RESEARCH COMMUNICATION

Characterization of rat spleen prostaglandin H D-isomerase as a sigma-class GSH transferase

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The prostaglandin H D-isomerase of rat immune accessory cells has been purified from spleen by a simple procedure, and its high specificity and activity [Urade, Fujimoto, Ujihara and Hayaishi (1987) *J. Biol. Chem.* **262**, 3820–3825] have been confirmed in an assay coupled to prostaglandin H synthase. The enzyme also decreases the formation of 12[S]-hydroxy-5,8,10-heptadecatrienoic acid formed by the synthase in the presence of GSH and

increases the overall rate of arachidonate oxidation. A partial amino acid sequence shows a strong relationship to GSH transferases of parasitic helminths and molluscs, indicating that it is the first example of a vertebrate sigma-class GSH transferase, and suggesting that certain helminth GSH transferases may be involved in prostaglandin synthesis.

INTRODUCTION

The GSH transferases (EC 2.5.1.18) are a group of catalytic/binding proteins usually involved in detoxication and excretion pathways. They have been classified on the basis of primary structure into five classes: alpha, mu, pi [1], theta [2] and sigma [3]. The theta class is considered the most ancient in evolutionary terms, occurring in bacteria, mitochondria and plants [4]. The sigma class occurs in mollusca (squid, octopus) and parasitic helminths (*Schistosoma*). Comparison of the three-dimensional structures suggests that the sigma class diverged from the ancestral (theta) class prior to the divergence of the alpha/mu/pi precursor [5]. In catalysis, GSH transferases generally have a broad specificity for hydrophobic electrophilic compounds, including both xenobiotic and endogenous metabolites [6]. For instance, the major GSH transferases of rat liver are able to catalyse not only the reduction of prostaglandin (PG)H₂ to PGF_{2α} but also its isomerization to PGD₂ and PGE₂ [7]. One GSH transferase, however, shows far greater specificity and activity in PG biosynthesis: the PGH D-isomerase isolated from rat spleen [8,9], the N-terminal sequence of which has a region of identity with a theta-class GSH transferase [2]. This enzyme was shown by immunohistochemistry to occur exclusively in immune accessory cells and mast cells [10,11], suggesting that it functions in PG biosynthesis associated with immune responses. Curiously, a more recent study of rat spleen GSH transferases failed to note the presence of this enzyme [12].

Another PGH D-isomerase that is GSH independent and structurally unrelated to the spleen enzyme has been isolated from rat brain [13]. Presumably this enzyme is involved in the synthesis of PGD₂ associated with the induction of sleep [14].

Recently, GSH, which is not a cofactor for PGH synthases (cyclo-oxygenases; EC 1.14.99.1), was shown to alter their catalytic outcome, causing the formation of a substantial amount of 12[S]-hydroxy-5,8,10-heptadecatrienoic acid (HHT) instead of PGs [15]. This observation suggested that PG synthesis may take place wholly within a lipid membrane compartment and cast doubt on whether a soluble, GSH-dependent PGH D-isomerase could play a role in immune-associated PG synthesis.

To clarify the role, if any, of the putative immune accessory cell PGH D-isomerase, we have developed a rapid purification method and demonstrated the enzyme's efficacy in an assay coupled to PGH synthase under approximately physiological conditions. In addition, further primary structure obtained surprisingly shows it to be a member of the sigma class of GSH transferases, which had not previously been found in a 'higher' organism and which raises important questions as to the function of related enzymes in parasitic helminths.

EXPERIMENTAL

Materials

PGs D₂, E₂, F_{2α} and 6-keto-F_{1α}, thromboxane B₂ and ram seminal vesicle PGH synthase (20 units/mg, 95% pure, constitutive type) were obtained from Calbiochem Novabiochem (Nottingham, U.K.). Aliquots of the PGH synthase were snap-frozen and stored at –70 °C. HHT, haematin, tryptophan, triphenylphosphine and bovine γ-globulin were obtained from Sigma (Poole, U.K.). Eicosanoid standards for thin-layer chromatography were prepared (1 mg/ml in ethanol) and stored at –20 °C. [1-¹⁴C]Arachidonic acid (50–62 mCi/mmol, 50 μCi/ml of ethanol) was obtained from Amersham International (Amersham, U.K.) and stored at –70 °C. Channelled silica gel thin-layer plates with a pre-adsorbent zone (LK6D) were obtained from Whatman (Maidstone, U.K.). Other chemicals were of analytical grade, and water was of mQ grade.

Purification of PGH D-isomerase

Spleens (total weight 2.6 g) from four male Sprague–Dawley rats were snap-frozen and stored at –70 °C. Upon thawing they were homogenized at 0–4 °C using a Teflon–glass homogenizer in buffer A [0.15 M NaCl/1 mM dithiothreitol/1 mM EDTA/30 mM bis-Tris adjusted with HCl to pH 6.60 at 22 °C (approx. pH 7 at 0–4 °C)]. The soluble fraction obtained by ultracentrifugation was applied under gravity to a column (2 cm × 2 cm) of DE-52 cellulose pre-equilibrated with buffer A,

and the unretarded (red) fraction was immediately applied to a column (1 cm $\times$ 2 cm) of S-linked GSH-agarose pre-equilibrated with buffer A. After washing with 8 ml of buffer A, the GSH transferases were eluted with 5 mM GSH/1 mM *S*-hexylGSH/100 mM Tris adjusted to pH 9.1 with HCl. The eluate was rapidly transferred at room temperature into buffer B [10% (v/v) glycerol/1 mM dithiothreitol/20 mM triethanolamine adjusted to pH 7.35 with HCl] using two Sephadex G-25 (PD-10) columns. The PGH Δ -isomerase was then purified by anion-exchange FPLC (Mono Q HR 5/5), eluting with a gradient of NaCl. The purified enzyme was analysed by reverse-phase HPLC using a 2.1 mm $\times$ 300 mm Brownlee-Aquapore column (Anachem, Luton, U.K.) at 0.13 ml/min using a gradient of 28–65% acetonitrile in 0.06% aqueous trifluoroacetic acid over 1 h and monitoring elution at 214 nm. The primary sequence of native and CNBr-fragmented protein was obtained by automated gas phase sequencing [2].

Assay of PGH metabolism

PGH Δ -isomerase activity was studied in a coupled reaction whereby PGH is both generated and metabolized. The following stock solutions were prepared. Solution I contained 0.3 M NaCl, 5 mM tryptophan, 1 μ M haematin and 20 mM sodium phosphate, pH 7.40. Solution II contained 5 mM EDTA and 5 or 30 mM GSH, adjusted to pH 6.0 with NaOH. Solution III contained 2 M citric acid. Solution IV contained 1 mg/ml triphenylphosphine in ethyl acetate. I and II were stored at -20°C ; III and IV were stored at $0-4^\circ\text{C}$. Assays were carried out in 0.5 ml silanized conical polypropylene capped vials. Assay mixtures were prepared at $0-4^\circ\text{C}$ and contained 20 μ l of enzyme sample and 5 μ l of solution II. [$1-^{14}\text{C}$]Arachidonic acid in ethanol was added to solution I (6 μ l/100 μ l), and 20 μ l of the mixture was added to each assay followed by 5 μ l of PG synthase. The reaction was then initiated by placing samples in a shaking water bath at 37°C for 3–4 min, and was stopped by placing in ice/water. Eicosanoids were immediately extracted with 250 μ l of a fresh mixture of solutions II and IV [1.5:1000 (v/v)] at $0-4^\circ\text{C}$. Half of each upper layer was analysed by thin-layer chromatography, using as solvent the upper phase (dried over sodium sulphate) of a mixture of ethyl acetate/2,2,4-trimethylpentane/acetic acid/water [110:50:20:100 (by vol.)] [16]. Eicosanoid standards (3 μ g) were run in separate channels. The standards were revealed with I_2 vapour, and 1 μ l of the remaining radioactive solution I was spotted on to each. Radioactivity was then quantified by two-dimensional scanning for 2 h using the Ambis radioanalytical system 415 (LabLogic, Sheffield, U.K.), and the radioactive fractions corresponding to arachidonic acid, HHT and PGs H_2 , D_2 , E_2 and $\text{F}_{2\alpha}$ were quantified and compared with control (buffer blank) samples. The triphenylphosphine in the extraction prevented decomposition of PGH_2 to PGD_2 or PGE_2 during extraction, thus preventing their over-estimation. However, up to 3% of the total ^{14}C found in $\text{PGF}_{2\alpha}$ resulted from the chemical reduction of PGH_2 .

RESULTS AND DISCUSSION

Purification

The purification of PGH Δ -isomerase as a major component of the GSH transferases of rat spleen (Figure 1) confirms the previous studies of Christ-Hazelhof and Nugteren [8] and Urade et al. [9]. It is unclear why the enzyme was not noted in a more recent study by Tsuchida and Sato [12]. The procedure described here is much simpler than the previously described method, being easily accomplished in 1 day. The DE-cellulose step can also be

Figure 1 Purification of PGH Δ -isomerase from the GSH transferase fraction by anion-exchange FPLC

A fraction containing soluble GSH transferases from 2.6 g of rat spleen was applied to a Mono Q HR 5/5 column in buffer B (pH 7.35) at a flow rate of 0.35 ml/min. Proteins (continuous line) were eluted with a gradient of NaCl (dashed line) and monitored at 280 nm (a). The peak containing the PGH Δ -isomerase activity (assayed in Figure 2) was analysed by reverse-phase HPLC (b). The dotted trace shows the comparable analysis of rat liver GSH transferase subunits. Details are given in the Experimental section.

omitted with little loss in yield (75 μ g/2.6 g of spleen). Since the enzyme only occurs in about 3% of the spleen cells [10], it is estimated to constitute 1.5–3.5% of the soluble protein in those cells. The enzyme elutes later than any other previously characterized rat GSH transferase upon reverse-phase HPLC (Figure 1b).

PGH Δ -isomerase activity

PGH isomerase (and reductase) activity has usually been measured using synthetic [^{14}C]PGH $_2$. However, it has recently been shown that PGH synthases (both constitutive and inducible types) yield a high proportion of HHT rather than PGH $_2$ in the presence of GSH [15]. Since the rat spleen enzyme is GSH dependent, it was considered important to examine the effect of the enzyme on PGD $_2$ formation in competition not only with the spontaneous degradation of PGH, but also with the GSH-dependent formation of HHT by PG synthase. The results

Figure 2 Activity of PGH α -isomerase

PG synthesis from [1^{14}C]arachidonate (initially $30\ \mu\text{M}$) was obtained using purified PGH synthase in a physiological buffer at 37°C for 3 min in the absence (a) or presence (b) of the major protein fraction separated by anion-exchange chromatography (Figure 1a). PGs were extracted and separated by thin-layer chromatography. The plates were scanned for radioactivity, and the counts/2 h in each track were normalized. Details are given in the Experimental section.

(Figure 2) show that the PGH α -isomerase specifically promotes PGD_2 formation ($\text{PGD}/\text{PGE} = 9.1$) and decreases the GSH-dependent formation of HHT by the PGH synthase. Furthermore, the presence of the isomerase appears to increase the metabolism of arachidonic acid, presumably by lowering the level of PGH_2 , which is thought to act as an alternative substrate for the PGH_2 synthase [15]. The concentration of the isomerase in the assay depicted in Figure 2 is only $1/8$ of that estimated to be present in the spleen dendritic cells, and the level of PGH_2 is approx. $1\ \mu\text{M}$. One may conclude that the PGH α -isomerase is well capable of catalysing the specific formation of PGD_2 under physiological conditions in the antigen-presenting cells and mast cells in which it occurs.

Structural characterization

N-terminal sequencing of the native PGH α -isomerase confirms its identity with the enzyme purified by Urade et al. [9] and provides additional residues to those published previously. Comparison of the sequence with those of other GSH transferases unexpectedly shows a remarkable degree of identity with enzymes of the sigma class, typified by GSH transferases isolated from molluscs (e.g. squid and octopus [5]) and parasitic helminths (e.g. *Schistosoma*, *Ascaris*, *Onchocerca* [17–19]). A CNBr fragment also appeared to correspond to the sigma-class C-terminal sequence. A comparison of these sequences with examples of GSH transferases of the other classes listed according to their three-dimensional structural characteristics [5,20] is shown in Figure 3. Several invariant residues occur in the sigma-class GSH transferase sequences shown: Phe-8, Glu-15, Asp-30, Trp-38, Lys-42 (numbering of the PGH α -isomerase) and an arginine residue six residues from the C-terminus. This is the first demonstration of a sigma-class GSH transferase occurring in a 'higher' organism.

Significance

The similarity between the sequence of the specific PGH α -isomerase and GSH transferases of parasitic helminths raises the possibility that some of the latter may also be involved in eicosanoid metabolism. The production of PGs by such blood/

GST	Class	N-terminus					C-terminus					% Identity
		1	10	20	30	40	48					
R.nPGI	(σ)	PNYKLLYFNMGRRAEIIIXYIFAYLDIKYEDHRIEQ-----ADWPKIKPTL-PF-G						ILKRPQTKL				
S.j28	σ	V..I...G...P.RM.LVAAGVEF..E..F-----Q.....I..G..						LSE.HA.PF				52.7
A.s	(σ)	M.Q...T...DI..LG.GARL..HQAGV.F..N.LKR-----E...AL..KT...						.AE..K.PY				50.9
S.m28	σ	MAGEHI.VI..DG...S.RMTLVAAGYD..E..SF-----Q.....I..G..						LGN..A.PF				49.1
SL11	σ	.S.T.Y.....CRML..VASVQ.Q.K..L-----E.TQF.TKM..C-H						.K..NN.AF				49.1
OV1	(σ)	MEK.T.T...G...V.RLL..LANVS..N..TR-----DE.KYL..RT...						.K...K.YF				47.4
1GSQ	σ	.K.T.H..PLM...LCRFVL.AHGEE.T.RVV.M-----..NL.A.M-YS-N						LK...VRDF				38.6
C.e	(σ)	MTL..T..DIH..L..P.RLLL.DKQVA...VTY-----EQ.AD...KM-I..						LN..AAINP*				38.6
A1	α	AEKP..H...A...M.STRWLL.AAGVEF.EKF.KS-----AE.LD.LRNDGYLMFO						.QPGSPR.P*				30.0
R.n3	μ	PMI.G.W.V..LTHP.RLLLE.T.SS..EK.YAMGDAPDYDRSQ.LNE.FK.GLDFF						MKSSRYLST*				21.2
P1	π	.P.TVV..PV...CAALRMLL.DQGQSWKEEVTV-----ET.QEGSLKA-SC-L						A.SPEYVN.*				19.3
R.n5	θ	VLE..LDLLSQPCRAI...KKNIPFQMHTV-----CLRKGEALSD-A.-A						LEAEVILK*				12.7

Figure 3 Comparison of the partial primary sequence of rat PGH α -isomerase with GSH transferases of the sigma and other classes

N-terminal and C-terminal sequences of PGH α -isomerase (R.nPGI) are aligned with GSH transferases of the sigma and other classes according to the three-dimensional structure for maximum similarity [5,20]. Data for the 28 kDa enzyme from *Schistosoma japonicum* (S.j28) and the 28 kDa enzyme from *S. mansoni* (S.m28) are from [17]. Data for enzymes from *Onchocerca volvulus* (OV1) and *Ascaris suum* (A.s) are from [18] and [19] respectively. Sequences of a squid lens crystallin (SL11), squid hepatopancreas enzyme (1GSQ), human GSTA1 (A1), human GSTP1 (P1) and rat GST subunit 3 (R.n3) are taken from [5], that of *Caenorhabditis elegans* (C.e) is from [21], and that of rat GST subunit 5 (R.n5) is from [4]. Conserved residues of the sigma class are in bold type; numbering corresponds to the PGH α -isomerase. Identical residues are denoted by a dot; spaces to accommodate tertiary structure similarity are denoted by hyphens, and an asterisk denotes sequence continuing past the conserved sigma-class C-terminus.

lymph-borne and intestinal parasites could be an important component of their mode of subversion or suppression of host immunity [22]. This view is supported, for example, in the case of lymphatic microfilariaes (elephantiasis) due to *Brugia malayi* and *Wuchereria bancrofti*, which are coated with PGE [23], which is well known to have profound effects on immunity [24,25]. Furthermore, elephantiasis may be treated with diethyl-carbamazine, a potent inhibitor of PGH synthase [26].

Investigation of the PGH isomerase activity of GSH transferases of a helminthic parasite, *Ascaridia galli*, which confirms this hypothesis, is presented in another paper [27]. If PG synthesis is a common function of helminth GSH transferases, their PGH isomerase activity should be considered as a potential target for therapy or prophylaxis. Since praziquantel, the major therapeutic agent for schistosomiasis, binds to and inhibits a GSH transferase of *S. japonicum* [28], its effect could be due to the inhibition of PG biosynthesis, in which case it can no doubt be rationally improved. Studies are under way to test for PGH isomerase activity in schistosomal GSH transferases.

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