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LIVER AND RED-CELL PORPHOBILINOGEN SYNTHASE IN THE ADULT AND FETAL GUINEA PIG

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SUMMARY

Studies were undertaken to compare adult and fetal guinea pig liver and red-cell porphobilinogen synthase (EC 4.2.1.24). Specific activities of the enzyme from both liver and red-cell sources are higher in the fetus than in the adult. Age–activity profiles for the enzyme from both tissue sources are described. Porphobilinogen synthase from adult and fetal liver and red blood cells was purified utilizing discontinuous preparative disc gel electrophoresis as the final purification step. The enzyme preparations behave identically on DEAE-cellulose column chromatography. They have the same electrophoretic mobility, isoelectric point and pH optimum. Equivalent K_m values are observed in the presence or absence of glutathione. Differences in inhibitory patterns between the various enzyme preparations are demonstrated by studies of inhibition by hemin, lead acetate and $HgCl_2$. Double-reciprocal plots for each of these inhibitors indicate certain kinetic differences between the adult and fetal liver and red-cell enzymes. These differences are abolished by the addition of reduced glutathione, in the presence of which inhibition is noncompetitive for all the enzyme preparations. Certain inhibitory properties of guinea pig porphobilinogen synthase have been shown to change as a function of age and tissue source, and an effect of added glutathione upon enzyme inhibition in vitro has been shown.

INTRODUCTION

Porphobilinogen synthase (EC 4.2.1.24) catalyzes the condensation of 2 molecules of δ-aminolevulinic acid to form the monopyrrole porphobilinogen [1–4], an intermediate in the biosynthesis of porphyrins and other tetrapyrroles. The enzyme has been at least partially purified from a wide variety of plant [5–7], animal [1, 3, 8–14] and microbial [4, 15–21] sources.

End-product inhibition of porphobilinogen synthase by hemin [4, 8, 15] and hematin [13, 14] has been reported, and Muthukrishan and coworkers [21, 22] postulated that the enzyme has a regulatory role in the heme biosynthetic pathway of *Neurospora crassa*. The inhibitory effect of Pb^{2+} upon porphobilinogen synthase has been shown in numerous organisms and tissues both in vitro and in vivo [23]. Appreciable inhibition of enzymic activity in human red-cell hemolysates has been demon-

Abbrevation: GSH, reduced glutathione

strated at Pb^{2+} concentrations that have been observed in urban environments [24, 25]. Glutathione restores enzymic activity in liver and kidney homogenates from Pb^{2+}-poisoned rabbits [26], and it has been inferred that the inhibition by Pb^{2+} is largely due to interference with enzyme sulfhydryl groups. Hg^{2+}, which inhibits a wide variety of enzymes presumably because of its ability to interact with sulfhydryl groups, has also been shown to inhibit porphobilinogen synthase both in vitro [1] and in vivo [27].

The pattern of porphobilinogen synthase development with age has been investigated in mouse liver [14], and it has been shown that the specific activity of the enzyme is higher in the fetus than in the adult. Fetal and adult enzymes also differ in stability to heat and proteolytic inactivation but appear to have the same electrophoretic migration, substrate affinity and immunological specificity. In the fetal liver porphobilinogen synthase participates in the biosynthesis of porphyrin moieties that are provided both to the liver cytochromes and to hemoglobin. After birth hemoglobin synthesis no longer occurs in the liver but is taken over by the bone marrow and spleen. No comparison has been made between fetal liver prophobilinogen synthase and its counterpart in the adult bone marrow or red blood cell. Nor has a developmental survey been reported for red-cell porphobilinogen synthase. It would be of interest to determine whether properties of the enzyme change as a function of both age and tissue source, possibly reflecting a changing role of the enzyme during development.

This communication compares the developmental pattern of guinea pig red-cell porphobilinogen synthase with that of the liver enzyme. Adult and fetal liver and red-cell enzyme preparations were studied with reference to possible physical and kinetic differences by acrylamide gel electrophoresis, isoelectric focusing and by comparison of K_m values calculated for the substrate. The different porphobilinogen synthase preparations have also been compared on the basis of the inhibitory action by hemin and by the heavy metals Pb^{2+} and Hg^{2+} in the presence or absence of reduced glutathione. Inhibition studies were undertaken with concentrations of Pb^{2+} that have been observed in the blood of human subjects without clinical signs of overt Pb^{2+} intoxication [25], and hemin concentrations used were below those recently employed in the therapy of human porphyria [36].

MATERIALS AND METHODS

Materials

Male and female Charles River guinea pigs were used. The time of gestation of pregnant animals was determined by counting the detection of a vaginal plug as day zero. δ-Aminolevulinic acid and hemin were purchased from Sigma Chemical Co., St. Louis, Mo. Reduced glutathione (GSH) and guanosine were obtained from Calbiochem, San Diego, Calif. Acrylamide, *N,N'*-methylene bis-acrylamide, and *N,N,-N',N'*-tetramethylethylenediamine were from Eastman Kodak Co., Rochester, N.Y. DEAE-cellulose (Whatman DE-52) was from W. and R. Balson, Kent, England. Ampholine (Carrier Ampholytes) was purchased from LKB Produkter-AB, Bromma, Sweden. All other reagents were analytical grade products obtained from several commercial sources.

Assays

Porphobilinogen synthase was assayed according to a modification of the method of Russell and Coleman [28]. A 1-ml reaction mixture contained at least 0.05 unit of enzyme, 100 μmoles of potassium phosphate buffer, pH 6.8, 10 μmoles of bicarbonate-buffered δ-aminolevulinic acid, and 20 μmoles of GSH, unless specified otherwise. After a 15-min preincubation at 37 °C, the reaction was started by the addition of substrate and continued for up to 1 h under air at ambient pressure. In inhibition studies, hemin, Pb^{2+} and Hg^{2+} were also added to the preincubation mixture. No evidence of precipitation was observed during the preincubation or incubation periods. The reaction was stopped by the addition of 1.0 ml of 10% trichloroacetic acid containing 0.1 M $HgCl_2$. The mixture was centrifuged, and 1.0 ml of Ehrlich's reagent, as modified by Mauzerall and Granick [29], was added to 1.0 ml of the supernatant fluid. Color was allowed to develop for 15 min, and absorbance was read at 555 nm in a Gilford spectrophotometer. Linear reaction rates were observed in the ranges of time and enzyme concentration used. A molar extinction coefficient for porphobilinogen of $6.2 \cdot 10^4$ [29] was employed, and one unit of porphobilinogen synthase activity is defined as the amount of enzyme necessary to form 1 μmole of porphobilinogen per h at 37 °C. Profiles of porphobilinogen synthase activity as a function of age were determined on red-cell hemolysates and crude liver homogenates prepared as described below. Inhibition studies and kinetic assays were performed on enzyme preparations purified through the DEAE-cellulose chromatography step.

Purine nucleoside phosphorylase (EC 2.4.2.1) was assayed in red-cell hemolysates by the method of Tsuboi and Hudson [30]. Protein determinations according to the method of Lowry et al. [31] were used with bovine serum albumin (Armour Pharmaceutical Co., Chicago, Ill.) as standard.

To determine whether the patterns of inhibition described in the double-reciprocal plots (Figs 6, 7, 8) were noncompetitive (common $1/S$ intercept) or mixed (dissimilar intercepts) tests of significance at the 95% confidence level were performed using least-squares techniques [32]. Enzyme units were adjusted to give the same activity in the presence or absence of GSH.

Analytical disc gel electrophoresis and isoelectric focusing in acrylamide gels

Polyacrylamide gel electrophoresis was carried out as described by Davis [33] in 5% and 7.5% gels made either in glass cylinders (0.5 cm × 8.0 cm) or in an Ortec slab electrophoresis apparatus. Approx. 100 μg of protein in 5% sucrose was applied to each gel, and electrophoresis was terminated when the tracking dye, bromphenol blue, had migrated 6 cm. Gels were either stained with coomassie blue or, in the case of cylindrical gels, sliced into 1-mm fractions and assayed for porphobilinogen synthase. Gel fractions were assayed as described above except that incubations were allowed to proceed overnight. Isoelectric focusing was performed in polyacrylamide gels as described by Wrighley [34]. Ampholine at a concentration of 0.2% in the pH range of 3.0 to 10.5 was employed with 0.4% ethanolamine and 0.2% H_2SO_4 as the cathode and anode solutions, respectively. Experiments were carried out by adding samples in 5% sucrose to the top of the gels and allowing them to focus for 4 h. The initial current per gel was 2 mA. At termination, the gels were cut into 1-mm slices and assayed for activity as described for the polyacrylamide gels. Identical

gels were divided into 3-mm sections and allowed to incubate for 2 h at room temperature in 0.5 ml of distilled water. pH profiles of the gels were obtained by measuring the acidity of these samples.

Purification of porphobilinogen synthase

Hemolysate and homogenate steps. Postnatal whole blood samples were obtained from guinea pigs by cardiac puncture. To obtain prenatal samples, pregnant animals were sacrificed by cervical dislocation, and the fetuses were removed, decapitated, and their blood pooled. The blood samples were collected in heparinized tubes, centrifuged at 2000 × *g* for 10 min and washed 3 times in 0.15 M NaCl. The cells were then lysed by the addition of 4 vol. of distilled water, followed by centrifugation at 2000 × *g* for 10 min to remove the stroma.

Livers were excised from either fetal or adult guinea pigs, washed with 0.15 M NaCl, and homogenized in a Waring blender for 30 s in a 25% (w/v) solution containing 0.15 M KCl and 0.025 M potassium phosphate buffer, pH 6.8. The liver homogenate was then subjected to centrifugation at 40 000 × *g* for 1 h, and the resultant supernatant fraction was heated at 60 °C for 10 min. The precipitate obtained by the heat treatment was spun down at 2000 × *g* for 15 min and discarded.

DEAE-cellulose step. Adult (greater than 800 g) and fetal (days 50–55 of gestation) red-cell hemolysates and liver heat-treated supernatant fractions were added to DEAE-cellulose columns (1.5 cm × 30 cm) which had been previously equilibrated with 0.025 M potassium phosphate buffer at pH 6.8. Protein was eluted at a flow rate of 1 ml/min by a 600-ml linear NaCl gradient (0–0.6 M) in potassium phosphate buffer at 0.025 M and pH 6.8 containing 5% sucrose. Fractions containing porphobilinogen synthase activity were pooled and concentrated to approx. 1.0 mg/ml in an Amicon concentrator (model 202) using a PM10 membrane.

Preparative acrylamide gel electrophoresis. Electrophoresis was carried out in an LKB 7900 Uniphor apparatus on a 6 cm long 5% acrylamide gel using the discontinuous Tris buffer system described by Davis [33]. However, the electrode buffer and elution buffer concentrations of Tris–glycine were increased to 0.17 M and 0.08 M Tris, respectively, in order to increase conductivity. An Ortec 4100 pulsed constant power supply was used and set initially at 450 V, 50 mA, and a pulse rate of 250 cycles. Approx. 4 mg of porphobilinogen synthase from concentrated DEAE-cellulose preparations were electrophoresed at one time. Bromphenol blue at 0.001% and sucrose at 5% were added to protein samples prior to their addition to the top of the gel. Fractions were collected at a flow rate of 20 ml/h, and those containing porphobilinogen synthase were concentrated by filtration as mentioned above. All purification steps were carried out at 0–4 °C.

RESULTS

Developmental pattern of porphobilinogen synthase

The pattern of red-cell and liver porphobilinogen synthase development with age is represented in Fig. 1. Specific activities of the fetal enzyme from both red-cell and liver sources are more than 2-fold higher than in the adult. Both tissues exhibit decreases in enzymic activity approx. 5 days prior to birth and reach plateaus at age 10 days. However, the patterns of activity are not identical in the perinatal period.

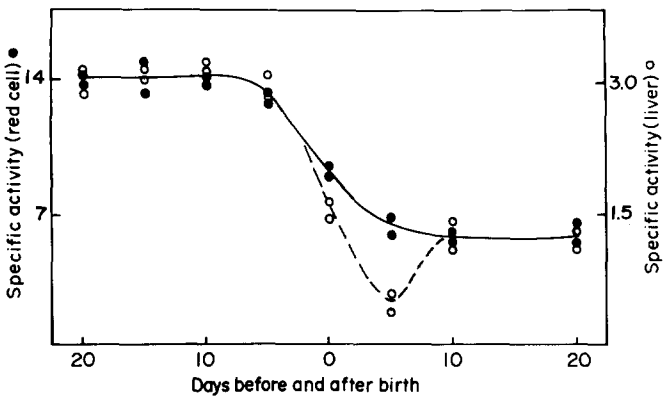

Fig. 1. Porphobilinogen synthase activity as a function of age in guinea pig liver and red blood cells. Enzyme activity was determined in crude liver homogenates and in red cell hemolysates as described in Methods. Red cell specific activity is expressed as units per ml of cells. Specific activity for liver is expressed as units per gram wet weight.

While specific activity of the red-cell enzyme gradually declines to adult levels by approx. 10 days of age, liver porphobilinogen synthase activity reaches a nadir shortly after birth and then increases to a constant specific activity at the same postnatal time period as the red-cell enzyme. In contrast, simultaneous assays of the red-cell enzyme purine nucleoside phosphorylase show no change in specific activity during the transition from fetal to adult life.

TABLE I

PURIFICATION OF PORPHOBILINOGEN SYNTHASE FROM ADULT AND FETAL GUINEA PIG LIVER AND RED BLOOD CELLS

All activities were determined in the presence of 20 mM GSH.

	Adult liver			Fetal liver		
Step	Volume (ml)	Total activity (units)	Specific activity (units/mg)	Volume (ml)	Total activity (units)	Specific activity (units/mg)
Homogenate	90	51.4	0.007	28	19.8	0.015
40 000 × *g* supernatant	54	43.3	0.015	20	20.5	0.033
Heat	40	35.9	0.029	16	17.0	0.053
DEAE-cellulose	8.3	19.1	0.91	15.7	14.4	0.65
Preparative disc gel electroforesis	2.2	11.5	2.60	3.4	11.2	1.60
	Adult red cell			Fetal red cell		
Step	Volume (ml)	Total activity (units)	Specific activity (units/mg)	Volume	Total activity (units)	Specific activity (units/mg)
Hemolysate	20	26.5	0.019	19	26.1	0.03
DEAE-cellulose	22	15.8	2.39	17	10.0	2.10
Preparative disc gel electroforesis	3.7	12.9	3.58	3.1	7.91	2.75

Purification and properties of porphobilinogen synthase

Porphobilinogen synthase was prepared from fetal and adult liver and red blood cells in essentially the same manner by using ion-exchange chromatography and preparative acrylamide gel electrophoresis as the final steps in the purification. The purification steps of the enzyme from the four different tissue sources are summarized in Table I. In liver preparations a heat precipitation step at 60 °C was employed prior to the DEAE-cellulose column chromatography without appreciable loss in total activity. However, this step could not be utilized in red-cell preparations of porphobilinogen synthase without loss of 80–90% of enzymic activity. Elution profiles of adult liver enzyme from DEAE-cellulose and preparative disc gel electrophoresis are shown in Fig. 2. One homogeneous enzymatic activity peak is eluted from the ion exchanger at a NaCl concentration of 0.25 M as well as from the discontinuous electrophoresis of 5% polyacrylamide at an R_F of approx. 0.57. The behavior of fetal liver and adult and fetal red-cell preparations of porphobilinogen synthase on DEAE-cellulose and on a discontinuous acrylamide gel system is the same as that of adult liver enzyme. All four enzyme preparations had the same R_F values in 5% analytical gels as well as in 7.5% gels (R_F = 0.23). The protein patterns of porphobilinogen synthase from adult and fetal liver and red blood cells before and after

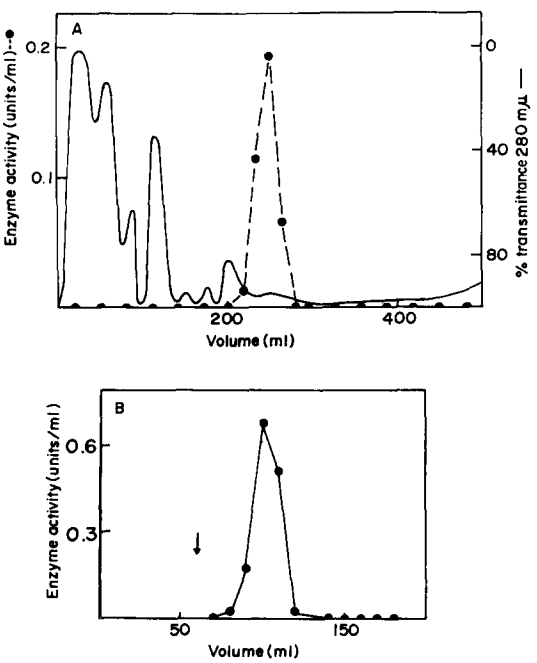

Fig. 2. Elution profiles of adult guinea pig liver porphobilinogen synthase from DEAE-cellulose (A) and preparative disc gel electrophoresis (B). Protein was eluted from the DEAE-cellulose column by a 0–0.6 M NaCl linear gradient. Fractions containing enzymic activity (200–250 ml range) were pooled and concentrated. The elution was monitored by an LKB Unicord II Absorbiometer using a 0.5-cm light path. The arrow in B indicates the fraction in which the bromphenol blue dye marker was eluted from the gel. Fractions in the range of 75–110 ml were pooled and concentrated. See Methods for details.

preparative disc gel electrophoresis (Fig. 3) indicate that all four enzyme preparations after final purification give essentially a single protein band migrating at the same velocity. Enzyme assays of the gel slices confirmed that the band contains porphobilinogen synthase activity. Furthermore, all four enzymes exhibited an isoelectric point of 4.8 as judged by the technique of isoelectric focusing in acrylamide gel. A broad pH optimum for all four enzyme activities was found in the range of 6.5 to 7.5. K_m values were calculated for the porphobilinogen synthase preparations and are given in the legends of Figs 6, 7 and 8. There are no major differences in K_m values among the four enzyme preparations, but in all instances values are slightly higher where GSH is present. The presence of GSH in the assays enhanced the activities of all porphobilinogen synthase preparations purified through the DEAE-cellulose chromatography step by 5-fold.

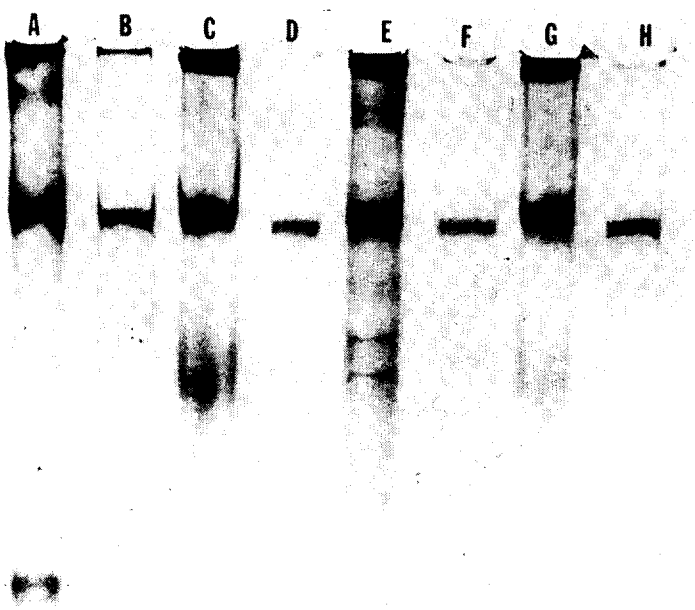

Fig. 3. Analytical polyacrylamide gel electrophoresis of liver and red cell porphobilinogen synthase from adult and fetal guinea pigs before and after preparative disc gel electrophoresis. 50–100 μg of protein were subjected to electrophoresis in 7.5% acrylamide in an Ortec slab apparatus, and gels were stained with coomassie blue. (A) Adult liver enzyme preparation before and (B) after preparative disc gel electrophoresis. (C) Fetal liver before and (D) after preparative disc gel electrophoresis. (E) Adult red cell before and (F) after preparative disc gel electrophoresis. (G) Fetal red cell before and (H) after preparative disc gel electrophoresis.

Hemin inhibition

Hemin inhibition curves for porphobilinogen synthase from adult and fetal liver and red blood cells (Figs 4A and 5A) appear to be hyperbolic, and all appear to reach saturation at the same concentration of inhibitor both in the presence and in the absence of GSH. At 50% maximum inhibition, hemin concentrations in the

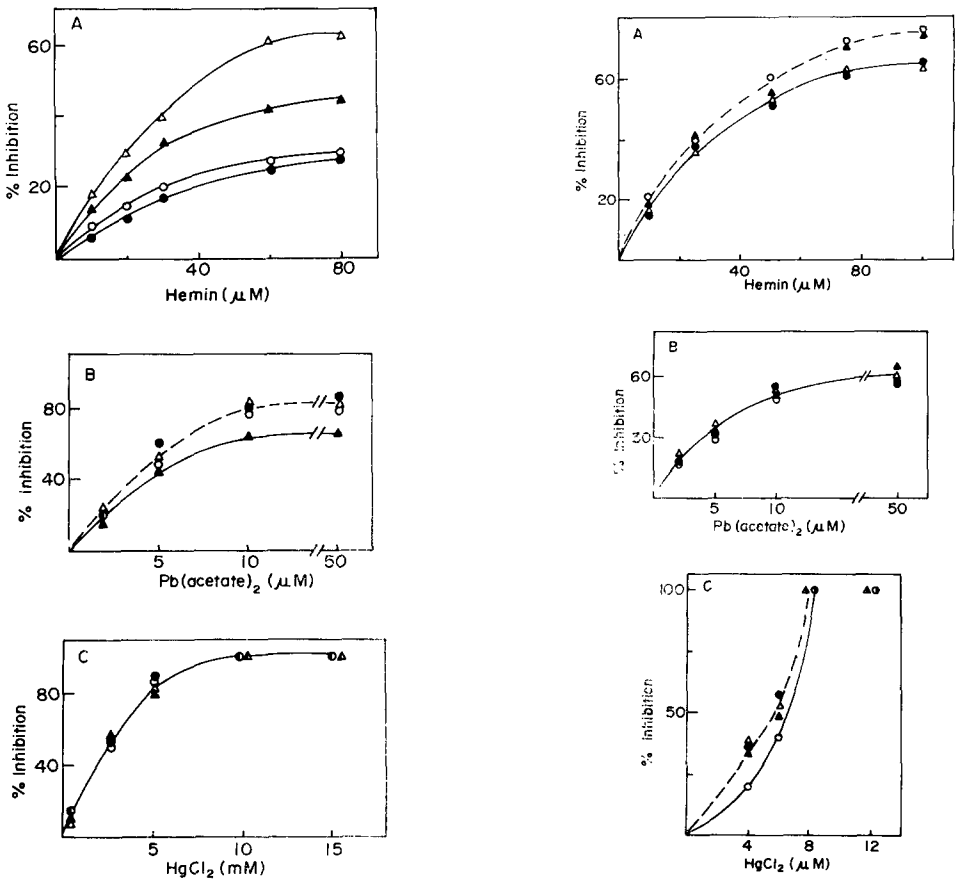

Fig. 4. Inhibition in the presence of 20 mM GSH of liver and red-cell porphobilinogen synthase from adult and fetal guinea pigs by hemin (A), lead acetate (B) and $HgCl_2$ (C). ●, adult liver; ○, fetal liver; △, adult red cell; ▲, fetal red cell.

Fig. 5. Inhibition in the absence of GSH of liver and red cell porphobilinogen synthase from adult and fetal guinea pigs by hemin (A), lead acetate (B) and $HgCl_2$ (C). ●, adult liver; ○, fetal liver; △, adult red cell; ▲, fetal red cell.

range of 19–25 μM were calculated from these curves. In the presence of GSH maximal inhibition by hemin is approx. 70%, 50%, 30% and 30% for adult red cell, fetal red cell, adult liver and fetal liver enzymes, respectively. However, without reducing agent in the assay mix 60–70% inhibition is observed at saturating concentrations of hemin for all four enzyme preparations. Kinetic studies of hemin inhibition are reproduced in Fig. 6. In the presence of GSH noncompetitive inhibition is observed for all four enzyme preparations, but in the absence of GSH inhibition is of the mixed type for fetal red cell porphobilinogen synthase and noncompetitive in the case of the adult red cell and both liver enzymes.

Lead acetate inhibition

In the presence or absence of GSH inhibition patterns of lead acetate appear to be hyperbolic with maximum saturation occurring at Pb^{2+} concentrations of

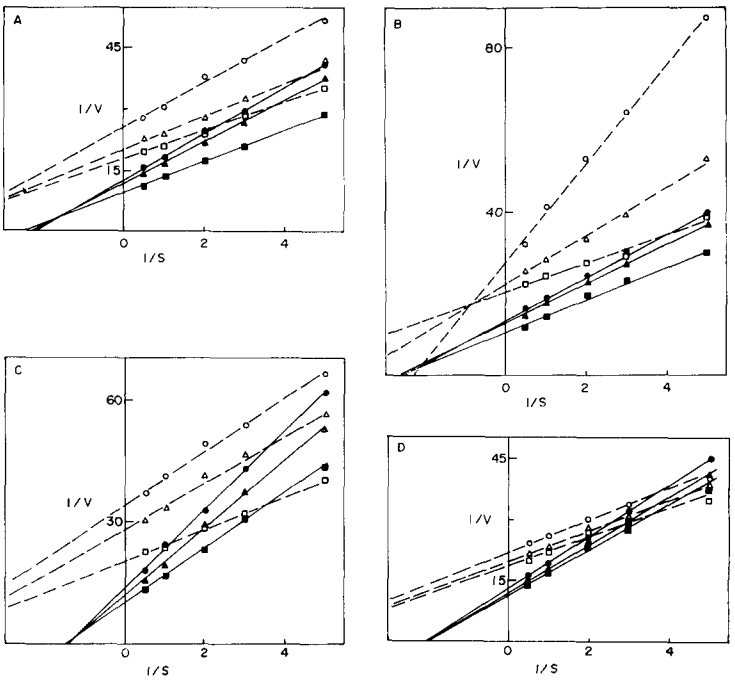

Fig. 6. Double-reciprocal plots varying the concentration of δ-aminolevulinic acid in the presence of hemin. Dashed lines are assays conducted without GSH, and solid lines are assays conducted in the presence of 20 mM GSH. (A) Adult red-cell enzyme; 0 μM hemin (■, □), 1 μM hemin (▲, △), 2.5 μM hemin (●, ○); K_m = 0.4 mM with GSH and 0.2 mM without GSH. (B) Fetal red-cell enzyme; 0 μM hemin (■, □), 1 μM hemin (▲, △), 2.5 μM hemin (●, ○); K_m = 0.4 mM with GSH and 0.2 mM without GSH. (C) Adult liver enzyme; 0 μM hemin (■, □), 1 μM hemin (△), 2 μM hemin (▲), 2.5 μM hemin (○) and 6 μM hemin (●); K_m = 0.7 mM with GSH and 0.2 mM without GSH. (D) Fetal liver enzyme; 0 μM hemin (■, □), 1 μM hemin (△), 2 μM hemin (▲), 2.5 μM hemin (○) and 6 μM hemin (●); K_m = 0.92 mM with GSH and 0.86 mM without GSH.

approx. 10 μM (Figs 4B and 5B). Maximum inhibition of 80–85% occurs for all enzyme preparations with the exception of the fetal red-cell enzyme (65%) in the presence of GSH. At 50% maximum inhibition, Pb^{2+} concentrations calculated from these curves were in the range of 3.6–4.4 μM. As shown in Fig. 7, inhibition is noncompetitive for all four enzyme preparations in the presence of GSH. However, in the absence of sulfhydryl reagent both red-cell enzyme preparations and the adult liver enzyme exhibit mixed inhibition, whereas fetal liver porphobilinogen synthase shows the noncompetitive type.

$HgCl_2$ inhibition

As shown in Fig. 4C, the pattern of inhibition by $HgCl_2$ in the presence of GSH is hyperbolic and equivalent for all four enzyme preparations. However, at higher concentrations of $HgCl_2$ total loss of activity results (at 50% maximum inhibition, $HgCl_2$ = 2 mM). Without GSH present (Fig. 5C), the inhibitory patterns for all four enzymes appear to be parabolic, and also concentrations of Hg^{2+} needed to achieve complete inhibition are approx. four orders of magnitude less than that

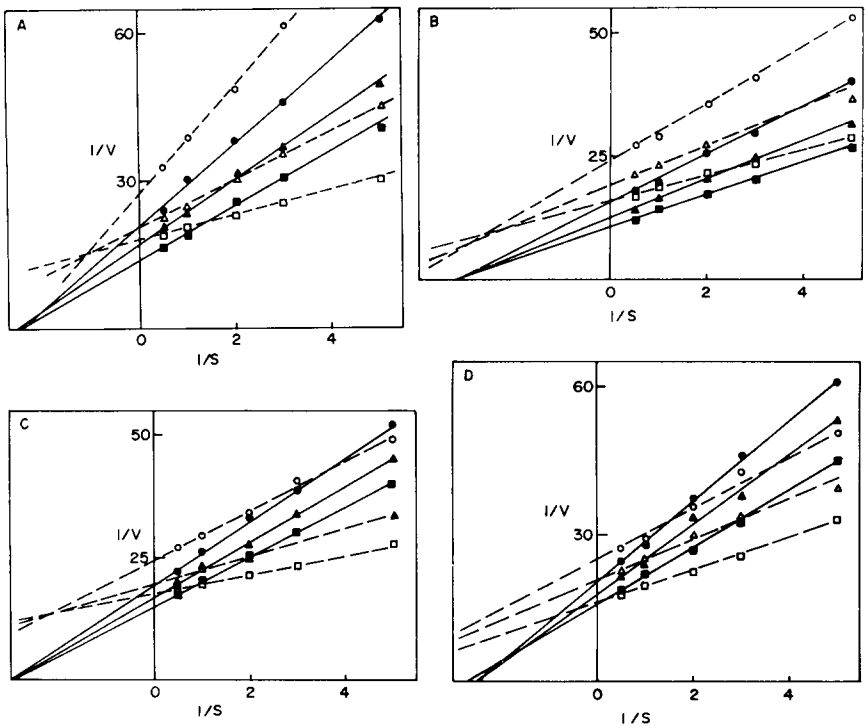

Fig. 7. Double-reciprocal plots varying the concentration of δ-aminolevulinic acid in the presence of lead acetate. Dashed lines are assays conducted without GSH, and solid lines are assays conducted in the presence of 20 mM GSH. (A) Adult red cell enzyme; K_m = 0.4 mM with GSH and 0.1 mM without GSH. (B) Fetal red-cell enzyme; K_m = 0.3 mM with GSH and 0.2 mM without GSH. (C) Adult liver enzyme; K_m = 0.3 mM with GSH and 0.1 mM without GSH. (D) Fetal liver enzyme; K_m = 0.4 mM with GSH and 0.2 mM without GSH; 0 (■, □), 0.5 μM (▲, △) and 1 μM (●, ○) lead acetate.

required in the presence of GSH. As shown in Fig. 8, in the presence of GSH adult and fetal liver and red-cell enzyme preparations show noncompetitive inhibition. Without GSH inhibition is competitive for fetal red cell porphobilinogen synthase and of the mixed type for the other three enzyme preparations. The types of inhibition by hemin, lead acetate, and $HgCl_2$ and values for K_i slope and K_i intercept for the four enzyme preparations are summarized in Table II. Activity in porphobilinogen synthase preparations optimally inhibited by Pb^{2+} or Hg^{2+} in the presence or absence of GSH could be recovered (90–100% of original activity) by dialyzing the metals away.

Additive inhibitory effects of Pb^{2+} and Hg^{2+} with the natural feedback inhibitor, hemin

Assays were conducted at a concentration of 60 μM hemin where maximum inhibition is observed for the four preparations of porphobilinogen synthase in the presence of GSH (Fig. 4A). Pb^{2+} and Hg^{2+} were added at concentrations that caused 50% inhibition in the absence of hemin. The results presented in Table III show that in the presence of hemin no further loss of activity of adult red cell porphobilinogen

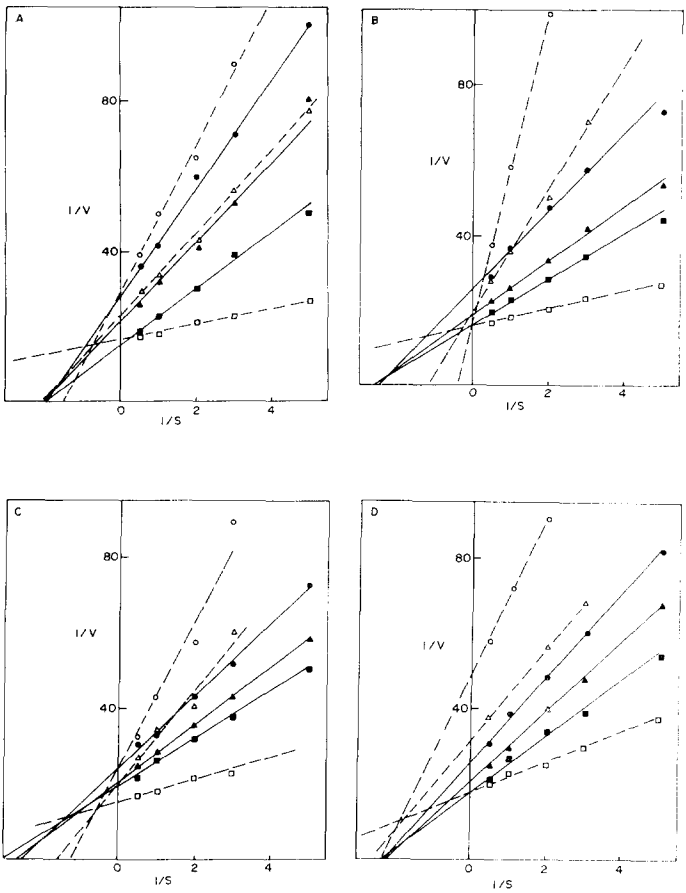

Fig. 8. Double-reciprocal plots varying the concentration of δ-aminolevulinic acid in the presence of $HgCl_2$. Dashed lines are assays conducted without GSH, and solid lines are assays conducted in the presence of 20 mM GSH. (A) Adult red-cell enzyme; K_m = 0.5 mM with GSH and 0.2 mM without GSH. (B) Fetal red-cell enzyme; K_m = 0.4 mM with GSH and 0.3 mM without GSH. (C) Adult liver enzyme; K_m = 0.3 mM with GSH and 0.2 mM without GSH. (D) Fetal liver enzyme; K_m = 0.4 mM with GSH and 0.2 mM without GSH; 0 μM (■, □), 4 μM (△), 6 μM (○), 0,4 mM (▲) and 1 mM (●) $HgCl_2$.

synthase results after the addition of lead acetate. In the case of the fetal red-cell enzyme, an 8% loss occurs, and an additional 50% decrease in activity is observed when lead acetate is added to the adult and fetal liver enzymes. $HgCl_2$ causes slight decreases in enzymic activity over hemin inhibition for adult and fetal red-cell enzymes, whereas further losses of 30% and 22% are obtained with adult and fetal liver porphobilinogen synthase, respectively.

DISCUSSION

As shown in Fig. 1, the specific activity of porphobilinogen synthase is high in fetal guinea pig liver and red cell and declines to adult levels shortly after birth.

TABLE II

KINETIC PARAMETERS OF HEMIN, Pb^{2+} AND Hg^{2+} INHIBITION OF PORPHOBILINOGEN SYNTHASE FROM ADULT AND FETAL GUINEA PIG LIVER AND RED BLOOD CELLS

Inhibition patterns were interpreted as noncompetitive, competitive or mixed from the double-reciprocal plots (Figs 6, 7, 8). The P values represent a test of the hypothesis of a dissimilar $1/S$ intercept. $P > 0.05$ indicated noncompetitive inhibition. Values for K_i slope and K_i intercept were determined by replotting slopes and intercepts, respectively. These values were not determined in the case of Hg^{2+} inhibition in the absence of GSH where inhibition was observed to be complex (Fig. 5C).

	Hemin		Pb^{2+}		Hg^{2+}	
	+GSH	−GSH	+GSH	−GSH	+GSH	−GSH
Adult liver						
Inhibition	Noncompetitive	Noncompetitive	Noncompetitive	Mixed	Noncompetitive	Mixed
P	>0.25	>0.5	>0.5	<0.01	>0.05	<0.01
K_i slope	12.5 μM	3.8 μM	3.2 μM	0.6 μM	2.0 mM	–
K_i intercept	17.2 μM	3.6 μM	3.6 μM	2.6 μM	4.0 mM	–
Fetal liver						
Inhibition	Noncompetitive	Noncompetitive	Noncompetitive	Noncompetitive	Noncompetitive	Mixed
P	>0.1	>0.25	>0.5	>0.25	>0.5	<0.05
K_i slope	44 μM	22 μM	2.8 μM	1.9 μM	2.0 mM	–
K_i intercept	40 μM	14 μM	3.3 μM	1.8 μM	2.0 mM	–
Adult red cell						
Inhibition	Noncompetitive	Noncompetitive	Noncompetitive	Mixed	Noncompetitive	Mixed
P	>0.1	>0.25	>0.5	<0.001	>0.1	<0.001
K_i slope	6.0 μM	5.1 μM	1.9 μM	0.3 μM	1.2 mM	–
K_i intercept	6.2 μM	5.5 μM	3.0 μM	1.9 μM	1.1 mM	–
Fetal red cell						
Inhibition	Noncompetitive	Mixed	Noncompetitive	Mixed	Noncompetitive	Competitive
P	>0.5	<0.001	>0.1	<0.001	>0.5	<0.001
K_i slope	10 μM	3.4 μM	2.0 μM	0.8 μM	1.4 mM	–
K_i intercept	7 μM	6.8 μM	2.3 μM	1.9 μM	1.6 mM	–

TABLE III

ADDITIVE EFFECTS OF Pb^{2+} AND Hg^{2+} UPON INHIBITION OF PORPHOBILINOGEN SYNTHASE BY HEMIN

Enzyme assays were carried out in the presence of 20 mM GSH. Hemin was present at a concentration of 60 μM. Lead acetate and $HgCl_2$ were added where indicated at concentrations of 5 μM and 2 mM, respectively. These concentrations gave 50% inhibition without hemin.

Inhibitor	Percent maximum activity			
	Adult liver	Fetal liver	Adult red blood cells	Fetal red blood cells
Hemin	74	72	32	55
Hemin + Pb^{2+}	37	36	32	47
Hemin + Hg^{2+}	44	50	28	52

The age–activity curves for liver and red-cell enzymes differ only in the immediate postnatal period when the liver activity alone exhibits a decline and subsequent rise. A similar pattern of activity has been observed by Doyle and Schimke [14] in developing mouse liver. They correlated the decrease in activity with a loss of hematopoietic cells in the liver. The rise in specific activity of the hepatic enzyme during the postnatal period is indicative of new enzyme production. This is consistent with the observed postnatal rise in various liver proteins such as cytochrome P450 [35] which require porphobilinogen synthase for the synthesis of their porphyrin moieties. The observed equivalence in specific activity for adult and fetal red-cell purine nucleoside phosphorylase indicates that the activity pattern of porphobilinogen synthase is not characteristic for all guinea pig red-cell enzymes.

As indicated in Table I, preparations of porphobilinogen synthase from adult and fetal guinea pig liver and red blood cells were obtained by utilizing discontinuous preparative disc gel electrophoresis as the final purification step. The enzyme has been previously purified to homogeneity by both ion-exchange chromatography [9–13, 15, 16] and by preparative acrylamide gel electrophoresis [14]. In the present studies the enzyme preparations from adult and fetal liver and red cells behaved similarly on DEAE-cellulose and in acrylamide gels, and the same pH optimum, isoelectric point and K_m values were observed. An argument for differences between the structure of fetal and adult porphobilinogen synthase in mouse liver has been made from the observation that the fetal enzyme is twice as active catalytically as the adult enzyme relative to its function as an antigen [14]. However, the specific activities of purified guinea pig porphobilinogen synthase of adult liver and adult red cells are slightly higher than the fetal sources (Table I), suggesting that the catalytic activity of the adult enzymes may be higher (assuming that all protein molecules are enzyme and all are active). The specific activities of purified preparations of guinea pig porphobilinogen synthase are more than 2-fold lower than those observed for either mouse [13, 14] or bovine [11] sources of the enzyme. This may be attributed to species differences.

In liver homogenates and red-cell hemolysates porphobilinogen synthase activity could be increased up to 2-fold by the addition of GSH, and upon further

purification from DEAE-cellulose columns the presence of thiol-activating agent was able to enhance activity by greater than 5-fold for all enzyme preparations. Concentrated fractions (1 mg/ml) of the four purified enzymes could be stored at −20 °C for up to 2 weeks without appreciable loss in activity when assayed in the absence of GSH. These findings suggest that the enzymes may exist in different oxidation states, and upon purification a shift occurs to a more inactive oxidized form which may be reversed by the addition of GSH. Wilson et al. [12] reported that appreciable amounts of porphobilinogen synthase could be measured in crude preparations of beef liver homogenates in the absence of reducing agents, but upon further purification very little enzymic activity was observed without such agents. Coleman [13] found similar behavior for mouse liver preparations of the enzyme, and Burnham and Lascelles [4] and Nandi et al. [15] reported that porphobilinogen synthase purified from *Rhodopseudomonas spheroides* has little or no activity without the addition of a thiol agent. From these comparisions it appears that the guinea pig enzymes, for which good activity is obtained without GSH after DEAE-cellulose chromatography, exist in a more active state in the absence of thiol agents than do the enzymes from bovine, mouse and bacterial species.

Because Hg^{2+} and Pb^{2+} complex with many ligands, including cysteinyl and histidyl side chains of proteins, there are many possible sites of interaction. Therefore, it is difficult to understand precisely the mechanism of inhibition. The situation can be further complicated by the addition of a thiol agent like GSH which can undergo mercaptide formation with heavy metals and reduce protein sulfhydryl groups. However, the presence of GSH does not significantly change either the shape of the inhibitory patterns of porphobilinogen synthase caused by Pb^{2+} and hemin or the concentrations needed to achieve maximum inhibition (Figs 4A, 4B, 5A, 5B). Unlike Pb^{2+}, the inhibition of porphobilinogen synthase by Hg^{2+} is complex, giving parabolic saturation curves, in the absence of GSH (Fig. 5C). When thiol agent is present, the inhibitory curves are changed to hyperbolas, and higher concentrations (greater than 1000-fold) are necessary to achieve maximum inhibition (Fig. 4C). Since GSH greatly affects Hg^{2+} inhibition of porphobilinogen synthase but has little effect on Pb^{2+} inhibition of the enzymes, variations in physical characteristics of the metals are probably responsible for this difference.

Although GSH has little effect on the binding of hemin to any of the enzymes, maximum inhibition of both adult and fetal liver porphobilinogen synthase and the fetal red-cell enzyme is decreased by the presence of the thiol-activating agent (Figs 4A and 5A). GSH has a more marked effect upon the inhibition of porphobilinogen synthase by Hg^{2+}, much higher concentrations being necessary to achieve 100% inhibition in the presence of the reducing agent. It has been reported [13] that Hg^{2+}, Fe^{2+} and Ag^{+} inhibit mouse liver porphobilinogen synthase only at metal concentrations that exceed the thiol-protecting agent. In the present studies with the guinea pig enzyme, 80–90% inhibition was observed with 5 mM $HgCl_2$ even though GSH was present at a concentration of 20 mM. Since maximum activation of porphobilinogen synthase could also be achieved at a concentration of 10 mM GSH, it is unlikely that loss of enzymic activity can be attributed to titration of GSH from enzyme by the metal. It is possible, however, that the reduced form of the enzyme may differ from the more oxidized state in affinity for the metal and/or that Hg^{2+} complexed with GSH is less inhibitory than Hg^{2+} alone (Fig. 5C).

Wilson et al. [12] and Komai and Neilands [18] have shown that chelating agents with affinity for divalent cations can inhibit porphobilinogen synthase isolated from bovine and microbial sources, respectively. The microbial enzyme molecule was found to contain Cu^{2+} by atomic absorption analysis [18]. Inhibition of the guinea pig enzyme by both Pb^{2+} and Hg^{2+} was shown to be reversible upon dialysis. Therefore, if guinea pig porphobilinogen synthase is a metalloenzyme, it is unlikely that inhibition by Pb^{2+} and Hg^{2+} is caused by the replacement of the essential metal yielding a less active or inactive enzyme.

Although no physiochemical differences were observed for porphobilinogen synthase isolated from adult and fetal guinea pig liver and red blood cells, several differences were observed from the inhibition studies. Hemin was found to be a more potent inhibitor of adult and fetal red-cell porphobilinogen synthase than of the liver enzyme preparations in the presence of GSH (Fig. 4A). Minimal further inhibition was observed by the addition of either Pb^{2+} or Hg^{2+} to adult and fetal red-cell enzymes already maximally inhibited by hemin, whereas additional inhibition was observed for adult and fetal liver porphobilinogen synthase preparations (Table III). The double-reciprocal plots (Figs 6–8) indicate further differences in inhibition patterns between the enzymes (Table II). These changes in inhibitory patterns indicate that the oxidized states of these enzyme preparations react differently with inhibitors. GSH, however, abolishes these kinetic differences. These observed effects of GSH in vitro leave unanswered the question of a possible role for thiol-activating agents in regulating enzyme activity in vivo. The observed discriminant inhibitory kinetic features of the enzyme preparations indicate that certain properties of guinea pig porphobilinogen synthase change as a function of age and tissue source.

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