EXPRESSION OF LIGANDIN AND GLUTATHIONE S-TRANSFERASE ACTIVITIES BY CELLS IN TISSUE CULTURE*

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SUMMARY

A wide distribution of glutathione S-transferase activity towards 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-dinitrobenzene has been detected in a range of non-transformed, transformed and hybrid cell lines. The levels of transferase activity are lower in these in vitro cell lines than are corresponding in vivo levels. A majority of the cell lines tested contain proteins that are antigenically related to rat liver glutathione S-transferase B (ligandin).

INTRODUCTION

The glutathione S-transferases (EC 2.5.1.8) are a major group of soluble liver proteins that effect cellular detoxification by catalyzing the conjugation of glutathione with a wide range of electrophilic compounds (1,2). Seven transferases have been separated from rat liver. These forms differ in substrate specificities and physical properties (3,4). The glutathione S-transferases have been calculated to represent some 10% of soluble rat liver proteins (5). Transferase B, or ligandin, as measured by quantitative immunoprecipitation comprises 4-5% of soluble protein in rat liver and 2% in small intestine and kidney (6,7). Ligandin also has been detected in rat ovary and testis (8,

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Abbreviations: DNCB = 1-chloro-2,4-dinitrobenzene, DCNB = 1,2-dichloro-4-nitrobenzene

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9), but not in plasma, bile, brain or other tissues (7,8,10). The protein has been detected by bromsulphthalein dye-binding measurement in fully developed amphibians, reptiles, birds and mammals including man, but is absent from gill-breathing vertebrates (11,12,13). Its levels are low in fetal liver but increase rapidly postpartum (8,12,14,15,16).

Rat liver ligandin, in addition to its glutathione S-transferase activity towards a range of substrates (4), binds a wide range of cellular metabolites in vivo including hematin, bilirubin, glucuronic acid, drugs such as tetracycline and bromsulphthalein, sulfate, steroids and their metabolites and several carcinogens and their metabolites (7,17). Ligandin has been shown recently to participate in Δ^5-3-ketosteroid isomerase reactions (18). Furthermore, ligandin and the glutathione S-transferases have been suggested to reduce the susceptibility of the liver to aminoazodye-, polycyclic aromatic hydrocarbon- and aromatic amine-induced carcinogenesis (17,19).

To date we have learned of no reports of the expression of ligandin by cells in established tissue culture systems. In consequence a range of established cell lines has been surveyed for glutathione S-transferases activity and antigenicity to rat liver ligandin in search of a system for the study of ligandin, its function and its role in chemically-induced carcinogenesis.

MATERIALS AND METHODS

Animals - Human liver was obtained from a Caucasian adult male within 24 hr of death from non-hepatic causes. Adult male Wistar albino rats were obtained from the Charles River Animal Laboratories, Wilmington, Mass., adult male Chinese hamsters from Chickline, Vineland, New Jersey and adult male Swiss mice from Hilltop, Scottdale, New Jersey.

Cell Treatment and Assay - Logarithmic phase or nearly confluent cells cultured in plastic flasks or glass Blake bottles were removed by trypsinization, washed in 10 mM potassium phosphate pH 6.5 containing 145 mM sodium chloride, resuspended in the same buffer and disrupted by sonication. The 100,000 gh supernatant was prepared and assayed for glutathione S-transferase activity with 1-chloro-2,4-dinitrobenzene (DNCB) or 1,2-dichloro-4-nitrobenzene (DCNB) as substrate using the method of Habig et al. (3). Protein concentration was determined by the method of Lowry et al. (20). Antigenicity towards rat liver ligandin was tested by the double diffusion method of Ouchterlony (21). The protein concentration for all samples was 5-10 mg/ml except for human fetal kidney 293-31 which was 1.8 mg/ml and tumor 293-31NP1 which was 4.0 mg/ml. Hybrid cell lines were prepared by the method of Croce et al. (22).

TABLE 1

GLUTATHIONE S-TRANSFERASE ACTIVITY AND ANTI-RAT LIVER LIGANDIN ANTIGENICITY IN STABLE CELL CULTURE SYSTEMS

	Cell System	Description		Glutathione S-Transferase Activity DNCB (μmoles/min/mg prot)	DCNB (μmoles/min/mg prot)	Antigenicity
IN VIVO						
	Rat Liver			1.093	0.035	+[b]
	Rat Kidney			0.292	nd[a]	+
	Human Liver			0.521	nd	+
	Hamster Liver			5.062	0.033	+
	Mouse Liver			4.930	0.074	+
NON-TRANSFORMED						
Mouse	TH02	Ouabain resistant BALB 3T3 Fibroblast (26)		0.239, 0.167[c]	0.004, nd	+
TRANSFORMED						
Rat	Fu5AH	Azaguanine-resistant hepatoma (27)		0.313, 0.094	0.005, nd	+
	HTC	Hepatoma Tissue Culture		0.344	0.003	
Human	293-31	Adenovirus 5-transformed fetal kidney (28)		0.146	nd	-
	293-31NP1	Adenovirus 5-transformed fetal kidney derived from nude mouse tumor		0.167, 0.135 0.229	nd, nd	-
	HT1080	Bone fibrosarcoma (29)		0.054, 0.042	nd, nd	-
Hamster	A3	Chinese Hamster Fibroblast		0.417, 0.735	0.007, 0.014	+
HYBRIDS						
Rat-Human	235D	Fu5AH x 293-31NP1		0.313, 0.600	nd, 0.012	+
	235H	Fu5AH x 293-31NP1	clone 1	0.164	0.002	+
			clone 2	0.200	0.003	+
Hamster-Human	324B	A3 x 293-31NP1	clone 1	0.167	0.004	+
			clone 2	0.208	0.004	+

(a) nd = not detected.
(b) + indicates positive cross-reactivity towards anti-rat liver ligandin.
(c) each value represents a separate cell culture.

Rat liver ligandin was purified by the method of Habig, Pabst and Jakoby (3) and rabbit antibody to rat liver ligandin by the method of Livingston (23).

RESULTS AND DISCUSSION

Table 1 shows the results of a survey of several non-transformed, tumor and somatic hybrid cell lines for glutathione S-transferase activities towards 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene. The activity levels of the transferases *in vivo* for several species and tissues are included for comparison. The data indicate a wide distribution of glutathione S-transferase activity in non-transformed, transformed and hybrid cell lines. In particular, the Chinese Hamster fibroblast line A3, the rat Hepatoma Tissue

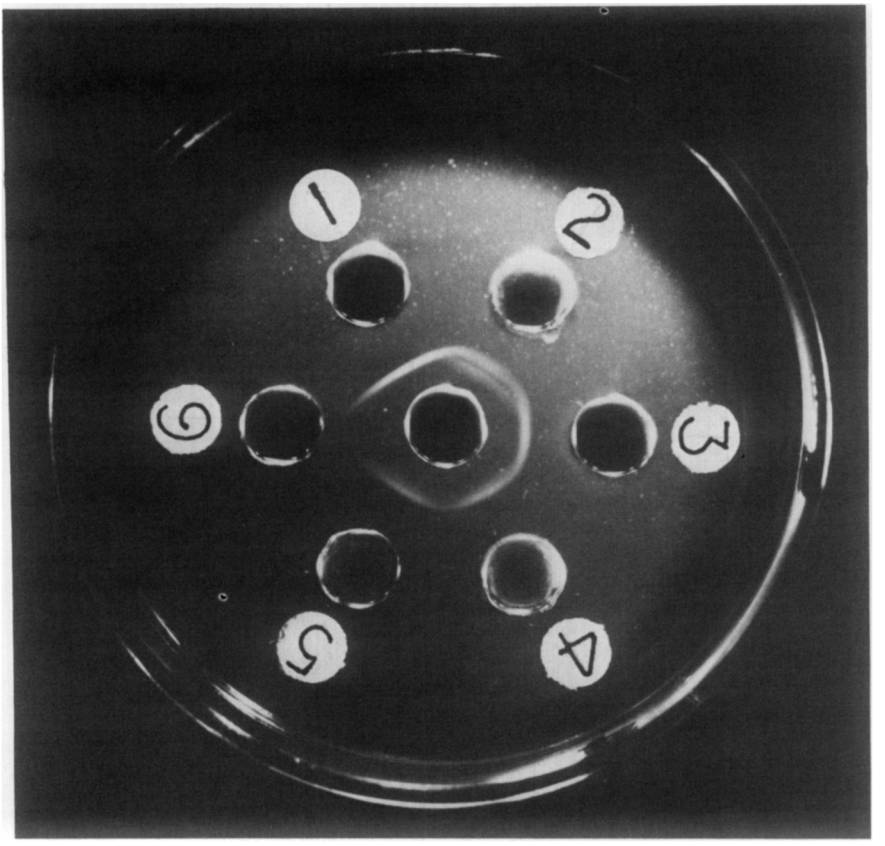

Figure 1. Ouchterlony double diffusion analysis of cytosols from several cell culture systems.
(CENTER) Rabbit anti-rat liver ligandin (4.0 mg protein/ml)
(1) Purified rat liver ligandin (0.2 mg/ml)
(2) Fu5AH (8.3 mg/ml)
(3) HTC (4.2 mg/ml)
(4) THO2 (10.8 mg/ml)
(5) 235D (6.4 mg/ml)
(6) 293-31NP1 (4.0 mg/ml)

Culture line HTC and the rat-human hybrid line 235D show high activity towards 1-chloro-2,4-dinitrobenzene relative to the other cell lines. The levels of the glutathione S-transferase activity in the transformed rat hepatoma lines is low compared with the levels of the enzyme in vivo (Table 1). This result agrees with the observations on solid tumors of Bannikov, Guelstein and Tchipysheva who demonstrated immunologically that ligandin is absent from poorly-differentiated hepatomas, anaplastic carcinomas and most adenocarcinomas

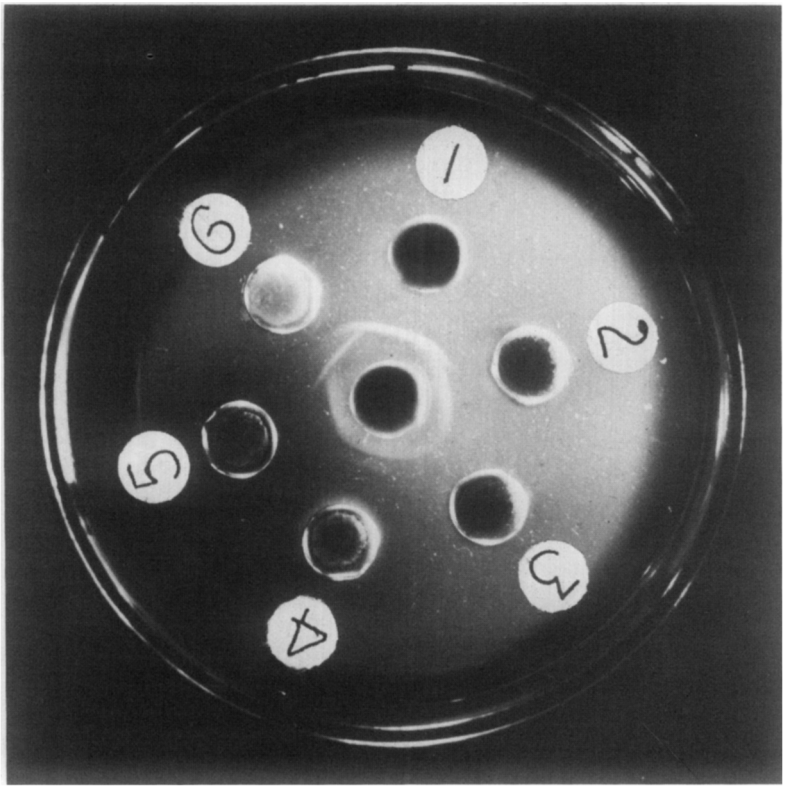

Figure 2. Ouchterlony double-diffusion analysis of several cell culture systems.
(CENTER) Rabbit anti-rat liver ligandin (4.0 mg protein/ml)
(1) Purified rat liver ligandin (0.2 mg/ml)
(2) Hamster liver cytosol (13.0 mg/ml)
(4) Mouse liver cytosol (19.0 mg/ml)
(5) Human liver cytosol (14.0 mg/ml)
(6) 235H cytosol (10.6 mg/ml)

(9). Another "h-protein", the "principal azodye binding protein" of rat liver also exhibits reduced levels in primary liver tumors (24).

The human cell lines show no detectable activity towards 1,2-dichloro-4-nitrobenzene (Table 1). This result is in agreement with the observations of Kamisaka et al. who showed that all the human liver glutathione S-transferases exhibit very low activity towards this substrate compared to the activity of transferases A and C in rat liver (3,25).

In Table 1 the rat-human hybrids 235D and 235H show a marked difference in transferase activity towards 1-chloro-2,4-dinitrobenzene. This result suggests that the different glutathione S-transferase forms may be subject to chromosomal segregation in these systems. Such cell hybrids may be suitable for the mapping of the transferase chromosomal loci and for studying the roles of any regulatory genes if they are involved in the expression of these proteins.

Figures 1 and 2 show the results of Ouchterlony double diffusion analysis of several of these cell systems using an antibody to rat liver ligandin. Purified rat liver ligandin forms a single precipitin band with purified anti-rat liver ligandin (Fig. 1) and does not cross-react with purified preparations of the non-antigenically related glutathione S-transferases A, C or AA of rat liver (data not shown). Fu5AH, HTC, TH02 and 235D cell lines cross-react with this antibody preparation and the precipitin bands fuse with that formed by purified rat liver ligandin (Fig. 1). The adenovirus 5 transformed human kidney cell line, 293-31NP1, and human bone fibrosarcoma, HT1080, show no such antigenicity. In Fig. 1 the cytosol from the rat hepatoma line Fu5AH shows a second precipitin band. This band, which does not form spurs with purified rat liver ligandin, indicates that the antiserum contains antibodies to more than one antigenic determinant on ligandin (21). In the case of human, hamster and mouse *in vivo* preparations and of the A3, 235H and 324B cells such bands represent the major cross-reaction observed (Fig. 2). These bands may indicate the presence of evolutionarily different but antigenically related glutathione S-transferases in the other animal species. The rat hepatoma line Fu5AH, the rat-human hybrid 235D and the mouse liver cytosol appear to contain two or more such proteins that are antigenically related to ligandin (Figs. 1 and 2). Fleischner *et al.* (8) recently reported cross-reactivity between Golden Hamster liver supernatant and antiserum against rat liver ligandin.

The stable cell lines shown in Table 1 may provide useful systems for studies of the interaction between carcinogens, steroids and the glutathione S-transferases and of the fate of these proteins in cellular transformation.

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