

Glucose 6-Phosphate Dehydrogenase Activatory Properties of Some Organic Compounds and Amino Acids

Bazı Organik Bileşikler ve Amino Asitlerin Glukoz 6-Fosfat Dehidrogenaz Aktivatör Özellikleri

Research Article / Araştırma Makalesi

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ABSTRACT

The aim of this study was to assess activatory potentials of 4'-(Methylsulfonyl)-acetophenone, 4-(methylsulfonyl) phenylacetic acid, tyrosine and phenylalanine on human erythrocyte glucose 6-phosphate dehydrogenase. For this purpose, initially erythrocyte glucose 6-phosphate dehydrogenase was managed to be purified 812-fold in a yield of 40.6% by using 2',5'-ADP Sepharose 4B affinity gel.

4'-(Methylsulfonyl)acetophenone, 4-(methylsulfonyl)phenylacetic acid, tyrosine and phenylalanine exhibited activatory effects on the enzyme. % Activity values for these compounds were determined by plotting activity percentage versus [Activator].

Key Words

Activator, amino acid, enzyme, glucose 6-phosphate dehydrogenase.

ÖZET

Bu çalışmanın amacı 4-(metilsülfonil)-asetofenon, 4-(metilsülfonil) fenilasetik asit, tirozin ve fenilalaninin insan eritrosit glukoz 6-fosfat dehidrogenaz üzerindeki aktivatör potansiyelini değerlendirmektir. Bu amaçla, öncelikle eritrosit glukoz 6-fosfat dehidrogenaz 2',5'-ADP Sepharose 4B afinite jeli kullanılarak 812-kat, %40.6 verimle elde edildi.

4-(Metilsülfonil) fenilasetik asit, tirozin ve fenilalaninin enzim üzerinde aktivatör etkileri gösterdi. Bu bileşikler için % aktivite-[Aktivatör] grafiği kullanılarak % aktivite değerleri belirlendi.

Anahtar Kelimeler

Aktivatör, amino asit, enzim, glukoz 6-fosfat dehidrogenaz.

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INTRODUCTION

Glucose 6-phosphate dehydrogenase (E.C.1.1.49; G6PD) is the key and first enzyme of the pentose phosphate metabolic pathway (PPP), catalyzing the conversion of glucose 6-phosphate to 6-phosphogluconate in the presence of NADP⁺. The NADPH produced by the G6PD and 6-phosphogluconate dehydrogenase in the PPP occurs widely in living cells, serves as an electron donor in reductive biosynthesis, notably of cholesterol and fatty acids, as well as in the synthesis of nitric oxide. NADPH is crucial in the protection of cells from oxidative stress. G6PD reaction is an important site of metabolic control [1-4]. NADP⁺ is reduced to NADPH by glutathione reductase in erythrocytes using glutathione as substrate. Glutathione prevents hemoglobin denaturation, preserves the integrity of red blood cell membrane sulfhydryl groups, and detoxifies hydrogen peroxide and oxygen radicals in the red blood cells [5,6]. A decrease in G6PD may result in NADPH and reduced glutathione deficiencies in erythrocytes; scarcity of reduced glutathione in erythrocytes causes early haemolysis in spleen [7].

Deficiency of glucose-6-phosphate dehydrogenase is one of the most common genetic abnormalities, affecting more than 150 million males. It has a polymorphic frequency that is second only to the hemoglobinopathies, 400 variants having been described [4,8]. G6PD deficiency mainly affects males; females can be carriers and/or can also suffer from G6PD deficiency although they are not as susceptible [9]. In G6PD deficiency, the NADP level in erythrocytes will be decreased. Reduced GSH cannot be regenerated in adequate amounts allowing peroxides to accumulate in erythrocytes, which results in hemolysis due to their oxidative effect on lipids of the red cell membrane [10]. Drug-induced hemolysis has attracted the most attention. Many antibiotics are being used in therapies. Numerous reports attest to the importance of drugs in causing hemolytic anemia and changing of enzyme activities (Figure 1) [10-13]. It has been reported that some increase and decline were found on human liver enzyme activity levels such as aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase [4,14].

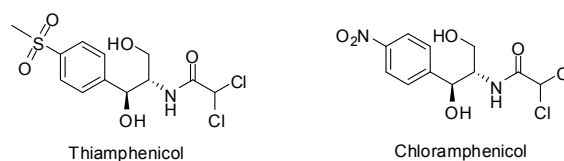


Figure 1. Structure of thiamphenicol and chloramphenicol.

When one molecule palmitate is synthesized in the biosynthesis of fatty acids, 14 NADPH molecules are used. Six of these 14 NADPH molecules come from the pentose phosphate pathway. Essentially, the pentose phosphate pathway is more active in adipose tissues compared with muscles, showing that the G6PD enzyme is very important in the biosynthesis of fatty acids [3,15]. NADPH plays an important role in initiating protein synthesis [16] and it is essential to the growth and proliferation processes, serving as hydrogen and electron source for a variety of reductive biosynthetic reactions, including the synthesis of fatty acids and cholesterol [17].

Many chemicals are being used in therapies which are potent inhibitor or activator of specific enzymes. Thus, we aimed in this study to purify G6PD from human erythrocytes and analyse the activatory potentials of 4'-(methylsulfonyl) acetophenone, 4-(methylsulfonyl)-phenylacetic acid, tyrosine and phenylalanine on activity of the enzyme (Figure 2).

MATERIALS AND METHODS

Materials

2',5'-ADP-Sepharose 4B was obtained from Pharmacia. All other chemicals were obtained from either Sigma Chem. Co. or Merck and they were of analytical grade.

Preparation of hemolysate

Fresh human blood was collected in endorph tubes with EDTA and centrifuged at 2500xg for 15 min and the plasma and leukocyte coat were removed by drip. After that, red cells were washed with KCl solution (0.16 M) three times, the samples were centrifuged at 2500 g for each time and supernatants were removed. The erythrocytes were hemolysed with 5 vol. of ice-cold water and centrifuged at 4°C, 10 000 g for 30 min to remove the ghosts and intact cells [6,7]. Ammonium sulphate (35-65%) precipitation was

made in hemolysate. Ammonium sulphate was slowly added for completely dissolution. It was centrifuged at 5000 g for 15 min and precipitate was dissolved in 50 mM of phosphate buffer (pH 7.0), then dialysed at 4°C in 50 mM K-acetate/50 mM K-phosphate buffer (pH 7.0) for 2 h with two changes of buffer [18].

Purification of G6PD by Affinity Chromatography

Dialysed sample was loaded on 2',5' ADP-Sepharose 4B affinity column and the gel was washed with 25 mL of 0.1 M K-acetate/0.1 M K-phosphate (pH 6.0), with 25 mL of 0.1 M K-acetate/0.1 M K-phosphate (pH 7.85), and finally, with 0.1 M KCl/0.1 M K-phosphate (pH 7.85). Elution was carried out with 80 mM K-phosphate + 80 mM KCl + 0.5 mM NADP⁺ + 10 mM EDTA (pH 7.85) solution at 20 mL/h flow rate. Eluates were collected in 2 mL tubes and each of their activity was separately calculated. All of the procedures were performed at 4°C [19]. Quantitative protein determination was spectrophotometrically measured at 595 nm according to Bradford's method, with bovine serum albumin as the standard [20]. SDS Polyacrylamide Gel Electrophoresis was made after the purification of enzyme according to Laemmli's method. It was carried out in 4% and 10% acrylamide concentration for stacking and running gel respectively, containing 0.1% SDS [21].

Measurements of G6PD Activity

G6PD activity was measured at 25°C according to Beutler's method which depends on the reduction of NADP⁺ by G6PD, at the presence of glucose 6-phosphate. In this spectrophotometric measurement, the reaction medium was maintained at 25 °C and contained 0.1 mM Tris-HCl (pH= 8.5) with 0.5 mM EDTA, 10 mM MgCl₂, 0.2 mM NADP⁺, and 0.6 mM (G6-P) in a total volume of 1 mL. One unit of enzyme (EU) activity was defined as the amount of enzyme reducing 1 μmol NADP⁺ per min at pH 8.5 [22].

In vitro studies for the compounds

We examined the activatory effects of four compounds including: 4'-(Methylsulfonyl)acetophenone (0.3-0.7 mM), 4-(methylsulfonyl)phenylacetic acid (0.3-0.7 mM), tyrosine (0.3-0.7

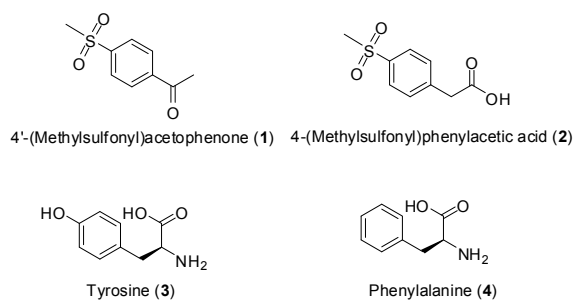


Figure 2. Structures of the investigated molecules 1-4.

mM) and phenylalanine (0.3-0.7 mM) (Figure 2). All compounds were tested in triplicate at each concentration used. G6PD enzyme activities were measured in the presence of different activator concentrations. Control cuvette activity was acknowledged as 100 % in the absence of the molecules. For each compound, an Activity%-[Activator] graph was drawn.

RESULTS AND DISCUSSION

Human G-6PD was purified by 2',5' ADP-Sepharose 4B gel affinity column. As shown in Table 1, specific activity was calculated for hemolysate and purified enzyme solution as 0.01 and 8.12 EU/mg proteins, respectively. We obtained a yield of 40.6% and a purification coefficient of 812 fold. Purification steps were controlled by SDS-PAGE. Serum albumin was used as the standard. Table 2 shows the obtained % activity values for compounds 1-4.

Currently, there are a lot of patient with G6PD deficiency disorder in some region of Turkey and in the world. G6PD deficiency is frequently seen in African, Mediterranean, Middle East and Far East nation sand their lineages with a frequency ranging from 5% to 40% [19,23].

Pamaquine was begun using in malaria therapy and it was determined to result in some severe side-effects in part of patients. Dark color urine, jaundice and anemia were observed in these patients. Afterwards, G6PD deficiency was determined in these individuals. Use of this and some other drugs cause the hemolysis connected with complication. These data showed that the enzymes and related reactions were important in maintenance of the life. The importance of G6PD in metabolism has

Table 1. Purification scheme of G6PD from human erythrocytes by 2',5'-ADP Sepharose 4B affinity gel chromatography.

Purification step	Activity (EU/ml)	Total volume (ml)	Protein (mg/ml)	Total protein (mg)	Total activity (EU)	Specific activity (EU/mg)	Yield (%)	Purification factor
Hemolysate	0.42	60	40.1	2406	25.2	0.010	100	1
Ammonium sulfate precipitation (35-65)%	1.13	9	1.27	11.43	10.17	0.89	40.36	89
2',5'-ADP Sepharose 4B affinity chromatography	3.41	3	0.42	1.26	10.23	8.12	40.6	812

been well known for many years. GSH is used by antioxidant defense mechanisms and its production requires NADPH to be synthesized in the pentose phosphate metabolic pathway in which G6PD and 6PGD participate [24,25]. For this reason, G6PD and 6PGD were considered as antioxidant enzymes [26].

Inhibitory effects of many compounds on G6PD enzyme activities in various animal species and human beings have been reported in many investigations. For example; It has been reported that thiamphenicol, amikacin, chloramine, chloramphenicol and CuSO_4 inhibit rainbow trout erythrocyte G6PD [4,27]. Several study reports concerning many drugs such as antibiotics, analgesic, anesthetic and metal ions were investigated on human G-6PD [28,29] and rainbow trout erythrocyte G6PD [30]. Although drugs are used in therapies, there is no report related with human glucose 6-phosphate dehydrogenase activity for compounds 1-4 used in our study. Therefore, in the present study, investigation of effects of compounds 1-4 on human erythrocyte G6PD was aimed. To this end, G6PD was purified from human erythrocytes by ammonium sulfate precipitation and 2',5'-ADP Sepharose 4B affinity chromatography, respectively.

In vitro studies were performed for determination of the activity % values. For compounds 1-4, activatory concentrations were determined by drawing the graph that is activity % vs concentration of activator (Figure 3). It is important that these compounds activated enzyme activity at low concentrations under *in vitro* conditions. It can be seen from Table 2 that these compounds are potent activator for human G6PD enzyme activity (Table 2).

4'-(Methylsulfonyl)acetophenone and 4-(methylsulfonyl) phenylacetic acid are frequently used in drug industry. Tyrosine and phenylalanine are also used in drug industry in addition to be present in the organism.

As shown Table 2, the activator concentrations causing up to 184 % activation were determined from Activity %-[A] graph. The obtained % activity values of 4'-(Methylsulfonyl) acetophenone, 4-(methylsulfonyl) phenylacetic acid, tyrosine and phenylalanine were $157 \pm 4\%$, $184 \pm 5\%$, $114 \pm 2\%$ and $144 \pm 4\%$, respectively.

As a result, G6PD was purified 812 times in a yield of 40.6% by using ammonium sulfate precipitation and 2',5'-ADP-sepharose 4B affinity gel. SDS polyacrylamide gel electrophoresis indicated a single protein band after the purification procedure (Table 1). % Activity values were determined for compounds 1-4. % Activity values of compounds are shown in Table 2. These compounds may be beneficial for the health of patients who have deficiency of glucose 6-phosphate dehydrogenase enzyme. According to these data, compounds 1-4 were found to be efficient activators. Owing to widely use of these compounds for both human and animals drug development; we thought that these molecules are important agents for G6PD activity.

Table 2. % Activity value obtained from Lineweaver-Burk graphs for G-6PD in the presence of compounds 1-4 with 0.5 mM.

Activator	% Activity
4'-(Methylsulfonyl)acetophenone (1)	157 ± 4
4-(Methylsulfonyl)phenylacetic acid (2)	184 ± 5
Tyrosine (3)	114 ± 2
Phenylalanine (4)	144 ± 4

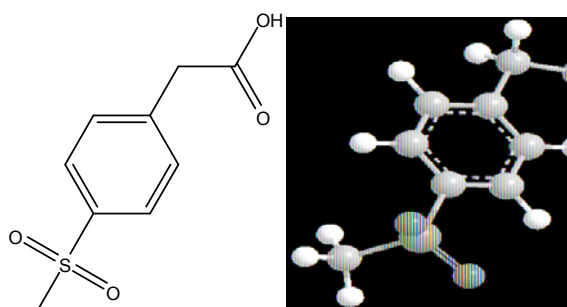


Figure 3. 4-(Methylsulfonyl) phenylacetic acid (**2**) molecular and 3D figure.

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