# 魚類の六炭糖燐酸酸化経路に関する研究I.


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# Studies on Hexose Monophosphate Shunt of Fishes—I. Properties of Hepatic Glucose-6-phosphate Dehydrogenase of Barracuda

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The enzymatic properties of glucose-6-phosphate dehydrogenase in the liver of barracuda, *Sphyraena pinguis*, were investigated and the results obtained were as follows:

Glucose-6-phosphate dehydrogenase was present in the liver and kidney of barracuda, yellow-tail and carp, and the existence of hexose monophosphate shunt in fish tissues was suggested by the presence of glucose-6-phosphate dehydrogenase, phosphogluconate dehydrogenase, phosphoglucose isomerase and fructose diphosphatase in these tissues. The enzyme was relatively active at pH 8.0 and was relatively stable at pH 7.0. Nicotinamide-adenine dinucleotide phosphate was necessary for the enzyme as coenzyme and the activity was activated by calcium, barium and magnesium ions and inhibited by mercuric ions. The apparent Michaelis constant was $4.5\times10^{-5}$ M for glucose-6-phosphate or $2.0\times10^{-5}$ M for nicotinamide-adenine dinucleotide phosphate.

Present knowledge indicates that the hexose monophosphate shunt is one of the major reaction sequences by which glucose is metabolized in many biological systems, including mammals, plants and microorganisms. This metabolic pathway which has been variously called "pentose phosphate pathway" or "WARBURG-DICKENS pathway" or "pentose cycle", plays such many physiological roles as the reproduction of reduced NADP necessary for synthesis of fatty acids and steroids, as the supply of D-ribose necessary for synthesis of nucleotides and nucleic acids, and as the oxidation of G6P independent of the tricarboxylic acid cycle.

There are very few reports about the studies on the hexose monophosphate shunt in fish[1], although it would seem that G6P would be an intermediate compound standing upon the crossroad of carbohydrate metabolism in fish, and metabolized via the hexose monophosphate shunt as well as the glycolytic pathway, like mammals. The present experiments were conducted, therefore, to demonstrate the existence of hexose monophosphate shunt in fish, and to examine some properties of glucose-6-phosphate dehydrogenase [D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49.] in fish liver, catalyzing the initial step of this shunt.

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Abbreviations used: G6P DH, glucose-6-phosphate dehydrogenase; G6P, glucose-6-phosphate; NADP, nicotinamide-adenine dinucleotide phosphate.

## Materials and Methods

**Materials.** Liver of fresh barracuda, *Sphyraena pinguis*, was used for the experiments.

**Preparation of enzyme solution.** After the liver was homogenized with 9 volumes of cold water for 5 minutes in POTTER-ELVEHJEM's glass homogenizer, the homogenate was centrifuged at 13,000×g for 30 minutes at 0–5°C. The supernatant solution was dialyzed against deionized water for few hours, and the dialyzed solution was used for the G6P DH assay.

**Measurement of activity.** Enzyme activity of G6P DH was assayed by the method of GLOCK and MCLEAN[2]: The reaction mixture contained, in a final volume of 2.5 ml, 5 μmoles of G6P, 0.25 μmole of NADP, 50 μmoles of $MgCl_2$, 25 μmoles of Tris aminomethane and 25–100 μg of the enzyme protein. The mixture was incubated at 25°C and the reaction was started by the addition of the last component of the system. The increase of optical density, which is due to the reduction of NADP, was measured at 340 mμ. The enzyme activity was expressed as μmoles of reduced NADP formed per 5 minutes. Optical density was measured by using a Hitachi Perkin-Elmer spectrophotometer equipped with a Hitachi recorder and a thermostatically controlled cell chamber. Unless otherwise stated, the reaction was carried out at pH 8.0 and 25°C for 5 minutes.

## Results and Discussion

**Time course.** As shown in Fig. 1, optical density increased linearly for 5 minutes in the complete reaction mixture. An omission of G6P or NADP from the complete system slowed down sharply the formation of reduced NADP, and therefore, optical density of the system was measured against a blank cuvette containing pure water instead of G6P. The enzyme activities in the following experiments were estimated during the first 5 minutes, because those were slightly deviated by time. The value of G6P DH activity in cell homogenate obtained by the assay method may be inaccurate, since the coexistence of phosphogluconate dehydrogenase results in too high an activity being obtained. $NADPH^+$ oxidizing reactions (glutathione reductase, methaemoglobin reductase and other flavoproteins) tend to compensate for this. However, phosphogluconate dehydrogenase was scarcely effective on G6P DH activity in the assay conditions, because of the low concentration of phosphogluconate and phosphogluconate dehydrogenase in the reaction mixture. As shown in Fig. 2, amounts of reduced NADP formed per 5 minutes under the above experimental condition, increased in proportion to the protein content. The change in optical density should not be more than 0.04 per minute according to our experiences, and the less or dilute enzyme solution must be taken, if necessary.

**Effect of pH.** The pH-activity curves of G6P DH in the liver of barracuda are shown in Fig. 3. The optimum pH of the G6P DH reaction was around pH 8.0, when the

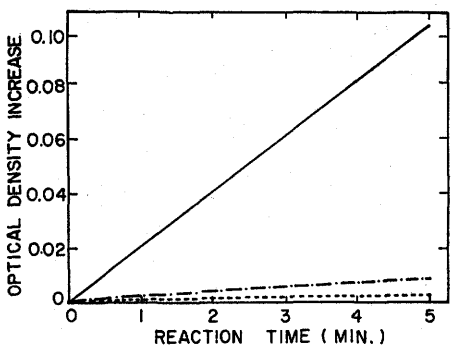


Fig. 1. Time courses of G6P DH in the liver of barracuda. Rate of reduction of NADP was spectrophotometrically measured at 340 mμ. The complete reaction mixture (2.5 ml) contained 5.0 μmoles of G6P, 0.25 μmole of NADP, 50 μmoles of $MgCl_2$, 25 μmoles of Tris and 27.5 μg of protein. Incubation was carried out at pH 7.5 and 25°C. ——, complete system; —·—, absence of G6P; – – –, absence of NADP.

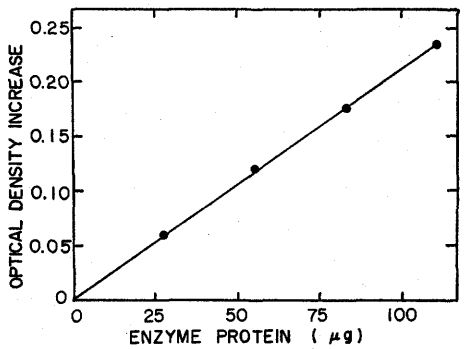


Fig. 2. Relation between the amount of the enzyme protein and increase in optical density. Incubation was carried out at pH 7.5 and 25°C for 5 minutes, and the components of the reaction mixture are as described in Fig. 1 except enzyme protein.

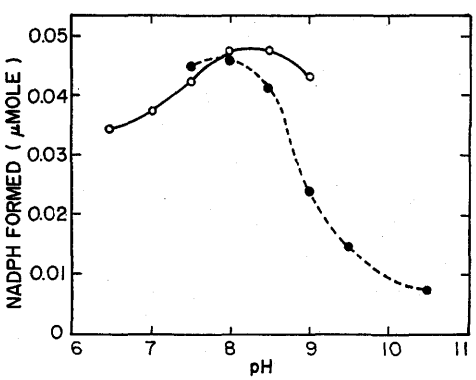


Fig. 3. Effect of pH on G6P DH activity in Tris and borate buffers. Incubation was carried out at 25°C for 5 minutes. ○——○, Tris buffer; ●------●, borate buffer.

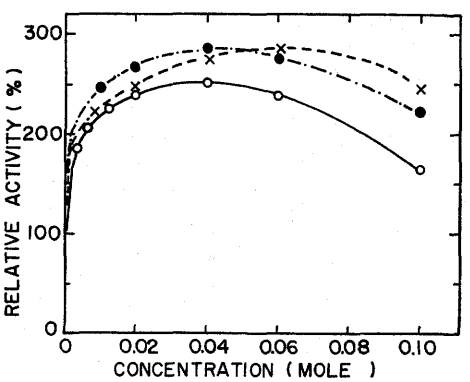


Fig. 4. Activation of G6P DH by magnesium, calcium and barium ions. ○——○, $MgCl_2$; ●–·–●, $CaCl_2$; ×------×, $BaCl_2$.

reaction was carried out at 25°C for 5 minutes in Tris or borate buffers. This value is in accord with those (pH 7.5–8.5) reported on various origins such as rat liver[2,3], *Escherichia coli*[4], *Leuconostoc mesenteroides*[5] and yeast[6].

**Effect of ions.** Effect of some ions on G6P DH activity is shown in Table 1. The enzyme was markedly activated by $Ca^{++}$, $Ba^{++}$ and $Mg^{++}$ ions, and inhibited completely by $Hg^{++}$ ions, as evident from the relative activity (%) shown in Table 1. As shown in Fig. 4, the optimum concentration of $Ca^{++}$, $Mg^{++}$ and $Ba^{++}$ ions for the activation of G6P DH was 0.04 M, 0.04 M and 0.06 M, respectively. Therefore, $MgCl_2$ was used as

**Table 1.** Influence of some ions on G6P DH activity.

| Ions | Concentration (M) | Relative activity (%) |
|---|---|---|
| Control | 0 | 100 |
| $Ca^{++}$ | 0.02 | 278 |
| $Ba^{++}$ | 0.02 | 264 |
| $Mg^{++}$ | 0.02 | 247 |
| $Mn^{++}$ | 0.02 | 142 |
| $PO_4^{---}$ | 0.02 | 83 |
| $Hg^{++}$ | 0.01 | 0 |

Metals were used as chloride.

the activating reagent at a concentration of 0.04 M in the assay procedure. Effects of metal ions such as $Ca^{++}$, $Mg^{++}$ and $Ba^{++}$ on G6P DH in the liver of barracuda are similar to those in other organisms[2,7], while G6P DH purified from *E. coli*[4] has been reported to be inactive in the absence of divalent cation.

**Apparent Michaelis constant.** Figs. 5 and 6 show the relationship between the reaction velocity and the concentration of G6P and NADP, respectively. The enzyme was saturated at about $1.0\times10^{-3}$ M of G6P and $1.0\times10^{-4}$ M of NADP. The apparent Michaelis constant was determined by the method of LINEWEAVER-BURK plots. Km was $4.5\times10^{-5}$ M for G6P or $2.0\times10^{-5}$ M for NADP.

**Coenzyme specificity.** The specificity of the enzyme with regard to coenzyme (NAD and NADP) was examined. As shown in Table 2, G6P DH in the liver of barracuda specif-

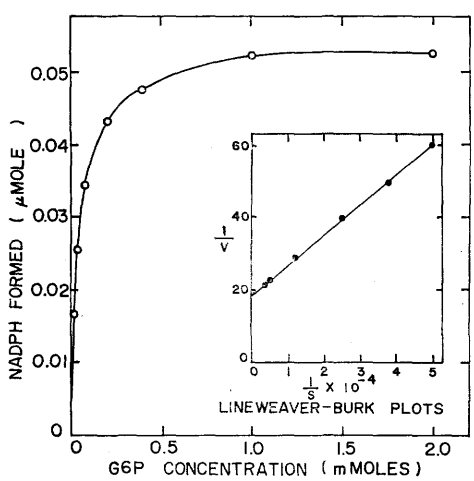


**Fig. 5.** Effect of G6P concentration on G6P DH activity. The reaction mixture contained 0.5 $\mu$mole of NADP, 50 $\mu$moles of $MgCl_2$, 25 $\mu$moles of Tris, 55 $\mu$g of protein and G6P.

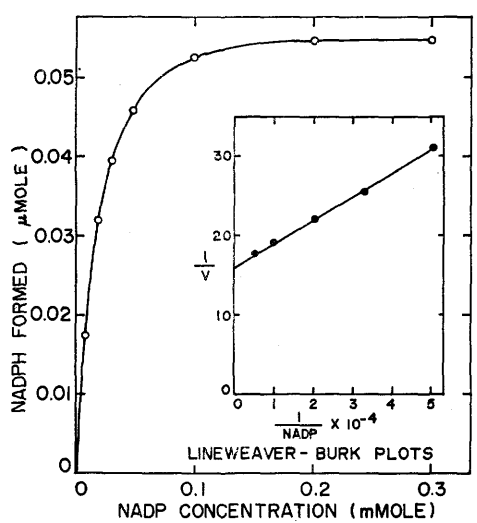


**Fig. 6.** Effect of NADP concentration on G6P DH activity. The reaction mixture contained 5.0 $\mu$moles of G6P, 50 $\mu$moles of $MgCl_2$, 25 $\mu$moles of Tris, 55 $\mu$g of protein and NADP.

ically reduced NADP, but NAD appears to be not replaced with NADP as coenzyme, and the same specificity was found in the enzyme from the liver of yellow-tail and the hepatopancreas of carp. While G6P DH from *L. mesenteroides*[4] has been reported to function with both NAD and NADP.

**Table 2.** Coenzyme specificity of G6P DH.

| Enzyme source | Relative activity (%) | | | |
|---|---|---|---|---|
| | NADP | | NAD | |
| | 0.0002 M | 0.001 M | 0.0002 M | 0.001 M |
| Liver of barracuda (1) | 100 | 103 | 0 | 0 |
| Liver of barracuda (2) | 100 | 104 | 0 | 0.4 |
| Liver of yellow-tail | 100 | | 0 | |
| Hepatopancreas of carp | 100 | | 1.4 | |

**pH stability.** The pH stability of the enzyme was examined at the range of pH 6.5–8.5 (Veronal buffer) and at 35°C. As shown in Fig. 7, the enzyme was relatively stable at pH 7.0, but unstable above pH 8.0.

SHATTON *et al.*[8] have recently reported that the liver of rainbow trout contains a highly active glucose dehydrogenase, reactive to both glucose and G6P. From the results of the report, purified glucose dehydrogenase of rainbow trout differs from crude G6P DH of barracuda in coenzyme specificity, pH optimum, and effect of $Mg^{++}$ ions. Therefore, more detailed studies on the properties of the purified enzymes of barracuda liver is required.

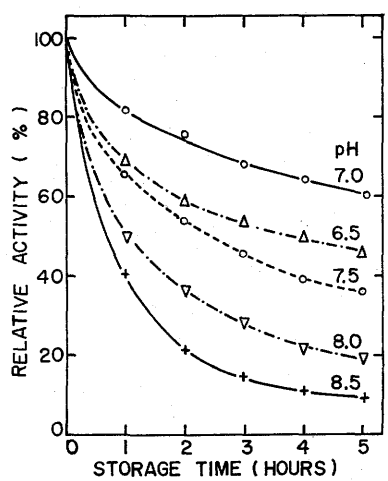


**Fig. 7.** pH stability of G6P DH. The enzyme solution was diluted with Veronal buffers (pH 6.5–8.5), and the solutions were stored at 35°C for 1–5 hours, then the activity was assayed.

We have reported the presence of phosphogluconate dehydrogenase, phosphoglucose isomerase and fructose diphosphatase, as well as G6P DH, in the liver and kidney of barracuda, yellow-tail and carp. Therefore, it was suggested that the presence of these enzymes in the fish tissues such as liver and kidney might be related to the existence of hexose monophosphate shunt in fish as in other organisms.

**Acknowledgements**

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