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Short communication

Kinetic properties of human placental glucose-6-phosphate dehydrogenase

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Abstract

The kinetic properties of placental glucose-6-phosphate dehydrogenase were studied, since this enzyme is expected to be an important component of the placental protection system. In this capacity it is also very important for the health of the fetus. The placental enzyme obeyed "Rapid Equilibrium Ordered Bi Bi" sequential kinetics with K_m values of 40 ± 8 μM for glucose-6-phosphate and 20 ± 10 μM for NADP. Glucose-6-phosphate, 2-deoxyglucose-6-phosphate and galactose-6-phosphate were used with catalytic efficiencies (k_{cat}/K_m) of 7.4×10^6, 4.89×10^4 and 1.57×10^4 $M^{-1}.s^{-1}$, respectively. The $K_m$app values for galactose-6-phosphate and for 2-deoxyglucose-6-phosphate were 10 ± 2 and 0.87 ± 0.06 mM. With galactose-6-phosphate as substrate, the same K_m value for NADP as glucose-6-phosphate was obtained and it was independent of galactose-6-phosphate concentration. On the other hand, when 2-deoxyglucose-6-phosphate used as substrate, the K_m for NADP decreased from 30 ± 6 to 10 ± 2 μM as the substrate concentration was increased from 0.3 to 1.5 mM. Deamino-NADP, but not NAD, was a coenzyme for placental glucose-6-phosphate dehydrogenase. The catalytic efficiencies of NADP and deamino-NADP (glucose-6-phosphate as substrate) were 1.48×10^7 and 4.80×10^6 $M^{-1}s^{-1}$, respectively. With both coenzymes, a hyperbolic saturation and an inhibition above 300 μM coenzyme concentration, was observed. Human placental glucose-6-phosphate dehydrogenase was inhibited competitively by 2,3-diphosphoglycerate ($K_i = 15 \pm 3$ mM) and NADPH ($K_i = 17.1 \pm 3.2$ μM). The small dissociation constant for the G6PD:NADPH complex pointed to tight enzyme:NADPH binding and the important role of NADPH in the regulation of the pentose phosphate pathway.

Keywords: Human placenta; Glucose-6-phosphate dehydrogenase; Substrate and coenzyme specificity; Inhibited by 2,3DPGA and NADPH

Abbreviations: Deamino-NADP, deamino nicotinamide adenine dinucleotide phosphate (oxidized form); 2-dG6P, 2-deoxyglucose-6-phosphate; 2,3-DPGA, 2,3-diphosphoglyceric acid; Gal6P, galactose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; G6P, glucose-6-phosphate; GSH, glutathione (reduced form); GSSG, glutathione disulfide (oxidized form); GSSGGR, glutathione disulfide reductase; NAD, nicotinamide adenine dinucleotide (oxidized form); NADP, nicotinamide adenine dinucleotide phosphate (oxidized form); NADPH, nicotinamide adenine dinucleotide phophate (reduced form); n_H: Hill constant.

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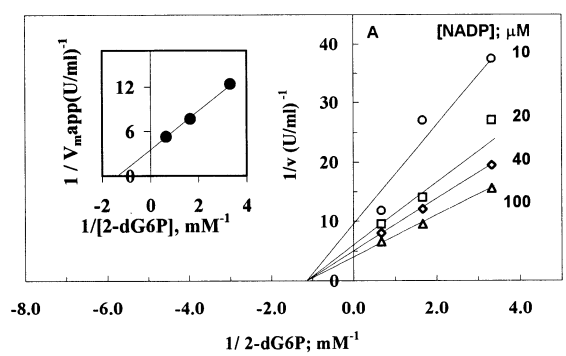
1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49, β-D-glucose-6-phosphate; $NADP^+$ oxidoreductase) is an enzyme that catalyzes the first step of the pentose phosphate pathway (PPP). PPP has three important functions: (i) production of reducing equivalents in the form of NADPH; (ii) production of pentose phosphates necessary for nucleotide biosynthesis and (iii) to serve as the route of entry of pentoses to the glycolytic pathway. NADPH serves as a hydrogen and electron donor for a variety of reductive reactions, including fatty acid and cholesterol biosynthesis. NADPH also has very important functions in the protection of the cell against oxidative agents by transferring its reductive power to oxidized glutathione (GSSG) via glutathione disulfide reductase (GSSGGR) [1],[2]. G6PD was discovered by Warburg and Christian in 1931 and was isolated in crystalline form by Noltmann et al. from brewer's yeast [3,4].

This cytosolic enzyme is widely distributed among microorganisms, plants and in different animal tissues [1–5]. Numerous reports are available regarding the reaction mechanism of G6PD but they have revealed inconsistencies and disagreements in the type of observed kinetics [5,6]. Some studies report classical Michaelis–Menten kinetics, others report sigmoidal kinetics for $NADP^+$ binding [3–6]. It is well known that in the presence of NADP the enzyme exists in the form of active dimers, trimers, tetramers and hexamers [7–10]. Kinetic properties of G6PD purified



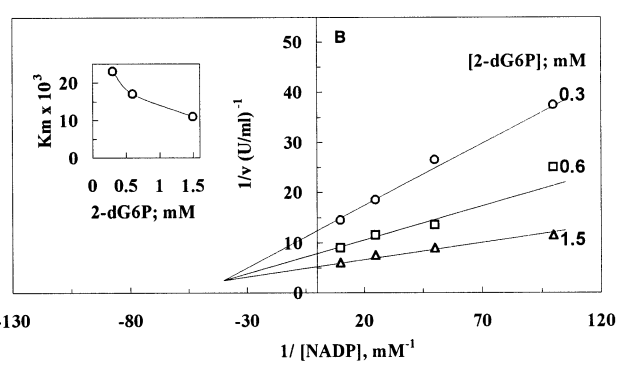
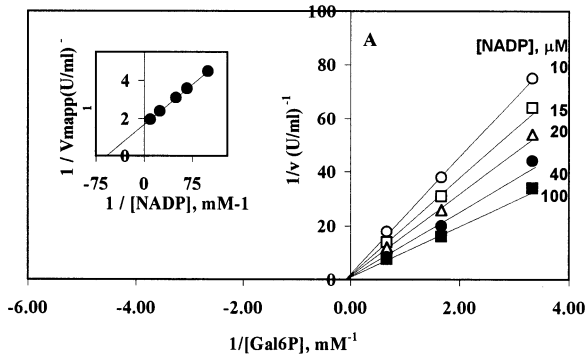


Fig. 1. Lineweaver–Burk plots for 2-dG6P and NADP at constant [NADP]: (–○–) 10, (–□–) 20, (–◇–) 40, and (–△–) 100 μM, (A) and at constant [2-dG6P]: (–○–) 0.3, (–□–) 0.6 and (–△–) 1.5 mM, (B). Double-reciprocal plots of V_m and K_m as a function of 2-dG6P inset to (A) and (B), respectively.



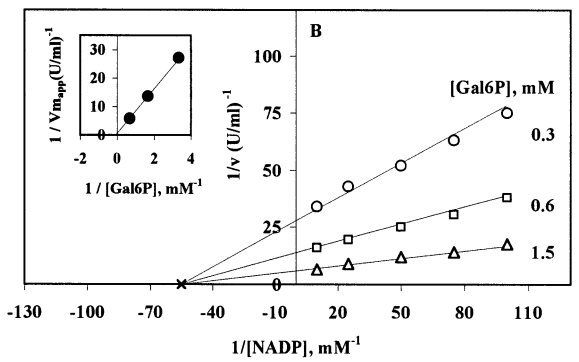


Fig. 2. Lineweaver–Burk plots for Gal6P and NADP at constant [NADP]: (–○–) 10, (–□–) 15, (–△–) 20, (–●–) 40, and (–■–)100 μM, (A) and at constant [Gal6P]: (–○–) 0.3, (–□–) 0.6 and (–△–) 1.5 mM, (B). Double-reciprocal plots of V_m as a function of NADP (inset to A) and 2-dG6P (inset to B).

from different sources such as erythrocytes, liver, kidney, brain have been published [5–20].

We have limited information about the biochemistry and physiology of human placenta. Elucidation of the kinetic mechanism of placental G6PD will increase our knowledge about the physiology of human placenta and its critical role in the health of the fetus. Glutathione (GSH) is very important for glutathione-S-transferase catalyzed detoxification reactions. In cells, GSH is strictly controlled by GSSGGR, at the expense of NADPH which is a product of G6PD activity. There are only a few reports on the purification of G6PD from human placenta and no report exists on its kinetics [21]. Studying the kinetic mechanism of human placental G6PD in detail will provide information about the control of the placental PPP, the biosynthesis of some physiologically important biomolecules and the status of placental detoxification in relation to the health of the fetus.

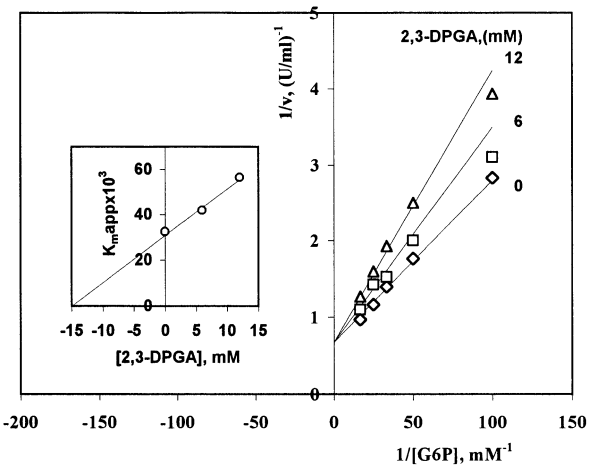


Fig. 3. Lineweaver–Burk plot for 2,3-DPGA inhibition. [2,3-DPGA], (–◇–) 0, (–□–) 6 and (–○–) 12 mM. Inset: $K_m$app vs. [2,3-DPGA].

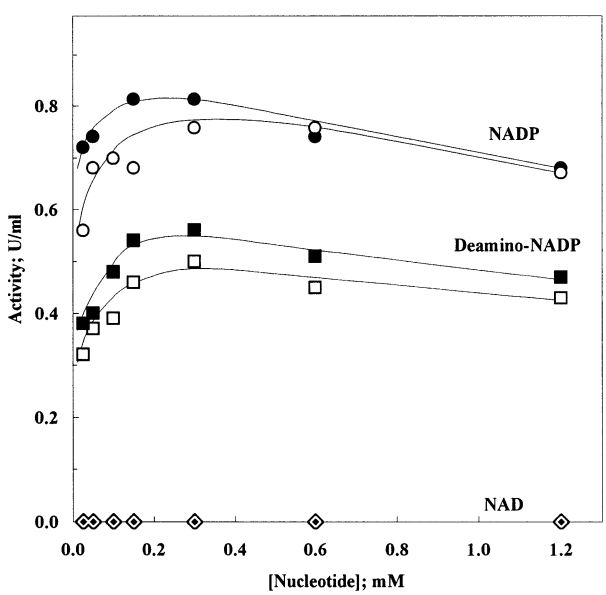


Fig. 4. Michaelis–Menten plots for nucleotides at constant [G6P]. [nucleotides], 40–1200 μM; empty symbols 0.3 mM [G6P]; filled symbols 0.6 mM [G6P]. NAD^+, (–◇–), (–◆–); deamino-NADP (–□–), (–■–); NADP, (–○–), (–●–).

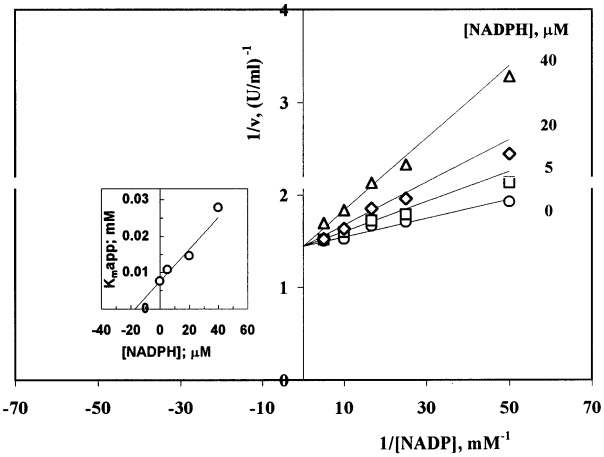


Fig. 5. Lineweaver–Burk Plot for NADPH inhibition. [NADPH], (–○–) 0, (–□–) 5, (–◇–) 20, and (–△–) 40 μM. Inset: $K_m$app vs. [NADPH].

2. Materials and methods

2.1. Materials

DEAE-Sepharose (fast flow), 2′,5′-ADP-Sepharose 4B, PBE 96 and PB 74 were from Pharmacia-LKB, Sweden; glycerol from Merck, Germany; 2-mercaptoethanol and Celite from BDH, UK; glucose-6-phosphate, galactose-6-phosphate

(Gal6P), 2-deoxyglucose-6-phosphate (2-dG6P), nicotinamide adenine dinucleotide phosphate ($NADP^+$ and NADPH), nicotinamide hypoxanthine dinucleotide phosphate (deamino-NADP), ε-aminocaproic acid (ε-ACA), nicotinamide adenine dinucleotide (NAD), 2,3-diphosphoglyceric acid (2,3-DPGA), ethylenediamine tetraacetic acid (EDTA) from Sigma, USA.

All other chemicals were standard products of Sigma or Aldrich, USA.

2.2. Placental G6PD

Placental G6PD was purified from full-term (40 weeks) human placenta using DEAE-Sepharose (fast flow), 2′,5′-AD-Sepharose 4B chromatography, and chromatofocusing on PBE 96 with PB 74, with 62% yield and had a specific activity of 87 IU mg^{-1} protein [22]. It was stored in 10 mM Tris-HCl, pH 7.4 containing 5 mM 2-mercaptoethanol, 2 mM EDTA, 1 mM ε-ACA and 40% (v/v) glycerol at -10°C.

2.3. Protein determination

Protein concentration was determined by the micromethod of Bradford, using bovine serum albumin as standard [23].

2.4. Kinetics of the human placental G6PD

G6PD activity was determined [24] at 37°C in 125 mM Tris-HCl, pH 7.4, containing 10 mM $MgCl_2$, and various concentrations of NADP, G6P, substrate or coenzyme analogues and inhibitors. The reactions were initiated by the addition of enzyme and the conversion of NADP to NADPH was followed by monitoring the change in absorbance at 340 nm, using a Milton–Roy 3000 spectrophotometer ($\varepsilon_{340} = 6.22$ $mM^{-1}cm^{-1}$). The activities were used in constructing Lineweaver–Burk and additional diagnostic plots to obtain K_m values for substrate, coenzyme, substrate and coenzyme analogues and K_i values for inhibitors [25].

3. Results and discussion

The aggregation state and the specific activity of G6PD depend on several factors such as enzyme, NADP, salt, RSH, Mg^{2+} (or Mn^{2+}) concentrations and pH [5–10,26]. The presence of RSH, high enzyme and Mg^{2+} (or Mn^{2+}) concentrations and low pH and ionic strength favour oligomeric, active, forms of G6PD (dimer, tetramer and hexamer) and the opposite conditions favour inactive monomers [5–20,26].

Sigmoidal or hyperbolic kinetics have been reported for G6PD in the intracellular medium and this property is reportedly lost upon purification [11]. The kinetics have been shown to depend on the storage and assay conditions [5–13,26,27]. It has been proposed that the sigmoidal kinetics in G6PD arise from hexamer–tetramer–dimer transitions in the enzyme. Aggregation states higher than dimer are responsible for sigmoidicity [5–14].

Under the storage and assay conditions given above, more than 95% of G6PD in our preparation existed in dimeric form [22]. The enzyme obeyed "Rapid Equilibrium Ordered Bi Bi" sequential kinetic model in the range, G6P = 20–300 μM and NADP = 10–200 μM [22]. This kinetic behaviour was in accordance with most of the G6PD from other sources [1,5]. The K_m values for G6P and NADP, obtained from Lineweaver–Burk plots, were 40 ± 8 and 20 ± 10 μM, respectively (Table 1) [22]. An intrinsic V_m value of 87 IU mg^{-1} protein was obtained from secondary plots of $1/V_m$ versus 1/G6P (or 1/NADP) (Table 1) [22]. These values are comparable with most of the kinetic parameters reported for G6PD from other sources [5–20].

Kinetic studies with substrate analogues have shown that G6PDs obtained from different sources (*Bacillus stearothermophilis* [28], rat testis [29] and human erythrocyte [13]) use these analogues with different efficiencies. The catalytic efficiencies (k_{cat}/K_m) of placental G6PD with G6P, 2-dG6P and Gal6P were 7.4×10^6, 4.89×10^4 and 1.57×10^4 $M^{-1}.s^{-1}$, respectively (Table 1). These data show that G6P is 150 and 500 times better as substrate than 2-dG6P and Gal6P. Among the substrate analogues 2-dG6P was three times better

Table 1
Kinetic parameter of G6PD from human placenta

Kinetic parameters: substrate, coenzyme, substrate and coenzyme analogues	V_m (IU mg^{-1} protein)	$K_m \pm$ S.D. (mM)	k_{cat} (s^{-1})	K_{cat}/K_m (M^{-1}.s^{-1})	$K_i \pm$ S.D. (mM)	$n_H \pm$ S.D.
Glucose-6-phosphate	87	0.040 ± 0.008	296	7.40×10^6	-	0.84 ± 0.15
NADP (G6P as substrate)	87	0.020 ± 0.010	296	1.48×10^7	-	0.86 ± 0.14
2-Deoxyglucose-6-phosphate	13	0.87 ± 0.06	43	4.89×10^4	-	-
Galactose-6-phosphate	46	10 ± 2	157	1.57×10^4	-	-
Deamino-NADP	29	0.0205 ± 0.0055	98	4.80×10^6	-	-
NADPH	-	-	-	-	0.0171 ± 0.0032	-
2,3-Diphosphoglycerate	-	-	-	-	15 ± 3	-

as substrate than Gal6P implicating the hydroxyl group on the fourth carbon is more critical than the hydroxyl group on the second carbon in determining selectivity. The K_m of 0.87 ± 0.06 mM for 2-dG6P was not affected by a change in the concentration of NADP (Fig. 1A and inset) but the reverse was not true; the K_m value for NADP decreased from 30 ± 5 to 10 ± 2 μM as the 2-dG6P concentration increased from 0.3 mM and approaching to a plateau at around 1.5 mM (Fig. 1B and inset). The K_m values for Gal6P and NADP were found to be 10 ± 2 and 20 ± 10 μM (Fig. 2A, B and the secondary plots as insets to Fig. 2A and B).

With G6P as the variable substrate, G6PD was inhibited competitively by 2,3 DPGA with a K_i of 15 ± 3 mM (Fig. 3 and inset).

G6PDs isolated from different sources are reported to use NAD as coenzyme [18,19,28,29]. NAD was neither a coenzyme nor an inhibitor for human placental G6PD indicating that this enzyme belongs to Class II type G6PDs (Fig. 4) [1]. Human erythrocyte G6PD utilizes deamino-NADP as coenzyme with ca. 60% efficiency compared to NADP [30–32]. The k_{cat}/K_m value for deamino-NADP as coenzyme in the human placental G6PD system was 4.80×10^6 M^{-1}.s^{-1}, corresponding to 32.4% of the native activity with NADP (Fig. 4, Table 1). Both NADP and deamino-NADP showed hyperbolic saturation upto 300 μM coenzyme concentration beyond which an inhibition was observed, regardless of the G6P concentration (Fig. 4).

All G6PDs isolated from different sources were inhibited by NADPH [1,5], with K_i values ranging from 6 (human platelet) to 200 μM (spinach leaf) [1,5]. Human erythrocyte G6PD K_i was found to be 9.1 μM [7]. Human placental G6PD was inhibited competitively by NADPH, with a K_i of 17.1 ± 3.2 μM (Fig. 5, inset), pointing to tight enzyme:NADPH binding and the important role of NADPH in the regulation of the pentose phosphate pathway which makes a critical contribution to many detoxification processes.

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