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PURIFICATION OF ENOLASE FROM HUMAN BRAIN TISSUE

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ABSTRACT

Neuron-specific enolase (NSE) and non-neuronal enolase (NNE) were purified using a small number of methods performed in the following order: DEAE-cellulose chromatography, diafiltration, preparative isoelectric focusing and gel-filtration. Pure isoenzymes were isolated in quantities, which would allow us to produce antisera against each form of the enzyme.

Key words: neuron-specific enolase, non-neuronal enolase, purification, DEAE-chromatography, preparative isoelectring focusing, electrophoresis

INTRODUCTION

Enolase (2-phospho-D-glycerate hydrolase, EC 4.2.1.11), a glycolytic enzyme, catalyzes the interconversion of 2-phosphoglycerate to phosphoenolpyruvate (6). Three distinct dimer isoenzymes of enolase exsist in mature brain tissue: γγ, αα, and αγ (2,4,5,17). Homodimer γγ predominantly present in neurons and neuroendocrine cells is also referred to as neuron-specific enolase (NSE) (18). Homodimer αα localized in the glial cells is called non-neuronal enolase (NNE) (10). Heterodimer αγ shown to be composed of one NSE subunit and one NNE subunit is called intermediate hybrid form (11).

NSE has been of clinical interest as a tumour marker in patients with neoplasms of the neuroendocrine cells, especially in those with neuroblastoma and small cell carcinoma of the lung (7,19,20).

The aim of the present study was to purify these two homodimer forms of enolase from human brain tissue by using a small number of steps and to obtain a sufficient quantity of the purified isoenzymes that could allow us to produce antisera against each form for a further study.

MATERIAL AND METHODS

Human brain tissue obtained at autopsy no more than 12h post mortem was minced and homogenized (1:3w/v) in a washing/equilibrating buffer (WEB): 10mM Tris.HCl (Merck, Germany) containing 5mM $MgSO_4$ (Merck, Germany) at pH 7,8. The homogenate was centrifuged at 100000xg, 4°C, for 60min and the supernatant was passed through a column (50x160) mm of DEAE-cellulose (Reanal, Hungary) previously equilibrated with the above described buffer. The column was eluted with a linear gradient of KCl from 0 to 0,6mol in WEB at 4°C.

Ultradialysis and concentration were performed at 4oC against WEB with ultrafiltration apparatus constructed by us (1) using SM/14549 20000D membranes (Sartorius, Germany).

Preparative isoelectric focusing experiments were performed in Rotophor Bio-Rad camera (BioRad, USA) using 25% glycerine (Merck, Germany) and 2% amfoline, pH 3-10 (BioRad) at 12W, 9-10mA, 1200-1300V, 4°C for 4 hours.

Gel-filtration was performed with a column (250x760mm) of Sephacryl S-200 (Pharmacia, Sweden) equilibrated with 50mM Tris.HCl buffer containing 5 mM $MgSO_4$, 0,1mM EDTA-Na, pH 7,3. The fractions exhibiting enzyme activity were pooled and concentrated with 40-80% ammonium sulphate.

Analytical electrophoresis was carried out on cellulose-polyacetate membranes (CPM) (Sartorius, Germany) using 20mM Tris.HCl pH 7,6 containing 10mM $MgSO_4$ and 0,1% human serum albumin (Sigma, USA) at 2-3 mA, 20°C for 50min with Sartophor electrophoresis camera (Sartorius, Germany). CPMs were developed according to the method of zymograms (20). The CPMs to be assayed were placed on agar film containing 2% agar; 200mM Tris.HCl pH 7,6; 7,3mM D-2-phosphoglyceric acid barium salt; 1mM NADH; 7,6mM ADP; 18,7mM MgSO4; 50mM KCl; 27,3 IU/ml lactate dehydrogenase, and 15 IU/ml pyruvate kinase.

The development was allowed to proceed for 15-20 min at 37°C. The region producing NADH oxidation was visually observed by the loss of fluorescence at 366nm.

The isoelectric focusing experiments in analytical mode

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were carried out with 150mM precots (pH 3-10 or 4-6) (Serva, Germany) in Sartophor electrophoresis camera.

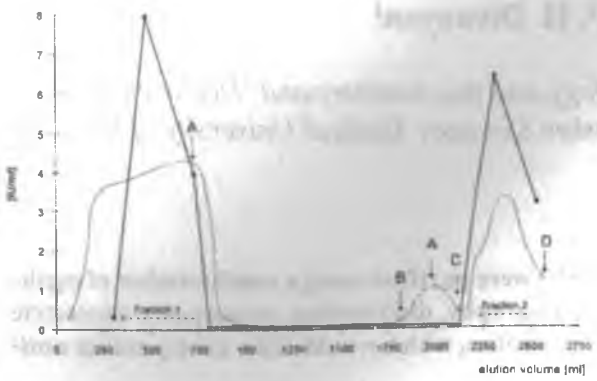

Fig. 1. Elution profile of enolase isoenzymes in human brain homogenate - DEAE-cellulose chromatography ------ collected fractions; —— relative protein content; ▪—▪ enolase activity profile

The precots were stained using non-specific methods for proteins (13) and tested for enolase activity (14). Enolase activity was spectrophotometrically estimated (VSU 2P, Germany) at 366nm, 37°C by coupling the reaction with pyruvate kinase and lactate dehydrogenase (Boehringer, Germany) (17).

Protein concentration was measured spectrophotometrically with the spectrophotometer of the same type at 280nm (15).

RESULTS AND DISCUSSION

The first step in the purification of enolase was by DEAE-ion-exchange chromatography. Fig. 1 presents the elution profile of the homogenate.

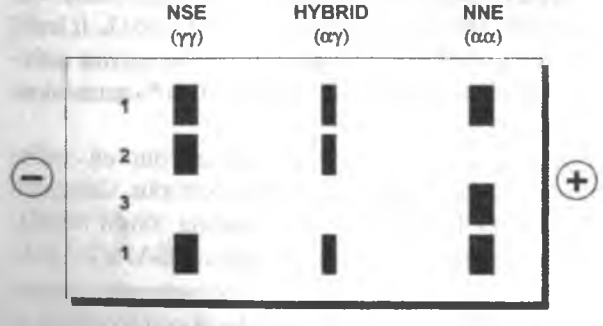

Fig. 2. CPM - electrophoretic scheme of enolase isoenzymes 1 - homogenate; 2 - peak 2; 3 - peak 1; 2(?) samples with 2,7-3,8 IU/ml enzyme activity were applied

Two distinct peaks of enolase activity were detected on the chromatogram: peak 1 produced by non-adsorbed enolase (fraction 1) which was thought to correspond to the αα-form of enolase (NNE) and peak 2 produced by the adsorbed enolase (fraction 2) thought to correspond to αγ + γγ isoforms (hybrid + NSE). In order to prove that fraction 1 contains exclusively NNE and fraction 2 - NSE + Hybrid form, these two fractions were separately ultrafiltrated and concentrated, then analyzed by CPM-electrophoresis (Fig. 2) (9). After the isoelectric focusing of fraction 1 in preparative mode a peak with maximal activity at pH 8,2 was obtained (Fig. 3).

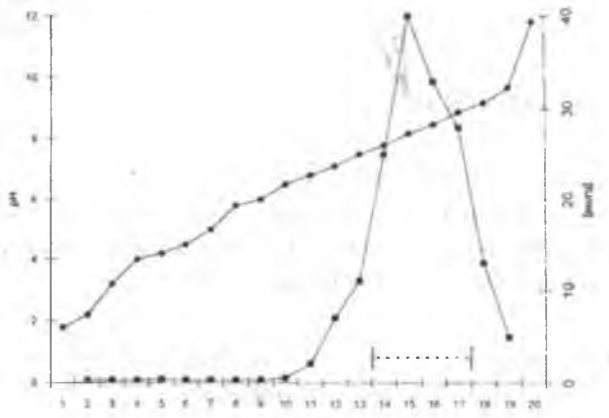

Fig. 3. Preparative isofocusing of fraction 1 ------ collected fractions; ▪—▪ enolase activity profile; ●—● pH gradient

Two peaks with maximal activity at pH 5,4 and 6,4 were obtained after isoelectric focusing of fraction 2 (Fig. 4).

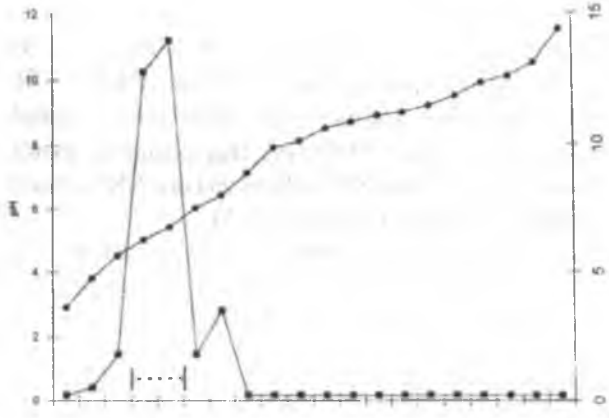

Fig. 4. Preparative isofocusing of fraction 2 ------ collected fractions; ▪—▪ enolase activity profile; ●—● pH gradient

The peak on Fig. 3 (fractions 14-17) and the peak 1 on Fig. 4 (fractions 3-5) designated as NNE and NSE, respectively, were further purified by gel-filtration (Fig. 5 and Fig. 6). The obtained fractions with maximal enzyme activity (NNE - fractions 22-33; NSE - fractions 25-35) were collected, precipitated with ammonium sulphate (40-80%) and kept at 4°C in excess of ammonium sulphate. Aliquotes from each preparation were ultradialysed against WEB (membrane 20000D) for analytic isoelectric focusing.

Each of the purified NNE or NSE preparations produces a single band with enolase activity after analytical isofocusing. The data about the specific activity of these two homodimeric isoenzymes are comparable with these reported by other authors (4,5,11,17).

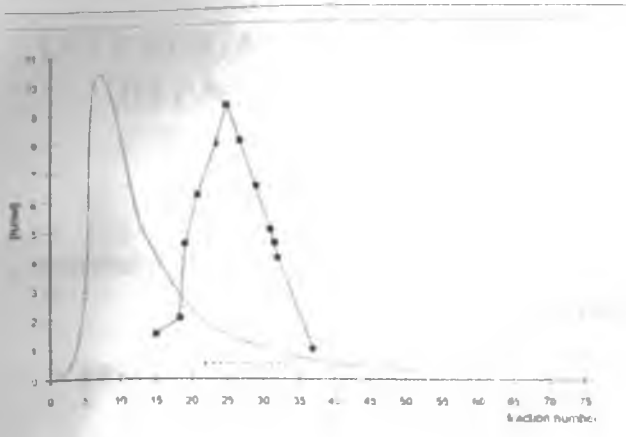

Fig. 5. Gel-filtration on Sephacryl S-200 of the peak from Fig. 3 ------ collected fractions; —— relative protein content; ▪—▪ enolase activity profile

The results from the present study reveal that good purification of two homodimeric isoforms of human brain enolase could be achieved using a small number of purification procedures such as DEAE-chromatography, diafiltration, isoelectric focusing, and gel-filtration whereas the procedures described by others (3,4,8,12,17) include 7-11 purification steps.

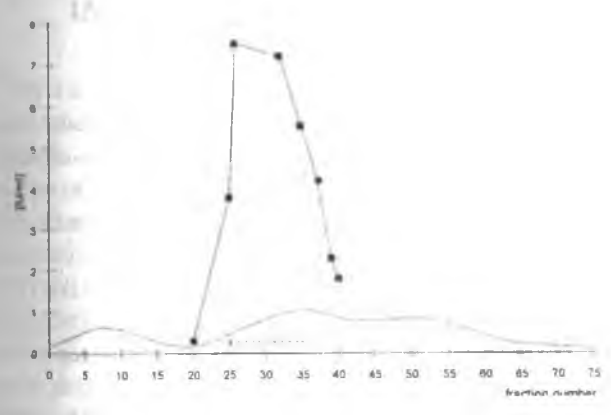

Fig. 6. Gel-filtration on Sephacryl S-200 of the collected fractions of peak 1 from Fig. 4 ------ collected fractions; —— relative protein content; ▪—▪ enolase activity profile

Using this scheme of purification we have obtained sufficient quantities of the purified isoenzymes (NSE and NNE) allowing us to produce specific antisera.

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