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Purification and kinetic characteristics of hepatic 6-phosphogluconate dehydrogenase (6PGD) from yellow catfish *Pelteobagrus fulvidraco*

[Sarı kedi balığı *Pelteobagrus fulvidraco* karaciğer 6-fosfoglukonat dehidrojenaz (6PGD)'ın saflaştırılması ve kinetik özellikleri]

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ABSTRACT

Objective: To purify and characterize hepatic 6-phosphogluconate dehydrogenase (6PGD) from yellow catfish and evaluate the effect of several metal ions (Cd^{2+}, Cu^{2+} and Al^{3+}) on the enzymatic activity *in vitro*.

Methods: The 2', 5'-ADP Sepharose 4B chromatography is used as the purification procedure; SDS-PAGE is used to assay the 6PGD purity and determine the molecular mass of the subunit; 6PGD activity is determined following rates of the conversion of $NADP^+$ to NADPH at 340 nm. The optimal temperature, pH and ionic strength is determined; The substrates and product kinetic, effect of metal ions are studied.

Results: The specific 6PGD activity is 5.75 U/mg, and its molecular weight is 50.1 kDa with a signal band. The optimum pH, temperature and ionic strength for the enzyme are pH 7.85, 60°C and 100 mM, respectively. The enzyme has the activation energy of 17.37 kcal/mol. The Km values of the enzyme for 6PGA and $NADP^+$ are 169.3 μM and 91.1 μM, respectively. The Vmax values of 6PGD with 6PGA and $NADP^+$ as the substrate are 5.1096 U/mg and 6.6776 U/mg, respectively. 6PGD is inhibited by NADPH in a competitive manner with the Ki value of 41.5 μM. The following metals, Cd^{2+}, Cu^{2+} and Al^{3+} show inhibitory effects on the enzyme. Cd2+ shows inhibitory effects in a competitive manner. The IC_{50} values of Cd^{2+} and Cu^{2+} are 0.16, 0.41mM, respectively, but the Al^{3+} shows almost no effects on the enzymatic activity.

Conclusion: The study can provide more information about purification and analysis of kinetic characterization of 6PGD in fish, and this is the first time that we study the in vitro influence of metal ions on 6PGD activity in fish.

Key Words: *Pelteobagrus fulvidraco*; 6PGD; purification; kinetic characterization; metal ions inhibition

Conflict of Interest: Authors have no conflict of interest.

ÖZET

Amaç: Bu çalışmanın amacı, hepatik 6-fosfoglukonat dehidrojenazı (6PGD), sarı kedi balığından saflaştırmak ve karakterize etmektir. Bununla birlikte, farklı metal iyonlarının (Cd^{2+}, Cu^{2+} ve Al^{3+}) enzim üzerine olan etkilerini incelemektir.

Metod: Saflaştırma için 2', 5'-ADP Sepharose 4B kromatografi prosedürü; 6PGD'nin saflığı ve moleküler ağırlığı için SDS-PAGE metodu; 6PGD aktivitesi ölçümü için $NADP^+$'nın NADPH 340 nm'de ölçümü kullanılmıştır. Optimum ısı, pH ve iyonik güç bulunmuştur. Substratlar ve ürün kinetiği için metal iyonlarının etkisi çalışılmıştır.

Bulgular: Özgül 6PGD aktivitesi, 5.75 U/mg, ve molekül ağırlığı, tek bir bant olarak 50.1 kDa bulunmuştur. Otimum pH, ısı ve iyonik güç, sırasıyla 7.85, 60°C and 100 mM olarak bulunmuştur. Enzimin aktivasyon enerjisi 17.37 kkal/mol'dür. 6PGA ve $NADP^+$'nin Km değerleri sırasıyla, 169.3 μM ve 91.1 μM olarak ve Vmax değerleri sırasıyla 5.1096 U/mg ve 6.6776 U/mg olarak gösterilmiştir. 6PGD, NADPH tarafından 41.5 μM Ki değeri ile kompetatif olarak inhibe edilmektedir. Cd^{2+}, Cu^{2+} ve Al^{3+}'un enzim üzerine inhibisyon etkileri gösterilmiştir. Enzimi Cd^{2+} kompetatif olarak inhibe etmektedir. Cd^{2+} ve Cu^{2+}'ın IC_{50} değerleri sırasıyla 0.16, 0.41mM olarak bulunmuştur. Al^{3+}'ün enzim aktivitesi üzerine bir etkisi gözlenmemiştir.

Sonuç: Çalışma balık 6PGD'sinin saflaştırılması ve kinetic özelliklerinin incelenmesi bakımından daha içerikli bir bilgi sunmaktadır. Balık 6PGD'si üzerine in vitro şartlarda metal iyonlarının etkisinin incelendiği ilk çalışmadır.

Anahtar Kelimeler: *Pelteobagrus fulvidraco*; 6PGD; saflaştırma; kinetic karekterizasyon; metal iyon inhibisyonu

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44) is a key enzyme of pentose phosphate pathway (PPP), which transfers 6-phosphogluconic acid (6PGA) into ribulose 5-phosphate and CO_2 with the NADPH production [1]. NADPH has very important functions in fatty acid biosynthesis [2], and can also protect the cell against oxidative stress by producing reduced glutathione (GSH) [3]. Due to its pivotal role in the PPP pathway [4], 6PGD received wide attention. 6PGD is widely available among animals, plants and microorganisms [5-9]. For the first time, the enzyme was purified from sheep liver [9], and then from other tissues, and subjected to extensive kinetic investigation [3,5-6,8,10-13]. However, based on the literatures available, information is scarce on the purification and analysis of kinetic characterization of 6PGD in fish.

In the past decades, increasing urbanization and industrialization of the region has resulted in increased contamination. Among environmental pollutants released into aquatic ecosystems, metals are of particular concern because some of them are necessary for important biological functions. However, they are toxic elements when their amounts exceed the requirement. Due to the two-sided effect, it is very important to evaluate the toxic effect of metal exposure on enzymatic activities [14]. At present, *in vivo* study indicated that tissue 6PGD was potential target for metal ions and functions to protect against metal toxicity [15,16]. However, to our knowledge, there is no information available involved in the *in vitro* effect of metal ions on 6PGD activity in fish.

Yellow catfish *Pelteobagrus fulvidraco* is widely distributed in lakes and reservoirs in China. Due to delicious meat and high market value, the culture of the species has increased rapidly in China. The present study is conducted to purify 6PGD from the liver of yellow catfish. The 2', 5'-ADP- Sepharose 4B affinity column was used to purify the enzyme The kinetic parameters and the influence of several metal ions on the enzyme activities *in vitro* were also evaluated.

Materials and Methods

Chemicals

In the present study, 2', 5'-ADP-Sepharose 4B was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Other chemicals, consisting of 2-mercaptoethanol (2-ME), $NADP^+$, NADPH, 6PGA, protein assay reagent, et al., were obtained from Sigma-Aldrich Chemical Co., MO, USA.

Fish husbandry and maintenance

100 healthy yellow catfish (body weigh 55 ± 5g) were obtained from a local fish dealer, and they were evenly distributed to four indoors circular tanks for 14-day acclimatization. During the acclimatization, yellow catfish were provided with a commercial Haid® feed at 2% of body weight daily. Each tank was provided with continuous aeration to maintain the dissolved oxygen level above saturation. Water in each tank was thoroughly replenished daily. Fecal matter was removed daily before feeding. We assure that the experiments performed on animals, animal care, and all protocols followed the ethical guidelines of Huazhong Agricultural University.

During the experiment, the water quality parameters were measured in the morning twice a week. Dissolved oxygen was more than 5.3 mg l^{-1}, pH=6.9~8.5, total ammonia–nitrogen 0.04–0.064 mg l^{-1}, and water temperature 25±3 °C.

Sampling

At the end of the 2-wk experiment, fish were starved for 24 h before sampling. Then they were killed by severing of the spinal cord. The liver was isolated immediately using sterile forceps in ice, quickly frozen in liquid nitrogen, and stored at -80 °C for subsequent analysis.

Purification of liver 6PGD

6PGD was purified according to the recently published protocols [17] with slight modification. The purification procedure consisted of 2', 5'-ADP-Sepharose 4B affinity chromatography. All the procedures were carried out at 4°C.

At first, the liver was homogenized in a glass-Teflon homogenizer with 3 volumes of buffer A (10 mM Tris-HCI buffer containing 5 mM 2-ME and 1 mM EDTA, pH 7.85) on the ice. The homogenate was centrifuged at 100,000 g for 60 min at 4°C. The supernatant was loaded onto 2'5'-ADP-Sepharose 4B column (1.6 cm×10 cm) pre-equilibrated with buffer A. The column was washed with the buffer A at the flow rate of 18 ml/h, by means of a peristaltic pump until the absorbance at 280 nm declined to 0.03 O.D. Then, the enzyme of G6PD was eluted with 10 mM Tris-HCl + 5 mM 2-ME + 1 mM EDTA + 0.2 mM $NADP^+$, pH 7.85. After G6PD was washed, 6PGD was eluted using buffer A containing 1 mM $NADP^+$, pH 7.85. The flow rates for washing and equilibration were reduced to 10.8 ml/h. Active 6PGD fractions were collected. Purification scheme of 6PGD from yellow catfish liver was shown in Table 1.

The determination of 6PGD activity

The hepatic 6PGD activity of yellow catfish was determined at 25°C by following rates of the conversion of $NADP^+$ to NADPH at 340 nm, following Beutler [18]. The reaction systems contained 100 mM Tris-HCl buffer, pH 7.85, 10 mM $MgCl_2$, 0.2 mM $NADP^+$, 1mM 6PGA and a suitable amount of the enzyme. The production of NADPH was measured every 10 s for 1 min at 340 nm at 25 °C. Assays were carried out in duplicates. One unit (U) of 6PGD activity was defined as the amount of enzyme that products 1μmol NADPH per minute, and expressed as U per mg of hepatic soluble protein. Protein concentrations were measured using bovine serum albumin as the standard, following Bradford [19].

Table 1. Purification of yellow catfish liver 6-phosphogluconate dehydrogenase.

Purification step	Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Total Activity (Units)	Special Activity (U/mg)	Yield (%)	Purification fold
100,000g supernatant	23	0.05	0.84	1.15	0.06	100	1
2′5′ADP-Sepharose Affinity eluate	5	0.115	0.02	0.575	5.75	50	95.8

SDS polyacrylamide gel electrophoresis (SDS-PAGE)

To assay the 6PGD purity and determine the molecular mass of the subunit, SDS-PAGE was adopted by Laemmli's procedure [20], with phosphorylase B from rabbit muscle (105 kDa), albumin from bovine serum (75 kDa), albumin from chicken egg white Grade VII (50 kDa), carbonic anhydrase from sheep liver (35 kDa), trypsin inhibitor from soybean (25 kDa), lysozyme from chicken (16 kDa). The acrylamide concentration of the stacking and the running gels was 3% and 10% containing 1% sodium dodecyl sulfate (SDS), respectively. The gel was stabilized in a solution containing 10% trichloroacetic acid, 50% propanol and 40% distilled water for 30 min. Then they were stained for 2 h in a solution of 10% acetic acid, 0.1% Coomassie Brilliant Blue R-250 containing 50% methanol, and 40% distilled water. The washing was carried out in the same solution without the dye until protein bands were cleared.

The determination of optimal temperature, pH and ionic strength

To determine the optimal temperature, 6PGD activity was assayed at different temperatures varying from 0°C to 85°C.

To determine the optimum pH, the 6PGD activity was measured in 100 mM Tris-HCl buffers with the pH range of 6.49~9.74.

To determine the optimal ionic strength, the activity was analyzed using different concentrations of Tris-HCl buffers in the range from 50mM to 300mM at a constant pH of 7.85.

Kinetic studies

For Km, Vmax and substrates kinetic type evaluation, Lineweaver-Burk curves were used [21]. To determine the K_m and V_m values of the $NADP^+$, the constant concentration of 1 mM 6PGA and various concentrations (0.05~0.2 mM) $NADP^+$ were used. To determine the Km and Vm values of the 6PGA, the constant concentration of 0.2 mM $NADP^+$ and various concentrations (0.25~1 mM) 6PGA were used. To determine the substrates kinetic type of the reaction, a matrix of substrate at (0.05~0.2 mM) $NADP^+$ and (0.2~0.6 mM) 6PGA was formed. The reactions were initiated by adding 6PGD to the matrix.

To determine the inhibition type and inhibition constant Ki of the production NADPH, a matrix of (0.025~0.1 mM) NADPH and (0.05~0.2 mM) $NADP^+$ was formed and the 6PGA were kept at constant concentration 1 mM. The Lineweaver-Burk curves and Dixon curves obtained were plotted for the determination of inhibitor type and K_i. The kinetic studies mentioned above were performed at the optimal pH value (100 mM Tris-HCl, pH 7.85) and at 25°C.

In vitro effects of metal ions

Cd^{2+} (0.04~0.2 mM), Cu^{2+} (0.1~0.7 mM), Al^{3+} (0.025~3.5 mM), were chosen to investigate their inhibitory effects. The determination was carried out in 100 mM Tris-HCl pH 7.85, 0.2 mM $NADP^+$, 1 mM 6PGA and a suitable amount of the enzyme at 25°C with varying concentration of each metal. The 6PGD activity was determined. An additional experiment without any inhibitors was used as the control (100% activity). Metal ions concentrations that produced 50% inhibition (IC_{50}) were calculated from the plots of activity (%) against metal ion concentration. In order to determine inhibition type of the Cd^{2+}, a matrix of (0.04~0.12 mM) Cd2+ and (0.05~0.15 mM) $NADP^+$ was used with the constant concentration of 6PGA at 1mM. The inhibition type was determined according to the Lineweaver-Burk double reciprocal plots.

Statistical analysis

Results are expressed as mean±standard deviation (SD).

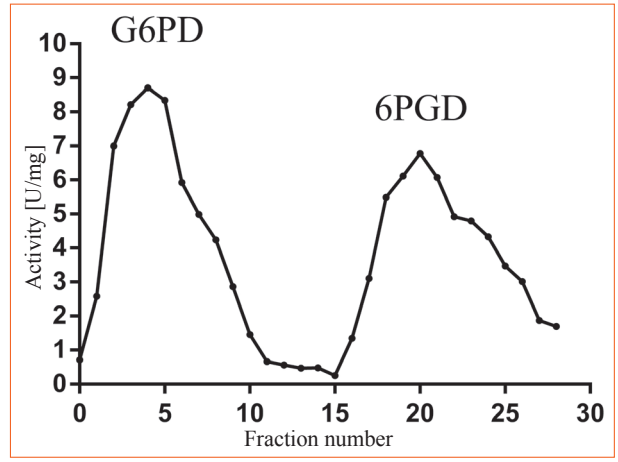

Figure 1. EThe elution profile of G6PD and 6PGD enzymes from liver of yellow catfish. The 2', 5'-ADP-Sepharose 4B column size, 1.6×10 cm; Eluant buffer, 10 mM Tris-HCl pH 7.85 containing 1 mM 2-ME and 1 mM NADP; flow rate, 0.18 ml/min. Fractions of 0.54 ml (3 min) were collected. The totall collection volume of activity 6PGD is about 5mL.

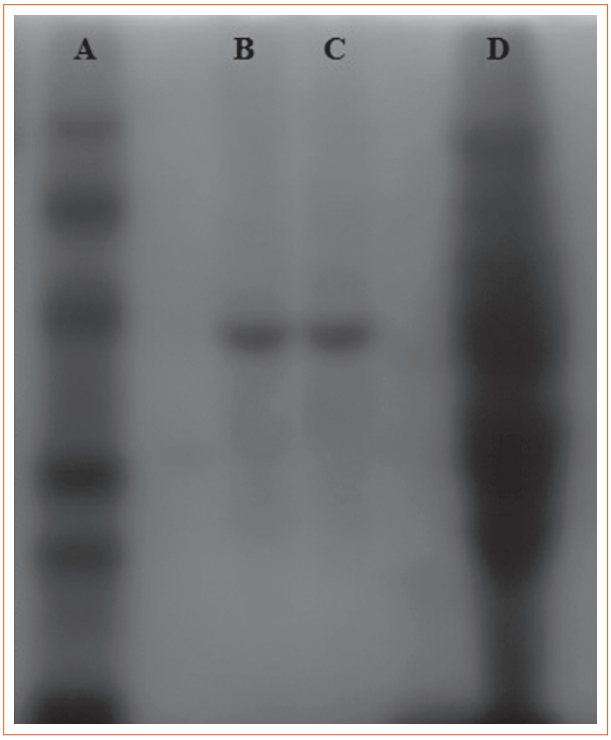

Figure 2. SDS-PAGE photograph, stained for 2 h in a solution of 10% acetic acid, 0.1% Coomassie Brilliant Blue R-250 containing 50% methanol, and 40% distilled water. Lane A, standard proteins, the MW of the band from top to bottom is phosphorylase B from rabbit muscle 105 kDa), bovine albumin 75 kDa), from hen egg albumin 50 kDa), carbonic anhydrase from sheep liver 35 kDa), trypsin inhibitor from soybean 25 kDa), Lysozyme from chicken muscle 16 kDa), respectively; Lane B and C, 2'5'ADP-Sepharose 4B; Lane D, 100,000g supernatant.

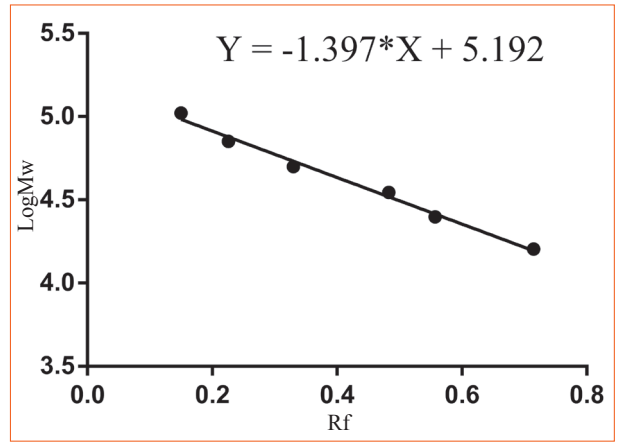

Figure 3. Standard Rf-LogMW graph of 6PGD using SDS-PAGE.

Results

Purification and kinetic parameters of 6PGD from yellow catfish liver

In the present study, the 2'5'-ADP Sepharose 4B chromatography was used to purify the 6PGD by the elution using buffer A containing 1mM $NADP^+$ (Fig. 1). The specific activity of 6PGD was 5.75 U/mg protein (Table. 1). The purity increased by the 95.8 fold, and the yield was about 50%. A single band was observed after SDS-PAGE (Fig. 2), indicating the enzymatic purity was high. We also calculated Rf values by Rf-logMW (molecular weight) picture using the method of Laemmli [20], based on the selected standard proteins (Fig. 3). 6PGD had a molecular weight of 50.1 kDa. The optimal temperature was 60°C (Fig. 4a). However, in our experiment, the physiological temperature of 25°C was used. Using the Arrhenius plot, Ea (activation energy) is 17.37 Kcal/mol (Fig. 4b). The activity was highest at pH 7.85 (Fig. 5a). The optimal ionic strength for the enzyme was 100 mM Tris-HCl buffer (Fig. 5b).

Kinetic behaviour of 6PGD

The K_m values of $NADP^+$ and 6PGA were 95.1 µM and 169.3 µM, and the V_m values of the $NADP^+$ and 6PGA were 6.6776 U/mg and 5.1096 U/mg, respectively (Fig. 6). The intersection points were on the left of the Y axis, indicating that the kinetic type of the reaction was a sequential mechanism (Fig. 7). The inhibition type of NADPH was the competitive inhibition type (Fig. 8a), and the K_i value of NADPH constant was found to be 41.5 µM (Fig. 8b). Table 2 presented several kinetic parameters of 6PGD.

The effects of metal ions at low concentration

The effects of the Cd^{2+}, Cu^{2+}, and Al^{3+} on the enzyme are show in the Fig. 9. The IC_{50} value of Cd^{2+} was 0.16 mM (Fig. 9a) and the inhibition type of the Cd^{2+} was the competitive inhibition (Fig. 9b). The IC_{50} value of Cu^{2+} was between 0.41 mM (Fig. 9c). However, the Al^{3+} showed nearly no effects on the enzyme (Fig. 9d).

Discussion

In the present study, we successfully purify 6PGD from liver of yellow catfish with the method of 2', 5'-ADP Sepharose 4B chromatorgraphy. This affinity gel is widely used to purify various $NADP^+$ affinity enzymes by using different elution buffers. For example, Williamson et al [22] successfully separated G6PD, ME (malic enzyme) and 6PGD from 2', 5'-ADP- Sepharose 4B chromatorgraphy with three different elution buffers, and G6PD could be eluted prior to 6PGD with the lower concentration $NADP^+$ elution buffer. Erat [23] also separated G6PD and GR (glutathione reductase) from this affinity gel using some different washing and elution buffers. In the study by Adem and Ciftci [17], G6PD, 6PGD, and GR enzymes could be well separated with three different elution buffers and G6PD could be eluted prior to 6PGD with the lower concentration $NADP^+$ elution buffer. In our study, G6PD could be eluted with the solution of 0.2 mM $NADP^+$ prior to 6PGD. Then, 6PGD was eluted when $NADP^+$ concentration was increased to 1 mM, with different elution solution concentration of $NADP^+$, and 6PGD could be successfully separated from G6PD.

In the present study, hepatic 6PGD specific activity purified from yellow catfish was 5.75 U/mg protein, lower

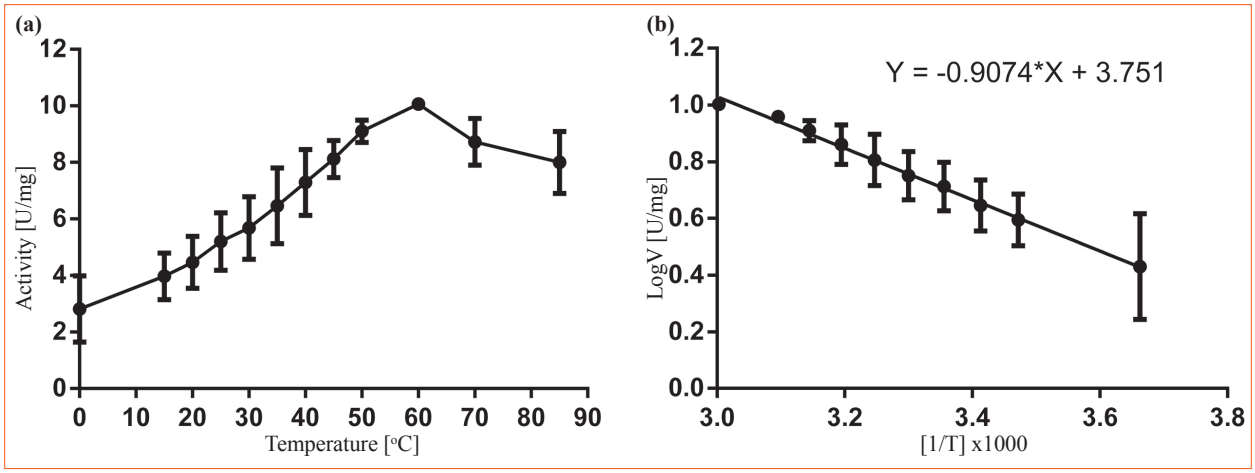

Figure 4. (a) The change of 6PGD activity related to temperature with the range of 0 and 85 °C with the addition of 1 mM 6PGA and 0.2 mM $NADP^+$. **(b)** The arrhenius plot with the determined Ea value of 17.37 kcal/mol. The slope of the line = -Ea/-2.303×R, where R is the gas constant equal to 8.314.

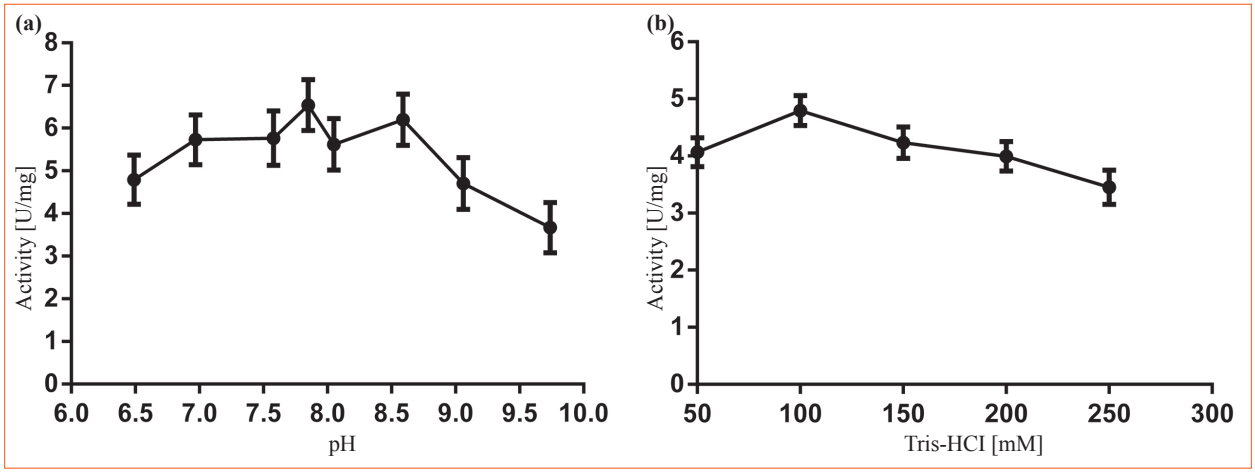

Figure 5. Effect of pH **(a)** and concentration **(b)** of Tris-HCl on the 6PGD activity purified from the liver of yellow catfish.

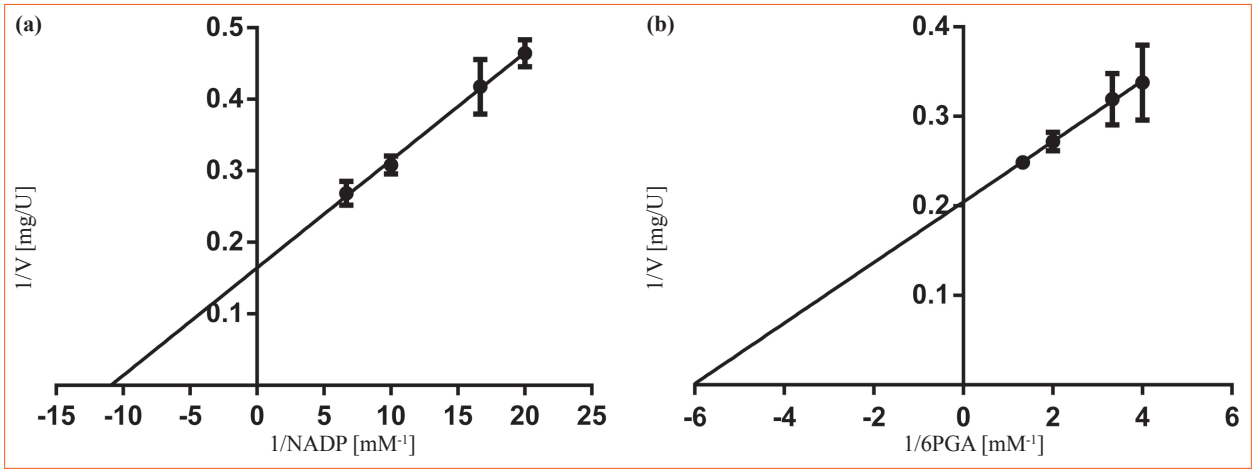

Figure 6. (a) Lineweaver-Burk graph with varied $NADP^+$ contents of 0.05, 0.10, 0.15 and 0.20 mM, respectively, at the fixed 6-PGA level. **(b)** Lineweaver-Burk graph with the varied 6PGA contents of 0.25, 0.5, 0.75 and 1 mM, respectively, at the fixed $NADP^+$ level.

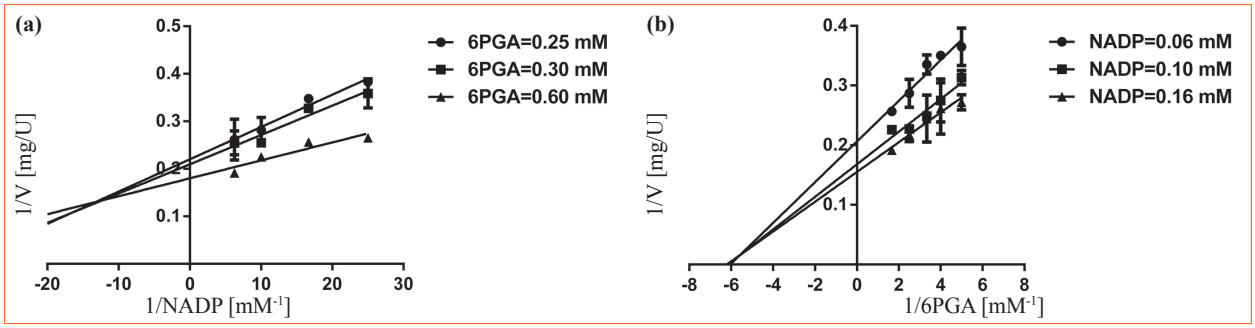

Figure 7. (a) Lineweaver-Burk graph of initial velocity against $NADP^+$ as varied substrate at different fixed 6PGA concentrations for the reaction catalyzed by 6PGD. **(b)** Lineweaver-Burk graph of initial velocity against 6PGA as varied substrate at different fixed $NADP^+$ concentrations for the reaction catalyzed by 6PGD.

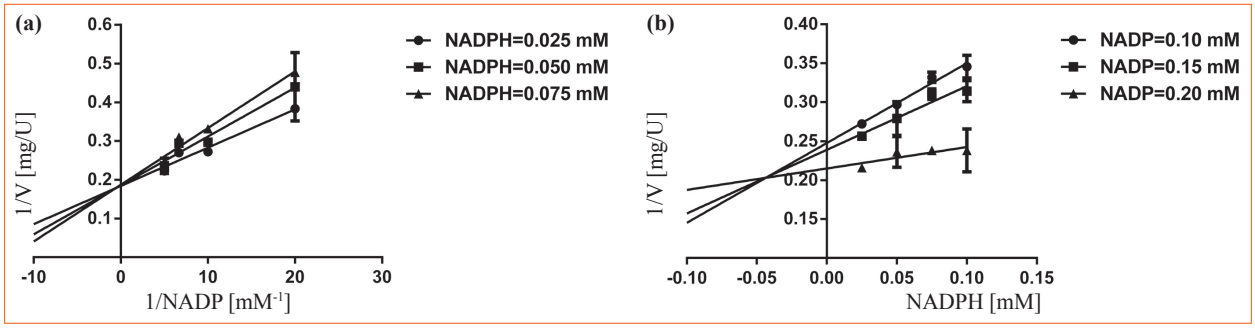

Figure 8. Lineweaver-Burk graph and Dixon graph to determine inhibit type and Dixon graph of NADPH. The substrate 6PGA) in 1mM constant concentration. **(a)** The initial velocity against $NADP^+$ as varied substrate at different fixed NADPH concentrations for the reaction catalyzed by 6PGD. **(b)** The initial velocity against NADPH as varied substrate at different fixed $NADP^+$ concentrations for the reaction catalyzed by 6PGD.

than those reported in other studies, such as15 U/mg protein in rat small intestine [10] and 25 U/mg protein in rat kidney [17]; but higher than that in rat liver (0.207 U/mg and rat kidney cortex 0.056 U/mg [24]). Ceyhan et al. [10] suggested that the specific activity of 6PGD showed great variability. For example, the specific activities of 6PGD from mammals were reported to range between 0.41 and 22.6 U/mg protein [5,8,9,13,25,26].

The molecular weight of the liver 6PGD from yellow catfish was 50.1 kDa by SDS-PAGE, similar to those in *Dicentrarchus labrax L*. liver [27], rat liver [13], rabbit mammary gland [25], human erythrocytes [26], sheep liver [28] and rat small intestine [10], lower than those in rat erythrocytes [5] and kidney [17], but higher than those in pig liver [8]. Generally speaking, dimer and/or tetramer is its active form for the enzyme, but dimer and tetramer possess different subunit mass. For example, all the 6PGD enzymes from mammalian sources belong to dimeric form, and 6PGD mass for each subunit varies between 38 and 65 kDa [5,8,9,13,25,26]. In contrast, the 6PGD enzymes from plants are either homotetrameric or homodimeric, and their subunits mass varies between 31 and 70 kDa [4,6,22,29-32]

Several studies suggested that 6PGD isolated from different sources showed a clear reliance on ionic strength [8,13,33]. For example, Ceyhan et al. [10] reported that the optimal pH values varied between 7.0 and 9.0 for the 6PGD from the rat small intestine when the concentration of phosphate buffer solution increased from 25 to 150 mM. Thus, the present study determined the optimum Tris-HCl concentration and pH, before determination of kinetic parameters. Based on the optimal buffer concentration of 100 mM, the optimum pH from liver of yellow catfish was measured to be 7.85, which was a little higher than rat erythrocytes [5], rat small intestine [10], pig liver [8], similar to *Drosophila melanogaster* [22], rat liver and kidney cortex [24]. In the present study, the optimal

Table 2. Kinetic parameters of 6-phosphogluconate dehydrogenase.

Parameters	Value±SD
K_m $NADP^+$ (mM)	0.0951±0.0052
K_m6PGA (mM)	0.1693±0.0053
V_m $NADP^+$ (U mg^{-1})	6.6776±0.8256
V_m6PGA (U mg^{-1})	5.1096±0.3003
K_{cat} $NADP^+$ (min^{-1} mg^{-1})	0.0701±0.0048
K_{cat}6PGA (min^{-1} mg^{-1})	0.0301±0.0008
K_i NADPH (mM)	0.0415±0.0049

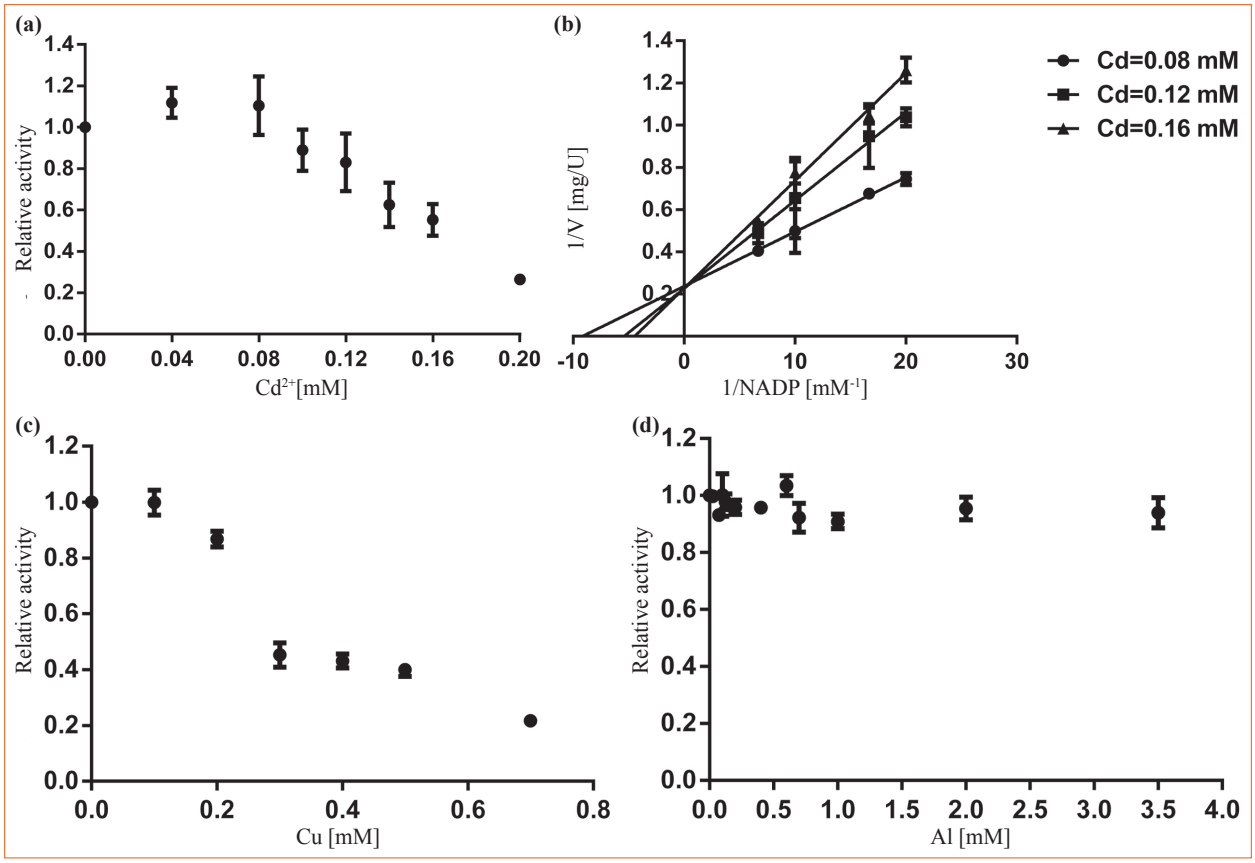

Figure 9. (a) Effects of the Cd^{2+} on 6PGD at the concentration 0.04~0.2 mM. **(b)** Lineweaver-Burk graph to determine inhibit type of Cd^{2+}. **(c)** Effects of the Cu^{2+} on 6PGD at the concentration 0.1~0.7 mM. **(d)** Effects of the Al^{3+} on 6PGD at the concentration 0.025~3.5 mM.

temperature of 6PGD enzyme was 60°C, higher than that in rat liver and kidney [24], rat small intestine [10], rat erythrocytes [5] and *Drosophila melanogaster* [22]. Our study also indicated that using the Arrhenius plot, Ea (activation energy) of 6PGD from liver of yellow catfish was determined to be 17.37 Kcal/mol. In contrast, Ceyhan et al. [10] reported 6PGD from rat small intestine had an Ea value of 7.52 Kcal/mol.

In the present study, the K_m values of $NADP^+$ and 6PGA were determined to be 95.1 μM and 169.3μM, respectively. K_m value of 6PGA was higher than that of $NADP^+$, indicating that the affinity of 6PGD to 6PGA was lower than that to $NADP^+$, in agreement with other studies [10,24,25,34,35]. The Vmax values of the $NADP^+$ and 6PGA were 6.6776 U/mg and 5.1096 U/mg. The catalytic efficiency (K_{cat}), defined as V_m/K_m, for $NADP^+$ and 6PGA was 0.0701 and 0.0301 min^{-1} mg^{-1} protein, respectively, suggesting that 6PGD was more efficient when using $NADP^+$ as the substrate. The inhibition type of NADPH was the competitive inhibition type, and the K_i constant of NADPH was 41.5 μM. NADPH had very important functions in fatty acid biosynthesis and in the protection of the cell against oxidative damage. Thus, the inhibition of 6PGD activity by NADPH was adverse to fish [5]. K_i values obtained for NADPH was 41.5 μM, which was similar with the study in rat kidney cortex [24], a little higher than rat small intestine [10], human erythrocytes [7] *Corynebacterium glutamicum* [36] and rat liver [24], but a little lower than rat erythrocyte [34].

In the present study, the effects of three typical metal ions on 6PGD activity are investigated. IC_{50} values were 0.16 and 0.41 mM for Cd^{2+} and Cu^{2+}; however, Al^{3+} showed almost no effect on 6PGD. In China, the concentration of waterborne, Cd^{2+} and Cu^{2+} in freshery water was limited to less than 0.005 and 0.01 mg/l, respectively, but there was no limit for Al^{3+} [37]. Hisar et al. [16] reported that the 6PGD activity was significantly diminished after Cd^{2+} exposure. In our laboratory, Tan et al. [38] reported that dietary Cd^{2+} level from 0.25 to 474.7 mg/kg significantly influenced the activities of hepatic glutathione peroxidase, alkaline phosphatase, lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) in yellow catfish. Hu et al. [39] reported that Cd^{2+} and Cu^{2+} inhibited G6PD activity in grass carp hepatic, the K_i constants of Cd^{2+} and Cu^{2+} are 4.8 mM and 0.26 mM, respectively; Chen et al. [15] reported that waterborne Cu exposure for 7 days significantly influenced hepatic SDH, malic dehydrogenase (MDH) and HL activities in yellow catfish. These inhibitions will in turn result in the change of some important physiological functions, based on the important role of NADPH in fatty acid synthesis and in the protection of cells against oxidative damage. On the other hand, the fish

meat is valuable food stuff of animal sources for human consumption. Under certain environmental conditions, metal elements accumulated in fish up to a toxic concentration will be dangerous and harmful for human health. Thus, it is impending to reduce the concentration of metal in contaminated lakes and rivers in China, for fishery and also human health.

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Conflict of Interest

There are no conflicts of interest among the authors.

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