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Solubilization, Purification and Properties of Hexose 6-Phosphate Dehydrogenase from Guinea Pig Liver Microsomes

(H6PD/guinea pig liver/solubilization and properties)

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The solubilization, purification and property of hexose 6-phosphate dehydrogenase from guinea pig liver microsomes is described. The enzyme was effectively solubilized by ammonium hydroxide in the presence of 0.5% detergent, and highly purified. The enzyme showed a different pH dependency for various substrates. The optimum pH for galactose 6-phosphate, glucose 6-phosphate and glucose was 8.5, 10.2 and more alkaline, respectively. *Km* values for the substrates were also changed, pH dependently. The enzyme exhibited the lowest *Km* values in an order of 10^{-6}M for glucose 6-phosphate at pH 7.0. The enzyme activity was inhibited by $CaCl_2$ and $HgCl_2$, but other metal compounds, steroids, pCMB and NADPH did not inhibit the activity. The antibody against the purified enzyme inhibited the enzyme activity of Triton X-100 treated microsomes while the inhibition of the enzyme activity of intact microsomes by antibody was very low.

H6PD is a microsomal enzyme widely distributed in mammalian and fish tissues. This enzyme appears to be identical with the microsomal glucose dehydrogenase (β-D-glucose : NAD (P)$^+$ 1-oxidoreductase, EC 1.1.1.47), and has properties quite distinct from the soluble G6PD (1). H6PD oxidizes G 6P, Gal 6P and hexoses including glucose, using either $NADP^+$ or NAD^+ as cofactor (2). It has been considered that this enzyme plays a physiological role in microsomes by supplying the reduced coenzymes for microsomal mixed-function oxidase systems (3, 4). Recently, it was reported that H6PD of rat liver was located on the luminal side of microsomal membranes (5). Therefore, whether H6PD is actually associated with the mixed-function oxidase system functioning on the cytoplasmic side of microsomal membrane remains to be determined.

On the other hand, Kahl (6) reported that NADPH-dependent aromatic ketone reductase was present in rat liver microsomes, and the enzyme could

Abbreviations : H6PD, hexose 6-phosphate dehydrogenase ; G6PD, glucose 6-phosphate dehydrogenase ; G6P, glucose 6-phosphate ; Gal 6P, galactose 6-phosphate ; NH_4OH, ammonium hydroxide ; pCMB, p-chloromercuribenzoate.

be activated using G6P or NADPH. Sawada and Hara (7) reported that NADPH-dependent aromatic ketone reductase occurring in liver microsomes of rabbit, rat and guinea pig showed a unique cofactor requirement, and that the addition of $NADP^+$ and G6P was as effective as that of the NADPH generating system, whereas adding NADPH alone produced lower activity. It was also reported that H6PD existed in liver microsomes of guinea pig and the unique cofactor requirement of aromatic ketone reductase was based on production of NADPH by H6PD, thereby indicating that one of the roles of H6PD was to supply NADPH for aromatic ketone reductase (8). However the properties and location of H6PD in liver microsomes of guinea pig have not yet been clearly elucidated.

We now report the solubilization, purification and some properties of H6PD. The intramembraneous location of H6PD in guinea pig liver microsomes using rabbit antiserum against the purified enzymes is also described.

MATERIALS AND METHODS

Reagents

Reagents were obtained from the following sources : pyridine nucleotides and G6P from Oriental Yeast Co. ; androsterone, dehydroepiandrosterone and Gal 6P from Sigma Chemical Co. ; phospholipase C from Boehringer Mannheim ; Ultrogel AcA 34 and Ampholyte from LKB Productor AB ; Sephadex G-200 from Pharmacia ; phosphate cellulose from Brown Co. ; DEAE cellulose (DE32) from Whatman Ltd. Human prostatic acid phosphatase was isolated according to the method of Sawada *et al.* (9). All other reagents were commercial products of the highest grade available.

Preparation of Microsomes

Male Hartley guinea pigs, weighing 200−300 g, were exsanguinated and the liver was immediately excised and thoroughly perfused with 0.15 M KCl and homogenized in 0.25 M sucrose. The microsomal fraction was separated by centrifugation according to the method of Sawada and Hara (7). The microsomal pellet was resuspended in 0.25 M sucrose at a protein concentration of 20 mg/ml. Protein was determined by the method of Lowry *et al.* (10) with bovine serum albumin as a standard.

Enzyme Assays

H6PD activity was assayed using a Hitachi 200 spectrophotometer equipped with a Hitachi 056 recorder. The assay mixture contained in a total volume of 2.5 ml ; 200 μmoles glycine-NaOH buffer (pH 10.2), 2 μmoles G6P, 0.2 μmoles $NADP^+$ or NAD^+ and 50 μl of enzyme preparation. The reaction was started by adding enzyme solution and the rate of coenzyme reduction was followed spectrophotometrically at 340 nm ($\varepsilon = 6.22 \times 10^3\ M^{-1} \cdot cm^{-1}$) and 37°C. One unit of activity is defined as the amount of enzyme required to produce 1 μmol of NADPH or NADH per min. G6PD activity was assayed

by the method of Chung and Langdon (11).

Treatment of Microsomes

The microsomal suspension was frozen at −45°C and thawed at 25°C. This procedure was repeated 5 times. The microsomal suspension was sonicated in a Kubota Insonator 200M for 20 min at 0°C and 2.5 A. The microsomal suspension was incubated with phospholipase C at 37°C for 30 min or with trypsin at 0°C for 16 hrs. The microsomal suspension was treated with Triton X-100 or desoxycholate at 0°C for 1 hr. NH_4OH treatment of the microsomes was conducted as follows ; pH of the microsomal suspension was adjusted with 4% NH_4OH at 0°C under constant stirring for 5 min, and the suspension was brought back to pH 6.5 by adding 25% acetic acid. After each procedure, the treated suspension was centrifuged at $105,000 \times g$ for 90 min at 4°C.

Purification of H6PD

The enzyme preparation, solubilized with 0.05% Triton X-100 at 0°C for 30 min and then NH_4OH at pH 10.7, was applied to a phosphate cellulose column (1.6×25 cm) which had been equilibrated with 10 mM phosphate buffer (pH 6.5). The column was washed with 280 ml of the same buffer, and H6PD was then eluted with 20 mM phosphate buffer (pH 7.0). The enzyme-rich fraction was concentrated by ultrafiltration with YM-10 filter (Amicon Co.), and the resulting solution was subjected to gel filtration through a Ultrogel AcA 34 column (1.2×70 cm) in a 20 mM Tris-HCl buffer (pH 7.5). The enzyme fraction was directly applied to a DE32 column (0.8×15 cm), equilibrated with 20 mM Tris-HCl buffer (pH 7.5), and the column was washed with 50 ml of the same buffer. The enzyme was eluted by using a linear gradient formed with 300 ml of 20 mM Tris-HCl buffer (pH 7.5) and 300 ml of 0.1 M NaCl in 20 mM Tris-HCl buffer (pH 7.5). The enzyme active fraction which was eluted at 0.05 M of NaCl concentration, was concentrated by ultrafiltration and stored at 4°C.

Soluble G6PD was purified as follows ; the homogenate of guinea pig liver was centrifuged at $105,000 \times g$ for 60 min and the pellet was discarded. The supernatant was fractionated with ammonium sulfate. After removing the precipitate formed at 30% saturation of ammonium sulfate, the supernatant was brought to 60% saturation and the so-obtained precipitate was collected by centrifugation at $10,000 \times g$ for 10 min, and dissolved in 10 mM phosphate buffer (pH 6.5). The solution was dialysed against the same buffer and the dialysate was placed on a phosphate cellulose column (1.6×40 cm). The non-absorbed fraction was collected and concentrated by ultrafiltration with a YM-10 filter. The fraction was subjected to gel filtration of Ultrogel AcA 34.

Polyacrylamide Gel Electrophoresis

Disc gel electrophoresis of the purified enzyme was performed according to

the method of Davis (12) using 7.5% polyacrylamide gel. Electrophoresis was carried out at 4°C with a constant current of 3 mA per tube. The gel was stained for protein with Coomassie brilliant blue R 250.

Determination of the Molecular Weight

Molecular weight was estimated by gel filtration (13) using the Sephadex G-200 column (1.1×90 cm), equilibrated with 10 mM Tris-HCl buffer (pH 7.5) containing 0.15 M KCl. The column was calibrated with cytochrome C (M. W. 12,900), ovalbumin (M. W. 45,000), bovine serum albumin (M. W. 68,000), human prostatic acid phosphatase (M. W. 104,000) and catalase (M. W. 230,000), as standards.

Isoelectric Focusing

Isoelectric focusing was conducted in a 110 ml of LKB focusing column containing 1% solution of ampholytes, pH 3.5—10, and 0—40% sucrose gradient at 4°C for 2 days at a constant voltage of 280 V. The column was then drained slowly through the bottom tubing and fractions of 2.5 ml were collected. The pH and enzyme activity of the fractions were determined.

Preparation of Antibody

Purified enzyme (1 mg/ml) was mixed with an equal volume of Freund's complete adjuvant, and 0.5 ml of the mixture was given intramuscularly to a rabbit. This injection was repeated 4 times, biweekly. Two weeks after the last injection, the serum was obtained and immunoglobulin fraction was prepared by fractionation with ammonium sulfate. The antibody was dialysed against 20 mM boric acid buffer (pH 8.0) containing 0.15 M KCl for 3 days. The normal immunoglobulin fraction was obtained from an unimmunized animal by the same procedure.

Immunological Study

Ouchterlony's immunodiffusion test was performed in 1.2% agarose gel containing 20 mM phosphate buffer and 0.15 M KCl. For the inhibition study with antibody, 0.2 ml of microsomal suspension (10 mg/ml protein), Triton X-100 treated microsomes and partially purified soluble G6PD were incubated with antibody and 50 μmoles Tris-HCl buffer at 20°C for 1 hr, and then the enzyme activity was assayed.

RESULTS

1. Solubilization of H6PD

The efficacy of various treatments on solubilization of H6PD from guinea pig liver microsomes is compared in Table I. Hydrolytic enzymes, trypsin and phospholipase C, did not solubilize the H6PD. Freezing and thawing released a small amount of the enzyme and sonication gave a moderate solubilization. Use of a high concentration of desoxycholate and Triton X-100 resulted in a maximum release of the enzyme and the activity was increased

TABLE I. *Solubilization of Microsomal Hexose 6-Phosphate Dehydrogenase by Various Treatments*

Treatment	Relative activity** recovered in	
	Supernatant	Pellet
25 μg/mg Trypsin	0	152
6 units/mg Phospholipase C	0	76
Freezing and Thawing	22	121
Sonication	85	30
0.05% Desoxycholate	66	73
1.0% Desoxycholate	225	12
0.05% Triton X-100	19	111
1.0% Triton X-100	183	88
NH_4OH (pH 10.7)	57	63
0.05% Desoxycholate + NH_4OH (pH 10.7)	103	78
0.05% Triton X-100 + NH_4OH (pH 10.7)	204	29

* The supernatant and pellet obtained after treatment of microsomes were assayed for dehydrogenase activity with G6P and $NADP^+$.
** Relative activity is given as a percentage of the activity recovered in the supernatant or pellet relative to that in untreated microsomes.

2-fold in comparison with that of the untreated enzyme in the microsomes. Treatment with NH_4OH solubilized a moderate amount of the enzyme and the maximum release of the enzyme was obtained at pH 10.7. The combination of alkaline treatment and 0.05% desoxycholate or 0.05% Triton X-100 treatment resulted in a considerable solubilization. The activity was comparable to that with 1% detergent treatments and the specific activities were enhanced to 0.033 and 0.040 unit/mg respectively, such being higher than those of 1% desoxycholate or Triton X-100 solubilized preparations 0.010 or 0.007 unit/mg, respectively. Consequently, this procedure was used for solubilization of H6PD from guinea pig liver microsomes.

2. *Purification of H6PD*

The yields and activities of the various fractions obtained during one purification procedure are summarized in Table II. The specific activity of

TABLE II. *Purification of Microsomal Hexose 6-Phosphate Dehydrogenase*

Purification step	Total protein (mg)	Total activity* (unit)	Specific activity (unit/mg)	Recovery (%)
Microsomes**	5290	15.9	0.003	100
Supernatant fraction after treatment with 0.05% Triton X-100 at pH 10.7	724	26.0	0.036	163
Phosphate cellulose chromatography	19.1	17.0	0.89	107
Ultrogel AcA34 chromatography	6.2	13.9	2.2	88
DE32 chromatography	1.0	5.6	5.8	35

* Activity was assayed at pH 10.2 with G6P and $NADP^+$.
** Starting material was 265 ml of microsomal suspension from guinea pig liver.

the final product was 5.8 unit/mg, which was about 2000-fold higher than that of the starting microsomes. The overall recovery of the activity from the microsomes was 35%. When physical homogeneity of the enzyme was examined by polyacrylamide disc gel electrophoresis, a single band was observed on electrophoresis, as shown in Fig. 1. Partially purified preparation of soluble G6PD had a specific activity of 0.03 unit/mg.

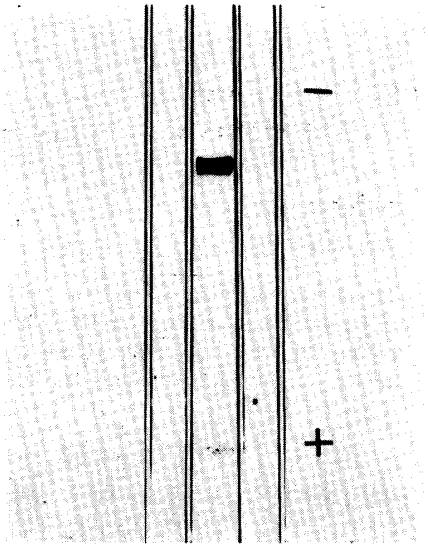

Fig. 1. Polyacrylamide gel electrophoresis of hexose 6-phosphate dehydrogenase.
Purified enzyme (10 μg) was run on 7.5% polyacrylamide gel and stained for protein.

3. Isoelectric Point and Molecular Weight

A symmetric peak with activity was obtained at pH 6.46, on isoelectric focusing. The apparent molecular weight of the enzyme which was estimated by gel filtration of Sephadex G-200 was 224,000.

4. Catalytic Properties

The enzyme oxidized various hexoses and their monophosphates in the presence of $NADP^+$ or NAD^+. The pH optimum for the H6PD varied with the substrate or cofactor used (Fig.2). $NADP^+$-dependent G6P dehydrogenase activity exhibited the maximum rate at pH 10.2, while the rate of NAD^+-dependent activity was increased up to pH 10.8. The dehydrogenase activity for glucose or Gal 6P showed the same pH optimum in the presence of NAD^+ and $NADP^+$, as cofactor. In the oxidation of G6P or Gal 6P, the enzyme activity with $NADP^+$ as cofactor was higher than that with NAD^+. However in the oxidation of glucose, the enzyme showed a higher activity in the presence of NAD^+ than that of $NADP^+$. The *Km* and *Vmax* values for these substrates were estimated under optimal and physiological pH conditions. As indicated in Table III, the affinity for G6P with both $NADP^+$ and NAD^+ as cofactor was elevated at pH 7.0, though *Vmax* values were reduced to one-eighth compared with those at pH 10.2. The *Vmax* value for G6P

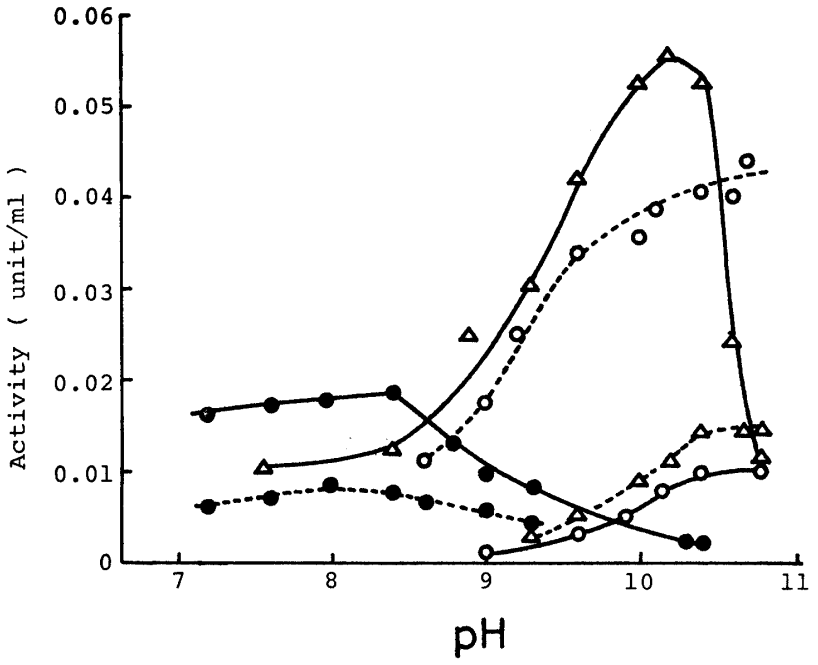

Fig. 2. Effect of pH on NAD^+- and $NADP^+$-dependent activity of hexose 6-phosphate dehydrogenase with different substrates.
The activity was measured with 160 μM NAD^+ (·····) or 80 μM $NADP^+$ (——) as cofactor and 0.8 mM G6P (△), 500 mM glucose (○) or 0.8 mM Gal 6P (●) as substrate.

TABLE III. *Kinetic Constants* of Hexose 6-Phosphate Dehydrogenase for Some Sugars under Different Conditions of Various pH*

Substrate	Cofactor**	pH 10.2		pH 7.0	
		Km (mM)	*Vmax**** (%)	*Km* (mM)	*Vmax**** (%)
G6P	$NADP^+$	0.31	100	0.009	13
G6P	NAD^+	0.043	16	0.004	2
Gal 6P	$NADP^+$	0.30	31	1.0	24
Glucose	NAD^+	5000	110	—	—

* *Km* and *Vmax* values were estimated from Lineweaver-Burk plots with 80 mM glycine-NaOH buffer, pH 10.2, or 80 mM phosphate buffer, pH 7.0.
** The concentrations of $NADP^+$ and NAD^+ were kept constant at 80 μM.
*** *Vmax* values are given as a percentage of that obtained with G6P and $NADP^+$ at pH 10.2.

with $NADP^+$ was 6 times higher than that with NAD^+, under both pH conditions. The *Km* and *Vmax* values for Gal 6P were slightly changed between pH 10.2 and pH 7.0, and those for glucose were extremely high at pH 7.0. The *Km* values for $NADP^+$ and NAD^+ with G6P as substrate were 8 and 2 μM at pH 10.2, respectively.

5. *Inhibition Studies*

The enzyme activity was inhibited 52% by 0.1 mM $HgCl_2$ and 57% by 1 mM $CaCl_2$, but was not effected by the addition of other metal ions such as

Mn^{2+}, Mg^{2+}, and Fe^{2+}, at a concentration of 1 mM. No effect on the enzyme activity was observed in the presence of 0.1 mM androsterone and dehydroepiandrosterone, and 1 mM pCMB. The effect of NADPH,a product of the enzyme reaction, was examined under various ratios of NADPH to $NADP^+$ (Table IV). The enzyme activity was inhibited only 13% by the ratio

TABLE IV. *Effect of NADPH Concentrations on Hexose 6-Phosphate Dehydrogenase**

$NADP^+$ (μM)	NADPH (μM)	Ratio $\frac{NADPH}{NADP^+}$	Inhibition (%)
12	26	2.16	13
12	50.9	4.24	6
12	100	8.33	13
24	17.2	0.72	0
24	33.3	1.39	0
24	96.8	4.03	13

* Enzyme activity was assayed in the reaction mixture containing 200 μmoles glycine-NaOH buffer (pH 10.2), 2 μmoles G6P, varying concentrations of $NADP^+$ and NADPH, and enzyme preparation. Corrections were made for controls without G6P.

2.16 of NADPH to $NADP^+$, but further inhibition of the enzyme activity was not observed with increases in the ratio.

6. Immunological Studies

The Ouchterlony's immunodiffusion test with the IgG against the purified H6PD showed a single precipitin line with the purified enzyme but none with the partially purified soluble G6PD (Fig. 3). The enzyme activity of Triton

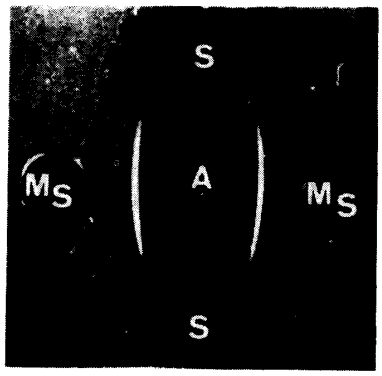

Fig. 3. Ouchterlony's immunodiffusion test using hexose 6-phosphate dehydrogenase antibody and the purified enzyme preparations. Anti-H6PD IgG was placed in center well (A), microsomal H6PD (Ms) and soluble G6PD (S) were in peripheral wells, and run for 17 hrs at 37°C.

X-100 treated microsomes was inhibited by the anti-H6PD antibody up to 75%, with increase in the amount of IgG (Fig.4). However, the inhibition of the enzyme activity of intact microsomes by the antibody was very low,

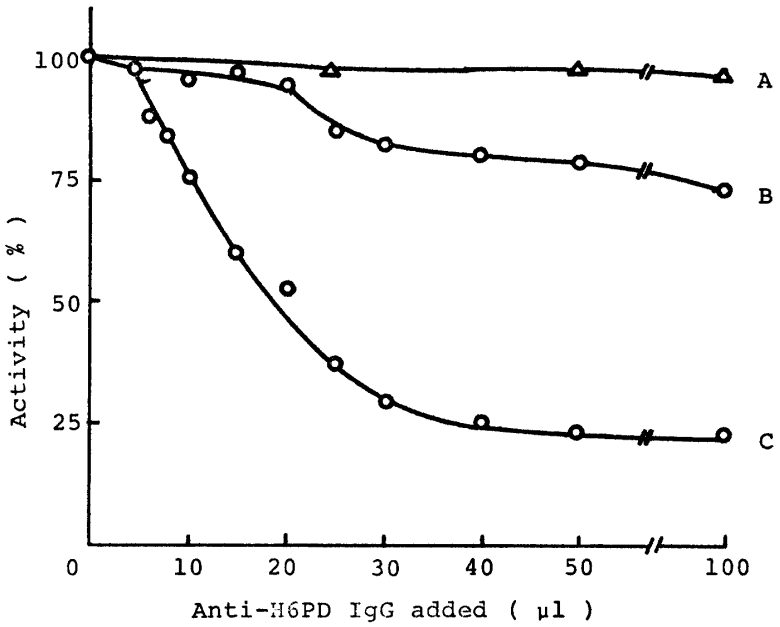

Fig. 4. Inhibition of hexose 6-phosphate dehydrogenase activity by anti-hexose 6-phosphate dehydrogenase antibody.
Soluble G6PD (A), intact microsomes (B) and Triton X-100 treated microsomes (C) were incubated with the IgG fraction (40 mg/ml) for 30 min in 10 mM Tris-HCl buffer (pH 7.5) containing 0.15 M KCl, and assayed for their activity. The enzyme activities are expressed in percentage of each enzyme fractions without the IgG fraction.

and the addition of the same amount of antibody which gave a maximum inhibition of the activity of Triton X-100 treated microsomes, resulted in only 26% inhibition of the activity of intact microsomes. The antibody was not inhibitory to soluble G6PD. It should be noted that the normal IgG from control rabbit serum did not effect the activity of either the intact or the Triton X-100 treated microsomes.

DISCUSSION

The activation of rat liver H6PD by desoxycholate, NH_4OH, glycine-NaOH (pH 8.0), Triton X-100 and sonic treatment has been reported by Hori and Takahashi (14). They also found that desoxycholate, NH_4OH and sonic treatment were effective for solubilization of the enzyme. We used various treatments to solubilize the enzyme from liver microsomes of guinea pig. The H6PD of guinea pig liver was moderately released by NH_4OH and sonic treatments from microsomes. The high concentration of detergents markedly solubilized the enzyme and the activity was increased 2-fold in comparison with the original enzyme activity in microsomes. Kuriyama (15) reported that the nucleoside diphosphatase was solubilized from rat liver microsomes by alkaline treatment in the presence of a low concentration of desoxycholate. We noted that the H6PD of guinea pig was effectively solubilized by NH_4OH in the presence of 0.05% desoxycholate or 0.05% Triton X-100, and had a higher specific activity. These values, 0.033 or 0.040 unit/mg are higher than values in the rat, 0.0086 unit/mg, by Emulgen 913 (16) or

0.012 unit/mg, by sonication (17). These results clearly show that NH_4OH treatment in the presence of detergents is a more effective solubilization procedure for the purification of the enzyme.

The purified enzyme preparation of H6PD had a specific activity 5.8 unit/mg and is approximately equal to that in the rat 10 unit/mg (17) or 3.36 unit/mg (16). The overall yield was 35%, these values being much the same as in the rat 13.2% (17) or 45% (16). Detergent was not required to keep the enzyme soluble throughout the process of column chromatography. This observation suggests that the enzyme behaves as a hydrophilic protein.

The molecular weight of guinea pig liver H6PD was 224,000, as estimated by gel filtration method. This is close to that of the rat 190,000 (17) or 210,000−220,000 (16) and is an excellent agreement with the value of 223,000 of human liver by Srivastava *et al.* (18). The isoelectric point of guinea pig liver H6PD 6.46 is strikingly close to that of the rat 6.4 (16).

The H6PD of guinea pig liver microsomes used either $NADP^+$ or NAD^+ as cofactor, and a similar cofactor requirement was observed in the rat (16, 17), ray (19) and rainbow trout (20). The pH optimum for G6P oxidation with $NADP^+$ as cofactor was at pH 10.2, and the value is close to that of rainbow trout pH 10.5 (20) and rat pH 10.8 (17) or pH 10.2 (16). Hori and Sado (17) reported that pH optimum of G6P dehydrogenase activity with NAD^+ as cofactor was at pH 11, in the rat. In the guinea pig, dehydrogenase activity for G6P with NAD^+ as cofactor was increased up to pH 10.8. In this study, however, as the enzyme activity was not assayed around pH 11, the extent of pH optimum of the enzyme is unknown. Hori and Sado (17) reported that the pH optimum with $NADP^+$ and Gal 6P or NAD^+ and Gal 6P in rat was 10.0 or 10.5, respectively. The enzyme activity of guinea pig in Gal 6P oxidation was independent of cofactors, $NADP^+$ or NAD^+. The pH optimum of Gal 6P was 8.5 and this value varies from that noted in the rat. Beutler and Morrison (2) reported that the pH optimum shifted to lower pH levels at lower Gal 6P concentrations. Even though the concentration of substrate used in our study differed from that used by others, there were no differences in pH optimum for G6P oxidation activity with $NADP^+$ as cofactor. These results suggest that there may be a variation in Gal 6P oxidation of individual species. As found in the rat (17) and rainbow trout (20), the affinity of guinea pig H6PD for G6P was considerably higher in physiological pH range than that in pH optimum. In the guinea pig, the *Vmax* value for G6P in physiological pH range was decreased to one-eighth compared with that in pH 10.2, and a similar tendency was seen in other species (2, 17).

Hori and Sado (17) reported that rat liver H6PD was not inhibited by steroid, but was somewhat inhibited by pCMB. The enzyme of guinea pig liver was not inhibited by steroids and pCMB. Like the enzyme in the rat, H6PD of the guinea pig was resistant to pCMB and dehydroepiandrosterone. H6PD was slightly inhibited by Mg^{2+} ion, an activator of soluble G6PD. NADPH, an inhibitor of soluble G6PD, inhibited slightly the H6PD. The

enzyme had a low *Km* value 9 μM for G6P with $NADP^+$ in physiological pH and was not inhibited by NADPH. On the basis of these observations, it is considered that one of the physiological roles of H6PD is to supply reduced coenzymes for the NADPH-dependent enzyme. In a previous report (21), we suggested that aromatic aldehyde-ketone reductase could serve as a steroid metabolizing enzyme in microsomes and there was an interaction between the reductase and H6PD. These suggestions may be supported by the result that H6PD activity was not inhibited by a steroid or NADPH.

In the immunodiffusion test, we found that the purified H6PD was clearly distinct from the soluble G6PD. The homogeneity of the enzyme was evidenced by the result that a single band was observed on the polyacrylamide electrophoresis, and a single precipitin line was observed between the antibody and purified enzyme on the immunodiffusion test. It has been reported that the antibody inhibited the H6PD activity of phospholipase or detergent treated microsomes, but had no effect on the enzyme activity of intact microsomes (5, 22). The incubation of Triton X-100-treated microsomes with antibody had an effect on the H6PD activity in the guinea pig, but the incubation of intact microsomes with antibody did not affect the H6PD activity. The enzyme was effectively released from microsomes by NH_4OH and detergent and the activity of intact microsomes was slightly inhibited by the antibody. These observations are in agreement with the findings in case of the nucleoside diphosphatase from rat liver microsomes described by Kuriyama (15). Therefore, the H6PD of guinea pig liver is probably located on the luminal side of the microsomal membrane.

This report concerns a study on the solubilization, purification and property of guinea pig liver H6PD. The NH_4OH and Triton X-100 treatments were found to be useful for solubilization of the enzyme. The location and the role of H6PD in microsomal membrane was discussed. The relationship between H6PD and NADPH requiring enzymes is the subject of ongoing studies.

REFERENCES

1) Kimura, H. and Yamashita, M. (1972) Studies on microsomal glucose 6-phosphate dehydrogenase of rat liver. *J. Biochem.* **71**, 1009–1014
2) Beutler, E. and Morrison, M. (1967) Localization and characteristics of hexose 6-phosphate dehydrogenase (Glucose dehydrogenase). *J. Biol. Chem.* **242**, 5289–5293
3) Kimura, K., Endou, H., Sudo, J., and Sakai, F. (1979) Glucose dehydrogenase (hexose 6-phosphate dehydrogenase) and the microsomal electron transport system. *J. Biochem.* **85**, 319–326
4) Stegeman, J. J. and Klotz, A. V. (1979) A possible role for microsomal hexose 6-phosphate dehydrogenase in microsomal electron transport and mixed-function oxygenase activity. *Biochem. Biophys. Res. Commun.* **87**, 410–415
5) Takahashi, T. and Hori, S. H. (1978) Intramembraneous localization of rat liver microsomal hexose 6-phosphate dehydrogenase and membrane permeability to its substrates. *Biochim. Biophys. Acta* **524**, 262–276
6) Kahl, G. F. (1970) Experiments on the metyrapone reducing microsomal enzyme system. *Naunyn Schmiedebergs Arch. Pharmakol.* **266**, 61–74

7) Sawada, H. and Hara, A. (1978) Studies on metabolism of bromazepam. VI. Reduction of 2-(2-amino-5-bromobenzoyl) pyridine, a metabolite of bromazepam, in the rabbit, rat, and guinea pig. *Drug Metab. Dispos.* **6**, 205–212
8) Sawada, H., Hayashibara, M., Hara, A., and Nakayama, T. (1980) A possible functional relationship between microsomal aromatic aldehyde-ketone reductase and hexose 6-phosphate dehydrogenase. *J. Biochem.* **87**, 985–988
9) Sawada, H., Sasaki, E., Asano, S., and Hara, A. (1978) Studies on human prostatic acid phosphatase. I. Effect of detergent and bovine serum albumin on hydrolysis of various substrates by purified prostatic acid phosphatase. *Yakugaku Zasshi* **98**, 1167–1172 (Eng. Abstr.)
10) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265–275
11) Chung, A. E. and Langdon, R. G. (1963) Human erythrocyte glucose 6-phosphate dehydrogenase. *J. Biol. Chem.* **238**, 2309–2316
12) Davis, B. J. (1964) Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.* **121**, 404–427
13) Andrews, P. (1965) The gel-filtration behaviour of proteins related to their molecular weights over a wide range. *Biochem. J.* **96**, 595–606
14) Hori, S. H. and Takahashi, T. (1977) Latency of microsomal hexose 6-phosphate dehydrogenase activity. *Biochim. Biophys. Acta* **496**, 1–11
15) Kuriyama, Y. (1972) Studies on microsomal nucleoside diphosphatase of rat hepatocytes. *J. Biol. Chem.* **247**, 2979–2988
16) Hino, Y. and Minakami, S. (1982) Hexose 6-phosphate dehydrogenase of rat liver microsomes. Isolation by affinity chromatography and properties. *J. Biol. Chem.* **257**, 2563–2568
17) Hori, S. H. and Sado, Y. (1974) Purification and properties of microsomal glucose 6-phosphate dehydrogenase (hexose 6-phosphate dehydrogenase) of rat liver. *J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool.* **19**, 515–529
18) Srivastava, S. K., Blume, K. G., Beutler, E., and Yoshida, A. (1972)Immunological difference between glucose 6-P dehydrogenase and hexose 6-P dehydrogenase from human liver. *Nature [New Biol.]* **238**, 240–241
19) Sado, Y. (1980) Properties of hexose 6-phosphate dehydrogenase from a Japanese ray, *Raja pulchra*. *J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool.* **22**, 156–163
20) Shatton, J. B., Halver, J. E., and Weinhouse, S. (1971) Glucose (hexose 6-phosphate) dehydrogenase in liver of rainbow trout. *J. Biol. Chem.* **246**, 4878–4885
21) Sawada, H., Hara, A., Hayashibara, M., Nakayama, T., Usui, S., and Saeki, T. (1981) Microsomal reductase for aromatic aldehydes and ketones in guinea pig liver. Purification, characterization, and functional relationship to hexose 6-phosphate dehydrogenase. *J. Biochem.* **90**, 1077–1085
22) Takahashi, T., Oka, K., and Hori, S. H. (1979) The latency of hexose 6-phosphate dehydrogenase activity in rainbow trout liver microsomes. *J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool.* **22**, 21–32