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Effects of Some Chemicals on G6PD Enzyme Purified by Affinity Chromatography

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme defect, being present in more than 400 million people worldwide. The clinical manifestations of G6PD deficiency are neonatal jaundice, and acute haemolytic anaemia, which is usually triggered by an exogenous agent. The aim of this study was to investigate the enhancing effect of some chemicals on G6PD. G6PD was purified from human erythrocyte by using ammonium sulphate precipitation and 2´,5´-ADP-Sepharose 4B affinity gel. The purified enzyme showed a single band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The effects of three different chemicals (theophylline ethylenediamine, pentoxifyllin, thiamphenicol glycinate hydrochloride) were investigated on the purified enzyme. These three chemicals promoted the enzyme activity.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP+ oxidoreductase EC 1.1.1.49; G6PD) is an enzyme that catalyses the first reaction in the pentose phosphate pathway. G6PD deficiency is an X-linked, hereditary genetic defect in the G6PD gene, resulting in protein variants with different levels of enzyme activity, that are associated with a wide range of biochemical and clinical phenotypes [1,2]. Enzyme deficiency in erythrocytes causes haemolytic anaemia. The pentose phosphate pathway (PPP) is the only source of NADPH in the erythrocytes and preserves the reduced form of glutathione. The main function of the pathway seems to protect the erythrocytes against oxidative damage that is caused by free radicals in the number of molecules in cells, including membrane lipids, proteins, and nucleic acids [2,3]. Formation of these harmful radicals is an occurring intracellular metabolic process [4]. If the generation of free radicals in cells impairs antioxidant defenses or exceeds the ability of the antioxidant defense system to eliminate them, oxidative stress causes [5]. The harmful effects of free radicals are checked by the cellular antioxidant defense system [6]. Antioxidant enzymes are indispensable in both maintaining cellular stability and scavenging free radicals [7,8]. Drug-induced haemolysis has attracted the most attention since G6PD deficiency

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was discovered in the mid-1950s as a result of researches carried out to disclose why some people digesting primaquine were sensitive to the hemolytic effects [9].

Many drugs and chemicals can increase the rate of free radicals formation in specific organs of the body. But, it is possible that some chemicals may activate G6PD enzyme and G6PD deficient persons may use these chemicals. For this purpose it was investigated the effects of three different chemicals on G6PD (Table 1).

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 analytical grade. Medical chemicals were from the Research Hospital of Suleyman Demirel University.

Preparation of the haemolysate

Fresh human blood who has not G6PD deficiency age: 36 male collected in EDTA was centrifuged (15 min, 2500 g). Preparation of the haemolysate was done as described by Ninfali [10].

Measurements of enzymes activities

G6PD was measured spectrophotometrically by monitoring the increase the rate of NADPH in 340 nm due the reduction of $NADP^+$ at 25°C as described by Beutler [11]. One unit of G6PD represents 1 μmol of $NADP^+$ utilized per minute.

Protein assay

Quantitative protein concentration was determined using Bradford method [12].

Ammonium sulphate fractionation, dialysis and purification of G6PD by affinity chromatography

Haemolysate was saturated by 35-65% $(NH_4)_2SO_4$. All assays and dialyses were done at 4°C in 50 mM K-acetate/50 mM K-phosphate buffer (pH 7.0) for 2 h with two changes of buffer according to Ninfali [10].

The dialysed enzyme solution was loaded on the 2',5' ADP-Sepharose 4B affinity column (1x10 cm). All other procedures were performed as described in the previous studies [13]. In eluates, activity of G6PD was determined in all fractions. It was not performed protein determination at 280 nm in eluates, since the $NADP^+$ absorbance masked the actual protein absorbance.

SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was done after purification of the enzyme. It was carried out in 10% and 4% acrylamide concentrations for the running and the stacking gel, respectively, containing 0.1% SDS according to Laemmli [13]. To the sample and standard 20 mg bovine serum albumin was applied to the electrophoresis medium. Gels were stained overnight in 0.1% Coomassie Brillant Blue R-250 in 50% methanol and 10% acetic acid, then destained with many changes of the

Table 1. Some properties of chemicals.

Name	IUPAC Name	Chemical Formula	Molecular Weight (g/mol)
Theophylline ethylenediamine	1,3-dimethyl-7H-purine-2,6-dione; 1,3-dimethyl-7H-purine-2,6-dione; ethane-1,2-diamine	$C_{16}H_{24}N_{10}O_4$	420.4
Pentoxifyllin	3,7-dimethyl-1-(5-oxohexyl)purine-2,6-dione	$C_{13}H_{18}N_4O_3$	278.0
Thiamphenicol glisinat HCl	D-d-threo-2-dichloroacetamido-1-(4-methylsulfonylphenyl)-1,3-propanediol	$C_{12}H_{15}Cl_2NO_3S$	356.2

same solvent without dye. The electrophoretic pattern was photographed (Figure 1).

In vitro studies

Theophylline ethylenediamine, pentoxifyllin, thiamphenicol glisinat hydrochloride were used as chemicals. Activity studies were done to determinate how to effect of these chemicals on G6PD. Activities were measured at 2.9 mM, 5.7 mM, 8.6 mM 14.3 mM, 17.1 mM cuvette concentration for theophylline ethylenediamine, 0.07 mM, 0.14 mM, 0.22 mM, 0.29 mM, 0.36 mM cuvette concentration for pentoxifyllin, and 0.033 M, 0.066 M, 0.099 M, 0.132 M, 0.165 M cuvette concentration for thiamphenicol glisinat hydrochloride. Regression analysis graphs were drawn by a statistical packing program on a computer.

RESULTS AND DISCUSSION

The purpose of the present study was to investigate the activating effects of some chemicals on erythrocytes G6PD as *in vitro*. For this reason, G6PD was purified from human erythrocytes by ammonium sulphate precipitation and 2', 5'-ADP Sepharose 4B affinity chromatography, respectively. Purification factor was 10.26 in a yield of 51.3%. Figure 1 shows the SDS-PAGE gel for the determination of purity of the enzyme from the one person under study. A high purity was obtained.

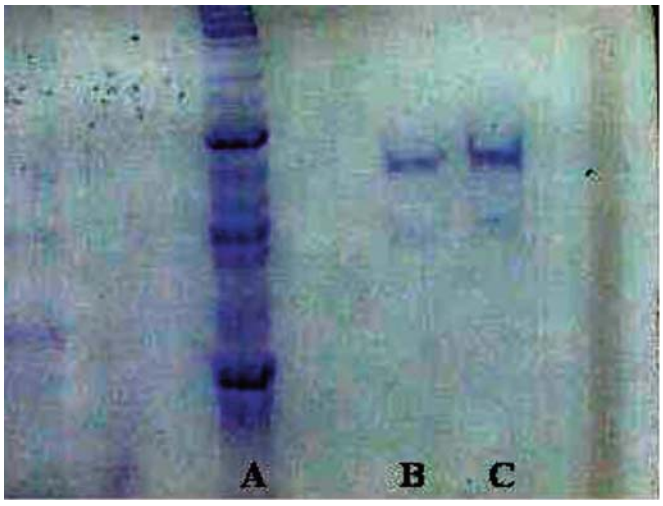


Figure 1. SDS-polyacrylamide gel electrophoresis of G6PD from human erythrocytes. Line A: mixture of standart proteins and approximately mol weight of them (*E. coli* β Galactosidase, 116000; rabbit phosphorylase B, 97400; bovine albumin, 66000; chicken ovalbumin, 45000; bovine carbonic anhydrase, 29000), line B and C: band for enzyme purified from human erythrocytes.

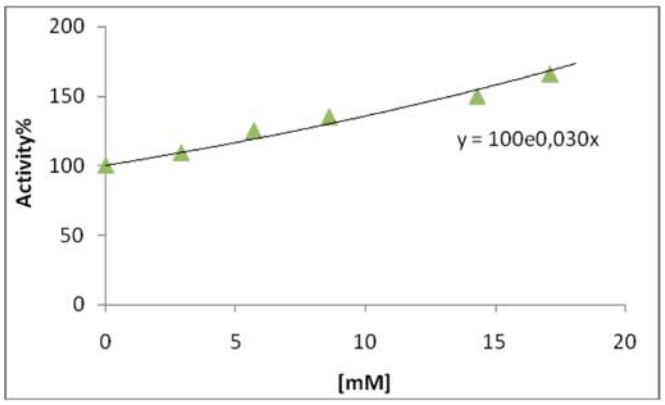
Theophylline ethylendiamine, pentoxifyllin, thiamphenicol glycinate hydrochloride were used as chemicals. It is seems that these chemicals activated G6PD enzyme (Figure 2). Table 2 shows concentrations of chemicals activating 50% and 100%.

Numerous reports attest to the importance of drugs in causing hemolytic anemia and changing of enzyme activities. This study is one of the rare researches through chemicals activating on G6PD. However, the genetic heterogeneity of G6PD deficiency means that a drug found to be safe in some deficient subjects may not be safe in all [14].

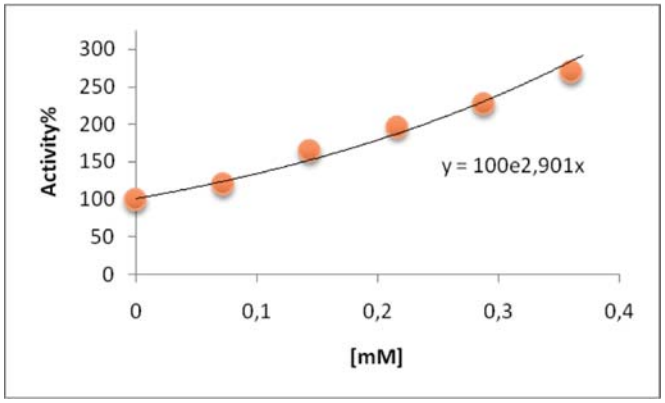
It is demonstrated the article that G6PD-deficient people may show persistence against the inhibitory effect of sodium cefuroxime, streptomycin, netilmycin and metamizol [15]. In another study, researchers determined that hexokinase and G6PD activities were found to be increased in cisplatin groups compared to control groups [16]. It was reached the conclusion that G6PD-deficient people may use these chemicals after different clinical studies.

Table 2. 50% and 100% activation values of chemicals.

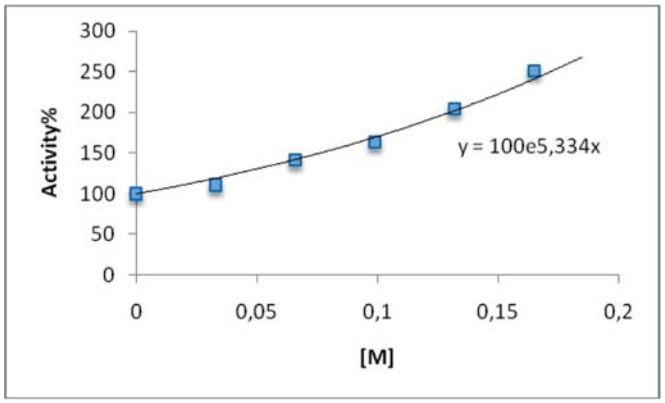
Chemicals	50% Activation	100% Activation
Theophylline ethylenediamine	13.52 mM	-
Pentoxifyllin	0.14 mM	0.24 mM
Thiamphenicol glisinat HCl	0.075 M	0.13 M



(a)



(b)



(c)

Figure 2. Regression analysis of theophylline ethylenediamine (a), pentoxifyllin (b), thiamphenicol glycinate hydrochloride (c).

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