PORCINE KIDNEY MICROSONAL CYSTEINE S-CONJUGATE N-ACETYLTRANSFERASE-
CATALYZED N-ACETYLATION OF HALOALKENE-DERIVED CYSTEINE S-CONJUGATES

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ABSTRACT:

N-Acetylation of xenobiotic-derived cysteine S-conjugates is a key step in the mercapturic acid pathway. The aim of this study was to investigate the N-acetylation of haloalkene-derived S-haloalkyl and S-haloalkenyl cysteine S-conjugates by porcine kidney cysteine S-conjugate N-acetyltransferase (NACt). A radioactive assay for the quantification of NACt activity was developed as a new method for partial purification of the enzyme, which was necessitated by the substantial loss of activity during the immunoaffinity chromatography method. 3-[(3-Cholamidopropyl)dimethylammonio]-1-propane-sulfonate, rather than N,N-bis[3-gluconamidopropyl]deoxycholamide, was used to solubilize the NACt from porcine kidney microsomes in the revised procedure. The partially purified NACt was free of detectable aminoacylase activity. Although low acetyl-coenzyme A hydrolase activity was observed, its effect on the assay was minimized by addition of excess acetyl-coenzyme A in the NACt assay mixture. Attempts to separate the residual hydrolase activity from NACt by different chromatographic procedures were either unsuccessful or lead to inactivation of NACt. Most of the cysteine S-conjugates studied were N-acetylated by NACt. Although the apparent K_m values for the cysteine S-conjugates studied differed by a factor of 2–5 (124–302 μM), a greater than 10-fold difference in the apparent V_max (0.75–15.6 mmol/h) and V_max/K_m (0.008–0.126 × 10^-3 I h^-1) values was observed. These data show that a range of haloalkene-derived cysteine S-conjugates serve as substrates for pig kidney NACt. The significant differences in cytotoxicity of these conjugates may be a result of more variable deacetylation rates of the corresponding mercapturates.

Mercapturic acid formation is a major pathway for the metabolism of electrophilic compounds and metabolites (Stevens and Jones, 1989). This pathway includes glutathione transferase-catalyzed glutathione S-conjugate formation, γ-glutamyltransferase- and dipeptidase-catalyzed hydrolysis of the glutathione S-conjugates to cysteine S-conjugates, and cysteine S-conjugate N-acetyltransferase (NACt; EC 2.3.1.80) 1-catalyzed N-acetylation of the cysteine S-conjugates to give mercapturic acids, which are excreted in the urine. Although mercapturic acid formation generally constitutes a detoxication pathway, some haloalkene-derived mercapturic acids undergo aminoacylase-catalyzed deacetylation to give cysteine S-conjugates, which are bioactivated by cysteine S-conjugate β-lyase (Commandeur et al., 1988; Boogaard et al., 1989; Anders and Dekant, 1994). Also, mercapturic acids derived from acrolein and 1,3-dichloropropane are bioactivated by sulfoxide formation and elimination of reactive intermediates (Hashmi et al., 1992; Park et al., 1992).

The N-acetylation of cysteine S-conjugates and of leukotriene E_4 is catalyzed by NACt (Barnsley et al., 1969; Green and Elce, 1975; Hagmann et al., 1986; Örning et al., 1986), which is apparently different from the N-acetyltransferase that catalyzes the N-acetylation of arylamines (Weber et al., 1990). NACt is a microsomal enzyme that requires acetyl-coenzyme A (CoA) as a second substrate whose activity is highest in kidney tissue (Duffel and Jakoby, 1982). The enzyme is localized on the cytoplasmic surface of the endoplasmic reticulum (Okajima et al., 1984), and its activity is highest in the straight portion of the renal proximal tubule (Heuner et al., 1991).

In previous studies, we developed a method for purification and characterization of porcine kidney NACt (Aigner et al., 1996), in which the enzyme was purified to homogeneity from pig kidney microsomes. Nevertheless, during chromatographic purification, >90% of the total activity was lost with the method described previously. As described earlier (Duffel and Jakoby, 1982), chromatographic purification was required to separate two major interfering activities, namely deacetylase (aminoacylase; EC 3.5.1.14), which catalyzes the hydrolysis of N-acetyl-cysteine S-conjugates, and acetyl-CoA hydrolase (EC 3.1.2.1), which may reduce the concentration of the cosubstrate acetyl-CoA in the NACt enzyme assay.

The objectives of this study were to develop simplified, time-saving purification procedure for NACt and to investigate the N-acetylation of a range of haloalkene-derived cysteine S-conjugates, which undergo β-lyase-dependent bioactivation to nephrotoxic metabolites (Anders and Dekant, 1998). The purification method described here includes the preparation of pig kidney microsomes, solubilization of the enzyme with 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate (CHAPS), and precipitation with polyethylene glycol (PEG) and does not include any chromatographic steps. The proce-
dure yielded partially purified NAcT that was free from aminocacylase activity. The effect of residual of microsomal acetyl-CoA hydrolysis activity in the enzyme fraction was minimized by addition of excess acetyl-CoA in the enzyme assay. The resulting enzyme fraction was used to investigate the kinetics of the NAcT-catalyzed N-acetylation of haloalkene-derived cysteine S-conjugates.

Experimental Procedures

Acetyl-CoA, 4-nitrobenzyl chloride, and L-cysteine hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO). [1-14C]Acetyl-CoA was obtained from Moravek Biochemicals (Brea, CA). Acetonitrile and methanol were obtained from Mallinckrodt Baker B.V. (Deventer, Holland). All other reagents used were of analytical grade. S-(4-nitrobenzyl)-L-cysteine was synthesized as described previously (Bachi and Ross-Petersen, 1972). S-(1,2,2-trifluoroethyl)-L-cysteine 1a, S-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine 1b, S-(2-bromo-1,1,2-trifluoroethyl)-L-cysteine 1c, S-(2-dibromo-1,1-difluoroethyl)-L-cysteine 1d, S-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-propyl]-L-cysteine 1e, S-(1,2-dichlorovinyl)-L-cysteine 2b, and S-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine 2e were obtained by synthesis, as described previously (McKinney et al., 1959; Odum and Green, 1984; Dohn et al., 1985; Finkelstein et al., 1994; Iyer and Anders, 1997). The structures of the cysteine S-conjugates are shown in Fig. 1. S-(pentachlorobutadienyl)-L-cysteine 2a, S-(pentachlorobutadienyl)-N-acetyl-L-cysteine, prepared as described by Nash et al. (1984), was heated in 6 N HCl for 16 to 20 h at 80°C. The solution was treated with charcoal and filtered, and the solution was evaporated to dryness. Residual HCl was removed by repeated addition of water and evaporation. The product was crystallized from ethyl acetate. The physical constants of the crystalline product were identical with reported values (Dekant et al., 1986).

Partial Purification of NAcT. Porcine kidneys were obtained from a local slaughterhouse and used immediately for purification of NAcT or stored at –80°C until used. Homogenization and all subsequent centrifugation steps were performed at 4°C. Renal cortex tissue (345 g) was homogenized in an Ultra-Turrax homogenizer (Janke and Kunkel, Staufen, Germany) in 370 ml of 250 mM sucrose containing 1 mM DTT. The crude homogenate was centrifuged at 8000g for 10 min, and the resulting pellet was homogenized in 240 ml of 250 mM sucrose containing 1 mM DTT. The resulting combined supernatant fraction was centrifuged at 13,800g for 15 min to remove the lysosomal fraction. Microsomes were sedimented by centrifugation at 100,000g for 60 min. Because the microsomal pellets, called microsomes (raw), contained substantial amounts of cytosolic proteins, especially when prepared in the presence of sucrose (Lambert, 1989), these contaminating proteins were removed by resuspending the microsomes in 150 mM Tris HCl (pH 8) with a Dounce (Wheaton, Millville, NJ) homogenizer (tight-fitting pestle). After a preliminary centrifugation step at 13,800g for 15 min, the microsomes were sedimented again at 100,000g for 60 min. Proteins were extracted from the microsomes as described by Aigner et al. (1996) with these modifications: microsomes (2 g total weight) were suspended in 50 ml of 250 mM sucrose containing 1 mM DTT and 200 mM KCl with a Dounce homogenizer (tight-fitting pestle) and then stirred at room temperature for 15 min. Fifty milliliters of 250 mM sucrose containing 1 mM DTT, 20 mM potassium phosphate buffer (pH 7), 200 mM KCl, and 10 mg/ml CHAPS were added, and the mixture was stirred for 15 min at room temperature. The resulting suspension (called microsomal protein fraction) was used for enzyme assays (NAcT, aminocacylase, and acetyl-CoA hydrolyase). After centrifugation at 100,000g for 60 min, 50% (w/v) PEG 6000 was added to the supernatant for a final concentration of 10% (w/v). Precipitated proteins were isolated by centrifugation at 18,000g for 30 min, and the resulting pellet was resuspended in 20 mM potassium phosphate buffer (pH 7) containing 1 mM DTT, 200 mM KCl, and 10% PEG 6000 (w/v) to remove coprecipitated proteins. The solution was centrifuged again at 18,000g for 30 min. A sample (0.5 g total weight) of the resulting pellet was suspended in 10 ml of 10 mM Tris HCl (pH 7.3) containing 30% (v/v) glycerol, 1 mM DTT, and 5 mg/ml CHAPS. After centrifugation at 18,000g for 30 min, the supernatant (230 µg protein/ml) was used for enzyme assays (called enzyme fraction).

Enzyme Assays. NAcT assay (nonradioactive). To determine the specific activity of the partially purified enzyme, samples were assayed as described previously with S-(4-nitrobenzyl)-L-cysteine as the substrate (Aigner et al., 1994). According to Duffel and Jakoby (1985), 1 U of NAcT activity is the amount of enzyme that catalyzes the formation of 1 nmol of product/min under the assay conditions.

NAcT assay (radioactive). To determine the kinetic constants for the N-acetylation of cysteine S-conjugates, a radioactive assay was developed based on the method described by Aigner et al. (1994). Cysteine S-conjugates in various concentrations (5–20 μM up to 1 mM, depending on the substrate) were incubated with the enzyme fraction (3.3 µg protein) in 100 mM potassium phosphate buffer (pH 7.0) and 760 µM acetyl-CoA (containing 157.5 mCi/mmol [14C]acetyl-CoA; specific activity = 55 mCi/mmol) in a final volume of 24.8 µL. The reaction mixture was incubated at 37°C for 1 to 60 min, depending on the substrate. The reaction was terminated by placing the reaction vessels in a boiling water bath for 1 min. The samples were then placed on ice for 2 min, and the precipitated proteins were removed by centrifugation at 13,800g for 30 min. A sample (5 µL) of the supernatant was applied to silica gel thin-layer chromatography (TLC) plates (200-µm thickness; Merck, Darmstadt, Germany), which were eluted with isopropanol/ethyl acetate/acidic acid/water (3:2:1). Because esterification occurs during storage, the TLC solvent system was prepared fresh before each use. The TLC plates were exposed to a radiographic film (BioMax MR; Kodak, Rochester, NY) for up to 1 month. Quantification of the signals was performed by densitometry with a flat bed scanner and the National Institutes of Health Image 1.61 program for Apple Macintosh.

Aminocacylase assay. Aminocacylase activity of the enzyme fraction was determined according to Aigner et al. (1994) with S-(4-nitrobenzyl)-N-acetyl-L-cysteine as the substrate. The assay was conducted under N-acetytransferase conditions. The enzyme fraction was assayed in a reaction mixture (total volume 60 µl) that contained 150 mM potassium phosphate buffer (pH 7.0), 1.6 mM acetyl-CoA, and 1.2 mM S-(4-nitrobenzyl)-N-acetyl-L-cysteine and was incubated at 37°C for 60 min. The reaction was terminated by placing the reaction vessels in a boiling water bath for 1 min; the precipitated proteins were removed by centrifugation at 13,800g for 30 min. A sample (25 µL) of the supernatant was collected for HPLC analysis. One unit of aminocacylase...
activity was defined as the amount of enzyme required for formation of 1 nmol of product/min under the assay conditions.

Acetyl-CoA hydrolase assay. The acetyl-CoA hydrolase activity of the enzyme fraction was determined under N-acetyltransferase conditions as follows: the partially purified enzyme was incubated at 37°C for 60 min in 150 mM potassium phosphate buffer (pH 7.0) containing 1.6 mM acetyl-CoA in a final volume of 60 μl. The reaction was terminated by placing the reaction vessels in a boiling water bath for 1 min. The precipitated proteins were removed by centrifugation at 13,800g for 30 min. A sample (25 μl) of the supernatant was taken for HPLC analysis according to the method of Rajgarhia et al. (1995). The isocratic elution described previously (Aigner et al., 1994) was changed to a linear gradient elution of 5 to 40% B [buffer A = acetonitrile; buffer B = 17.5% (v/v) methanol containing 50 mM potassium phosphate (pH 4)]. One unit of acetyl-CoA hydrolase activity was defined as the amount of enzyme required for formation of 1 nmol of product/min under the assay conditions.

Protein Determination. The protein content was determined by amino acid analysis. The concentration of protein in the final enzyme fraction was 230 μg/ml.

Calculations. The log P values (n-octanol/water partition coefficients) were calculated with the ChemProp program running in ChemDraw version 4.5 (CambridgeSoft Corp., Cambridge, MA). The program uses the fragmentation method of Ghose and Crippen (1986, 1987) to calculate log P values from the structures of the compounds.

Results
To investigate the N-acetylation of haloalkene-derived cysteine S-conjugates, a simplified, time-saving method for the purification of NAcT was developed. No differences in yield of enzyme activity were observed when using kidneys immediately after receipt or after storage at −80°C for up to several months, respectively. Microsomes from porcine renal cortex, which were obtained by differential centrifugation according to Duffel and Jakoby (1982), contained aminocysteine activity (data not shown) that could be minimized by isolating the primary microsomal pellet in buffer that lacked sucrose (Lambert, 1989). After solubilization with CHAPS, the resulting microsomal protein fraction exhibited a residual specific activity of 1.9 U/mg of the cystosolic aminocysteine (Table 1). In our purification procedure published previously (Aigner et al., 1996), we used different detergents in concentrations of 2.5 × critical micellar concentration (cmc)2 and N,N-bis[3-gluconamidopropyl]deoxycholamide provided the best solubilization properties. On treatment with this detergent, a 45% loss of enzyme activity was accepted. In the current procedure, CHAPS (5 mg/ml; 1 × cmc) was used to solubilize NAcT from the pig kidney microsomes. After CHAPS treatment, the supernatant contained 85% of the NAcT activity and therefore provided best results among the different detergents under investigation (data not shown). As with the observations of Lehner and Kuksis (1993), who purified acyl-CoA hydrolase from rat intestinal microsomes, microsomal NAcT activity was totally lost in the presence of CHAPS greater than its cmc level. Additional purification was performed by addition of PEG to a final concentration of 10%. Coprecipitated protein, which included ~60% of acetyl-CoA hydrolase activity, was then removed by an additional step with 10% PEG. Similar results were obtained when PEG was used in the presence or absence of buffer. Because additional purification by two subsequent chromatographic steps led to a dramatic loss of enzyme yield, primarily caused by removal of fractions with lower specific activity (Aigner et al., 1996), the precipitated protein was resuspended and used for kinetic experiments (enzyme fraction: 230 μg protein/ml). NAcT activity of the enzyme fraction was not impaired by storage at −80°C for several months, whereas it was reduced to 69% when kept at 4°C for 72 h.

Enzyme activities, which may interfere with the N-acetylation of cysteine S-conjugates, such as aminocysteine and acetyl-CoA hydrolase, were determined under NAcT assay conditions. The properties of the partially purified porcine kidney NAcT enzyme fraction, which was used in the kinetic studies, are summarized in Table 1. The resulting enzyme fraction was free from aminocysteine activity, and the specific activity of NAcT (86.5 U/mg) was comparable with that reported previously (Aigner et al., 1996) after two additional chromatographic steps (80.4 U/mg). Further removal of acetyl-CoA hydrolase activity was not successful or led to inactivation of NAcT. An excess of acetyl-CoA was added to the NAcT assay to minimize the hydrolase-catalyzed loss of acetyl-CoA.

A range of haloalkene-derived cysteine S-conjugates (Fig. 1) was studied as substrates with the modified radioactive assay, as described in Experimental Procedures to investigate N-acetylation by the enzyme fraction. Linearity of product formation varied within 1 to 60 min, depending on the substrate. A representative progress curve for the NAcT-catalyzed N-acetylation of conjugate 1c is shown in Fig. 2A. In this case, the reaction rate declined by 12 min; therefore, the incubation time for this substrate was 2 min in all experiments to calculate the kinetic constants. The Michaelis–Menten graph of the enzyme reaction with substrate 1c is shown in Fig. 2B. No N-acetylation of conjugate 2c was observed. The apparent Km and Vmax values as well as the Vmax/Km values of the substrates studied are summarized in Table 2.

The relationship between the Vmax/Km values and the log of the n-octanol/water partition coefficient (log P) of the cysteine S-conjugates studied was examined. Although no general correlations were observed, there was an excellent correlation (r2 = 0.96) between Vmax/Km values and log P for the acetylation of the S-(2-halo-1,1,2-trifluoroethyl)-l-cysteine conjugates 1a, 1b, and 1c (Fig. 3).

Discussion
The objectives of this study were to develop a simplified purification procedure for NAcT and to investigate the N-acetylation of haloalkene-derived cysteine S-conjugates. Cysteine S-conjugate formation plays an important role in the bioactivation of nephrotoxic haloalkenes (Anders and Dekant, 1998). After initial glutathione transferase-catalyzed glutathione S-conjugate formation, hydrolysis of the glutathione S-conjugates affords the corresponding cysteine S-
conjugates, which may undergo cysteine conjugate β-lyase-catalyzed bioactivation to reactive intermediates or may undergo NAcT-catalyzed N-acetylation to mercapturic acids. The balance between these two competing reactions is an important determinant of the nephrotoxicity of haloalkenes, because mercapturic acids cannot undergo bioactivation by the pyridoxal phosphate-dependent β-lyase. Hence, three enzymes, namely NAcT, β-lyase, and aminoacylase, catalyze key steps in the detoxication or bioactivation of haloalkenes.

Investigation of the kinetics of NAcT-catalyzed N-acetylation may be confounded by the presence of interfering enzyme activities. Aminoacylases, which catalyze the hydrolysis of mercapturic acids, serve as a cysteine S-conjugate-regenerating system and, thereby, alter apparent $K_m$ and $V_{max}$ values. This effect may not be recognized when data are analyzed using Michaelis-Menten kinetics. The analysis of the catalytic mechanism of NAcT, which requires the determination of the kinetics of product inhibition, may also be confounded by the presence of interfering enzyme activities. These effects were most striking when NAcT activities of pig kidney microsomes, which were prepared according to the method of Duffel and Jakoby (1982), were studied. The apparent $K_m$ values for S-(4-nitrobenzyl)-l-cysteine increased with the degree of purification, as described previously (Aigner et al., 1996) and may be in part attributable to the loss of aminoacylase activity during purification.

The kinetics of the NAcT-catalyzed N-acetylation of S-(1,2-dichlorovinyl)-l-cysteine 2b and S-(1,2,3,4-pentachlorobutadienyl)-l-cysteine 2a by rat liver and kidney microsomes were reported recently (Birner et al., 1997). The investigators concluded that N-acetylation in both liver and kidney is catalyzed by one enzyme, as shown using monophasic Eadie-Hofstee plots. Furthermore, the participation of the same enzyme in both liver and kidney was postulated, although the apparent $K_m$ values differed ~5-fold and the $V_{max}$ values differed as much as 20-fold. In addition to the presence or absence of inhibitory or activating factors in liver and kidney microsomes, the observed differences may also be attributable to relative rates of acetylation and aminoacylase-catalyzed hydrolysis in the two microsomal preparations.

Microsomes prepared by differential centrifugation in presence of sucrose contain substantial amounts of cytosolic proteins (Lambert, 1989). Removal of enzyme activities that interfere with NAcT, such as aminoacylase and acetyl-CoA hydrolase, was successful after solubilization with N,N-bis[3-gluconamidopropyl]deoxycholamide, PEG sedimentation, and an additional chromatographic step (Duffel and Jakoby, 1982). Because chromatographic purification led to a substantial loss of NAcT-activity (Duffel and Jakoby, 1982; Aigner et al., 1996), the method presented here is based on preparation of microsomes, solubilization with CHAPS, and PEG sedimentation without chromatographic fractionation. Therefore, the method is simple and time-saving, yielding a partially purified NAcT enzyme fraction with a specific activity (NAcT) comparable with that of Aigner et al. (1996). The overall enrichment of NAcT using this method was ~60-fold. The resulting enzyme fraction was free of aminoacylase. Excess of acetyl-CoA was used in the enzyme assay to minimize the effect of residual acetyl-CoA hydrolase.

All substrates studied were N-acetylated by NAcT, except conjugate 2c (Table 2). The failure to detect N-acetylation of conjugate 2c may be attributed to its rapid cyclization to 2-[1-(fluoromethoxy)-2,2,2-trifluoroethyl]-4,5-dihydro-1,3-thiazole-4-carboxylic acid (Iyer and Anders, 1997). Also, conjugate 2c is not nephrotoxic in rats, apparently because of its cyclization to a thiazole that is not a substrate for β-lyase (Iyer et al., 1997). Although the apparent $K_m$ values differed little (~2-fold) among the cysteine S-conjugates studied, the of $V_{max}$ values differed 20-fold. Similarly a 40-fold range of $V_{max}/K_m$ values was observed.

The apparent $K_m$ values for the N-acetylation of haloalkene-derived S-haloalkyl and S-haloalkenyl cysteine S-conjugates determined in this study (porcine kidney NAcT) are in the same order of magnitude as those of some S-alkyl and S-benzyl cysteine S-conjugates (rat kidney NAcT) reported by Duffel and Jakoby (1982). When calculating $V_{max}$ values (Table 2) in units of nanomoles per minute per milligram protein as given previously, maximum rates of catalysis are ~10-fold lower in comparison with the S-alkyl and S-benzyl derivatives. Whether the differences in reaction rates are caused by the different extents of purification (394 U/mg for the rat enzyme) or caused by species differences, structural properties, and/or other factors remains to be elucidated.

The structural properties that govern the rate of N-acetylation of cysteine S-conjugates have not been investigated in detail. Duffel and Jakoby (1982) found a good correlation between the log $V_{max}/K_m$ for N-acetylation and Hansch $\pi$ constants for S-ethyl-, S-(1-propyl)-, S-benzyl-, and S-(1-butyl)-l-cysteine. In this study, the $V_{max}/K_m$ for the N-acetylation of S-(2-halo-1,1,2-trifluoroethyl)-l-cysteine conjugates increased in the order F (1a) < Cl (1b) < Br (1c) and showed a good correlation with the log $P$ values of the conjugates (Fig. 3). Hence, for these cysteine S-conjugates, lipophilicity appears to exert an effect on catalytic efficiency. The observed correlations, however, were found for a limited number of compounds, and the data need to be extended to determine whether log $P$ values or other free-energy
The cysteine S-conjugates were incubated with purified porcine kidney NAcT, and product formation and kinetic constants were measured as described in Experimental Procedures. The structures of the cysteine S-conjugates are shown in Fig. 1. Data are shown as mean ± S.D.; n = 4.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (nmol/h)</th>
<th>$V_{max}/K_m$ ($10^{-5}$ l/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-(1,1,2,2-Tetrafluoroethyl)-l-cysteine 1a</td>
<td>302 ± 3</td>
<td>2.3 ± 0.03</td>
<td>0.008</td>
</tr>
<tr>
<td>S-(2-Chloro-1,1,2-trifluoroethyl)-l-cysteine 1b</td>
<td>211 ± 3</td>
<td>5.78 ± 0.05</td>
<td>0.027</td>
</tr>
<tr>
<td>S-(2-Bromoo-1,1-difluoroethyl)-l-cysteine 1c</td>
<td>179 ± 7</td>
<td>13.9 ± 0.4</td>
<td>0.078</td>
</tr>
<tr>
<td>S-(2,2-Dibromo-1,1-difluoroethyl)-l-cysteine 1d</td>
<td>203 ± 22</td>
<td>6.2 ± 0.4</td>
<td>0.030</td>
</tr>
<tr>
<td>S-(2-(Fluoromethoxy)-1,2,3,3,3-pentafluoropropyl)-l-cysteine 1e</td>
<td>241 ± 30</td>
<td>3.4 ± 0.3</td>
<td>0.014</td>
</tr>
<tr>
<td>S-(Pentachlorobutadienyl)-l-cysteine 2a</td>
<td>124 ± 19</td>
<td>15.6 ± 1.2</td>
<td>0.126</td>
</tr>
<tr>
<td>S-(1,2-Dichlorovinyl)-l-cysteine 2b</td>
<td>273 ± 47</td>
<td>0.75 ± 0.07</td>
<td>0.003</td>
</tr>
<tr>
<td>S-[2-(Fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-l-cysteine 2c</td>
<td>—</td>
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<td>—</td>
</tr>
</tbody>
</table>

* No activity detected; <0.1 nmol/h after exposition for 1 month.

![Fig. 3. Relationship between log $V_{max}/K_m$ and calculated log P values of substrates 1a–c.](image)

Relationships can be used to predict NAcT-catalyzed acetylation of cysteine S-conjugates. Correlations between molar volumes and log $P$ are observed for the aminoacylase-catalyzed hydrolysis of mercapturates, but the correlations extend also only over a narrow range of compounds (Uttamsingh et al., 1998).

The rates of acetylation, as measured by apparent $V_{max}$ or $V_{max}/K_m$ in this study, followed the order 1d > 1b > 1a, whereas their cytotoxicity in isolated renal proximal tubular cells followed the order 1a ≈ 1b > 1d (Boogard et al., 1989). In contrast with the results found in this study, the investigators did not detect N-acetylation of conjugates 1a and 1b in renal proximal tubular cells in the presence of (aminooxy)acetic acid to inhibit β-lyase. Studies in whole cells may be confounded by the presence of aminoacylases; in renal proximal tubular cells, the deacetylation of the mercapturic acids of conjugates 1a, 1b, and 1d followed the order 1a ≈ 1b > 1d (Boogard et al., 1989). These data indicate that conditions that favor the highest intracellular concentrations of conjugates 1a, 1b, and 1d are associated with the highest cytotoxicity. Additional evidence for this hypothesis is given by the observation of Commandeur et al. (1988), who investigated the metabolism of N-(trideuteroacetyl)-l-cysteine S-conjugates of 2,2-difluorotylenes in the rat. After i.p. administration of N-(trideuteroacetyl)-labeled mercapturate of 1d, 31% of the dose was excreted unchanged and 28% was excreted as the unlabeled mercapturic acid, indicating extensive N-deacetylation and racemization in vivo. In contrast, <1% of the labeled mercapturates of conjugates 1a and 1b was found, and <2% of those mercapturates was excreted as unlabeled mercapturate, which may indicate a higher deacetylation rate. In conclusion, the kinetic data reported here on conjugates 1a, 1b, and 1d do not differ as much as the cytotoxic effects of these compounds. Because cysteine S-conjugates 1a to 1e are substrates for NAcT with kinetic parameters in the same order of magnitude, nephrotoxicity is apparently influenced primarily by deacetylation rates of the corresponding mercapturic acids, which may differ significantly.

In summary, a partially purified NAcT that catalyzes the N-acetylation of several haloalkene-derived cysteine S-conjugates has been identified. Furthermore, the data on the kinetics of the N-acetylation of the cysteine S-conjugates along with data on the kinetics of the deacetylation of the corresponding mercapturic acids reported in previous studies provide insights into the relative cytotoxicity of the cysteine S-conjugates.

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**References**


