SEX AND SPECIES DIFFERENCES IN GLUTATHIONE S-TRANSFERASE ACTIVITIES

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CONTENTS

SUMMARY

Glutathione S-transferases (GSTs) are one of the important enzymes in terms of not only drug metabolism but also physiological functions. The marked sex difference in GST activity has been found in rat and mouse liver cytosol, and such differences in rat liver are suggested to be primarily due to the differences in the subunit composition of GSTs in both sexes. In addition, GST activities of rat liver cytosol are known to be largely influenced by treatment with inducers such as phenobarbital and 3-methylcholanthrene and various hormones. GSTs are widely distributed in mammalian species, and multiplicity of GST has been demonstrated so far. The present review also describes multiple forms of GST from the viewpoint of enzymology and immunology.

ABBREVIATIONS

GST	=	glutathione S-transferase
GSH-Px	=	glutathione peroxidase
CDNB	=	1-chloro-2,4-dinitrobenzene
DCNB	=	1,2-dichloro-4-nitrobenzene
EA	=	ethacrynic acid
tPBO	=	trans-4-phenyl-3-buten-2-one
ENPP	=	1, 2-epoxy- 3- (p-nitrophenoxy) propane
PB	=	phenobarbital
3-MC	=	3-methylcholanthrene
SDS	=	sodium dodecyl sulfate

I. INTRODUCTION

The glutathione S-transferases (GSTs; EC 2.5.1.18) are a family of multifunctional enzymes that play significant roles in the conjugation of glutathione with a variety of xenobiotics or have the Se-independent GSH peroxidase activity towards lipid peroxides. Also they possess functions as binding protein and carrier proteins for several materials such as cholic acids, steroid hormones, carcinogens and heam /1,2,3/ The multiple forms of GSTs have been reported so far. The GST isozymes were originally named on the basis of assumed specificities for

functional groups of electrophilic substrates and referred to as GSH S-aryltransferase, GSH S-alkyltransferase, GSH S-alkenetransferase, GSH S-epoxidetransferase /1/. This nomenclature was, however, abandoned as soon as it became evident that isolated transferases had overlapping substrate specificities towards a variety of different electrophilic compounds. In 1974, Jakoby et al. /4-6/ have designated the GST forms in rat liver cytosol as GSH S-transferases AA, A, B, C D and E on the basis of their reverse order of elution from a column of carboxymethylcellulose. Since all the cytosolic GST forms are dimeric combinations of various distinct subunits, Mannervik and Jensson /7/ have suggested that the name of the isozymes should reflect their respective subunit composition, and then proposed a new nomenclature for GSTs according to the subunit composition, in which the subunits, Ya, Yb1, Yb2, and Yc, are designated as L, A, C, and B by a single letter of the alphabet, respectively. Hence the six basic transferases, A B, ligandin, C, D, and AA in rat liver cytosol correspond to A_2, BL, L_2, AC, C_2 and B_2, respectively. However, it soon became clear that variants of rat GSTs may be too numerous to be covered by the 26 letters of the alphabet. Thus the nomenclature has more recently been changed so that the subunits of the well-characterized GSTs from rat cytosol, L, B, A and C, are now named subunits 1, 2, 3 and 4 respectively, by Arabic numerals in the chronological order of its characterization /8/. Even though the basic question as to how many distinct subunits are present in all rat tissues still remains to be answered, eight subunits from 1 to 8 have been defined so far /9, 10). The GST isozymes are identified by their constituent subunits. The cytosolic GST each comprises two subunits and the multiple forms that exist arise from dimeric combinations of a limited number of subunits /7, 11, 12/. A homodimer of subunit 1 should be referred to as GST 1-1, whereas a heterodimer of subunits 1 and 2 should be called GST 1-2, and so on. Microsomal GST is distinct in that it is composed of polypeptides (Mr 14000) which have little or no similarity to cytosolic subunits /13-16/.

It has recently become to be clear that GSTs are closely implicated in the bioactivation as well as inactivation process of many xenobiotics /3/. On the other hand, it has been also known from a long period of time that the sensitivity, drug metabolizing enzymes or toxicity to xenobiotics might be different from both sexes and among species

The purpose of the present paper, therefore, to describe the sex and

species differences in GST activities, which are thought to be one of very important factors in the view of drug metabolism and toxicity.

II. SEX DIFFERENCE

2.1 Subunit-composition of GST isozymes

The sex differences in hepatic GST activities have been shown in the rat /17-23/ and mouse /24, 25/, but not in hamster (our unpublished data), marmoset (our unpublished data), guinea pig /22/, lizard /22/, man /22/, goldfish /22/, duck /22/ and frog /22/.

In adult rat, liver, GST activity has been demonstrated to be significantly higher in males than in females /17, 18/. We have also recognized the sex difference in hepatic GST activity in rats and showed that the extent of sex difference depends on the substrate used /19/ (Table 1).

Male rats showed about 1.6-fold higher activity towards DCNB than that in females, however, the activities towards CDNB were almost the same. CDNB is a good substrate for various isozymes of transferases

TABLE 1

The activities of glutathione S-transferases and glutathione peroxidase in male and female rat hepatic cytosols

Substrate	Male (M)	Female (F)	M/F
CDNB	927.3 ± 9.3	847.9 ± 30.1	1.09
DCNB	46.5 ± 1.0	29.8 ± 1.1**	1.56
Hydrogen peroxide	128.3 ± 9.3	214.1 ± 7.7**	0.60
Cumene hydroperoxide	209.3 ± 16.9	343.4 ± 14.4**	0.61

The figures are expressed in μunits per mg of cytosolic protein. Values are means ± S.E.M. for five animals, with evaluation by Student's t-test. *$P < 0.05$, **$P < 0.01$ vs males.
Data taken from reference /19/.

but DCNB is available for only GST 3-4 type isozymes containing 3(Yb_1) subunits, as a substrate /4, 7/. Other investigators have also reported that there was little sex difference in GST activities in rat liver when CDNB was used as a substrate /17, 18/, however, a significant sex difference was seen in GST activities towards DCNB /17, 18, 20/, sulfobromophthalein /17/, and styrene oxide /26/.

Glutathione peroxidase (GSH-Px) is present as at least two isozymes in rat liver /27/. One is selenoenzyme GSH-Px (Se-GSH-Px) which utilizes hydrogen peroxide as well as many organic hydroperoxides as substrates. The other is non-selenium dependent GSH-Px (non-Se-GSH-Px) which cannot use hydrogen peroxide as a substrate. Table 1 also shows that the GSH-Px activities of females were approximately 2-fold higher than those of males towards both substrates.

Higher activity towards cumene hydroperoxide of GSTs in female rats has also revealed by indicating that the peroxidase activity of GSTs which was retained on GSH affinity column was higher in females than in males /19/. These results suggest that the sex differences in cytosolic GSH-Px activities are mainly due to Se-GSH-Px, but also in part to non-Se-GSH-Px. Since non-Se-GSH-Px activity is attributed to certain isozymes of GSTs /28/, it is likely that the activities of transferase having peroxidase activity are higher in female rat liver than in males /19/.

During development, rats more than seven weeks old exhibit significantly higher GST activity in males than females /21/. As no sex difference in microsomal GST activities was seen at any age during development /21/, the sex difference in GST activities appears to be specific for cytosolic GSTs.

Analysis of GST isozymes by the SDS-polyacrylamide gel electrophoresis showed a marked difference in the relative compositions of 1(Ya), 3/4(Yb) [3(Yb_1),4(Yb_2)] and 2(Yc) subunits between males and females (Figure 1). In males, 3/4(Yb) was the most abundant followed by 1(Ya) and 2(Yc), while Yb was the lowest in females. The sex difference in GST activities in adult rat liver may be thus due to the difference in the relative proportions of the GST subunit composition in males and females /21/.

Hales and Neims /18/ demonstrated that the amount of GST-B in the cytosol from male rat liver was significantly lower than that in females by immunoprecipitation with anti-GST-B antibody. However, they purified GST-B by CM-cellulose column chromatography, based

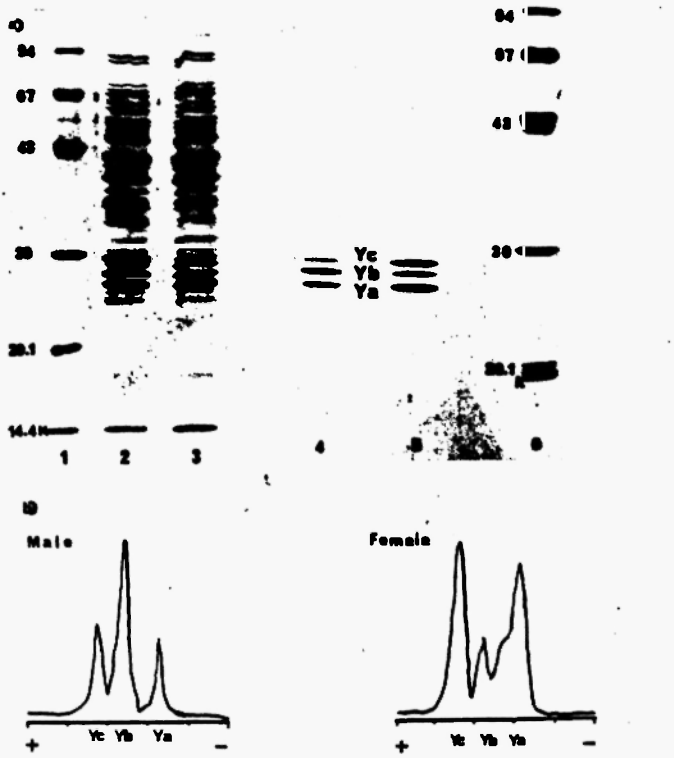

Fig. 1: SDS-polyacrylamide gel electrophoresis of transferase fractions obtained on GSH-affinity chromatography of male and female rat hepatic cytosols. (a) Electrophoresis was carried out in 12.5% acrylamide resolving gel. Lanes 1 and 6, marker proteins for molecular weight determination (products of Pharmacia Fine Chemicals: phosphorylase b 94K; bovine serum albumin, 67K; ovalbumin, 43K; carbonic anhydrase, 30K; soybean trypsin inhibitor, 20.1K; α-lactalbumin, 14.4K); 2 and 3, male (9.8μg) and female (10μg) cytosols, respectively; 4 and 5, male and female transferases obtained from the GSH-affinity column, respectively. The Ya, Yb(Yb1/Yb2), and Yc bands are indicated. (b) Densitometric scanning of lanes 4 and 5 was performed at 550 nm. Data taken from reference /19/.

on the method of Habig et al. /4/, therefore, GST-B which they used would have contained both GST 1-2 and 1-1. The relative amounts of the activities in each GST fraction obtained on chromatofocusing were quantitatively determined by summing the activities of the individual fractions (Table 2). The activities of the GST 3-4 type, such as 3-3, 3-4, and 4-4, and of neutral/acidic enzymes were found to be higher in males, and those of the GST 1-2 type, 1-1, 1-2, and 2-2, were higher in females. Two groups of GST 1-2 and 3-4 are immunologically distinguishable from each other /7/. Then, a distinct difference was also seen in the degree of inhibition of GST activities between males and females on immunotitration with anti GST 1-2 or 3-4 antiserum /19/

In animals other than the rat, mouse liver GST activities are higher in males than in females /24, 25/. Among three forms, MI, MII, MIII, of mouse liver GSTs, the major form, MII, is sex-specific /24, 25/ and the levels are approximately 10-fold higher in males than those of females /24/. Sex difference in cytosolic GST activities in mouse liver is suggested to be due to a major difference in the content of this sex-specific subunit (m_2) between males and females /24/ Three forms in mouse liver GSTs all appear to be dimers of identical subunits /24, 29/, in contrast to the basic rat hepatic isozymes which include heterodimeric structures in addition to homodimers /7/.

Rabbit hepatic cytosolic GST activities have exhibited no distinct sex difference /30, 31/, while they have significant individual difference /30/.

2.2 **Drug Induction**

The GSTs have been reported to be induced by treatment with phenobarbital (PB) /20, 32-35/ and 3-methylcholanthrene /3-MC/ /32-34, 36/, which are well-known enzyme inducers having different properties on drug-metabolizing enzymes. GST isozymes are also known to be induced by PB and 3-MC in a different manner /33/. We have examined the effect of treatment with PB and 3-MC on hepatic cytosolic GST activities using three-week-old rats, which exhibited no sex difference in GST activities, and seven-week-old rats, which showed a marked sex difference in enzyme activities (Table 3) Young rats exhibited no significant sex difference in GST activities, and there was little or no sex difference in the enzyme activities of rats treated with

TABLE 2

Distribution of male and female glutathione S-transferase activities of each isozyme fraction separated by chromatofocusing

	Basic GST isozymes					Neutral/
	1-1	1-2	2 2	3-3	3-4	4-4	acidic GSTs
Male (%)	10.4	5.6	8.4	2.7	12.4	18.2	42.3
Female (%)	15.9	25.0	8.9	0.9	9.5	13.0	26.8
Male/Female	0.65	0.22	0.93	3.0	1.31	1.40	1.58

Each isozyme fraction of GSTs obtained on chromatofocusing was pooled and each pooled transferase activity towards CDNB was measured. Total recoveries of transferase activities of each isozyme fraction separated by chromatofocusing from males and females were 97.4% and 69.9%, respectively.
Data taken from reference /19/.

TABLE 3

Effect of phenobarbital and 3-methylcholanthrene treatment of male and female rats on hepatic cytosolic glutathione S-transferase activity

Treatment	Glutathione S-transferase activity		M/F
	Male	Female	
Exp.1 Young rats (3 weeks old)			
Control (saline)	0.58 ± 0.01	0.52 ± 0.03	1.11
PB	1.43 ± 0.04 (247)	1.22 ± 0.07* (234)	1.17
Control (corn oil)	0.59 ± 0.05	0.51 ± 0.01	1.16
3-MC	1.04 ± 0.05 (176)	1.18 ± 0.06 (232)	0.88
Exp.2 Adult rats (7 weeks old)			
Control (saline)	0.74 ± 0.02	0.61 ± 0.02**	1.22
PB	1.89 ± 0.07 (255)	1.24 ± 0.05*** (204)	1.52
Control (corn oil)	0.62 ± 0.01	0.47 ± 0.02***	1.32
3-MC	1.15 ± 0.03 (188)	1.06 ± 0.06 (265)	1.08

Glutathione S-transferase activity was assayed with 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. Each value represents means ± S.E. obtaine *P < 0.05, **P < 0.01, ***P < 0.001 vs. male.
Data taken from reference /21/.

PB and 3-MC. In contrast, with adult rats, a sex difference in GST activity was obviously found. The extent of the inducibility of GST by PB in males is much greater than in females. Conversely, 3-MC induced GST in females is to a much greater extent than in males. Therefore PB treatment made the difference between males and females become more marked, and the sex difference in GST activities disappeared after 3-MC treatment. We have measured the GST activities from male and female using CDNB as a substrate, because CDNB is accepted to be the best and useful substrate towards all GST isozymes /3/. Also, it must be noted that the degree of these sex differences in GST activities may be largely dependent on the substrate used. The quantitative change in GST isozymes induced by PB and 3-MC has been demonstrated that PB treatment increased GST 1-2 type GSTs more than 3-4 type in amounts, and 3-MC did not affect 3-4 type GSTs but increased markedly 1-2 type GST /21/.

2.3 Hormonal Influences

GST activities are subject to modulation by hormonal influences. Ovariectomized rats had markedly increased the GST activity /37/. The decrease of male GST activity by estradiol administration was more evident in castrated rats than in normal rats, while testosterone administration to castrated males had no effect /37/. Hypophysectomy significantly increased GST activities in female rats, but not in male rats /18/.

The levels of the sex-specific GST form, MII, in male mouse liver are decreased to those in females by castration, and those in females increased to those in adult males by testosterone administration /24/.

III. SPECIES DIFFERENCE

3.1 Tissue Distribution

GST activities have been detected in other tissues as well as liver including lung /38-40/. GST activity is generally greater in hepatic than in extrahepatic tissues /41/. However, GST activity towards the K-region epoxide benz(a)anthracene 5,6-oxide has been, exceptionally

shown to be more than 2-fold greater in the lung than in the liver in the rat /42/ and the monkey /43/. Hepatic GST activity has been found in all the species so far examined /22, 41, 44/. Grover and Sims /22/ have investigated the hepatic GST activities in eight animal species including mouse, rat guinea pig and human, and indicated the marked species difference in their activities.

3.2 Hepatic Activities

Hepatic cytosolic multiple forms of GST have been purified to apparent homogeneity from the rat /4, 45-48/, mouse /24, 29 49/, man /50, 51/, rabbit /30, 52, 53/, hamster /54/, sheep /55/, chicken /56/, monkey /44/, bovine /57/, dog /58, 59/, camel /60, 61/ and carp /63/. Wheldrake et al. /63/ have reported that inter-strain variations exist in mouse liver GST activity, but it is also demonstrated by other investigators /24, 25/ that no significant inter-strain differences in the properties of the individual forms of mouse liver GSTs.

Species differences in GST isozymes exhibiting non-Se-GSH-Px activity between rat and guinea pig liver have been described /64/ Furthermore, non-Se-GSH-Px in guinea pig liver is suggested to be the primary functional GSH-Px /64/, since Se-GSH-Px activity is very low in guinea pig liver /65, 66/.

As shown in Figure 2, there was a marked species difference in the activities of hepatic GSTs in seven animal species when the enzyme activity was assayed under the same conditions of substrate and GSH concentration. These species differences in the hepatic GST activities have been also reported by other investigators /22/. Interspecies differences are known to be dependent on the substrate studied (Figure 2) /67/. For instance, of the seven species, the rat had the highest GST activity towards tPBO, but the lowest activity with CDNB. The liver of rat has been reported to possess much higher GST activities towards styrene oxide /68/, but the lower activity towards CDNB /67/ than that of rabbit.

Figure 3 shows SDS-polyacrylamide gel electrophoresis in various animal species. It has become apparent that the subunit compositions of GSTs are different among animal species. There was also marked species difference in elution profile of GST activity on column chromatography. The elution patterns from rats, mice and rabbits were quite different from hamsters and guinea pigs /69/.

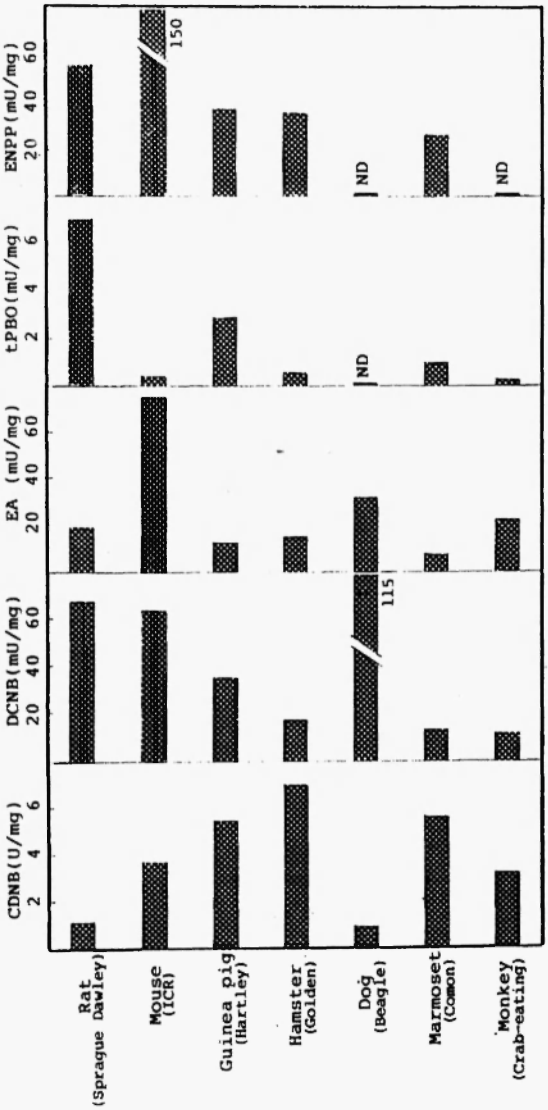

Fig. 2: The specific activity of GSTs in the liver cytosol from several animal species.

Fig. 3 SDS-polyacrylamide gel electrophoresis of cytosolic proteins from several animal livers before and after (b) affinity chromatography on a column of S-hexylglutathioneSepharose 6B. (a) cytosol (lanes 1-7, 10μg each), (b) fractions by affinity column (lanes 1-7, 1 μg each). Lane 1; Rat, 2; mouse, 3; Dog, 4; Guinea pig, 5; Marmoset, 6; Monkey, 7; Hamster, St; marker proteins (molecular weight determination, Mix; a mixture of Ya, Yb1/Yb2 and Yc subunits obtained from S-hexyl/glutathione affinity column.

3.3 Immunological Relationship Between GST Isozymes from Different Animal Species

It was recently further proposed by Mannervik et al. /70/ that GSTs from different mammalian species can be grouped by the criteria of their amino acid composition, N-terminal sequences, substrate specificities, sensitivities to inhibitors and immunochemical properties, into three distinct species-independent classes named Alpha, Mu, and Pi, that are thought to be the products of three gene families /70-72/. The Alpha class involves the GST isozymes composed of subunits 1 and 2, and Mu class is associated with 3, 4 and 6 subunits. The Pi class is a family of isozymes which contain only subunit 7. Table 4 represents the summary of immunoreactivity obtained from Western blot analysis. These subunits show protein band by SDS-polyacryl-amide gel electrophoresis, but do not represent the number of GST isozymes. Anti GST 1-1 to 4-4 antiserum cross-reacted with GSTs from various animal species, but the anti GST-P(7-7) antibody immunoreacted with only mouse and dog liver GSTs. It is also noteworthy that GSTs having common antigenicity to rat subunit 7 may be major forms in dog liver /58/. The GST 7-7 (also named GST-P) is almost undetectable in normal rat liver, while found in high concentration in pre-neoplastic nodules /73/. Thus GST 7-7 has recently attracted particular attention as a new marker enzyme for (pre)neoplastic lesions arising during chemical carcinogenesis in rat liver /73/ and hamster pancreas /74/ and human colonic carcinoma /75/. In more recent years, the liver cytosol of rhesus monkeys has been reported to also contain all three class of GSTs that are very similar to the human liver GSTs (Alpha, Mu and Pi) /76/.

Since GST is an important enzyme for the detoxification of xenobiotics, the drastic species differences in substrate specificity of this enzyme system may become an important concern in choosing a laboratory animal for the evaluation of biochemical transformation and toxicity or carcinogenesis.

IV. CONCLUSIONS

GSTs catalyze the conjugation of GSH with various xenobiotics. The GSH conjugates are usually less toxic than their parent compounds and

TABLE 4

Crossreactivity of liver cytosolic GSTs from several animal species with Western blot analysis

Species	Subunit	MW($x10^3$)	Antibodies to rat liver GST 1-1	2-2	3-3	4-4	7-7
Rat[a)]	Ya(1)	26.5	+	-	-	-	-
	$Yb_2(3)$, $Yb_2(4)$	27.5	-	-	+	+	-
	Yc(2)	28.5	-	+	-	-	-
Mouse	Ym_1	26.0	-	-	-	-	+
	Ym_2	27.0	+	+	-	-	-
	Ym_3	28.0	-	-	+	-	-
Dog[a)]	Yd_1	26.0	-	-	-	-	+
	Yd_2	27.0	+	+	-	-	-
	Yd_3	28.0	-	-	+	-	-
Guinea pig	Yg	27.0	-	-	+	+	-
Hamster	Yh_1	27.0	+	+	-	-	-
	Yh_2	28.0	-	+	+	+	-
Marmoset	Yma_1	26.5	+	+	-	-	-
	Yma_2	28.0	-	-	-	-	-
Monkey	Ymo_1	26.5	+	+	-	-	-
	Ymo_2	28.0	-	-	-	-	-

[a)]Data taken from reference /58/.

are readily excreted in bile or in urine as their corresponding mercapturic acids. However, in some cases, the conjugation with GSH has been implicated in the activation of certain xenobiotics to mutagenic and carcinogenic electrophiles /77-85/, and there are evidences that GSH conjugates of a variety of compounds and/or their corresponding cysteine conjugates are nephrotoxic /86-90/.

Among several experimental animals, rat and mouse possess a marked sex difference in hepatic cytosolic GST activities. The sex difference in GST activity in rats might be due primarily to the quantitative difference of each isozyme between males and females. The relevance of these sex difference in GST activities to hepatic toxicity remains unclear.

Even though several forms of GST which differ in their structural and immunological properties are present in tissues of various animal species including human, the relationships among them generally appear complex. In view of the observed species variations regarding the multiple forms and the substrate specificity of glutathione S-transferases, further investigations of this group of enzymes from other closely related species seem necessary for a better understanding of their physiological functions.

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