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Can Glutathione-S-Transferases Function as Intracellular Heme Carriers?

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The possibility that glutathione-S-transferases can serve as heme carriers in cells was studied via the following two characteristics: the ability to bind hemin reversibly and the coordination between heme and glutathione-S-transferases level in the cell. Two erythroleukemic cell lines that can be induced to synthesize hemoglobin were studied, K-562 and Friend murine erythroleukemia cells. It was found that hemin-associated glutathione-S-transferase tends to lose its native structure as expressed by partial irreversible inhibition of glutathione conjugation activity. In K-562 cells, a small increase in heme synthesis was induced, but under no condition could glutathione-S-transferase be elevated. In addition, introduction of high hemin from without caused large hemoglobin production but did not induce changes in the glutathione-S-transferase content. Dimethyl sulfoxide-induced Friend murine erythroleukemia cells synthesized a large amount of endogenous hemin that had to be transported from the mitochondria for hemoglobin synthesis. Although a concomitant increase in glutathione-S-transferase level (20–40%) was observed, it was only short-lived, unlike hemin, which continued to increase. These data indicate a lack of correlation between glutathione-S-transferase and hemin or hemoglobin levels. Finally, dimethyl sulfoxide-induced cells were treated with succinyl acetone to inhibit heme synthesis. These cells showed the same increased levels and time-dependent pattern of glutathione-S-transferase as untreated cells. A similar phenomenon was observed when different substrates were used to measure the activities of glutathione-S-transferases. These results raise doubts about the possibility of glutathione-S-transferases functioning as heme carriers in cells.

Key words: S-transferase, heme transport, erythroleukemic cells, inhibited glutathione conjugation

Hemin serves as a key molecule in many basic functions of the cell. The final step of heme synthesis, namely, the insertion of iron by the enzyme ferrochelatase, is carried out in the inner mitochondrial membrane [1]. Hemoproteins functioning within the mitochondrial membrane probably receive their heme in this location, as has been shown for cytochrome C [2,3]. Since not all hemoproteins are mitochondrial (hemoglobin [Hb], catalase, peroxidase, etc.), how does heme reach its other functional locations? The most trivial way that comes to mind would be heme transport by passive diffusion. However, being a hydrophobic molecule, heme has been shown to partition in membranes and thus become hazardous to biological structures and functions related to membrane compo-

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nents [4–6]. In light of the above, one would expect one or more carrier molecules to be involved in transport of heme from the mitochondria to the sites of its function.

Several studies have implicated the involvement of cytosolic proteins in transport of heme from the mitochondria [7–9]. Based on the following characteristics glutathione-S-transferases (GSTs) seem most suitable for heme transport: GSTs were shown to be able to bind hemin [10–12] and are also widespread in many organisms and tissues, such as liver, intestine, kidney, and erythrocytes. In some cases such as in liver cells, a collection of isoenzymes exists; in others, such as in mature erythrocytes, only one form of the enzyme has been reported [11,13]. In experiments carried out in vivo, labeled cellular heme was found to be associated with a number of cytosolic fractions; the major one was suggested to be GST [14,15]. Finally, recent, in vitro studies demonstrated acceleration of heme transport from the mitochondria by GST [16,17].

The main function attributed to date to GSTs is their participation in the process of detoxification by conjugating glutathione (GSH) with electrophylic xenobiotics [13]. Regarding this function, GSTs were reported to respond to the presence of toxic drugs by elevated synthesis [18,19]. It is therefore expected that, if GSTs serve as heme carriers, their synthesis will be similarly linked to heme levels in cells. In addition, as carriers, GSTs should be resistant to heme binding, a condition not always met in proteins that bind hemin. For example, spectrin was shown to lose its tetrameric structure by binding hemin [20]. The above characteristics, namely, efficiency as a carrier and coordination with ligand level, have not been studied in the GSTs and are the topic of this paper.

Most of the work on GSTs has been carried out in hepatic cells; however, the most massive heme synthesis occurs in erythroid cells. The erythroid model systems Friend murine (MEL) and human erythroleukemic K-562 cells can both be induced to synthesize Hb by different agents [21–24]. These cells could thus serve as an ideal model for showing the possible coupling between heme and GST by following the response of the enzyme's activity to induction of heme synthesis.

MATERIALS AND METHODS

Materials

Fetal bovine serum, Dulbecco's modified Eagle's medium (supplemented with glucose 4.5 g/liter), RPMI 1640, glutamine, and penicillin-streptomycin solutions were purchased from Biological Industries Kibbutz, Beit Haemek, Israel. The commercial enzyme GST and its substrates, 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-epoxy-3-(p-nitrophenoxy)propane (EPX), p-nitrobenzyl chloride (NBC), and GSH; in addition to hemin, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide, butyric acid, thymidine, and succinyl aceton (SA) were obtained from Sigma Chemicals Co. (St. Louis, MO). Spectroscopic-grade dimethyl sulfoxide (DMSO) was from Merck. All other reagents were of analytical grade.

Culture Conditions

MEL cells were maintained in suspension culture in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum; 2 mM glutamine, and penicillin-streptomycin, 100 U/ml and 0.1 mg/ml, respectively. Incubation was carried out at 37°C under a water-saturated atmosphere of 95% air and 5% CO_2. Stock cells were diluted every 3–4 days to approximately 5×10^4 cells/ml. For experimental use, cultures

were diluted every 1 or 2 days to 1–2 × 10^5 cells/ml to maintain logarithmic growth. For induction of differentiation, MEL cells were grown for different time periods in the presence of 1.5% DMSO. In experiments in which hemin synthesis was inhibited, MEL cells were grown in the presence of 1 mM SA, and fetal calf serum was reduced to 3%.

K-562 human erythroleukemia cells were maintained in suspension culture in RPMI 1640 medium supplemented and incubated as the MEL cells, except that HEPES, pH 7.35, at a final concentration of 10 mM was added to the growth medium. Experimental cultures were diluted, if needed, only every 3–4 days, since the generation time of these cells was longer (30 h) than that of the MEL cells (14 h). For induction of Hb synthesis, K-562 were grown for different time periods in the presence of several inducers: thymidine 0.1 mM, butyric acid 1 mM, or hemin at different concentrations.

Cell Disruption

After growth for appropriate times, cells were counted and washed twice with phosphate-buffered saline (PBS), then diluted to the order of 10^7 cells/ml and disrupted by sonication for 30 sec. This sonicate was used with no further treatment for hemin and GST determinations in MEL or K-562 cells. In experiments designed to determine reversibility of heme binding to GST, by addition of hemin and globin to the cytosol, the sonicated suspension was further centrifuged for 2 h in an ultracentrifuge at 100,000*g*, and the supernatant was used for the determinations.

Measurements of Enzymatic Activities

GST conjugation activity was determined, unless otherwise stated, by using CDNB as substrate. The reaction was carried out at 25°C according to Simons and Vander Jagt [25]. The method is based on the reaction between GSH and CDNB catalyzed by GST, resulting in formation of a GSH-CDNB conjugate, which is followed by its light absorbance at 340 nm. GST activity with EPX and NBC as substrates was determined at 37°C by following the light absorbance at 360 nm [26] and at 310 nm [27], respectively. Acetylcholinesterase (AChE) activity was determined at 37°C in a concentrated sonicate (8 × 10^7 cells/ml), since specific activity was low, according to the method of Ellman et al. [28]. AChE catalyzes the hydrolysis of acetylcholine to thiocholine. The rate of production of thiocholine was measured by following spectrophotometrically, at 412 nm, the reaction of the latter with DTNB, which produces 5-thio-2-nitrobenzoic acid. In all cases, one unit of enzyme activity is defined as formation of 1 μmol product per minute.

Other Methods

Globin was prepared from fresh red blood cells essentially according to Rossi-Fanelli et al. [29], with a slight modification in which acetone HCl solubilization was repeated twice. Hb in induced MEL or K-562 cells was determined on the supernatant of Nonidet P-40 lysed cells according to Hunt and Marshal [30].

Hemin was measured in cell sonicates according to Morrison [31], with some modifications described previously [6]. In short, hemin was converted quantitatively into porphyrin by use of oxalic acid as an acidifier and a reducing agent. For quantitation of porphyrin, the solutions were excited at 403 nm and their emission intensity was measured at 602 nm. Hemin and Hb at known concentrations were similarly converted into porphyrins and used as standards. Protein was determined using the Lowry method [32], with bovine serum albumin as standard.

Instruments

Fluorescence measurements were carried out using a Hitachi Perkin-Elmer model 44B fluorimeter. Spectrophotometry was performed with Carry 219 and Varian Techtron 635 spectrophotometers. Centrifugations were carried out in a Sorval RC-5C or a Beckman L2 50B ultracentrifuge. Cell disruption was achieved using an MSE sonicator.

RESULTS

Inhibition and Reversibility of GST Activity by Hemin and Globin, Respectively

Binding of hemin to GST was reported to compete with binding of other ligands and thus inhibit GST activity as measured by conjugation of CDNB and GSH [11]. Based on this information, the first set of experiments was designed to test if the inhibition of the GST conjugation reaction caused by hemin could be completely reversed. Reactivation of GST was achieved by in vitro addition of globin, since in erythroid cells the main task of the transporter molecule is expected to be the transfer of heme to the globin molecule for Hb synthesis. The source of GST in these experiments was the supernatant cytosol of MEL cells grown and treated as described in Materials and Methods. Hemin was added to the cytosol at two concentrations 10 and 100 μM. The mixtures were incubated on ice (to prevent temperature-dependent protein denaturation) for different time periods, after which GST activity was measured. To measure the reversibility of GST activity, globin was added to an aliquot of each heme-cytosol mixture (at a final concentration of 100 μM) at different times after the addition of hemin and further incubated on ice for 5 h. GST activity of the globin-containing mixtures was measured and compared to the parallel aliquots not containing globin. The results, which are illustrated in Figure 1, demonstrate that inhibition of GST activity was dependent on hemin concentration; a smaller inhibition (35%) was caused by 10 μM hemin compared with 100 μM (79%). These levels of inhibition were reached already after 5 min incubation of GST with hemin, and no additional reduction in activity could be seen after longer incubation times, up to 21 h. Reversibility, on the other hand, was dependent on the time of the incubation with hemin. After 5 min incubation with 10 μM hemin, 100% reversibility was achieved; with 100 μM hemin, only 81% of the GST activity could be reversed. Longer incubation times with the hemin, from 30 min to 21 h, in addition to a higher hemin concentration, caused growing irreversibility until 21 h incubation with 100 μM hemin brought about only a 51% reversibility of activity in the presence of globin. Thus it can be seen that the irreversible loss of GST's ability to catalyze conjugation upon binding of hemin is both time- and hemin concentration-dependent.

Correlation Between Heme Level and GST Activity

In this set of experiments, K-562 and MEL cells were induced to synthesize Hb so that we could search for a possible concomitant elevation in GST activity. In each case, the activity of GST was measured under conditions of increased heme levels and compared with the control cells, in which no elevation of cytosolic hemin occurred.

GST activity in K-562 cells. According to previous reports, K-562 cells can be induced to synthesize Hb, and thus hemin, by butyric acid and thymidine [22,24]. Cells were grown for 7 days in the presence or absence of inducers as described in Materials

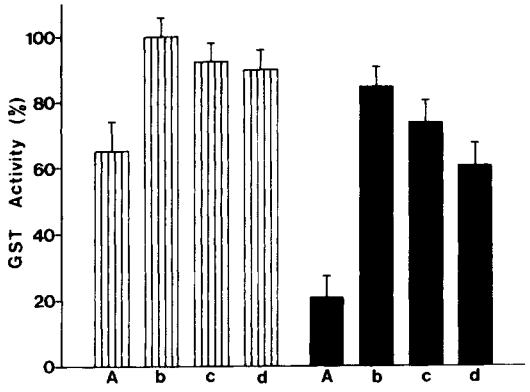

Fig. 1. Reversibility of GST activity inhibited by hemin. GST activity in supernatant was set as 100%. For reversibility measurements, 100 μM globin was added (see text). A, Activity of GST after addition of hemin only. b–d, Activity of the appropriate samples incubated with hemin for time periods listed below followed by incubation with globin for additional 5 h: 5 min (b), 30 min (c), 21 h (d). Striped columns: In the presence of 10 μM hemin. Solid columns: In the presence of 100 μM hemin.

TABLE I. GST Activity in K-562 Cells Induced by Different Agents*

Treatment	GST activity (%) Day 2	GST activity (%) Day 7	Hb (%) (day 2)
Control	100	100	100
	(19)	(22)	(37)
Thymidine 0.1 mM	96	102	140
	(12)	(17)	(48)
Butyric acid 1 mM	104	81	94
	(11)	(7)	(34)
Hemin 1 μM	105	96	150
	(15)	(6)	(43)
Thymidine 0.1 mM	114	97	169
+ hemin 1 μM	(17)	(24)	(34)
Butyric acid 1 mM	108	72	141
+ hemin 1 μM	(7)	(17)	(28)
Hemin 30 μM	88	105	656
	(10)	(12)	(50)

*The SD of the measurements is given in parentheses. One hundred percent GST activity of control culture in the absence of any inducers represents 0.254 units/mg protein. One hundred percent Hb per cell in control culture represents 0.32 pg/cell.

and Methods. At various time intervals, samples were removed from the different cultures and treated for measurements of Hb and GST activities (see Materials and Methods). The K-562 cells employed in our experiments showed no synthesis of Hb above the constitutive level in the presence of butyric acid alone and a small induction, up to two times control level, with thymidine. On the otherhand, in the presence of externally added hemin, the cells were able to synthesize Hb proportionally to the amount of hemin added (see Table I). In an effort to increase mitochondrial hemin, low external hemin (1 μM) combined with either of the above-mentioned inducers was used. As is shown in Table I, the inducers did affect to some extent the internal heme as indicated by elevation of Hb in the induced cells, but the effect of various combinations

was additive rather than synergistic, resulting in low internal hemin under all conditions employed. In parallel, GST synthesis in these cells was followed, but, as is demonstrated in Table I, under no condition and at no time was this enzyme elevated (within experimental error).

Since it turned out that under all conditions employed the endogenous production of hemin in this cell line remained low, K-562 cells were used to follow GST production when hemin was introduced from without and therefore has to be transported from the outer membrane to cellular organelles. For this set of experiments, the hemin concentration range was elevated to 30 μM. As is shown in Table I, at this hemin concentration, no elevation of GST activity was observed after 2 or even 7 days. This could reflect a failure of the cells to incorporate the hemin introduced from outside. However, measurements of hemin concentrations in the cells demonstrated a tenfold elevation of internal hemin within 3 h (0.17×10^{-9} mol/mg protein in control and 1.64×10^{-9} in the presence of 30 μM hemin in the medium); therefore, influx of hemin into the cells does occur. The above results demonstrate that, under two different conditions of low internal heme synthesis and high externally introduced hemin, the K-562 cell system failed to show any elevation in GST activity.

GST in MEL cells grown with DMSO. We turned to the MEL cell system in which, according to available information, a high internal heme synthesis can be achieved by induction with DMSO [21]. MEL cells were grown as described in Materials and Methods in the presence or absence of 1.5% DMSO. At different times after initiation of differentiation, aliquots were removed, and the levels of GST, hemin, and Hb in the cells were determined. Figure 2 shows that cellular hemin began to rise during the first 24 h after induction with DMSO, whereas elevation of Hb could be detected only after 48 h. These results, although showing an earlier increase than in other reports, follow the same basic pattern: an earlier elevation of heme as opposed to a lag in the onset of globin synthesis [33,34]. Like hemin, GST conjugation activity was increased already after 24 h, and the level measured was 120–140% that of uninduced control cells. During the next days, hemin and Hb continued to rise, while GST activity began to decline back to control level. The inset in Figure 2 demonstrates the differences in the patterns of heme and GST synthesis by following the average daily rate of increase in their concentration. Although the rate of heme synthesis continued to rise up to day 7, a net increase in GST production occurred only during the first 24 h after induction. Between the third and fifth days, the average rate of concentration changes fell below zero, demonstrating that GST degradation at that period was greater than its synthesis. On the other hand, the changes in the hemin concentration were positive all along. For quantitative comparison of available hemin and GST, their average amounts were calculated. After the first 24 h of induction, when no Hb was produced, the elevated hemin could be considered "free" or non-Hb hemin. This amount was calculated to be 0.2×10^{-9} mol/mg protein. The activity of GST at the same time, translated into concentration units, as based on the specific activity of a commercially available GST preparation, was 0.03×10^{-9} mol/mg protein (1 mg = 44 units as calculated on the basis of CDNB as substrate). Thus the estimated molar concentration of non-Hb hemin, at that specific time, is six- to seven-fold greater than that of GST.

AChE is another enzyme reported to be elevated after induction of MEL cells with DMSO [33]. To find the order of magnitude of GST elevation relative to enzymes that have no correlation with heme transport, the activity of the two enzymes in DMSO-

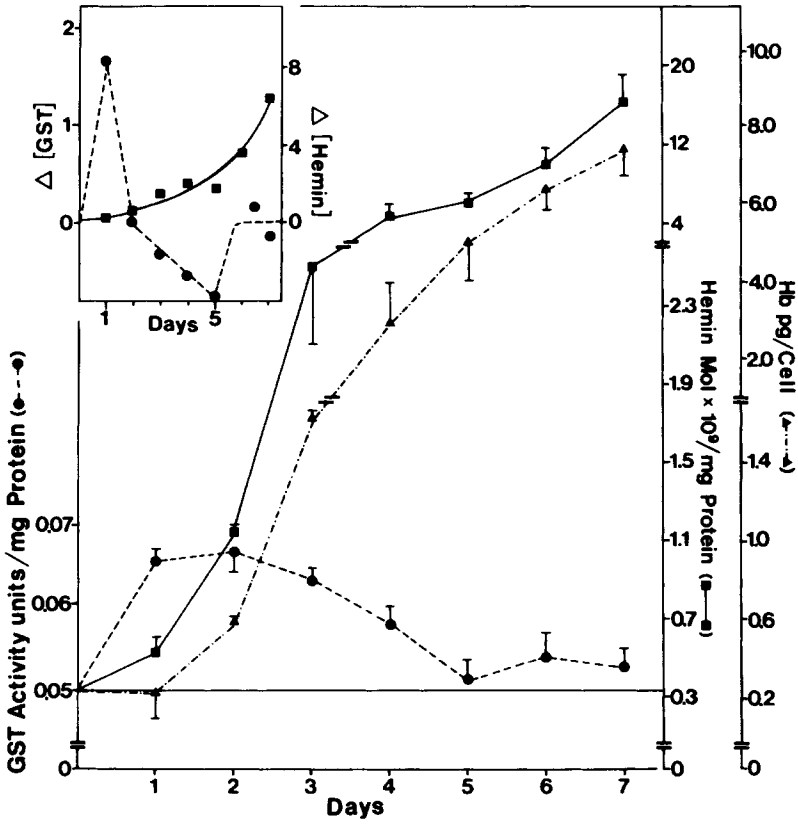

Fig. 2. GST activity and hemin and Hb contents in MEL cells induced by DMSO. The full line with no symbols represents the average control levels (in the absence of DMSO) of the three parameters measured: GST activity, 0.0488 (SD 0.0069) units/mg protein; hemin, 0.33×10^{-9} (SD 0.074×10^{-9}) mol/mg protein; Hb, 0.235 (SD 0.098) pg/cell. To simplify the representation, the three coordinate scales were matched so that the different control numbers merge into one line. **Inset:** Rates of GST and hemin production. Symbols identical to those in the main figure. Δ(GST), the numbers represent 1×10^{-2} units activity/mg protein/day. Δ(hemin), the numbers represent 1×10^{-9} mol/mg protein/day.

induced cells was compared. The results in Table II demonstrate that the elevation in AChE activity at different times after induction with DMSO was much greater (260–340%) than that observed with GST. In addition, AChE continued to rise between days 2 and 7, whereas GST activity declined and returned to control level after the fourth day.

Since the elevated hemin can inhibit GST (Fig. 1), the possibility arose that the increased level of GST observed after induction with DMSO (Fig. 2), represented only a fraction of the total GST activity in the cell. To test this assumption, MEL cells were grown in the absence or presence of a high hemin concentration in addition to DMSO. The results, as demonstrated in Table II (second column), show that, in the presence of 30 μM hemin added to the medium, only a minor reduction in GST activity was observed. To estimate the lost fraction of GST activity due to heme binding under conditions of internal heme synthesis, globin was added to cell cytosols of induced and uninduced cultures, and the mixtures were incubated on ice for 20 h. As is demonstrated

TABLE II. GST and AChE Activities in MEL Cells*

Treatment	GST (day 2)	GST + globin (day 2)	AChE (day 2)	GST (day 4)	GST (day 7)	AChE (day 7)
Control	100	121	100	100	100	100
	(11)	(5)	(16)	(15)	(15)	(12)
DMSO	130	148	260	127	106	342
	(12)	(10)	(12)	(8)	(8)	(14)
DMSO + hemin 30 μM	120	—	—	123	107	—
	(2)			(20)	(15)	

*The SD of the measurements is given in parentheses. One hundred percent GST activity in control culture, in the absence of any inducers, represents 0.0505 units/mg protein. One hundred percent AChE activity represents 0.0053 units/mg protein. Globin concentration when present was 100 μM.

in Table II (third column), globin brought about only a small elevation in GST activity (the order of 20%), and this elevation was equal in cultures with or without DMSO. Thus the above experiments demonstrate that the measured elevation in GST activity observed after induction with DMSO, namely, 120–140%, does represent the actual order of activity in the cell's cytosol.

MEL Cells Grown With DMSO and SA

Since MEL cells did show some elevation of GST correlated to elevated heme production, we further examined the GST activity in an extreme situation in which no heme is synthesized. The possible coordinated expression of hemin and GST synthesis was tested by the following approach. Internal heme synthesis was inhibited by SA in cells induced to differentiate by DMSO. SA is a known inhibitor of the second enzyme in the heme synthesis pathway, namely, δ-aminolevulinic dehydrase [36].

Serum contains heme binding proteins, so addition of serum may supply some hemin to the growing cells. Fetal bovine serum, according to measurements carried out in our laboratory, contains approximately 6 μM hemin. Therefore, media containing 10% fetal bovine serum also includes 0.6 μM hemin. To lower the amount of external hemin to a minimum, the growth conditions used in these experiments were as follows: 1 mM SA added 24 h prior to addition of DMSO and 3% fetal bovine serum in the media instead of 10%. These conditions allowed a very close to normal growth rate in control cells. With DMSO, even in the presence of SA, the typical morphological changes of differentiated cells did occur, although Hb synthesis was inhibited. Table III summarizes the Hb, hemin, and GST levels in the cells grown with and without SA in the presence or absence of DMSO. After induction of 24 h with DMSO, the level of hemin did not rise when SA was present, as compared to the typical rise seen in the culture including DMSO only (see Fig. 2). In parallel, table III demonstrates that, in the presence of SA and DMSO, GST activity did rise to a level similar to that of the control grown with DMSO alone inspite of the lack of hemin production.

Comparison of GST Activity as Expressed by Different Substrates

Mature red cells have been shown to carry one type of GST only; detailed information on the possible existence of GST isoenzymes in precursor red cells is less clear [11]. If several isoenzymes exist, the specific activity of each one, with various substrates, may differ. Thus the contribution of those serving as hemin transporters to

TABLE III. GST Activity and Hemin and Hb Contents in DMSO-Treated MEL Cells With and Without SA*

Treatment	GST activity (%)	Hemin (%)	Hb (%)
Control	100	100	100
	(5)*	(10)	(11)
DMSO	125	125	89
	(6)	(11)	(12)
SA	93	62	65
	(7)	(16)	(19)
DMSO + SA	123	65	60
	(9)	(2)	(15)

*GST activity was measured 24 h after addition of DMSO. The SD of the measurements is given in parentheses. Controls with no addition are considered 100%, and their measurements are as follows: GST activity, 0.0464 units/mg protein; hemin, 0.244×10^{-9} mol/mg protein; Hb, 0.13 pg/cell.

total activity, when measured by CDNB as substrate, may be low. In such a case, activity of the cytosol measured by CDNB would underestimate a unique increase in the transporter isoenzyme level. Since up to this point only CDNB was utilized as substrate, further experiments were designed in which the activity of GST was measured simultaneously with three different substrates: CDNB, EPX, and NBC. DMSO-induced MEL cells were grown in the presence or absence of SA as described in the previous section. GST activity was measured after 24 h induction with DMSO, and the results of these studies are summarized in Table IV. It can be seen that with all three substrates DMSO-induced cells showed increased GSTs activity compared with untreated cells. However, for both EPX and NBC, the increase in total activity was even lower than the increase expressed using CDNB. Moreover, inhibition of heme synthesis with SA had no effect on the GSTs activity measured by all three substrates.

DISCUSSION

GST can release bound hemin as demonstrated in the present study and also in a previous work by Tipping et al. [10], who replaced hemin with other ligands. However, the slow loss of conjugation activity of the hemin–GST complex points at denaturation of

TABLE IV. GST Activity in DMSO-Grown MEL Cells With and Without SA as Determined With Different Substrates*

	Substrate		
Treatment	CDNB	EPX	NBC
Control	100	100	100
	(12)*	(10)	(18)
DMSO	123	109	109
	(10)	(11)	(12)
DMSO + SA	125	110	112
	(9)	(8)	(15)

*GST activity was measured 24 h after addition of DMSO. The SD of the measurements is given in parentheses. Controls with no addition are considered 100%, and and their activities (units/mg protein) with the various substrates are as follows: CDNB, 0.0463; EPX, 0.00563; NBC, 0.00734.

the enzyme. In other words, prolonged incubation with hemin partially inactivates GST. It was recently observed that heme binding proteins such as actin and spectrin undergo hemin-induced denaturation whereas other proteins, such as albumin, which have a biological function as heme carriers, are very resistant to hemin-induced conformational changes [37]. Still, the fact that hemin-bound GSTs lose stability with time does not rule out their ability to act as heme transporters for a limited time prior to denaturation.

The data from the K-562 system demonstrate that hemin transported from the external membrane, even at high levels, does not activate GST synthesis. In addition, low levels of internal heme produced in the mitochondria and therefore required to be transported to the cytosol also do not induce any changes in the GST synthesis. Nevertheless, GST may act as a transporter for mitochondrial hemin only when large quantities of hemin have to be transported from the mitochondria. The latter possibility was tested using the MEL cell system, in which high mitochondrial heme production could be induced. As learned from the study performed on MEL cells, high internal heme synthesis causes only a small concomitant induction in GST activity (Fig. 2, Tables III, IV). The comparison of the molar concentrations of the hemin and GST as described in Results demonstrates that the number of GST molecules present, although smaller, is within the same order of magnitude of non-Hb hemin. Therefore, the rate of heme turnover may be high enough to allow only a small additional amount of induced GST to take care of the cell's need for heme transport from the mitochondria to other organelles. Accumulation of an unusual high cytosolic free hemin occurs only on the first day, when the increased amount of hemin cannot yet be transfered to globin because of the lag in its synthesis (Fig. 2) [33,34]. During this time, when no transfer of hemin to globin occurs, the function of GST as heme carrier is expected to be independent of the kinetic characteristics of the system, and enough GST should be available for storage of the newly synthesized hemin. Our calculations show that this is not the case, and thus, even if it is destined to be a carrier, GST is not the only cytosolic heme transporter. However, the fact that the increased rate of GST production occurred concomitantly with the first day when a dramatic free heme pool build-up takes place may reflect a correlation between GST and heme transport.

At this point, it seemed that the best way to decide whether the increase in GST activity is connected to its function as a heme transporter would be to separate heme synthesis from the differentiation process. This was achieved by totally inhibiting heme production using SA, which is considered to be the most potent and specific inhibitor of the heme synthesis pathway [36]. It should be stressed that, since the heme molecule is vital for all cell processes, a minimal supply of hemin had to be introduced to keep the cells functional, that is, growing and differentiating normally. Under these conditions, in the presence of SA, the differentiation process continued normally, as was shown also by Beaumont et al. [38], but heme synthesis was completely inhibited. The data in Table III show clearly that, despite the fact that heme synthesis is inhibited, GST synthesis does occur. In addition, the level of elevation of GST after induction with DMSO in the presence of SA is the same as that observed in the control culture grown in the presence of DMSO alone. Since the same picture emerged with use of different substrates (Table IV), the results of this study do not indicate the existence of any particular GST isoenzyme that may serve as hemin carrier. Moreover, elevated synthesis is not unique to GST alone and can be demonstrated for other enzymes having no direct functional correlation with heme (AChE in Table II). Thus it seems that the increase in GSTs

activities is connected to the general phenomenon of elevation in enzymes activities in differentiating cells.

In summary, this study has demonstrated that with time hemin inhibits GST irreversibly from functioning as catalyst in the conjugation of toxic molecules and GSH. The fact that hemin binding induces irreversible conformational changes in the protein molecule makes GST undesirable as a carrier molecule functioning in repeated binding events. In addition, unlike its conjugation function [18,19], GST synthesis is in no way linked to the need of the cell to transport heme between cellular organelles. The above conclusions point out that GST is unlikely to be the specific cellular heme carrier. Interestingly, based on a different approach, Boyer et al. [39] also expressed doubts about the hemin carrier function of GST.

REFERENCES

1. Mckay R, Druyan R, Getz GS, Rabinowitz M: Biochem J 114:455, 1969.
2. Hennig B, Neupert W: Eur J Biochem 121:203, 1981.
3. Enosawa S, Ohashi A: Biochem Biophys Res Commun 141:1145, 1986.
4. Chou AC, Fitch CD: J Clin Invest 66:856, 1980.
5. Avissar N, Inbal A, Rabizadeh E, Shaklai M, Shaklai N: Biochem Int 8:113, 1982.
6. Kirschner-Zilber I, Rabizadeh E, Shaklai N: Biochim Biophys Acta 690:20, 1982.
7. Yoda B, Israels LG: Can J Biochem 50:633, 1972.
8. Neuwirt J, Ponka P, Borova J: Biochim Biophys Acta 264:235, 1972.
9. Tipping E, Ketterer B: Biochem J 195:441, 1981.
10. Tipping E, Ketterer B, Christodoulides L, Enderby G: Biochem J 157:461, 1976.
11. Harvey JW, Beutler E: Blood 60:1227, 1982.
12. Vander Jagt DL, Hunsaker LA, Garcia KB, Royer RE: J Biol Chem 260:11603, 1985.
13. Jakoby WB: Adv Enzymol 46:383, 1978.
14. Ketterer B, Srai KS, Christodoulides L: Biochim Biophys Acta 428:683, 1976.
15. Davies DM, Liem HH, Johnson EF, Muller-Eberhard U: Biochem J 202:211, 1982.
16. Husby P, Srai KS, Ketterer B, Romslo I: Biochem Biophys Res Commun 100:651, 1981.
17. Senjo M, Ishibashi T, Imai Y: J Biol Chem 260:9191, 1985.
18. Arias IM, Fleischner G, Kirsch R, Mishkin S, Gatmaitan Z: Arch Biochem Biophys 188:287, 1978.
19. Pearson WR, Windle JJ, Morrow JF, Benson AM, Talalay P: J Biol Chem 258:2052, 1985.
20. Liu S-C, Zhai S, Lawler J, Palek J: J Biol Chem 260:12234, 1985.
21. Marks PA, Rifkind RA: Annu Rev Biochem 47:419, 1978.
22. Andersson LC, Jokinen M, Gahmberg CG: Nature 278:364, 1979.
23. Rutherford TR, Clegg JB, Weatherall DJ: Nature 280:164, 1979.
24. Rowley PT, Ohlsson-Wilhelm BM, Farley BA: Blood 65:862, 1985.
25. Simons PC, Vander Jagt DL: Anal Biochem 82:334, 1977.
26. Fjellstedt TA, Allen RH, Duncan BK, Jakoby WB: J Biol Chem 248:3702, 1973.
27. Pabst MJ, Habig WH, Jakoby WB: Biochem Biophys Res Commun 52:1123, 1973.
28. Ellman GL, Courtney KD, Anders V, Featherstone RM: Biochem Pharmacol 7:88, 1961.
29. Rossi-Fanelli A, Antonini E, Caputo A: Biochim Biophys Acta 30:608, 1958.
30. Hunt RC, Marshall LM: J Cell Sci 38:315, 1979.
31. Morrison GR: Anal Chem 37:124, 1965.
32. Lowry OH, Rosenbrought NJ, Farr AL, Randall RJ: J Biol Chem 193:265, 1951.
33. Sassa S: J Exp Med 143:305, 1976.
34. Lo SC, Aft R, Mueller GC: Cancer Res 41:864, 1981.
35. Concience JF, Miller RA, Henry J, Ruddle FH: Exp Cell Res 105:401, 1977.
36. Eberet PS, Hess RA, Frykholm BC, Tschudy DP: Biochem Biophys Res Commun 88:1382, 1979.
37. Solar I, Shaklai N: Biochim Biophys Acta (in press).
38. Beaumont C, Deybach JC, Grandchamp B, Da Silana V, De Verneuil H, Nordmann Y: Exp Cell Res 154:474, 1984.
39. Boyer TD, Zakim D, Vessey DA: Biochem Pharmacol 32:29, 1983.