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Purification of 6PGD enzyme from rat erythrocytes

Yusuf Temel [1], Mehmet Çiftçi[2]
[1] Department Of Health Services. Vocational Schools, , Bingöl, Turkey
[2]Department of Chemistry, Art and Science Faculty, Bingöl, Turkey
E-mail: ytemel@bingol.edu.tr

Abstract: Pentose phosphate pathway consists of two stages including oxidative and non-oxidative. The aim of this metabolic pathway occurring in the cytosol to produce for reductive biosynthesis events NADPH and in the structure of various compounds ribose 5-phosphate. 6-Phosphogluconate dehydrogenase (6PGD; E.C. 1.1.1.44) enzyme is involved in the synthesis of metabolic intermediates vital importance for life. For example, the 6PGD is an important enzyme in pentose phosphate pathway that catalyzes the second step of the oxidative metabolic pathways. Herein, we purified the 6PGD in 61.5% yield from rat erythrocytes with a specific activity of 1,37 EL) mg~[1] using 2',5'-ADP sepharose 4B affinity chromatography one step. As a result of purifications, the overall purification was determined to be approximately 244,7 fold. A single band and obtained approximately 59,8 kDa was observed from SDS-polyacrylamide gel electrophoresis.

INTRODUCTION

There are two main functions of the pentose phosphate pathway in cells, which are the synthesis of ribose 5-phosphate that are required for DNA, RNA and ribonucleotide synthesis of the reducing power NADPH which is used in reduction reactions [1]. Also, phosphorylated carbohydrates such as erythrose-4-phosphate are synthesized which is vital for synthesis of aromatic amino acids and vitamins and sedoheptulose-7-phosphate that as a component of the bacterial celi wall [2].

6-Phosphogluconate dehydrogenase enzyme (6PGD; E.C.1.1.1.44) catalyzes conversion reaction 6-phosphogluconate to D-ribulose 5-phosphate in the presence of $NADP^+$ which is third step of the pentose phosphate pathway [3]. NADPH is one of the products resulting from these reactions plays a role biosynthesis of fatty acid, cholesterol, L-ascorbic acid, nitric oxide, the reduction of peroxides, drug and xenobiotic detoxification and reduction of glutathione in cells [2,4]. Reduced glutathione (GSH) and GSH-dependent enzymes protect celi versus intemal and the external of the origin of the toxic compounds and reactive oxygen species (ROS) [5]. Hence 6PGD is classified in the antioxidant enzyme class [6]. İn the absence of the enzyme is determined hemolytic anemia, deficiency in the number of reticulocytes, jaundice and hemolytic epidemic events. also, observed increase in pyruvate kinase activity and decrease in GSH level. Consequently shortens the life time of the erythrocytes [6].

6PGD enzyme has been purified many resources from bacteria to mammals. This enzyme was first purified from sheep liver by Villet and Dalziel [7], then it has been purified and caracterizated many resources such as human erythrocytes [8,16], rat liver, mammary gland of rabbit [15], pork liver [9], parsley leaves [10], rat erythrocytes [11], rat heart and lung tissues [12], chicken liver [13], cat erythrocytes [14] and rainbow trout giıı [17].

İn the present study having great importance in metabolism 6PGD enzyme was purified in a single step using affinity chromatography of rat erythrocytes.

RESULTS AND DISCUSSION

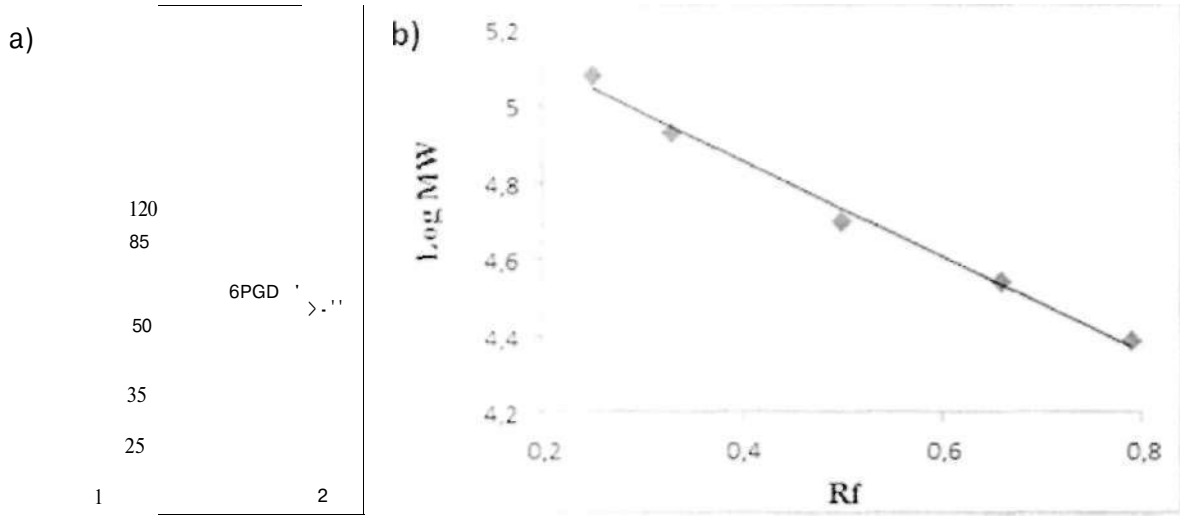
The pentose phosphate pathway enzymes of 6PGD has an important role in the metabolism. Even though less severe hemolytic cases were detected in the absence of enzymes. 6PGD is an inhibitor of phospho-glucose isomerase and it is accumulation in celi leads to inhibits glycolysis path. For this reason, it may be used the target enzyme in antimicrobial chemotherapys [21]. 6PGD enzyme was purified from rat erythrocytes and purification of enzyme was performed by single step which is 2',5'-ADP Sepharose 4B affinity gel chromatography. The enzyme was obtained to have a specific activity of 1.37 EU/mg protein with a yield of 61,5% and 244.7 of purification fold (Table 1).

FULLTEXT

Table 1. Purification table of 6PGD of rat erythrocytes.

Purification Steps	Activity (EU/ml)	Protein (mg/ml)	Total Activity (EU)	Total Protein (rrıg)	Specific Activity (EU/mg)	Yield (%)	Purification fold	Totalv volume (mi)
HemoTysate	0.0695	" 12.35	0.208	37.05	0.0056	ÎÖÖ	1	3
2',5'-ADP Sepharose- 4B chromatography	0.0642	0.0467	0.128	Ö.0934	Ö37	6ÎTö	244.7	2

Until now, enzyme has been purified using different methods such as DEAE cellulose, CMC-cellulose and ammonium sulfate precipitation methods of mammals tissue [7], high-speed centrifugation, ammonium sulfate precipitation, and 2',5'-ADP Sepharose 4B affinity chromatography methods of using from rat erythrocytes [11], using 2',5'-ADP Sepharose 4B affinity chromatography by single step of rat heart and lung tissue [12], ammonium sulphate precipitation, 2',5'-ADP Sepharose 4B affinity chromatography and Sephadex G-200 gel filtration chromatography of chicken liver [13], 2',5'-ADP Sepharose 4B affinity chromatography of cat erythrocyte [14], ammonium sulphate precipitation and 2',5'-ADP Sepharose 4B affinity chromatography of human erythrocyte [16]. Ali these steps have been used costly and requires a long time. İn the present study were obtained in high purity enzyme in a short time and at low cost by single step. The purity of the 6PGD enzyme obtained from rat erthrocyte was controlled by SDS-PAGE method.
Purity of enzyme was determined by the observation of a single band in the gel (Figüre 1a). The molecular mass of the subunit of the enzyme was calculated to be about 59,8 kDa by this method. İn different study reported that the molecular vveight of the native enzyme was found to by Sephadex G-200 chromatography at 111 kDa and it was a homodimeric structure [11]. İt is reported from various resources in the literatüre that the purified 6PGD enzyme is homodimeric or homotetrameric structure and the subunits molecular masses are between 33 and 60 kDa [7-17], The molecular weight of rat erythrocyte 6PGD enzyme is similar to the rat liver and rabbit mammary gland. SDS-PAGE method was used for purity and determine subunits molecular weight of the enzyme (Figüre 1a). Using Standard protein and 6PGD enzyme, R_f values were calculated. and R_fLog MW graph was prepared by Laemmli procedure, obtained molecular vveight of approximately 59,8 kDa for 6PGD enzyme (Figüre 1b).



Figüre 1. a) SDS-polyacrylamide gel electrophoresis of 6PGD purified by affinity gel (Lane 1: Standard proteins; Lane 2: 6PGD) and b) Standard graph used for the determination of molecular weight of 6PGD enzyme by the method SDS-PAGE

MATERIAL AND METHOD

Chemicals

The chemicals used were purchased from Sigma and Merck. 2',5'-ADP Sepharose 4B was purchased from Pharmacia.

Preparation of hemolysate

Fresh blood samples from rats, placed in EDTA-containing tubes, were centrifuged (15 min, 2.500xg) and plasma were removed. The precipitate (red blood cells) vvashed three times with KCl (0.16 M) and hemolyzed with 5 volume of cold-water. Then, to remove the celi membranes, and intact cells 30 minutes centrifugation at 10.000xg were performed. AH procedures were carry out at 4°C.

2',5'-ADP Sepharose 4B Affinity Chromatography

The dried 2',5'-ADP Sepharose 4B gel (2 g) was vveighed for 10 mi column volume. The gel vvashed vvith 300 mi distilled vvater. The gel was treated vvith 50 mM KH_2P0_4 +1 mM EDTA + 1mM DTT (pH 7.3) buffer and packed in column (10 cm). And the gel vvas equilibrated same buffer. After the sample was passed through the column, the elution column was vvashed with equilibration buffer until the absorbance difference 0.05 in 280'nm. Elution vvas carried out with 80 mM K-fosfat + 10 mM EDTA + 80 mM KCl + 5 mM $NADP^+$ (pH 7.3). Ali procedures vvere carry out at 4°C.

Activity determination

The activity of 6PGD enzyme was measured according to Beutlers method [18].

Protein determination

To quantify the amount of proteins in the samples, Bradford method [19] has been relied on, by vvhich the amount of proteins vvere determined spectrophotometrically at 595 nm using boving serum albumin as a Standard protein.

SDS polyacrylamide gel electrophoresis

6PGD enzyme purities vvere guranted using SDS polyacrylamide gel electrophoresis by Laemmli method vvhich described previously [21] 10% and 3% of acrylamide used for both running and stacking gels respectively vvith 0.1 % of SDS.

Conculation

İn conclution, we purified 6PGD enzyme from rat erythrocytes by a single step vvhich is 2',5'-ADP sepharose 4B affinity chromatography. Thus, we have obtained the enzyme in high purity, economic and effective method and in very short time compared vvith other studies. Hereby, the study vvould be investigation of the effect of organic compounds on purified 6PGD enzyme activity from rat eryhtrocytes in the future.

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