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Do the major human glutathione *S*-transferases have fatty acid ethyl ester synthase activity?

Takashige Suzuki, Matthew S. Kovacs and Philip G. Board

Molecular Genetics Group, John Curtin School of Medical Research, Australian National University, GPO Box 334, Canberra, A.C.T. 2601, Australia

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Two fatty acid ethyl ester (FAEE) synthase isoenzymes purified from human myocardium were reported to be glutathione *S*-transferases (GST) [(1989) Proc. Natl. Acad. Sci. USA 86, 4470–4473; and (1989) J. Clin. Invest. 84, 1942–1946]. In the present study, the FAEE synthase activity of several purified and well characterized human GSTs were examined with ethanol and [^{14}C]oleic acid as substrates. Three isoenzymes, GST1, GST2 and GST3 which are members of the evolutionary classes μ, α and π, respectively, were studied and failed to show any significant synthesis of FAEE after 45 min incubation at 37°C. FAEE synthase activity and GST3 activity in human placental extracts can be readily separated by ion exchange chromatography on DEAE cellulose. Thus the results show that FAEE synthase activity is not a feature of the major GSTs found in human tissues. The two FAEE synthase isoenzymes isolated by Bora et al. may have been co-purified with GST isoenzymes or these FAEE synthases may be members of the GST super family that have low specific activity in conventional GST assays and have not been previously described.

Glutathione *S*-transferase; Fatty acid ethylester synthase

1. INTRODUCTION

It has recently been reported [1] that a fatty acid ethyl ester (FAEE) synthase isoenzyme purified from human myocardium had a very similar N-terminal sequence to that of an acidic human glutathione *S*-transferase (GST). The FAEE synthase also had GST activity and was able to catalyse the conjugation of GSH and 1-chloro-2,4-dinitrobenzene. In addition, another FAEE synthase isoenzyme purified from human myocardium also displayed GST activity [2]. These results suggested that some of the FAEE synthase isoenzymes seemed to be members of the GST family.

The GSTs are products of a large gene family and catalyse the conjugation of GSH with a wide range of electrophilic substrates including many known mutagens and carcinogens. The cytosolic GST isoenzymes can be classified into at least three evolutionary classes termed α, μ and π in mammalian species [3]. In humans, GST1 from liver, GST4 (muscle-specific isoenzyme) and GST5 (brain-specific isoenzyme) have been shown to be members of the μ evolutionary class [4,5]. GST2 purified from human liver and GST3 purified from lung, placenta and erythrocytes are members of the α and π evolutionary classes, respectively [4,6–8].

Correspondence address: T. Suzuki, Molecular Genetics Group, John Curtin School of Medical Research, Australian National University, GPO Box 334, Canberra, ACT 2601 Australia

Abbreviations: GST, glutathione *S*-transferase; GSH, reduced glutathione; FAEE, fatty acid ethylester

In order to determine if FAEE synthase activity is a common feature of all human GSTs or if it is confined to particular classes or isoenzymes, we have determined the FAEE synthase activity of several highly purified human GST isoenzymes that represent examples of the three main evolutionary classes of cytosolic GST. In addition, we have followed the FAEE synthase activity of human placental extracts during the purification of GST3, the major GST isoenzyme expressed in placenta, and the GST isoenzyme with the closest N-terminal sequence to that reported for the myocardial FAEE synthase [1].

2. MATERIALS AND METHODS

2.1. *Materials*

[^{14}C]Oleic acid (52 Ci/mol) and [9,10(n)-^{3}H] oleic acid (5.0 Ci/mmol) were obtained from Amersham. Oleic acid (sodium salt) and ethyl oleate were purchased from Sigma, and silica gel 60 F-254 TLC plates were from Merck. Ethyl [^{3}H]oleate was synthesized by the method of Mogelson and Lange [9]. *S*-Hexylglutathione was prepared according to previously described methods [10].

2.2. *Enzyme assay*

The FAEE synthase activity was measured with [^{14}C]oleic acid and ethanol as substrates [9]. [^{3}H]Ethyl oleate was used for estimation of [^{14}C]ethyl oleate recovery [9], and the average recovery was 46.7% in the present studies. Blanks without samples were prepared for each assay and the average non-enzymatic synthesis of FAEE was found to be 352 pmol/h/ml in our assay system at 37°C. GST activity was determined spectrophotometrically [11] using GSH and 1-chloro-2,4,-dinitrobenzene as substrates.

2.3. *Tissue homogenate and purified GST*

Tissue homogenates were prepared with 20 mM sodium phosphate

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Table I

FAEE synthase and GST activities of purified human GSTs and rat tissues

	FAEE synthase[a]	GST[b]
Purified human GST		
GST1	<0.01[c]	58.3
GST2	<0.01[c]	17.4
GST3	<0.01[c]	16.4
Human placenta	0.016	0.013
Rat tissue		
Heart	0.096	0.04
Liver	0.655	0.87
Pancreas	218.2	0.09
Testes	0.022	1.21
Stomach	0.240	0.10
Intestine	7.90	0.23

[a] The enzyme activity is expressed as nmol/h/mg of protein
[b] The enzyme activity is expressed as μmol/min/mg of protein
[c] ^{14}C radioactivity in the assay was less than 60 dpm for 45 min incubation

buffer, pH 7.4, from liver, heart, pancreas and testis of male Wistar rats. The homogenates were centrifuged at 2000×*g* for 10 min and the supernatants used in the enzyme assays. Human GST isoenzymes, GST1 type 1 and GST2 type 1, were purified from human liver as previously described [4]. The GST3 isoenzyme was isolated from placenta by the methods described by Mannervik and Guthenberg [10] with the exception that glutathione agarose [12] was used in the affinity chromatography step rather than hexylglutathione agarose. Protein concentration was measured by well established methods [13,14].

3. RESULTS

Because previous studies [15] had demonstrated that FAEE synthase activity was very high in rat pancreas, different volumes of rat pancreas homogenate were used to evaluate the sensitivity of the FAEE synthase assay system. The enzyme activity and protein concentration were positively correlated. In addition, at a protein concentration of 35 μg, the rate of FAEE synthesis was time-dependent up to 90 min at 37°C. Rat tissue homogenates showed different FAEE synthase activities (Table I), and these results largely confirmed previously published data [15]. It is evident from these results that FAEE synthase activity and GST activity are not correlated in these tissues.

The purified human GST isoenzymes we tested (GST1, GST2, GST3), did not have any significant FAEE synthase activity (less than 0.01 nmol/h/mg) (Table I). Human placenta shows low FAEE activity (Table I) and this activity was followed during the purification of GST3, the major cytosolic GST expressed in that tissue. As shown in Fig. 1, the FAEE synthase activity and the GST activity were clearly separated during chromatography on DEAE cellulose at pH 8.

It was previously reported [1] that GSH stimulated myocardial FAEE synthase activity. Since *S*-substituted glutathione derivatives such as *S*-hexylglutathione are

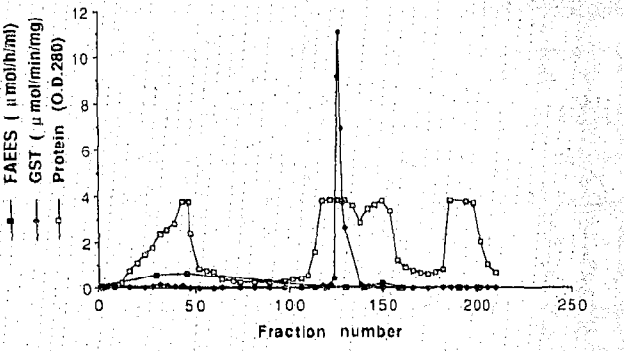

Fig. 1. Fractionation of GST and FAEES activities from human placenta by DEAE cellulose ion exchange chromatography at pH 8.

strong inhibitors of GST [16], we determined the effect of *S*-hexylglutathione on FAEE synthase activity in rat pancreas and liver extracts. FAEE synthase activity was not inhibited by *S*-hexylglutathione at concentrations up to 2 mM, but as expected the GST activity was substantially decreased (I_{50} < 0.2 mM).

4. DISCUSSION

Two FAEE synthase isoenzymes (major and synthase I forms) purified from human myocardium have been reported to have properties suggesting that they are members of the glutathione transferase family [1,2]. The N-terminal sequence (23 amino acids) of the major synthase was 82% identical to that of human GST3 [1,4,5]. In addition, the major synthase had GST activity with GSH and 1-chloro-2,4-dinitrobenzene as substrate [1]. In a separate study of the isozyme termed synthase I, FAEE synthase activity and GST activity were shown to co-purify after chromatography on DEAE cellulose, Sephadex G-100 and hexylglutathione agarose [2]. These two reports therefore suggested that major and synthase I FAEE synthases of human myocardium may be GST isoenzymes. In particular, the homology between the amino terminal sequence of the major FAEE synthase with the GST3 isoenzyme suggested that the synthase may be a member of the π evolutionary class.

The results obtained from the present study have shown that it is unlikely that major GST isoenzymes in human or rat tissues have significant FAEE synthase activity. Firstly, the lack of correlation between synthase and GST activities in rat tissue extracts suggests that these activities are independent in that species. Secondly, in direct assays of high purified GST1, GST2 and GST3, the major human cytosolic GST isoenzymes that also represent the μ, α and π evolutionary classes, we could not detect significant FAEE synthase activity. This indicates that FAEE synthase activity is not a significant property of the major human GST isoenzymes. The clear separation of FAEE synthase and GST activities in human placental extracts by DEAE

cellulose chromatography clearly suggests that in this tissue, these activities result from different enzymes.

We also found that *S*-hexylglutathione, a potent inhibitor of GST, did not affect the activity of FAEE synthase in rat tissues suggesting that these two enzymatic activities are the properties of different proteins. However, these data do not exclude the possibility that a single protein may have two distinct active sites capable of independent catalytic activity and differential inhibition.

The results obtained by Bora et al. [1,2] have several possible explanations. For example, it is possible that the FAEE synthases from human heart represent tissue-specific forms of GST that have relatively low glutathione transferase activity and have not previously been identified and characterized in our previous studies of human heart GSTs [4]. Alternatively, the purified myocardial FAEE synthases [1,2] may have been contaminated by small amounts of a GST which has similar physical properties to those of the FAEE synthases.

Recently, a FAEE synthase was purified from human pancreas and its N-terminal amino acid sequence indicated that it is a member of the lipase family [17]. Thus, further studies of the FAEE synthases in different tissues will be needed to confirm the relationships between the FAEE synthases, GST and lipase.

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