were obtained from postoperative material from the Department of Vascular, General, and Transplantation Surgery of Wroclaw Medical University in accordance with the Polish legal requirements under a license issued by the Commission of Bioethics of Wroclaw Medical University.

Purification of Human Muscle-Specific Enolase

Human β-enolase was isolated according to the method of Witkowska et al. [13] with some modifications. Briefly, frozen human striated muscle was homogenized with deionized water containing 3 mM $MgSO_4$ and the protease inhibitors PMSF (phenylmethylsulfonyl fluoride) and aprotinin (2 μg/ml). The homogenate was centrifuged at 4500 × *g* for 30 min. and the supernatant was filtered through gauze and heated to 53–54°C for 3 min, cooled to 4°C, and centrifuged at 9000 × *g* for 45 min. The supernatant was treated with 60–80% saturated ammonium sulfate and the precipitated proteins were centrifuged at 9000 × *g* at 4°C for 45 min. The pellet was dissolved in buffer A (20 mM Tris-HCl buffer (2-amino-2(hydroxymethyl)-1,3-propanediol), pH 9.0, containing 3 mM $MgSO_4$ and 1 mM β-mercaptoethanol (β-ME)), dialyzed overnight against the same buffer, and applied to a DEAE-Sephadex A-50 column (30 × 3 cm) equilibrated with buffer A, which was also used for elution. Enolase was not retained under these conditions and fractions containing enolase activity were collected and precipitated with ammonium sulfate. The pellet was dissolved in buffer B (10 mM phosphate (Na^+) buffer, pH 6.4, with 3 mM $MgSO_4$) and, after dialysis against buffer B, was run on a CM-Sephadex C-50 column (10 × 3 cm) equilibrated with the same buffer. The protein was eluted with a pH gradient of 6.4–9.0. Fractions with enolase activity were pooled, concentrated and, after dialysis against buffer A, fractionated on a QAE-Sephadex column (5 × 1.6 cm) in buffer A. The main peak, containing about 90% enolase activity, was collected and precipitated by dialysis in 80% ammonium sulfate. The pellet was dissolved in 7.5 mM imidazole-HCl buffer, pH 6.8, containing 2.5 mM $MgSO_4$, 50 mM NaCl, and 50% glycerol and stored at 4°C for several months without loss of activity.

Purification of *K. pneumoniae* Enolase

The purification of enolase from *K. pneumoniae* bacterial cells required a simplification of the procedure used for human muscle-specific enzyme. Bacterial cells were grown at 37°C for 24 h in TSB without shaking, then centrifuged and washed with PBS (phosphate buffered saline). In the next step the cells were resuspended in 10 mM Tris-HCl buffer, pH 7.8, containing 1 mM $MgSO_4$, 0.5 mM β-ME, 1% glycerol, and the protease inhibitors 4 mM PMSF and aprotinin 2 μl/10-ml. The suspension was treated with ultrasound for 30 min at 0˚C with a Vibra-Cell YC-130PB (LaboPlus). The disrupted cell suspension was centrifuged at 4000 × *g* for 45 min at 4°C to remove cell debris. The supernatant, obtained by low-speed centrifugation, was centrifuged at 100,000 × *g* for 1 h to separate the envelope fraction and the supernatant was used as the crude extract for further purification.

Bacterial enolase from the crude extract was precipitated by dialysis against an 80–100% saturated AS (ammonium sulfate) solution at 4°C. The pellet was collected after centrifugation at 8000 × *g* at 4°C for 30 min, dialyzed against 20 mM Tris-HCl buffer, pH 7.8, containing 1 mM $MgSO_4$ and 1 mM β-ME (buffer C), and fractionated on a Sephadex G-100 column (100 × 1.8 cm) at 4°C. Fractions with enolase activity were eluted with buffer C, collected, and precipitated overnight by dialysis against an 80–100% saturated AS solution, pH 7.0, at 4°C. The pellet was centrifuged at 8000 × *g* for 30 min at 4˚C, resuspended in 20 mM Tris-HCl buffer, pH 9.0, containing 1 mM $MgSO_4$ and 1 mM β-ME (buffer D), and dialyzed overnight at 4°C against large volumes of the same buffer. The dialyzed proteins were loaded to a DEAE Sephadex A-50 column (30 × 3 cm) equilibrated with buffer D. Bacterial enolase was adsorbed on an anion exchanger under these conditions. Bound proteins were eluted with a linear gradient of 0-0.5 M NaCl in the same buffer. Fractions with enolase activity were collected and concentrated by ultrafiltration through a 30-kDa cutoff membrane (Amicon, Millipore). In the next step the preparative purification of enolase was performed in a Prep Cell apparatus model 491 (Bio-Rad). According to the manufacturer's instructions, a gradient of concentrations of a polyacrylamide gel system containing 10 ml of 12%, 38 ml of 10%, 32 ml of 8%, and 10 ml of 6% resolving gels and 12 ml of 4% stacking gel were prepared. A 37-mm-ID tube was set up for electrophoresis using an electrode buffer containing 43 mM imi-

dazole and 35 mM HEPES (N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid). The same buffer was used in the cathode and anode reservoirs as well as for elution. The enolase sample containing 70–80 mg of protein in 1.5 ml of electrode buffer was applied for resolution and electrophoresis was run at 100 V. The elution of proteins with the electrode buffer was started when the bromophenol blue indicator band reached the base of the separating gel. Fractions with enolase activity were pooled and concentrated using a 10-kDa cut-off membrane (Amicon, Millipore). The homogeneity of the enzyme was determined by SDS-PAGE. The enolase preparation was dialyzed against 20 mM Tris-HCl buffer, pH 7.8, with 1 mM $MgSO_4$ and stored at –80°C.

SDS-Polyacrylamide Gel Electrophoresis

SDS-PAGE was performed using a mini gel apparatus (Biometra). Samples of protein were applied to 10% acrylamide resolving gels [14] using an electrode buffer containing 25 mM Tris, 192 mM glycine, and 0.1% SDS for 45 min at 200 V. After electrophoresis, the gels were stained with 0.25% Coomassie Brilliant Blue R 250 in 10% acetic acid and 40% methanol and destained using a solution of 5% methanol and 7.5% acetic acid in water. Under these conditions the molecular weight of the enolase subunit was estimated using the Vilber Lourmat System and BIO 1D+ software.

Molecular Weight Determination of Native *K. pneumoniae* Enolase and Human β-enolase by Polyacrylamide Gel Electrophoresis in Native Conditions

The molecular weight of native enolase was determined by electrophoresis in non-denaturing systems according to the Sigma manual bulletin. A sample of 10 µg of enolase and non-denatured protein molecular-weight markers were characterized by 7%, 8%, 9%, and 10% polyacrylamide gels. Resolution was performed in 5 mM Tris-HCl buffer, pH 8.3, containing 38 mM glycine. After determining the R_f of the protein in each gel relative to the tracking dye, values of 100×[log-(R_f × 100)] were plotted against the percent gel concentration for each protein (R_f is the protein electrophoretic mobility). From these plots, individual slopes were determined for each protein and the logarithm of the negative of the slope was plotted against the logarithm of the molecular weight of each protein. From this linear plot, the molecular mass of the native enolase was determined.

Protein Concentration Determination

Enolase concentration was determined spectrophotometrically at 280 nm using the absorption coefficient $A_{0.1\%}$= 0.89 established for 1 mg/ml of rabbit muscle enolase [1].

Enolase Activity Assay

Bacterial enolase activity was assayed spectrophotometrically at 240 nm at room temperature as the increase in PEP concentration in a standard assay containing 50 mM imidazole-HCl buffer, pH 7.8, with 1 mM $MgSO_4$, 0.4 M KCl, and 1 mM 2-PGA as a substrate. For measuring human enolase activity, the pH of the assay medium was 6.8 and contained 3 mM $MgSO_4$. One unit of enolase activity was defined as the amount of protein which catalyses the synthesis of 1 µmol of PEP from 2-PGA in 1 min under these conditions. The molar absorption coefficient for PEP was taken as 1.52 $M^{-1}cm^{-1}$ [11].

Results

Purification of Enolases

The purification of bacterial and human muscle-specific enolases is summarized in Table 1. Enolase from human striated muscle was purified from crude extract using standard chromatographic techniques. Nine mg of 83-fold purified enolase with a specific activity of 75 U/mg and 15% recovery was obtained from 150 g of muscle tissue. Such a procedure applied to bacterial cells gave inhomogeneous enolase of low activity. Therefore, the heat-treatment step was omitted and the fractionation on CM-Sephadex and QAE-Sephadex columns was replaced by preparative gel electrophoresis for bacterial proteins. The procedure for the isolation and purification of enolase from bacterial cells is demonstrated in Fig. 2. The enolase from the cytosol of *K. pneumoniae* was purified from the crude cell extract by protein precipitation in 80–100% saturated ammonium sulfate, two chromatographic steps, and preparative electrophoresis in non-denaturing conditions. The elution profile obtained during fractionation of the

Table 1. Purification of *K. pneumoniae* enolase and human muscle-specific enolase

Tabela 1. Bilans oczyszczania enolazy cytosolowej z komórek bakteryjnych *K. pneumoniae* i mięśniowoswoistej enolazy ludzkiej

Step (Etap)	Total protein [mg] (Całkowita ilość białka)	Total activity [U] (Aktywność całkowita)	Specific activity [U/mg] (Aktywność specyficzna)	Purification (fold) (Stopień oczyszczenia)	Yield [%] (Wydajność)
Human β-enolase					
Crude extract	5000	4500	0.9	1	100
Heat treatment	3000	4200	1.4	1.6	93
60–80% $(NH_4)_2SO_4$ precipitation	750	3225	4.3	4.8	72
DEAE-Sephadex A-50	108	2270	21	23	50
CM-Sephadex C-50	48	1440	30	33	32
QAE-Sephadex	9	675	75	83	15
K. pneumoniae enolase					
Crude sonic extract	3276	1441	0.41	1	100
80–100% $(NH_4)_2SO_4$ precipitation	790	987.5	1.25	3.05	68.5
Sephadex G-100	280	588	2.1	5.15	40.8
DEAE-Sephadex A-50	33	293.7	8.9	21.7	20.4
Preparative electrophoresis	6.5	202.8	31.2	76.1	14.1

K. pneumoniae crude extract by Sephadex G-100 gel chromatography is presented in Fig. 3A. In this step, substantial amounts of ballast proteins were removed. Fractions with enolase activity were applied to the DEAE-Sephadex A-50 column. The adsorbed enolase on the anion exchanger was eluted with a linear gradient of 0–0.5 M NaCl (Fig. 3B). Fractions with significant enolase activity were pooled, concentrated, and, after dialysis, continuous preparative electrophoresis in non-denaturing conditions on a Prep-Cell 491 system followed. The elution profile from the preparative electrophoresis is presented in Fig. 3C. The fractions with enolase activity formed major and minor peaks of activity, but only the main peak contained electrophoretically homogenous protein (Fig. 4, lane 3). This pure enzyme preparation was used for molecular studies. The enolase from *K. pneumoniae* was purified 76-fold with a specific activity of 31.2 U/mg and has been stored at –80°C for one year with no apparent loss of activity (multiple freezing and thawing steps were avoided).

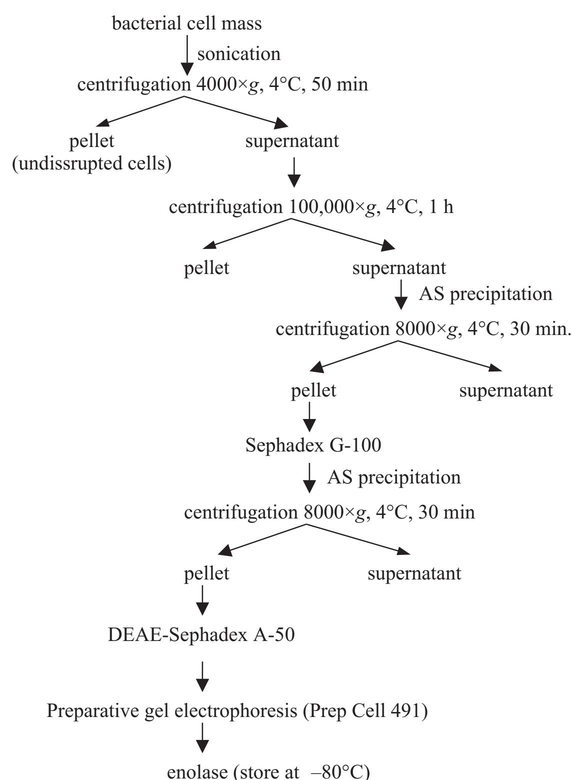

Fig. 2. Schedule of enolase preparation from the cytosol of *K. pneumoniae* cells

Ryc. 2. Schemat izolacji enolazy z cytozolu komórek *K. pneumoniae*

Molecular Properties

The molecular mass of native *K. pneumoniae* enolase was determined by electrophoresis in a non-denaturing system using molecular mass markers and various percentages of polyacrylamide gels. The molecular mass of the native bacterial enolase was found to be 94 kDa (Fig. 5). The molecular weights of the monomers of *K. pneumoniae* enolase and human β-enolase determined by electrophoresis in SDS-PAGE were 47 kDa and 45 kDa, respectively (Fig. 4). Because the molecular mass of the native bacterial enolase was 94 kDa, the enolase from *K. pneumoniae* has a dimeric structure. The single band of protein observed

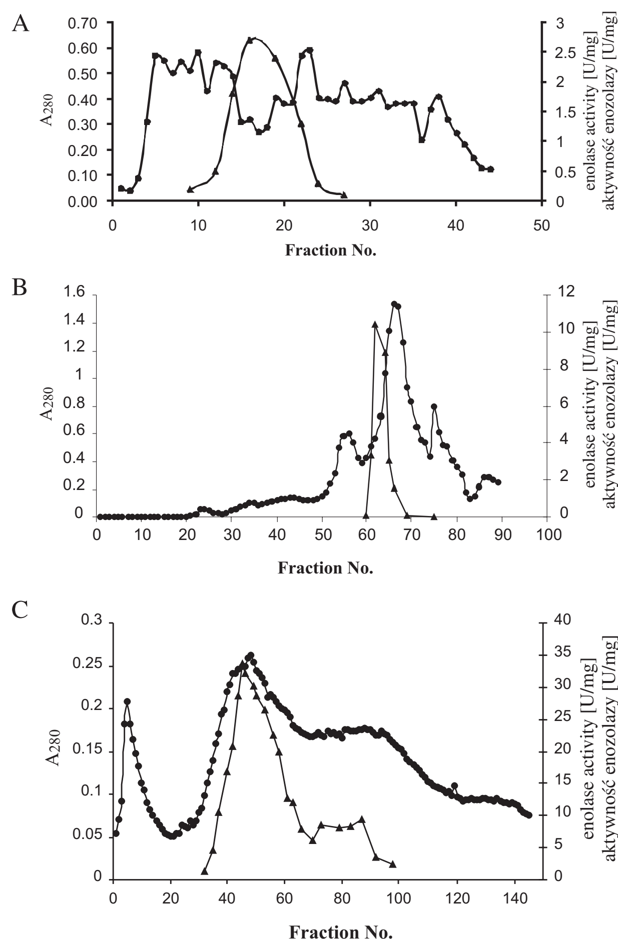

Fig. 3. Purification of *K. pneumoniae enolase*. (A) Sephadex G-100 gel chromatography of *K. pneumoniae.* Enolase. About 700 mg of protein sample was applied to the column. (B) Elution profile of 270 mg of protein sample fractioned on DEAE Sephadex A-50 column. (C) *K. pneumoniae* enolase purification by continuous preparative non-denaturing electrophoresis using a Prep Cell apparatus model 491 (Bio-Rad). 70–80 mg sample was applied on top of the stacking gel. (●) protein profile determined at 280 nm, (▲) enolase specific activity

Ryc. 3. Oczyszczanie enolazy z komórek bakteryjnych *K. pneumoniae.* (A) Rozdział ok. 700 mg białek wyizolowanych z cytosolu pałeczek *K. pneumoniae* w kolumnie z żelem Sephadex G-100.(B) Profil elucji bakteryjnych białek cytosolowych (ok. 270 mg) po rozdziale w kolumnie z żelem DEAE Sephadex A-50. (C) Rozdział białek metodą elektroforezy preparatywnej w warunkach natywnych. Próbki białek ok. 70 mg rozdzielano z użyciem aparatu Prep Cell model 491 (Bio-Rad). (●) profil elucji białek, (▲) aktywność specyficzna enolazy

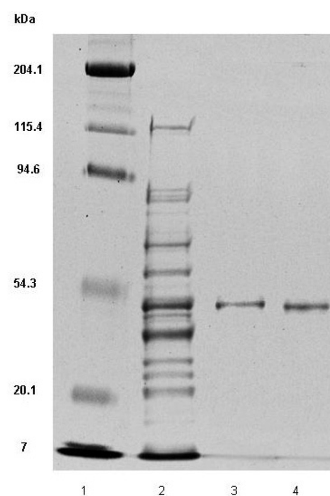

Fig. 4. SDS-PAGE analysis of *K. pneumoniae* enolase purification: lane 1) protein M_w standards; lane 2) 25 μg of partially purified bacterial enolase after DEAE-Sephadex fractionation; lane 3) 8-μg sample of bacterial enolase after preparative electrophoresis; lane 4) 8-μg sample of human muscle enolase after the QAE-Sephadex step

Ryc. 4. Analiza oczyszczania enolazy bakterii *K. pneumoniae* metodą elektroforezy SDS-PAGE. Ścieżka 1: markery mas cząsteczkowych (Bio-Rad), 10 μg, ścieżka 2: białka po częściowym oczyszczeniu na DEAE-Sephadex A-50 (25 μg), ścieżka 3: enolaza po rozdziale metodą elektroforezy preparatywnej (8 μg), ścieżka 4: mięśniowo-specyficzna enolaza ludzka po oczyszczeniu na QAE-Sephadex

after SDS-PAGE analysis (Fig. 4, lane 3) suggested that identical subunits formed a dimeric molecule. Similar properties were observed for human β-enolase (Fig. 4, lane 4).

Discussion

The method of enolase purification from human muscle summarized in Table 1 involves thermal denaturation of the crude protein extract at a temperature of 53–54°C, precipitation of the proteins with 60–80% saturated AS, and ion-exchange chromatography [12, 15–17]. The conditions for ion-exchange chromatography in our experiments were established on the basis of the pI 7.72 value for human β-enolase [18].

The method used to obtain homogenous human β-enolase had low efficiency when applied to the cytosolic enzyme from *K. pneumoniae* cells. Therefore, in the first steps of bacterial enzyme purification (Table 1), the heat treatment was omitted because enzyme activity was lost. For the precipitation of bacterial enolase from the protein mixture, AS was needed in a degree of saturation of 80–100%, higher than for the human muscle enzyme (60–80%). After precipitation, similar efficiency of purity was obtained, namely about 70% in both cases. The ion-exchange chromatography on the DEAE-Sephadex column resulted in a significant amount of colorful protein adsorption under equilibration conditions of the column with

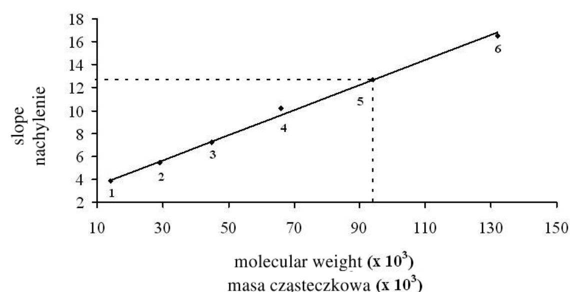

Fig. 5. Determination of the native molecular mass of *K. pneumoniae* enolase by polyacrylamide gel electrophoresis in nondenaturing conditions. Protein standards: (1) bovine lactate albumin 14.2 kDA, (2) carbonic anhydrase 29 kDa, (3) chicken eggs albumin 45 kDa, (4) monomer BSA 66 kDa, (6) dimer BSA 132 kDa(5) enolase from the cytosol fraction of *K. pneumonie* cells

Ryc. 5. Wyznaczanie masy cząsteczkowej natywnego enzymu metodą elektroforezy w warunkach niedenaturujących. Białka standardowe: (1) albumina z mleka wołowego 14.2 kDa, (2) anhydraza węglanowa 29 kDa, (3) albumina z jaja kurzego 45 kDa, (4) monomer BSA 66 kDa, (6) dimer BSA 132 kDa, (5) enolaza cytosolowa komórek *K. pneumoniae*

a Tris-HCl buffer of low ionic strength and high alkaline pH, similar to the case for human muscle-specific enolase purification. Human β-enolase was eluted under these conditions with elution buffer, but bacterial enolase was adsorbed on the DEAE-Sephadex column and was subsequently eluted using a gradient of NaCl. This stage of the purification of bacterial enolase permitted obtaining a degree of purity similar to that obtained from human muscle, i.e. 21.7 and 23, respectively. The yield of this process was lower by about half for the enolase from *K. pneumoniae* (20.4%) than for human β-enolase (50%). The ion-exchange chromatography step on the CM-Sephadex column was omitted in the bacterial enzyme purification because the yield of the procedure was decreased due to the instability of the catalytic activity. In the last step of purification, preparative electrophoresis was performed. This procedure can be especially useful in the isolation of enolase from bacterial cells for a good efficiency of the purification process. We obtained homogeneous protein with 76-fold purity and with a final yield of 14%. Similar results were reported for the purification of enolase from *Escherichia coli* [19] and *Streptococus mutans* cells [20]. The specific activity of homogenous enolase from *K. pneumoniae* was about 50% lower than that of the human muscle enzyme, but similar values were obtained for the enolases from other bacterial and fungal pathogens [8, 12].

Similarly to other glycolysis enzymes, enolase has been shown to have been highly conservative during evolution [5]. Comparison of the amino-acid composition and sequences shows 40–90% identity among enolases from different species [3]; therefore, most eukaryotic enolases have similar subunit molecular weights, ranging from 82 to 100 kDa. The native molecule is usually a homo- or heterodimer [1, 16, 21], but octameric forms have been reported for enolases from a variety of bacterial strains, such as *Streptococcus mutans*, *S. pneumoniae*, *Bacillus subtilis*, and *Thermotoga maritima* [7, 8, 20, 22]. The monomeric molecular mass of *K. pneumoniae* enolase determined by SDS-PAGE was 47 kDa. This is consistent with earlier findings for the enzyme from other sources and is in agreement with the subunit size found for other prokaryotic enolases obtained from various bacterial [22, 19, 24] and fungal strains [25, 15]. The subunit molecular mass established for human β-enolase was 45 kDa, this result being similar to that reported by Cali et al. [32]. The molecular masses of native *K. pneumoniae* and human β-enolase were estimated to be 94 kDa and 90 kDa, respectively, suggesting that both enzymes are composed of two identical subunits.

In conclusion, the results reported in this paper represent a starting point for investigating the kinetic properties of the obtained bacterial enolase for a better knowledge of glycolysis in *K. pneumoniae* cells. These results will be presented in the second part of these studies.

Acknowledgments

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