Glutathione S-transferases in rat olfactory epithelium: purification, molecular properties and odorant biotransformation

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The olfactory epithelium is exposed to a variety of xenobiotic chemicals, including odorants and airborne toxic compounds. Recently, two novel, highly abundant, olfactory-specific biotransformation enzymes have been identified: cytochrome *P*-450olf1 and olfactory UDP-glucuronosyltransferase (UGT_{olf}). The latter is a phase II biotransformation enzyme which catalyses the glucuronidation of alcohols, thiols, amines and carboxylic acids. Such covalent modification, which markedly affects lipid solubility and agonist potency, may be particularly important in the rapid termination of odorant signals. We report here the identification and characterization of a second olfactory phase II biotransformation enzyme, a glutathione S-transferase (GST). The olfactory epithelial cytosol shows the highest GST activity among the extrahepatic tissues examined. Significantly, olfactory epithelium had an activity 4–7 times higher than in other airway tissues, suggesting a role for this enzyme in chemoreception. The olfactory GST has been affinity-purified to homogeneity, and shown by h.p.l.c. and N-terminal amino acid sequencing to constitute mainly the Yb_1 and Yb_2 subunits, different from most other tissues that have mixtures of more enzyme classes. The identity of the olfactory enzymes was confirmed by PCR cloning and restriction enzyme analysis. Most importantly, the olfactory GSTs were found to catalyse glutathione conjugation of several odorant classes, including many unsaturated aldehydes and ketones, as well as epoxides. Together with UGT_{olf}, olfactory GST provides the necessary broad coverage of covalent modification capacity, which may be crucial for the acuity of the olfactory process.

INTRODUCTION

Glutathione S-transferases (GSTs; EC 2.5.1.18) constitute a gene superfamily of xenobiotic-metabolizing enzymes which bind various ligands and catalyse the nucleophilic addition of glutathione (γGlu-Cys-Gly) to diverse electrophilic substrates (for reviews, see refs. [1–4]). These enzymes underlie drug detoxification and protection against peroxidative damage in the liver and other tissues [1]. Their conjugation products may undergo further metabolism to the respective mercapturates and mercaptans [1,2]. Cytosolic GSTs are abundant soluble homo- or hetero-dimers consisting of polypeptides of about 25 kDa. Another form, microsomal GSTs, have 17 kDa subunits attached to the endoplasmic reticulum membrane [1,2,5].

Based on sequence similarity, mobility on SDS/PAGE and isoelectric points, rat cytosolic GSTs are divided into four classes: (i) Alpha, which includes the Ya (or 1), Yc (2) and Yk (8) subunits; (ii) Mu, including the Yb_1 (3), Yb_2 (4), Yb_3 (6) and Yn (11) subunits; (iii) Pi, the Yp (7) subunit; and (iv) the more recently discovered Theta, including subunits 5, 12 and Yrs [6,7]. These and some additional GST subunits have been further characterized by molecular cloning [2,8–12]. Dimerization occurs within, but not across, class barriers.

GSTs are biotransformation enzymes, akin to cytochromes *P*-450 and UDP-glucuronosyltransferases (UGTs). Olfactory epithelium has recently been shown to have high concentrations and unique types of the latter enzymes. The olfactory biotransformation enzymes have been proposed to underlie the termination of odorant signals, as well as the protection of the sensory cells against airborne toxic compounds [13–15]. Even though cytochrome *P*-450s and UGTs are broad-spectrum enzymes, they only act upon odorants with certain chemical structures and functional groups. GST action may broaden the biotransformation scope of the sensory tissue, Indeed, GST immunoreactivity has been detected in the rat olfactory mucosa using antibodies against several hepatic isoenzymes [16]. Furthermore, olfactory GST enzyme activity has been reported towards 1-chloro-2,4-dinitrobenzene (CDNB) in rats [17], cattle [18] and humans [19], and towards styrene oxide in the rat [20] and the dog [21].

To obtain more detailed information at the molecular level regarding olfactory GSTs, we have purified the enzymes to homogeneity from the olfactory epithelium. The chemosensory tissue is shown to have very high levels of the enzyme, with a specific isoenzyme spectrum dominated by the Yb_1 and Yb_2 subunits. Odorants are found to be efficient substrates for the olfactory enzyme. These findings suggest a possible involvement of GSTs in the olfactory process.

MATERIALS AND METHODS

Enzyme purification

Olfactory and respiratory nasal mucosa (including epithelium and subepithelium), as well as other tissues, were freshly dissected from 5–7-week-old male Wistar rats (Weizmann Institute Animal Breeding Facility). Tissues were collected and stored for up to 2 h in ice-cold buffer A (20 mM Tris/HCl, pH 8.0, 2 mM EDTA, 1 mM phenylmethanesulphonyl fluoride, 100 mM NaCl). The tissues were then transferred to 3 vol. of ice-cold buffer B (20 mM Tris/HCl, pH 8.0, 2 mM EDTA, 1 mM phenylmethane sulphonyl fluoride) and homogenized with a Polytron for 1 min at half-maximal speed. Bones and large aggregates were removed by a brief centrifugation step (5 min, 1200 *g*, 4 °C). Membranes were precipitated by centrifugation at 27000 *g* for 30 min at 4 °C, and the supernatant was filtered through glass wool and subjected to high-speed centrifugation (100000 *g*, 1 h, 4 °C). The super-

Abbreviations used: GST, glutathione S-transferase; UGT, UDP-glucuronosyltransferase; CDNB, 1-chloro-2,4-dinitrobenzene.
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natant (cytosolic fraction) and pellet (microsomal fraction) were divided into aliquots, quick-frozen in liquid nitrogen and kept at −80 °C. GSTs were purified using glutathione–agarose affinity column chromatography according to [22].

H.p.l.c. separation of GST monomers

Affinity-purified GSTs were separated by reverse-phase h.p.l.c. as described in [23]. For analytical preparations, GSTs from liver or from olfactory epithelium were separated on a 25 cm × 4.6 mm C18 Vydac column (Alltech, Deerfield, IL, U.S.A) with a Waters h.p.l.c. system (Milford, MA, U.S.A.). The solvents were water (solvent A) and 0.1 % (v/v) trifluoroacetic acid in acetonitrile (solvent B). A linear gradient was run from 35 % to 70 % (v/v) solvent B for 60 min with a flow rate of 0.9 ml/min. Detection was by u.v. absorption at 215 nm.

Preparative separations of olfactory GSTs were performed on an HP1090 liquid chromatograph using an HP1040 diode array detector and a data processing unit integrator. An affinity-purified fraction (430 μg) was separated by applying solvent B gradients of 35–55 % for 60 min followed by 55–80 % for 30 min.

Protein microsequencing

Amino acid N-terminal sequencing was carried out on an automatic pulse liquid gas-phase protein microsequencer (Model 475A) equipped with an on-line h.p.l.c. phenylthiohydantoin-amino acid analyser (model 120A) and a data acquisition and processing unit (Model 900) (all from Applied Biosystems Inc, Foster City, CA, U.S.A.).

Enzyme assays

A GST spectrophotometric assay was performed as described [24] in a volume of 1 ml, with 1 mM glutathione and 1 mM CDNB (Sigma Chemical Co., St. Louis, MO, U.S.A.) as an electrophile, at 23 °C for 5 min. The reaction was stopped by the addition of acetic acid to a final concentration of 0.2 M [25]. Odorant substrates (1 mM; Sigma Chemical Co. and Aldrich Chemical Co.) were studied by a t.l.c. assay which was similarly performed in a volume of 50 μl. An aliquot of 10 μl of reaction products was loaded on a 0.2 mm thick t.l.c. silica gel 60 F_{254} plate (Merck, Darmstadt, Germany) and developed using butan-l-ol/acetic acid/water (12:3:5, by vol.) for 3 h [25]. The air-dried plate was stained with ninhydrin (0.25 %, w/v, in acetone). For quantitative assays, 0.25 μCi of [^{3}H]glutathione (1 mCi/ml; 1Ci/mmol; NEN–du Pont) was included in the reaction. The stained bands were excised and the product was extracted with 1 ml of 1 M NaOH for 15 min, followed by neutralization with 100 μl of concentrated acetic acid and scintillation counting. Localization of the product t.l.c. bands for weak odorant substrates was aided by parallel chromatography of a reaction performed under saturating conditions (10 mM glutathione and odorant; 30 min reaction at 37 °C).

General methods

SDS/PAGE [26] and silver staining [27] were used to analyse the purified GST. Protein content was determined by the method of Bradford [28] using BSA as a standard.

Molecular cloning

RNA was prepared from rat olfactory epithelium by the urea/LiCl method [29]. The PCR primers designed to amplify rat Yb GSTs were: (1) 5′ primer: CCCAATCTGCCCTAC-TTAATTGATCG; and (2) 3′ primer: GC(G/C)AAACTC-CCGGACTTCTTCTA(T/G)AC. Single-stranded DNA synthesized from 0.5 μg of total RNA was amplified by the PCR method, using 0.2 mM of each of the deoxynucleotides, 100 pmol of each primer, 2.5 units of *Taq* DNA polymerase and the supplier's buffer (Promega, Madison, WI, U.S.A.), in a volume of 100 μl. The amplification was performed in a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA, U.S.A.) programmed as follows: 94 °C/1 min, 55 °C/2 min, 72 °C/2.5 min. Amplification was performed over 40 cycles, followed by a last extension at 74 °C for 7.5 min. The PCR product was subcloned into a pCR1000 vector (Invitrogene, San Diego, CA, U.S.A.) and sequenced by the dideoxy chain-termination method using a Sequenase II kit (United States Biochemicals, Cleveland, OH, U.S.A.).

RESULTS

The level of GST activity in various rat tissues was measured using CDNB as a substrate (Table 1). The olfactory cytosols had the second highest activity value, about 46 % of that in liver. Other tissues, such as brain and kidney, had activities within the range 7–22 % of that in liver. Notably, olfactory epithelium had an activity 5–10 times higher than in other airway tissues

Table 1 GST activity towards CDNB in cytosols from various rat tissues

Cytosols (5–20 μg of protein) from rat tissues, including olfactory and respiratory epithelia, were assayed for GST activity by spectrophotometry. The results are means ± S.D.s of 3–12 experiments. The liver activity was taken as 100 %.

Tissue	CDNB conjugation activity (nmol/min per mg of protein)	(%)
Liver	1280 ± 254	100.0
Kidney	284 ± 57	22.2
Brain	105 ± 25	8.2
Olfactory epithelium	594 ± 184	46.4
Respiratory epithelium	162 ± 12	12.7
Lung	84 ± 12	6.5

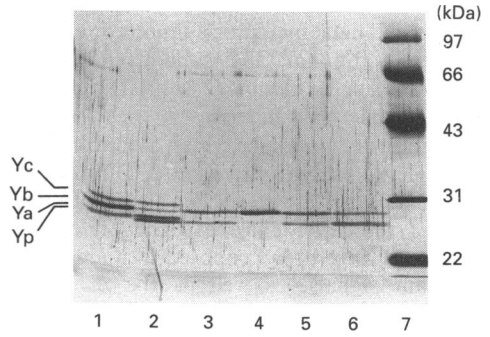

Figure 1 SDS/PAGE of affinity-purified GSTs from various rat tissue cytosols

Glutathione–agarose-affinity-purified polypeptides (1.5 μg) were electrophoresed on SDS/12.5 %-polyacrylamide gels and silver-stained. Samples were from the following sources: lane 1, liver; lane 2, kidney; lane 3, brain; lane 4, olfactory epithelium; lane 5, respiratory epithelium; lane 6, lung. Molecular masses of the standards (lane 7) are shown on the right.

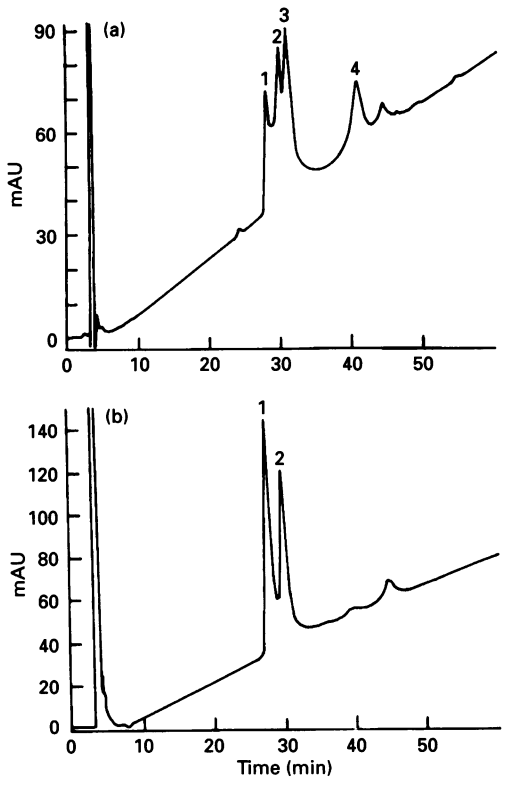

Figure 2 H.p.l.c. separation of liver and olfactory GST monomers

Dimeric GSTs from (**a**) liver (70 μg) and (**b**) olfactory epithelium (40 g) were injected for separation on a C_{18} reverse-phase h.p.l.c. column. Liver monomers were identified according to the published profile [23] as: 1, Yb_1; 2, Yb_2; 3, Yc; 4, Ya. mAU, milli-absorbance units.

Yb_1	PMILGYW*NV*RGL*T*HPIRL*LL*EYTD*SS*YE*E*KRY*A*MG
Yb_4	AMILGYW*NV*RGL*T*HPIRL*LL*EYTD*SN*YE*E*KRY*V*MG
SEQ1.1	PMILGY?NV?GLT?PI?LLLEYT
SEQ1.2	PMILGYWNVRGLTHPIRLLLlYyT
	i e t
	e
Yb_2	PMTLGYW*DI*RGLAHAIRL*F*LEYTD*TS*YE*D*KKY*S*MG
Yb_3	PMTLGYW*DI*RGLAHAIRL*L*LEYTD*S*SYE*D*KKY*T*MG
SEQ2.1	PMTLGYWDIRGLAHAIRLFLE
	i rnv t l

Figure 3 N-terminal amino acid sequences of olfactory GSTs and their similarity to liver GSTs

H.p.l.c. peaks were collected and subjected to N-terminal amino acid sequencing. The sequences labelled SEQ1.1 and SEQ1.2 are duplicate determinations of the protein in peak 1 of Figure 2. The sequence labelled SEQ2.1 is for the protein in peak 2 of Figure 2. Lower-case letters indicate ambiguity in the sequence. Rat GSTs were divided into two groups according to their sequence similarity. Differences within a group are underlined, while differences between groups are italicized.

(respiratory epithelium and lung). The high activity of rat olfactory epithelium is contributed mainly by the cytosolic GSTs and not by the microsomal form, since parallel assays conducted on a 100000 *g* olfactory membrane fraction showed only negligible GST activity (results not shown).

Specific patterns of GST subtype expression are known for several tissues (reviewed in [1–4]). In order to obtain information about the GST isoenzymes expressed in the olfactory epithelium, the olfactory epithelial cytosols were further purified using glutathione–agarose affinity columns. The eluted enzymes showed discrete band patterns on SDS/PAGE with silver staining (Figure 1) suggesting a successful purification. The olfactory GSTs included a prominent band that co-migrated with the liver Yb isoenzymes, with only minor amounts of Ya and negligible Yc. The GSTs of the nasal respiratory epithelium were composed of isoenzymes co-migrating with Yb as well as with Yp.

In order to further analyse the identity of the olfactory GSTs, they were separated into their monomeric subunits by reverse-phase h.p.l.c. [23]. Figure 2 shows the elution profiles for liver and olfactory cytosolic GSTs. It can again be seen that liver contains a greater variety of subunits than does the olfactory epithelium. The latter contains mainly two types of polypeptide, and these two major olfactory peaks co-migrate with liver GST peaks identified as Yb_1 and Yb_2 [23]. Four such analyses were conducted, using fractions from different purifications.

The identities of the two major h.p.l.c. peaks were confirmed by N-terminal protein microsequencing (Figure 3). The first h.p.l.c. peak was found to be identical to Yb_1 and the second to Yb_2, in agreement with the h.p.l.c. retention profile.

Although the N-termini of the olfactory GSTs are identical to those of the liver Yb_1 and Yb_2 subunits, there is no evidence that the sequences are identical throughout. Therefore we examined the olfactory GSTs using molecular biological techniques. PCR primers were designed with regard to conserved regions, to amplify all of the Yb transcripts simultaneously. These oligonucleotides flank a 413 bp variable region, allowing a more accurate discrimination between the isoenzymes. The amplified fragment could originate only from reverse transcription of mRNA, because it spans few introns. Genomic DNA contamination would generate a larger band, easily distinguishable from the RNA-generated fragment. Total olfactory epithelial RNA was reverse-transcribed into cDNA, and amplified by PCR. When analysed, the fragments showed the expected lengths. The PCR product was subcloned and a partial nucleotide sequence was determined for 16 independent clones. The sequences of the olfactory clones were practically identical to the published sequences of either Yb_1 [8] (2 clones) or Yb_2 [9] (14 clones).

The identities of the olfactory GST enzymes were further verified by restriction endonuclease analysis of the PCR-generated DNA fragment. Yb_1, Yb_2 and Yb_3 are all expected to yield a fragment of an identical size of 413 bp, but each of the sequences has a different restriction pattern (Figure 4a). As seen in Figure 4(b), the major product (60%) corresponded to the Yb_2 digestion pattern, while only 32% corresponded to that expected for the Yb_1 sequence. In one of the two RNA preparations examined a Yb_3 digestion pattern [10] was also detectable at a level of 4%.

In order to assess the functional significance of olfactory GSTs, we studied their ability to catalyse glutathione conjugation to various odorants. Many GST substrates contain $\alpha\beta$-unsaturated carbonyl structures (R–C=C–C=O) [1]. Over 20 such compounds were selected, including both aldehydes and ketones. All of them have been reported to be functional odorants [30] or are homologues of such established odorants. A representative odorant analogue, limonene oxide, belonging to another class of GST substrates (epoxides), was also studied. For the purpose of screening many potential odorant substrates, we devised a t.l.c. assay with ninhydrin staining of the product (Figure 5), based in part on a published paper chromatography protocol [25]. Table

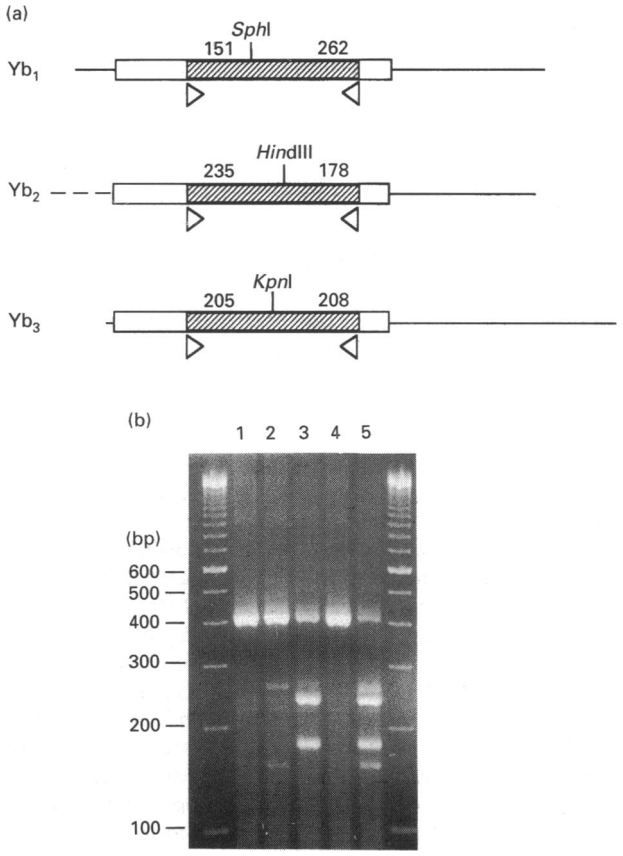

Figure 4 PCR analysis of rat olfactory Yb GSTs

(**a**) Expected pattern of restriction enzyme analysis of rat liver Yb PCR product. The known cDNAs are expected to yield a 413 bp fragment (hatched) upon PCR amplification using Yb primers (open arrows), which lies in the coding region (open box). Each product should give the indicated fragments (in bp) when digested with the indicated enzyme. The enzymes were chosen to cut uniquely once in each sequence. (**b**) Restriction enzyme pattern of olfactory Yb PCR products. PCR amplification of olfactory cDNA was performed and a sample of the reaction was digested with the following restriction enzymes: lane 1; none, lane 2; *Sph*I (Yb_1-specific); lane 3; *Hin*dIII (Yb_2-specific); lane 4; *Kpn*I (Yb_3-specific); lane 5, *Sph*I and *Hin*dIII. The outside lanes are molecular size markers (bp).

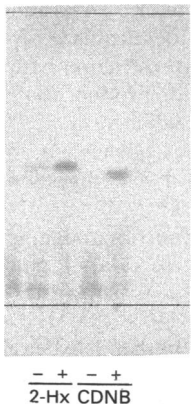

Figure 5 T.l.c. assay for the ability of odorants to serve as GST substrates

T.l.c. separation of glutathione–odorant conjugates is shown. GST reactions were carried out with (+) or without (−) the addition of olfactory epithelium cytosol. The products were separated from the substrates and stained with ninhydrin. 2-Hx, 2-hexenal.

Table 2 Odorants tested as substrates for olfactory GSTs

The indicated odorants were tested for their ability to serve as substrates for the olfactory GSTs. The t.l.c. assay was performed as described. Odorants designated as non-substrates did not give a detectable band upon staining even under saturating conditions (see the Materials and methods section).

Name	Odour
Odorants that are olfactory GST substrates	
t-2-Pentenal	Orange
t-2-Hexenal	Leafy
t-2-Heptenal	'Green'
t-2-Nonenal	Fatty
S-Carvone	Caraway
R-Carvone	Spearmint
Mesityl oxide	Honey
t-4-Phenyl-3-buten-2-one	Rhubarb
5-Methyl-3-hexen-2-one	Roasted
3-Methylbutyl aldehyde	Herbaceous
Citral	Lemon
t-Cinnamaldehyde	Spicy
3-Penten-2-one	Fruity
Limonen oxide	Lemon-like
3-Methyl-2-cyclohexen-1-one	Medicinal
Odorants that are not olfactory GST substrates	
Coumarin	Hay
Jasmone	Jasmine
2-*o*-Methoxycinnamaldehyde	Spicy/floral
5-Methyl-2-thiophenecarboxaldehyde	Roasted
Pulegon	Camphor

Table 3 Activity of olfactory epithelium cytosol towards odorants

The Table gives quantification of the activity assay of Figure 5. In order to demonstrate GST activity of various odorants under the same conditions, a large amount (50 μg/assay) of cytosol was used; therefore the specific activity towards the highly active substrate CDNB is considerably lower than presented in Table 1, due to enzyme saturation. The results are means ± S.D. of triplicate reactions.

Odorant	Conjugation activity (nmol/min per mg)
CDNB	71.6 ± 5.0
t-2-Hexenal	53.1 ± 8.6
t-Cinnamaldehyde	21.7 ± 9.3
Mesityl oxide	4.3 ± 0.5
Citral	3.4 ± 0.4
S-Carvone	2.2 ± 1.0
Limonene oxide	1.3 ± 0.3

2 shows the results of an initial screen to determine which of these compounds were efficient substrates for the olfactory epithelial GST enzyme.

To obtain more quantitative information, conjugation to [^{3}H]glutathione was performed for six selected substrates, representing different classes (Table 3). In addition to CDNB, a known efficient substrate for GSTs in other tissues, the odorants *trans*-2-hexenal and *trans*-cinnamaldehyde were found to be the best substrates.

DISCUSSION

We have identified a third class of biotransformation enzymes in the rat olfactory epithelium, complementing the previous discovery of olfactory-specific cytochrome *P*-450olf1 [13,15,44] and

olfactory UGT [14,44]. GST, similar to UGT, is a phase II biotransformation enzyme, which conjugates hydrophilic moieties to hydrophobic substrates, thereby effecting their inactivation and clearance. In the olfactory epithelium, biotransformation enzymes have been proposed to constitute a mechanism for odorant signal modulation [13–15,31]. We propose here that olfactory GSTs mediate the covalent modification of odorants, the agonists of olfactory neurons, thereby effecting their neutralization and clearance. GST has similarly been proposed to act on neuronal ligands, affecting the uptake, release and transport of various chemicals in the brain [32]. Since odorants have highly variable chemical configurations, a diversity of enzyme types may be necessary to afford an efficient signal termination mechanism. Olfactory GSTs may contribute importantly to this required diversity.

We report here that the rat olfactory epithelium has the highest GST activity towards CDNB among all extrahepatic tissues tested. This activity is ~ 4 times higher than that in the adjacent respiratory epithelium, which resides in the same nasal cavity and is exposed to the same concentrations of inhaled chemicals. This finding supports a potential role of the enzyme in chemosensory function. Similar results were recently reported in bovine olfactory epithelium [18], which contains 48% of the GST activity in the liver and an activity 11 times higher than that in the respiratory epithelium. Comparable GST activities in olfactory epithelium and liver were also seen in the dog, using styrene oxide as a substrate [20], although the measured olfactory/respiratory ratio activity was lower. Another previous report examined the GST activity in the entire nasal epithelium in the rat, including both olfactory and respiratory turbinates, and therefore did not reveal the relatively high olfactory GST activity [17,20]. Interestingly, the level of glutathione, the common substrate for all GST-catalysed reactions, is also higher in the olfactory epithelium than in the brain [33]. The level of GST activity was measured in mature (5–7-week-old) male Wistar rats. It will be interesting to find out whether there are differences in olfactory GST expression between rat strains and sexes, and during development as for some other rat tissues (reviewed in [34]).

To compare the activities of numerous odorants under the same conditions, we used an assay based on conjugation with radioactive [^{3}H]gluthatione as a second substrate, rather than the broadly used spectrophotometric methods [24]. This assay utilizes a standard set of conditions common to diverse substrates, even for those not suitable for spectrophotometry, and provides accurate quantification. This could allow rapid screening of many uncharacterized substrates for any tissue of interest.

Trans-cinnamaldehyde was found to be a good substrate for the olfactory epithelial GSTs. This substrate has previously been reported to result in high GST activity in liver also [35]. In general, class-Mu GSTs have been shown to react with $\alpha\beta$-unsaturated carbonyl compounds and epoxides. The Yb_2 homodimer (4-4) is more active than the Yb_1 homodimer (3-3) towards *t*-4-phenyl-3-buten-2-one (24-fold), 4-hydroxynonenal (2.6-fold) and *t*-stilbene oxide (20-fold) [1]. At present we have no information on the substrate preferences of the individual GST isoenzymes in the olfactory tissue. However, since olfactory tissue contains mainly Yb_3, it is likely that much of the presently reported substrate profile is attributable to this GST species.

The spectrum of GST subunits expressed in the olfactory epithelium is different from that of many other tissues. It is composed almost exclusively of class Mu, namely Yb subunits. Other tissues examined here had a more heterogeneous mixture of Ya, Yb, Yc and Yp. Profiles that are more similar to that reported here for olfactory epithelium have been found in rat heart [36,37] and testis [38], both expressing mainly the Yb isoenzymes. Another tissue that has a relatively high abundance of the Yb form (mainly Yb_3) is the brain [32]. The predominance of the Yb forms of GST in rat olfactory epithelium is also supported by immunohistochemistry [16], where antibodies against the A and C forms (Yb_1 and Yb_2) reacted strongly with the olfactory epithelium, while those against the B (Ya/Yc) and E forms had a considerably weaker reactivity. At this stage we do not know whether the enzyme in the olfactory epithelium is composed of homo- or hetero-dimers. Further characterization, using high-performance liquid chromatofocusing and immunoreactivity assays, should help us to obtain this information.

Olfactory GSTs are shown here to be mainly two subclasses of Yb, namely Yb_1 and Yb_2. This is shown by several independent methods: (a) the mobility of the purified subunits in h.p.l.c.; (b) amino acid sequence analysis of the subunits; (c) DNA sequencing of PCR-generated clones; and (d) restriction enzyme analysis of the PCR products. This last method constitutes a general approach for rapid GST typing, which is more sensitive and accurate than Northern analysis. Thus many tissues may be screened with an oligonucleotide pair derived from conserved sequences of an entire GST class, and subclasses discerned and quantified by their different restriction patterns.

Immunohistochemical methods have been used to visualize GSTs in rat olfactory and respiratory tissues [36]. The staining was localized to a rather broad apical layer and a narrower basal layer of the olfactory epithelium, as well as to the subepithelial Bowman's glands. A similar pattern of labelling was seen by histochemical localization of glutathione [39]. This is consistent with the olfactory epithelial localization of other biotransformation enzymes, such as cytochrome *P*-450 and UGT [14,16,40–42]. The most plausible interpretation of the olfactory epithelial labelling for all of these enzymes is localization to the supranuclear and basal feet layers of the glia-like olfactory supporting cells [14,42]. Notably, in the brain, GST is similarly localized in glial cells, but not in neurons [32,43].

The olfactory biotransformation enzymes studied so far cover a range of odorant configurations and functional groups. Thus UGT has been shown to glucuronate many aliphatic and aromatic alcohols [14,44], and could potentially act also on thiols, amines and carboxylic acids [45]. Olfactory GST broadens the range of potential odorant substrates, by acting on unsaturated aldehydes and ketones. Many widely used odorants, such as citral and *R*- and *S*-carvone belong to this group. Odorants that do not originally contain an active functional group may be modified by the action of olfactory cytochrome *P*-450 [46,47] prior to conjugation by GST or UGT. Cytochrome *P*-450-catalysed hydroxylation would thus generate UGT substrates, and some of these alcohols could then be further oxidized to aldehydes, ketones and epoxides, all of which are substrates for GST.

GST has been reported to have a function parallel to its catalytic activity: the binding of various hydrophobic ligands [4]. In this capacity, some GST species have been termed 'ligandins' [1]. At present, there is no direct evidence for such a function for GST in the olfactory epithelium. However, as GSTs of the Yb class are known to bind steroids [32], it is possible that such bulky hydrophobic compounds, some of which constitute behaviourally significant odorants, are bound to olfactory GST as an intracellular carrier. In this function, GST may complement the activity of odorant-binding protein [48–50] and pheromone-binding protein [51,52], proposed to be extracellular odorant carriers.

A major question to be elucidated by future research is the fate of odorant glutathione conjugates in the olfactory epithelium.

Such products may undergo further metabolism, ending up as thiol derivatives [2]. These, in turn, could be acted upon by UGT to produce the respective glucuronates. In this case, the final biotransformation products, which would accumulate in the endoplasmic reticulum lumen, might be externalized by a vesicular secretion mechanism as previously proposed [53]. If not further modified, glutathione conjugates would accumulate in the cell's cytoplasm, as they are practically membrane-impenetrable. To emerge into the extracellular space (a prerequisite for efficient clearance), odorant glucuronates would have to be transported across the plasma membrane. This might be achieved by a mechanism akin to that of multidrug resistance proteins [54]. Such a mechanism would have to be of a rather broad specificity in order to accommodate the diverse chemical structures of odorants.

We thank Dr. Chen-Pei D. Tu and Dr. Iriving Listowsky for helpful discussions. We are grateful to Dr. Yechiel Shai and his group and to Ida Oren for their help in h.p.l.c. purifications, and to Dr. Menachem Rubinstein and Natan Tal for protein sequencing. This research was supported by grants from the National Institutes of Health, the Human Frontier Science Program Organization, the U.S.–Israel Binational Science Foundation (with Dr. Chen-Pei D. Tu, The Pennsylvania State University, PA, U.S.A), the Fund for the Basic Research of the Israeli Academy of Sciences, the Leo and Julia Forchheimer Center for Molecular Genetics, and the Ebner Family Biomedical Research Foundation.

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Received 30 October 1992/4 January 1992; accepted 15 January 1993