CONCISE REPORT

Binding of Heme by Glutathione S-Transferase: A Possible Role of the Erythrocyte Enzyme

By John W. Harvey and Ernest Beutler

Human erythrocyte glutathione S-transferase activity is inhibited, probably competitively, by hemin with a $K_i$ of $10^{-7}M$. It is postulated that glutathione S-transferase functions physiologically as a hemin-binding and/or transport protein in developing erythroid cells.

A GROUP OF PROTEINS that have in common the ability to bind reduced glutathione (GSH) and a wide variety of hydrophobic compounds have been isolated and characterized from rat and human liver.1,2 If the hydrophobic compounds bear sufficiently electrophilic atoms (usually carbon), these proteins will catalyze a conjugation reaction between the compound and GSH.3 They have therefore been classified, based on their enzymatic activities, as glutathione S-transferases or GSH S-transferases.

The functions of GSH S-transferases may be classified into two general categories. As binding proteins, they function intracellularly on a broad scale in the solubilization and transport of substances, much as albumin functions extracellularly.3 An example is the binding of bilirubin. One of the GSH S-transferases in rat liver, designated transferase-B, has been shown to be identical to the bilirubin-binding protein or ligandin. This protein is believed to be important in the uptake and transport of bilirubin in hepatocytes.3

The second major function is believed to be the protection of cellular constituents from electrophilic, xenobiotic chemicals.4 The preferential reaction of the electrophilic agent with GSH through the enzymatic action of GSH S-transferase prevents its reaction with other cellular nucleophiles. In addition to the protective enzymatic properties of GSH S-transferases, they may also detoxify certain extremely reactive substances by the direct covalent binding of the electrophilic agent to the proteins.5 Although GSH S-transferases have been studied most extensively and occur in highest concentrations in liver, these enzymes are present in a wide variety of tissues.4 A form of the enzyme, designated GSH S-transferase $p$, exists in human erythrocytes.5 It is immunologically distinct from the multiple forms of GSH S-transferase found in the human liver.5 The physiologic role of this enzyme is not known. It has been suggested that the location of the enzyme in erythrocytes is ideal for the removal of circulating xenobiotics.5 One might also consider that GSH S-transferase occurs in erythrocytes primarily for the protection of erythrocytes against electrophilic compounds, rather than serving a general protective function in the body.

MATERIALS AND METHODS

Forty milliliters of fresh venous human blood was collected in EDTA and freed of leukocytes as previously described.10 Erythrocytes were washed 3 times in 0.9% NaCl, and packed erythrocytes were lysed with 5 vol of cold 10 mM potassium phosphate buffer (pH 7.2). The hemolysate was frozen with dry ice in acetone, thawed, and centrifuged at 20,000 g for 20 min at 5°C to remove erythrocyte membranes. The supernate was removed and dialyzed overnight at 5°C against 2 liters of 29 mM potassium phosphate buffer (pH 7.2) and recentrifuged at 20,000 g for 20 min.11

The hemolysate supernate was passed through a 1 x 10 cm GSH-linked epoxy-activated sepharose 6B column11 at a flow rate of approximately 10 ml/hr.11 The column was washed for 1 hr with 20

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and its concentration was determined. When hemin was added to the GSH S-transferase assay, the ethanol concentration in the cuvette increased to a final concentration of 3%. The addition of ethanol alone did not affect the enzyme activity. The inhibitor constant ($K_i$) for hemin was determined from a Dixon plot, obtained by varying the hemin concentration using 2 different CDNB concentrations (0.5 mM and 1.0 mM).

**RESULTS**

The sequence of addition of reactants in the GSH S-transferase assay was determined to be important in that the enzyme is rapidly inactivated by CDNB in the absence of GSH (Fig. 1). For this reason, GSH was mixed with the buffer containing CDNB immediately before enzyme addition.

The addition of low concentrations of hemin to the standard assay system containing 0.5 mM CDNB resulted in dramatic inhibition of enzyme activity (Fig. 2). A $K_i$ of $10^{-7}$ M for hemin was determined in a second experiment by plotting the reciprocal of reaction rate ($1/V$) versus hemin concentration at 0.5 mM and 1 mM CDNB concentrations (Fig. 3). The fact that the two lines intersect well above the baseline suggests that the inhibition of activity with CDNB by hemin may be competitive in nature.

**DISCUSSION**

The iron atom is reduced to the ferrous state for incorporation into the protoporphin molecule to syn-
HEME BINDING BY GSH S-TRANSFERASE

1.0 mM CDNB

0.5 mM CDNB

10

-1

[HEMIN] x 10^-7 M

Fig. 3. Dixon plot of the reciprocal of GSH S-transferase activity (1/V) versus hemin concentration utilizing 0.5 mM and 1.0 mM CDNB concentrations. Lines of best fit were determined by least squares linear regression analysis.

thesize heme at the heme synthetase (ferrochelatase) step. Once formed, heme must be translocated from mitochondria to the site of hemoglobin synthesis in the cytoplasm. Endogenously synthesized heme does not freely penetrate the inner mitochondrial membranes, and consequently, the transport of heme across this membrane appears to be a potential rate-limiting step in hemoglobin synthesis. The efflux of heme from mitochondria depends on the presence of cytosolic protein. Uncommitted cytoplasmic heme (probably as hemin) inhibits cellular and mitochondrial uptake of iron and promotes the synthesis of globin chains.

Bovine serum albumin has been shown to have sufficient binding affinity to enhance efflux of heme from isolated mitochondria. The $K_d$ of bovine serum albumin for hemin has not been reported, but it has been shown to have a considerably lower affinity for hemin than does human serum albumin, which has a $K_d$ for hemin of $2 \times 10^{-4} M$. Based on the $K_d$ of $10^{-7} M$ determined for erythrocyte GSH S-transferase, it appears that the affinity of GSH S-transferase for hemin approximates that of bovine serum albumin.

Hemin is poorly soluble in aqueous solutions at body pH and tends to form large aggregates at concentrations as low as $10^{-7} M$. Inasmuch as GSH S-transferase binds hemin at $10^{-7} M$, a potential function of GSH S-transferase is to keep hemin solubilized.

Previous studies in rats have indicated that ligandin (GSH S-transferase-B) may be important in the transport of heme from mitochondria to the cytoplasm. Erythrocyte GSH S-transferase appears to bind hemin with an affinity equal to, if not greater than, that of ligandin in rat liver and GSH S-transferase proteins present in human liver. It is of interest in this respect that bilirubin, a nonsubstrate ligand that is metabolized and excreted by the liver, is bound poorly by erythrocyte GSH S-transferase compared to liver forms of GSH S-transferase.

These considerations make it seem plausible that erythrocyte GSH S-transferase may function physiologically as a heme-binding and/or transport protein.

REFERENCES

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JW Harvey and E Beutler