Purification and characterization of prostaglandin-H E-isomerase, a sigma-class glutathione S-transferase, from *Ascaridia galli*

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Comparison of partial primary sequences of sigma-class glutathione S-transferases (GSH) of parasitic helminths and a GSH-dependent prostaglandin (PG)-H D-isomerase of rat immune accessory cells suggested that some of the helminth enzymes may also be involved in PG biosynthesis [Meyer and Thomas (1995) Biochem. J. **311**, 739–742]. A soluble GSH transferase of the parasitic nematode *Ascaridia galli* has now been purified which shows high activity and specificity in the GSH-dependent isomerization of PGH to PGE, comparable to that of the rat spleen enzyme in its isomerization of PGH to PGD, and similarly stimulates the activity of prostaglandin H synthase. The enzyme subunit is structurally related to the rat spleen enzyme and sigma-class GSH transferases of helminths according to the partial primary sequence. The data support the hypothesis that some sigma-class GSH transferases of helminth parasites are involved in PG biosynthesis which, in the case of PGE, is likely to be associated with the subversion or suppression of host immunity. A PG-H E-isomerase of comparable specificity and activity has not previously been isolated.

INTRODUCTION

Prostaglandin (PG) E is best known as a potent modulator of T helper cell cytokine secretion, which may result in immunosuppression or the development of a Th2-type response [1,2]. Some unicellular parasites (e.g. *Entamoeba histolytica* [3], *Hypoderma lineatum* [4]) and the intracellular pathogen, *Leishmania major* [5], are associated with stimulation of PGE formation in monocytes/macrophages. In multicellular (helminth) parasites there is evidence of endogenous prostanoid synthesis, the inhibition of which may lead to parasite elimination concomitant with strong immunological reaction [6]. Indeed, in the case of *Brugia malayi* and *Wuchereria bancrofti*, the microfilariae are coated with PGE [7]. It is also becoming clear that tissue invasion by certain tumours may be related to the stimulation of macrophage PGE synthesis which inhibits macrophage production of the endogenous inhibitor of tissue-destructive metalloproteinases [8,9].

Despite the obvious importance of PGE in immune function, particularly in disease states, an enzyme clearly involved in its specific synthesis from PGH has not hitherto been isolated, although glutathione S-transferase (GSH)-dependent enzymes specifically catalysing PG-H E-isomerization have been partially purified from bovine and sheep vesicular gland microsomes [10,11]. A GSH transferase showing low, but apparently specific, PG-H E-isomerase has been isolated from human brain [12], which may be related to the involvement of PGE in sleep–wake regulation [13].

A structural comparison of a specific, highly active PG-H D-isomerase (a sigma-class GSH transferase), occurring in rat immune accessory cells with other GSH transferases, suggested a close relationship with GSH transferases of helminth parasites [14], indicating a possible novel role for the latter in PG synthesis as an immunosubversive strategy. Purification and analysis of the GSH transferases of the intestinal parasitic nematode, *Ascaridia galli*, now presented, characterize for the first time a highly active, specific PG-H E-isomerase.

EXPERIMENTAL

Purification of PG-H E-isomerase

A. galli, adult worms were obtained [15] and stored at −70 °C. A mass of 2 g was homogenized using an Ultra Turrax in 4 vol. of buffer A: 0.2 M NaCl/1 mM dithiothreitol (DTT)/1 mM EDTA/50 μM PMSF/1 μM leupeptin/1 μM pepstatin/20 mM Na-phosphate, pH 7.0. The soluble fraction plus suspended lipid, obtained by centrifugation, was snap-frozen and kept at −70 °C. Upon thawing it was re-homogenized using a Dounce homogenizer and applied at 0.3 ml/min to a 2.5 ml column of S-linked GSH–agarose equilibrated with buffer A. After washing with 10 ml of buffer A, the GSH transferases were eluted with 35 mM GSH/1 mM EDTA/0.1 M Tris/HCl, pH 9.5. The GSH eluate (2.8 ml), which contained 99.5–100% of the original 1-chloro-2,4-dinitrobenzene (CDNB) GSH transferase activity, was dialysed twice against 1 litre of buffer B: 10% (v/v) glycerol/1 mM DTT/20 mM Tris/HCl, pH 8.0, and applied at 0.5 ml/min to a MonoQ (HR5/5) FPLC column equilibrated in buffer B. The column was eluted at 0.35 ml/min with a linear gradient of NaCl in buffer B. After assay of CDNB GSH transferase and PGH isomerase activities, and subunit analysis by reverse-phase HPLC and SDS/PAGE, fractions were frozen. Portions of the fractions containing specific PG-H E-isomerase activity were thawed, mixed with an equal volume of buffer C: 10% (v/v) glycerol/2 mM DTT/2 mM EDTA/50 mM Tris/HCl, pH 7.60, containing 2.4 M ammonium sulphate, and applied at 0.3 ml/min to a polyPROPYL A column equilibrated in buffer C containing 1.2 M ammonium sulphate. The column was

Abbreviations used: GSH, glutathione S-transferase; PG, prostaglandin; CDNB, 1-chloro-2,4-dinitrobenzene; HHT, [12*s*]hydroxy-heptadeca-5,8,10-trienoic acid; DTT, dithiothreitol.

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Table 1 Effect of *A. galli* fractions on PG synthesis

Arachidonic acid (30 μM) was incubated with PGH synthase and 0.5 mM GSH in the presence or absence of fractions from *A. galli* and the products separated and quantified [14]. A 10 μl volume of each fraction was included in each assay; final volume 52 μl. Protein in the cytosol and fractions, unbound and eluted from GSH–agarose, were estimated using the Bradford reagent [18]; protein in the other fractions was estimated from the A_{280} assuming 1.0 equalled 1 mg/ ml. * Indicates specifity of the PGH isomerase.

Fraction		Protein (μg/ml)	Change in compound compared with control (plus/minus % of total c.p.m.)						PGE/PGD*
			AA	HHT	PGH	PGD	PGE	PGF	
Control		0	24.0	8.8	4.7	9.8	25.4	24.6	2.6
Cytosol		3300	+0.9	−5.2	−4.2	−5.4	+12.6	+4.7	8.6
GSH–agarose									
Unbound		2680	+21.3	+3.6	−4.6	−3.2	−7.1	−2.7	2.9
GSH-eluate		220	−9.9	−2.3	−4.6	−9.7	+26.4	+2.8	10.2
MonoQ fraction	2	14	−18.6	−4.4	−2.6	−4.1	+19.0	+13.4	7.7
	11	10	−20.9	+1.2	+2.0	+1.2	+8.8	+10.4	3.1
	13	6	−11.7	+1.1	+0.6	+3.2	+1.6	+6.6	2.1
	18	10	−4.3	−5.7	−1.5	+12.4	+9.1	−7.3	1.5
	21	8	−12.6	−3.0	−2.6	−4.7	+42.6	−16.1	13.4
	22	9	−14.0	−8.3	−4.2	−5.5	+49.6	−14.2	17.5
	23	6	−21.0	−2.4	−1.1	−4.7	+49.9	−17.6	14.8
	24	5	−15.9	−5.9	−1.1	−9.3	+53.1	−18.3	> 50
	25	4	−13.3	−0.8	0	−1.3	+20.5	−3.3	5.4
HIC fraction	5	0.7	−6.3	−3.8	+1.7	−2.3	+21.7	−10.9	10.8
	8	0.7	−10.9	−1.9	+2.1	−0.8	+19.0	−7.5	6.9

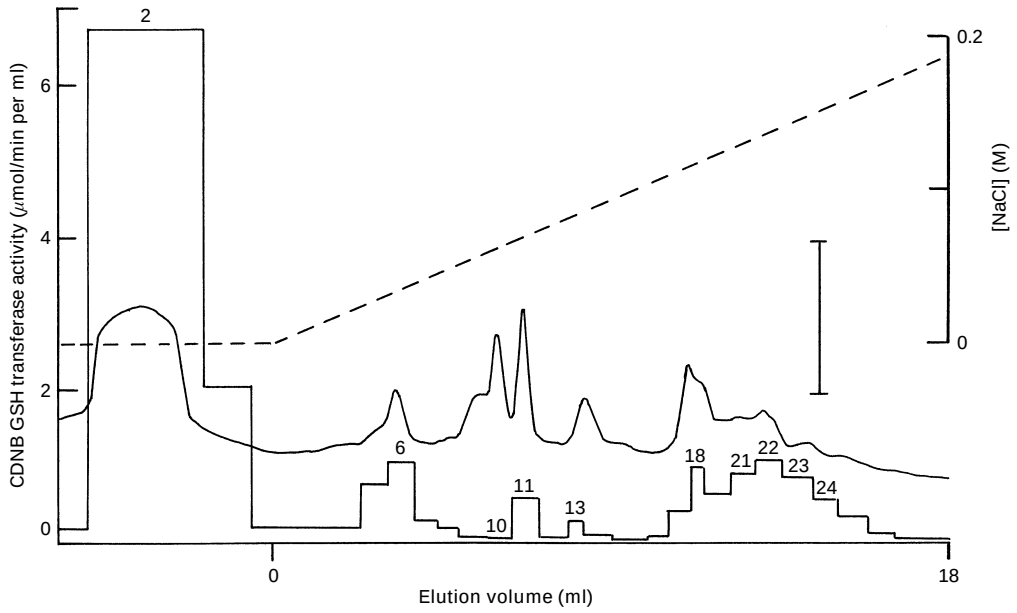

Figure 1 Fractionation of *A. galli* GSH–agarose eluate by anion-exchange chromatography

The GSH eluate of *A. galli* proteins retained by GSH–agarose was fractionated using MonoQ FPLC. Protein elution was monitored by A_{280} (continuous trace). All fractions were assayed for CDNB GSH transferase activity at 37 °C [19] (histogram), and selected fractions (numbered) were assayed for effects on PG synthesis (see Table 1) and analysed by reverse-phase HPLC (see Figure 2) and SDS/PAGE (not shown).

eluted with a decreasing gradient of ammonium sulphate in buffer C. Steps prior to the MonoQ separation were done at 0–4 °C.

PG-H isomerase assays

PG-H isomerase activity was measured using [1-^{14}C]arachidonate and purified ram seminal vesicle PG-H synthase, under approximately physiological conditions of pH, temperature and ionic strength for 3–4 min, and analysed by TLC with radioactive scanning [14]. Since PGE_2 is only slightly separated from thromboxane B_2 in the solvent system used routinely, portions of samples from several experiments were separated using an alternative solvent: chloroform/methanol/acetic acid/water (90:8:1:0.8 by vol.) [16]. The main peak of radioactivity co-chromatographed with PGE_2 in each case.

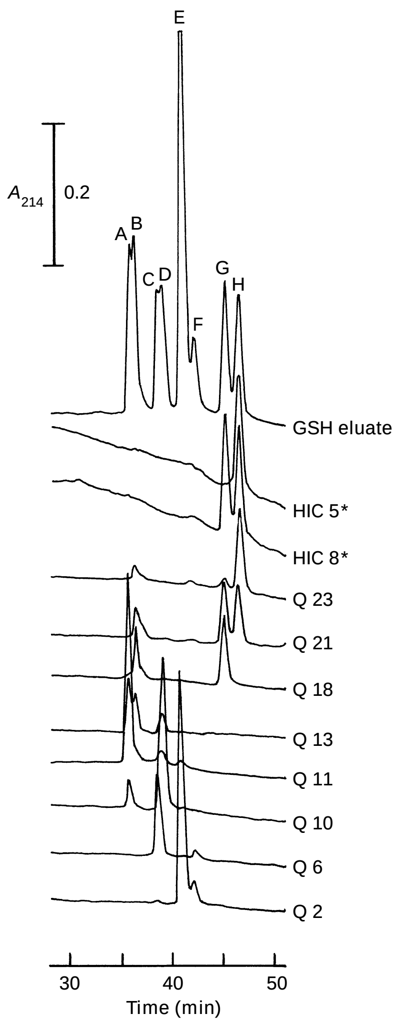

Figure 2 Reverse-phase HPLC analysis

The GSH–agarose eluate and fractions from anion-exchange and hydrophobic-interaction chromatography (Figures 1 and 2) were analysed by microbore reverse-phase HPLC with a gradient of 28–65% (v/v) acetonitrile in 0.6% (v/v) aqueous trifluoroacetic acid over 1 h, and elution was monitored at 214 nm [14]. An asterisk indicates a five times more sensitive absorbance scale. Eight subunits are distinguished, termed A–H in order of elution.

Primary structure determination

N-Terminal and tryptic peptide primary structure were obtained by automated gas-phase sequencing [17].

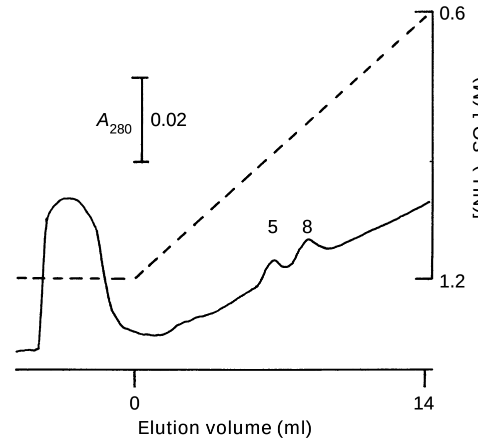

Figure 3 Hydrophobic-interaction chromatography of fractions showing specific PG-H E-isomerase activity

Portions of fractions 21–25 from MonoQ FPLC were thawed, pooled and further separated by hydrophobic-interaction chromatography using a column of PolyPROPYL A. The fractions obtained were assayed for effects on PG synthesis (Table 1) and analysed by reverse-phase HPLC (Figure 2) and SDS/PAGE.

RESULTS

Purification of PG-H E-isomerase

The soluble fraction plus suspended lipid ('cytosol') of *A. galli* displayed PG-H E-isomerase activity and to a lesser extent PG-H F-reductase activity (Table 1). Essentially, all the PGH metabolizing activity together with all the CDNB GSH transferase activity was retained by, and subsequently eluted from, GSH–agarose. The GSH–agarose eluate was resolved into multiple fractions by anion-exchange FPLC and most showed CDNB GSH transferase activity (Figure 1). Eight subunits (A–H) were distinguished by reverse-phase HPLC (Figure 2). Their relative mobilities (kDa) by SDS/PAGE (not shown) were: A, 25.7; B, 26.4; C, 26.0; D, 28.3; E, 24.3; G, 26.4; and H, 25.7 (subunit F was not analysed). Analysis of the effect of selected MonoQ fractions upon PGH (Table 1) showed specific PG-H E-isomerase activity in the latest eluting fractions (MonoQ 21–25) preceded by material also showing PG-H D-isomerase activity (MonoQ fraction 18). The fraction not retained by the anion-exchanger (MonoQ 2) showed both PG-H E-isomerase and PG-H F-reductase activity similar to that of rat GSH transferase 1-1 [20]. Since the fractions showing the most active and specific PG-H E-isomerase (Q21–25) contained varying amounts of two subunits, G and H, a portion of each was pooled and further fractionated

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                            1        10        20        30        40                  80        90
A. galli   subunit H         TKYKLTYFDLRGLGEGARLIFHQAGVQFEDHRITQEEWPNLKPK     YLGRQFGLVPKDPIEEA VDAIYDA
A. suum    GST1             MPQ.......I.................K...N.LKR.D..A....  (R)........AG.PTM...Q..S.F.Q
A. galli   subunit A          G..V...AI...A.PI.ML
A. galli   subunit C         VG......NAT.RA.
A. galli   subunit E         VN.......A..RA.A..MX....N
A. galli   subunit G         .........F...................

                                  130           150        160          200                  Identity (%)
A. galli   subunit H           DIVIPASDRR      TGSGFLVGKSLTWADLAIFDR     NYLAERPK
A. suum    GST1             (K)EVAV..R.KH  (K)S..EYM....V.....V.T.S  (K)KW......                 66.7
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Figure 4 Primary sequence analysis

The PG-H E-isomerase subunit H was purified from MonoQ fractions 22–25 by reverse-phase HPLC as in Figure 2. A portion was subjected to N-terminal sequencing and the remainder was hydrolysed with trypsin. The tryptic digest was fractionated by reverse-phase HPLC and several peptides sequenced. The sequences were compared with over 100 GSH transferase sequences showing a close relationship to several nematode sigma-class GSH transferases. The best fit with GST1 of *Ascaris suum* [22] is shown. Subunit G was also purified by HPLC and the N-terminal sequence obtained. Sequences of subunits A, C and E were obtained from MonoQ fractions 11, 6 and 2 respectively. Identical residues are denoted by a dot.

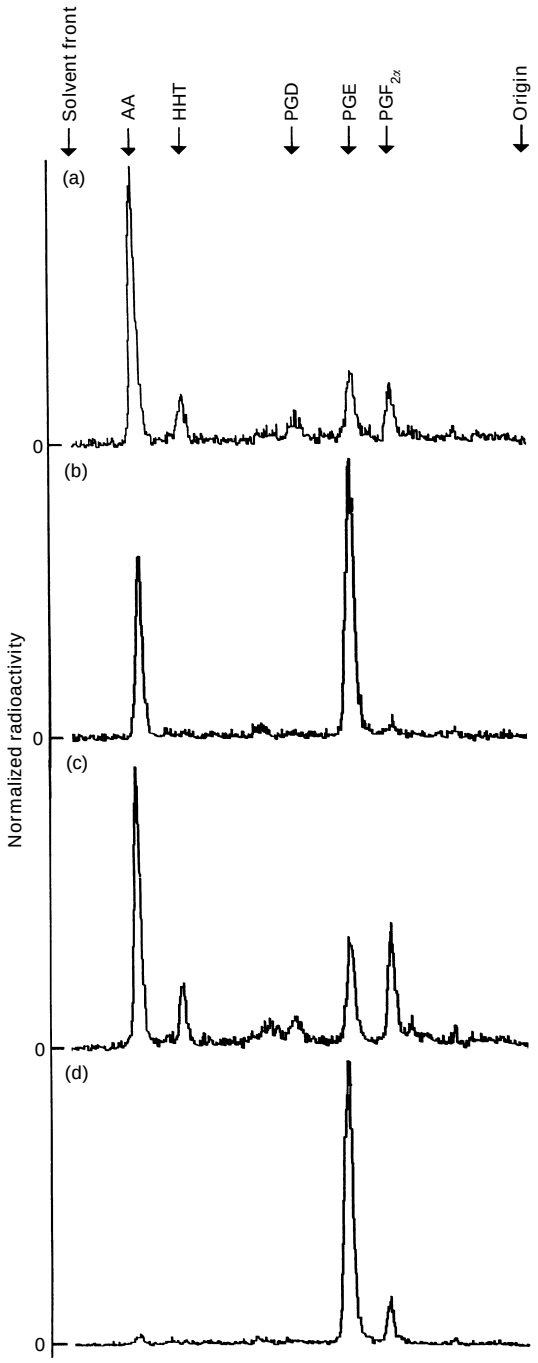

Figure 5 Effect of MonoQ fractions 22–25 on PG synthesis

PG synthesis from [1-^{14}C]arachidonate (initially 30 μM) was obtained using purified PGH synthase in a physiological buffer at 37 °C in the absence (**a**,**c**) or presence (**b**,**d**) of approx. 2 μg/ml of pooled MonoQ fractions 22–25 and either 3 mM (**a**,**b**) or 0.5 mM (**c**,**d**) of GSH. Products were separated by TLC and analysed by scanning for radioactivity. Counts/2 h for each track were normalized [14]. Individual assays are shown, typical of several similar experiments.

by hydrophobic-interaction chromatography. Two peaks were obtained, HIC 5 and HIC 8 (Figure 3). The former contained only subunit H, the latter contained approximately equal amounts of subunits G and H (Figure 2), consistent with the presence of a heterodimer. The absorbance in the unretained fraction was found not to be due to protein. Both fractions HIC 5 and HIC 8 showed specific PG-H E-isomerase activity. Slightly higher activity in HIC 5 suggested that the activity resides mainly (if not totally) in subunit H (Table 1). Separation of the two forms, HIC 5 and HIC 8, resulted in substantial loss of protein. The activity in these fractions appears less impressive. However, the protein concentration is so low that the estimated specific activity of 2 μmol/min per mg of protein, which is generated at a PG-H concentration of about 1.5 μM, represents approx. twice that of the rat spleen PG-H D-isomerase at this PGH concentration (deduced from data in [21]). It is concluded that *A. galli* subunit H is a GSH transferase having high activity and specificity in the isomerization of PGH to PGE.

Assuming similar A_{214} for the different subunits, subunit H constituted 9.9 % (110 μg) of the total GSH eluate, the bulk of which was recovered in MonoQ fractions 20–25, and represented 0.06 % of the protein obtained from the centrifugal supernatant of the *A. galli* homogenate.

Partial primary sequence determination

The N-terminal sequence and tryptic peptide sequences obtained from the subunit H peak is similar to other nematode sigma-class GSH transferases, particularly *Ascaris suum* GST1 with which it is aligned in Figure 4. Also shown are the N-terminal sequences of subunits A, C, E and G.

Effect of GSH

Studies on the effect of GSH were conducted with a pool of MonoQ fractions 22–25. Its specific stimulation of PGE formation in the presence of 3.0 or 0.5 mM GSH is clearly evident in Figure 5, which also shows its inhibition of [12*S*]hydroxyheptadeca-5,8,10-trienoic acid (HHT) formation and stimulation of arachidonate utilization comparable to that observed with the PG-H D-isomerase [14]. In order to prevent overestimation of enzyme-directed PGE formation, the presence of triphenylphosphine in the extraction solvent directed any PGG and PGH decomposition during the analysis to PGF. Even so, the specificity of PGE formation is remarkable (PGE:PGD = 18; PGE:PGF = 3–5, at about 5 μg/ml enzyme).

The K_m for GSH obtained with CDNB as second substrate was 75 $\pm$ 5 μM, similar to those of other GSH transferases of the alpha, mu and pi classes [23] but lower than those reported for *Schistosoma* sigma-class GSH transferases [24]. In the absence of GSH, no effect on PG synthesis was detected, however, the PG-H E-isomerase activity was still prominent when only 10 μM GSH rather than 500 μM was included in the assay; PGE:PGD = 8.5 and 17.5 respectively.

Inhibition

Since rat spleen PG-H D-isomerase activity with CDNB was sensitive to inhibition by Indocyanine Green [21], its effect was studied in our PG synthase system. Indocyanine Green (8 μM) completely inhibited both specific PG-H isomerase activity and stimulation of arachidonate utilization by either the rat spleen or *A. galli* enzymes (results not shown).

DISCUSSION

On the basis of structural similarity with the PG-H D-isomerase of rat immune accessory cells, some sigma-class GSH transferases of parasitic helminths were predicted also to exhibit PGH

isomerase activity [14]. The PG-H E-isomerase, GSH transferase subunit H of *A. galli* described herein shows similar high levels of specificity and activity to the rat enzyme, similarly reduces the formation of HHT and stimulates PGH synthesis, is similarly sensitive to inhibition by Indocyanine Green, and, according to primary structure, is also a sigma-class enzyme. The hypothesis is thus confirmed in one case.

To our knowledge, the *A. galli* subunit H is the first high-activity, high-specificity PG-H E-isomerase to be purified. Although whether *A. galli* actually produces PGE remains to be determined.

Since PGE is known for its potent physiological and immunological effects, questions immediately arise as to whether similar enzymes occur in parasitic helminths responsible for human diseases, and whether their modulation might provide novel therapy or prophylaxis. There is strong evidence of PGE formation by *Brugia* and *Wuchereria* (elephantiasis) [7] and *Taeniae taeniaeformis* [25], and evidence of eicosanoid synthesis in *Onchocerca* (river blindness) [6] from which GSH transferases of related structure have been obtained [26].

Effects of helminthiases upon human immunity in terms of immunosuppression or the inappropriate stimulation of either humoral (T-helper lymphocyte 2 cytokines) or cellular (T-helper lymphocyte 1 cytokines) adaptive immunity [27–29] is currently under intense study. Since PGs are associated with alterations in cytokine secretion pattern [1,2], the current data suggest that further study of helminth PG synthesis might also be useful.

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