Cysteinesulfonate and \( \beta \)-Sulfopyruvate Metabolism

PARTITIONING BETWEEN DECARBOXYLATION, TRANSAMINATION, AND REDUCTION PATHWAYS

(Received for publication, September 21, 1987)

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\( \beta \)-Cysteinesulfonate (\( \beta \)-cysteate) is present in plasma, urine, and tissues in concentrations comparable to that of \( \beta \)-cysteinesulfinate, the primary oxidative metabolite of \( \beta \)-cysteine. Although cysteinesulfonate is known to be decarboxylated to taurine by cysteinesulfinate decarboxylase, the occurrence and importance of other metabolisms has not been examined. The present studies indicate that cysteinesulfonate partitions in vivo between decarboxylation and transamination; the latter reaction is catalyzed by aspartate aminotransferase and yields \( \beta \)-sulfopyruvate. Whereas \( \beta \)-sulfopyruvate, the product of cysteinesulfinate transamination, decomposes spontaneously, \( \beta \)-sulfopyruvate is stable and is reduced by malate dehydrogenase to \( \beta \)-sulfolactate. When \( \beta \)-[\( \text{\( ^{14} \text{C} \))} \text{Cysteinesulfonate} \) is given to mice, 60-75% is decarboxylated to taurine and about 25% is excreted in the urine as \( \beta \)-sulfolactate. \( \beta \)-Sulfo[\( \text{\( ^{14} \text{C} \))} \text{Cysteinesulfonate} \) pyruvate is found to partition about equally between \( \beta \)-sulfolactate and cysteinesulfinate formation; >90% of the latter is decarboxylated. Parenterally administered \( \beta \)-sulfo[\( \text{\( ^{14} \text{C} \))} \text{Cysteinesulfonate} \) is mostly excreted in the urine, but 12% is metabolized via \( \beta \)-sulfopyruvate and cysteinesulfinate to \( ^{14} \text{CO}_2 \) and taurine. \( \beta \)-Sulfopyruvate is not excreted, and only traces of sulfoacetate, perhaps formed by oxidative decarboxylation, are detected. These studies establish that cysteinesulfonate, \( \beta \)-sulfopyruvate, and \( \beta \)-sulfolactate are reversibly interconverted in vivo. Since only cysteinesulfonate is directly metabolized to \( ^{14} \text{CO}_2 \), the rate of \( ^{14} \text{CO}_2 \) formation from \( \beta \)-[\( \text{\( ^{14} \text{C} \))} \text{Cysteinesulfonate} \) is a valid measure of total cysteinesulfinate decarboxylase activity in vivo; use of this assay permits inhibitor effects to be accurately determined in intact mice. Thus, whereas in vitro assays indicate that \( \beta \)-methyleneproline inhibits brain, liver, and kidney cysteinesulfinate decarboxylase by 0, >60, and 90%, respectively, in vivo studies with \( \beta \)-[\( \text{\( ^{14} \text{C} \))} \text{Cysteinesulfonate} \) show net metabolic inhibition is about 40%.

Although many pathways contribute to cysteine metabolism (1), oxidation of cysteine to cysteinesulfinate, a reaction catalyzed by cysteine dioxygenase, initiates the quantitatively important pathways of cysteine catabolism in most mammals (2, 3). Cysteinesulfinate does not accumulate (4) but is either decarboxylated to hypotaurine by cysteinesulfinate decarboxylase (5, 6) or is transaminated to \( \beta \)-sulfopyruvate by aspartate aminotransferase (AAT) (7, 8). The partitioning of cysteinesulfinate between these pathways shows both tissue and species specificity. Hypotaurine is efficiently oxidized to taurine in a poorly characterized reaction that may (9) or may not (10) be enzyme catalyzed. \( \beta \)-Sulfinylpyruvate spontaneously decomposes to pyruvate and sulfite (7), and the latter is then oxidized to sulfate by sulfite oxidase.

It is notable that \( \beta \)-cysteinesulfonate (\( \beta \)-cysteate) does not participate in the metabolism outlined and is thus not generally considered an important metabolite of cysteine (11). Nevertheless, cysteinesulfonate is a normal constituent of urine and plasma (12, 13) and is present in mammalian brain at about 50% of the concentration of cysteinesulfinate (14). Although cysteinesulfonate has received considerable attention as a possible central nervous system neurotransmitter (see Refs. 15 and 16 and references therein), the origins and metabolism of the cysteinesulfonate pool are poorly defined. The finding that cerebellar cysteinesulfinate and cysteinesulfonate levels are both low in spontaneously hypertensive rats (17) and that both decline substantially and in parallel in 3-acetylpiperidine-treated rats (18) suggests that biosynthesis of the two compounds may depend on similar processes or precursors. In vivo formation of \( [\text{\( ^{35} \text{S} \))} \text{Cysteinesulfonate} \) from \( [\text{\( ^{35} \text{S} \))} \text{Cysteine} \) has, in fact, been reported (19) and (denied (20, 21)), but specific enzymatic synthesis of cysteinesulfonate from either cysteine or cysteinesulfinate has not yet been shown in vitro. Conversely, the synthesis of cysteinesulfonate from PAPS and aminoaacrylate, a serine metabolite, has been clearly shown in vitro (22) but is of doubtful quantitative importance in vivo (23). Several nonbiosynthetic sources of the cysteinesulfonate pool have also been considered. Cysteinesulfonate is readily absorbed from the gut (24), and there is evidence that cyst(e)ine and the cyst(e)aryl residues of proteins are partially oxidized during food processing (24–26). Cysteinesulfonate excretion, and presumably formation, is increased by radiation therapy (27), but the importance of this oxidation at background levels of radiation is unknown.

While the origins of cysteinesulfonate are obscure, several in vitro and in vivo studies indicate that cysteinesulfonate is decarboxylated to taurine by cysteinesulfinate decarboxylase (5, 6, 28). The in vitro transamination of cysteinesulfonate by the cytoplasmic and mitochondrial isoforms of AAT (c-AAT and m-AAT, respectively) is also clearly established (8, 29). More recently, we reported that the transamination product, \( \beta \)-sulfopyruvate, is reduced to \( \beta \)-sulfolactate in vitro by NADH and malate dehydrogenase (30). The direct metabolism of \( \beta \)-sulfopyruvate to \( ^{14} \text{CO}_2 \) has been suggested on the basis of limited studies with rats (3) but has not been directly dem-

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\[ K_{\text{m}} \] V, maximum reaction rate with second substrate at the concentration specified; HPLC, high pressure liquid chromatography; TLC, thin layer chromatography.
onstrased in mammalian systems (31). In the present studies, we have quantitated the partitioning of cysteinesulfonate and $\beta$-sulfopyruvate among these pathways in vivo and have established that L-[1-$^{14}$C]cysteinesulfonate is a valid and useful probe of cysteinesulfinate decarboxylase activity in intact animals. 2

EXPERIMENTAL PROCEDURES

RESULTS

Cysteinesulfinate, Cysteinesulfonate, and $\beta$-Sulfopyruvate As Substrates of AAT Isozymes—Although the activity of AAT toward cysteinesulfinate and cysteinesulfonate is established (7, 8, 29, 33, 34), kinetic studies directly comparing these substrates with aspartate under physiological conditions of pH and co-substrate concentration have not been reported. In the present studies, the relative activity of rat liver c-AAT and m-AAT toward the several substrates was compared at pH 7.4 (Table I); the a-ketoglutarate concentration was held constant at 1.0 mM to approximate its concentration in the cytoplasm and mitochondrial matrix. Under these physiologically relevant conditions, the activity of c-AAT toward aspartate exceeds that toward cysteinesulfinate and cysteinesulfonate by 5- and 100-fold, respectively, on a $V/K_{\text{m}}$ basis; most of the difference is attributable to the high $K_{\text{m}}$ values for cysteinesulfinate and cysteinesulfonate. For the same reason, activity of m-AAT toward aspartate exceeds that toward cysteinesulfinate and cysteinesulfonate by 7- and 46-fold, respectively, on a $V/K_{\text{m}}$ basis.

In the presence of 1.0 mM L-glutamate, $\beta$-sulfopyruvate is transaminated by both c-AAT and m-AAT to form L-cysteinesulfonate. As shown in Table I, the $K_{\text{m}}$ of $\beta$-sulfopyruvate with c-AAT is about 70% of that reported for oxaloacetate, whereas the $K_{\text{m}}$ of $\beta$-sulfopyruvate with m-AAT is slightly higher than that of oxaloacetate. At saturation, oxaloacetate is transaminated 9- and 1.6-fold faster than $\beta$-sulfopyruvate by c-AAT and m-AAT, respectively. As noted previously (7, 31), $\beta$-sulfopyruvate is unstable, and cysteinesulfinate and cysteinesulfonate are the predominant metabolites. At pH 7.4 in phosphate buffer, the normal AAT reaction exhibits a $K_{\text{m}}$ of 6.74 where $K_{\text{m}}$ = [a-ketoglutarate][L-aspartate]/[L-glutamate][oxaloacetate] (37). Under similar conditions with L-cysteinesulfinate and $\beta$-sulfopyruvate replacing L-aspartate and oxaloacetate, the apparent $K_{\text{m}}$ is 8.91 ± 0.38 (“Experimental Procedures,” Miniprint).

In Vivo Decarboxylation and Transamination of Radiolabeled Cysteinesulfonate—Mice administered [1-$^{14}$C]cysteinesulfonate (0.25 or 1.0 mmol·kg$^{-1}$) metabolize 60–75% of the compound to $^{14}$CO$_2$ in 6 h (Fig. 1). Mice given [3-$^{14}$C]cysteinesulfonate (1.0 mmol·kg$^{-1}$) convert less than 0.2% of the compound to $^{14}$CO$_2$ (Fig. 1). Animals given [1-$^{14}$C]- and [3-$^{14}$C]cysteinesulfonate excrete 20–30% and 60%, respectively, of the administered radioactivity in their urine within 24 h. Urine from mice given [1-$^{14}$C]cysteinesulfonate (0.25 mmol·kg$^{-1}$) was fractionated on small columns of Dowex 1 (“Experimental Procedures,” Miniprint): cysteinesulfinate and $\beta$-sulfopyruvate constitute 21 ± 2% and 79 ± 2% of the contained radioactivity, respectively. $\beta$-Sulfopyruvate was not detected in the urine. On the basis of the total [1-$^{14}$C]cysteinesulfonate administered, about 70% is decarboxylated to $^{14}$CO$_2$ (and unlabeled taurine), about 20% is transaminated to $\beta$-sulfopyruvate, reduced to $\beta$-sulfolactate and recovered as such in the urine, and about 5% is recovered in the urine as cysteinesulfonate. A small part of the dose (<10%) is unaccounted for and presumably represents unexcreted urinary metabolites. Urine from animals given [3-$^{14}$C]cysteinesulfonate contains [1-$^{14}$C]taurine in addition to radiolabeled cysteinesulfonate and $\beta$-sulfolactate; no $\beta$-sulfopyruvate and only traces of sulfocacetate are detected. Although $\beta$-sulfolactate is the predominant radiolabeled species present, it is noted that $[\beta^{14}C]$taurine is equilibrated with large tissue pools and is therefore excreted slowly. In a similar study, the 24 h urine from mice administered L-[3-$^{14}$S]cysteinesulfonate (0.25 mmol·kg$^{-1}$) contained 14–18% cysteinesulfonate, 2–3% taurine, 80–

TABLE I

<table>
<thead>
<tr>
<th>Isozyme</th>
<th>Substrate</th>
<th>$K_{\text{m}}$</th>
<th>$V/K_{\text{m}}$</th>
<th>Relative rate $V/K_{\text{m}}$</th>
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<tr>
<td></td>
<td></td>
<td>mM</td>
<td>v</td>
<td></td>
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<tr>
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<td>0.91</td>
<td>0.78</td>
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<tr>
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<td>0.91</td>
<td>0.12</td>
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<td>Experiment 2</td>
<td>Oxaloacetate</td>
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<tr>
<td>Cytoplasmic</td>
<td>$\beta$-Sulfopyruvate</td>
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<td>$\beta$-Sulfopyruvate</td>
<td>0.029</td>
<td>0.63</td>
<td>21.7</td>
</tr>
</tbody>
</table>

1 A preliminary report of some of these studies has appeared (32).
2 Portions of this paper (including “Experimental Procedures,” part of “Results,” and Fig. 4) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
3 The cytoplasmic a-ketoglutarate concentration is 0.35 and 0.13 mM in liver from fed and fasted rats, respectively; whereas the mitochondrial a-ketoglutarate concentration is 1.35 and 0.54 mM in liver from fed and fasted rats, respectively (36). The cytoplasmic L-glutamate concentration is 2.67 and 1.94 mM in liver from fed and fasted rats, respectively. The mitochondrial L-glutamate concentration is 0.95 and 1.24 mM in liver from fed and fasted rats, respectively (36).
FIG. 1. Formation of $^{14}$CO$_2$ from radiolabeled L-cysteinesulfonate in vivo. Mice were given L-[1-$^{14}$C]cysteinesulfonate (solid symbols) or L-[3-$^{14}$C]cysteinesulfonate (open symbols) and were immediately placed in metabolic chambers. Respiratory $^{14}$CO$_2$ was collected for 6 h; urine was collected for 24 h. Radiolabeled cysteinesulfonate was given by intraperitoneal injection at a dose of 1.0 mmol·kg$^{-1}$ (circles, three mice) or by subcutaneous injection on the back of the neck at a dose of 0.25 mmol·kg$^{-1}$ (triangles, six mice). Lines show means ± SD.

85% $\beta$-sulfolactate and about 0.5% sulfoacetate. To verify that taurine peptides did not account for the peaks identified as $\beta$-sulfolactate or sulfoacetate, urine samples were hydrolyzed in 3 M HCl at 110 °C for 24 h; there were no significant changes in the relative amounts of the compounds detected.

In Vivo Transamination and Reduction of $\beta$-Sulfopyruvate—Mice given $\beta$-[35S]sulfopyruvate (0.05 or 0.1 mmol·kg$^{-1}$ in separate experiments) excrete 40–60% of the administered radioactivity in the urine after 24 h and excrete 75–85% of the radioactivity after 72 h. The 72 h urine samples were analyzed for $\beta$-sulfopyruvate and its probable metabolites by chromatography on Dowex 1; the results, expressed as percentage of dose, were similar for the two $\beta$-sulfopyruvate dosages. Radiolabeled taurine, cysteinesulfonate, $\beta$-sulfolactate, and sulfoacetate accounted for 41 ± 8, 3 ± 1, 55 ± 8, and 4 ± 2% of the excreted radioactivity, respectively. No $\beta$-sulfopyruvate was found in any urine sample by direct enzymatic assay or by chromatography. The identification and quantitation of taurine, cysteinesulfonate, and $\beta$-sulfolactate were confirmed by HPLC. Since sulfoacetate elutes as a broad peak on both ion-pair and SAX-column HPLC, the presence of sulfoacetate in the urine samples could be confirmed only qualitatively and semiquantitatively.

Mice given $\beta$-sulfo[1-14C]lactate (0.1 mmol·kg$^{-1}$) metabolize about 30% of the compound to $^{14}$CO$_2$ in 6 h (Fig. 2A, upper curve). In seven additional control mice, respiratory $^{14}$CO$_2$ accounted for 32 ± 8% of the administered radioactivity (data not shown). Urine collected for 24 h contains 50–60% of the radioactivity administered; cysteinesulfonate, $\beta$-sulfolactate, and $\beta$-sulfoacetate accounted for 3, 0, and 97% of the urinary radioactivity, respectively.

Metabolism of $\beta$-Sulfolactate—Sulfo[1-14C]lactate was prepared by malate dehydrogenase-mediated reduction of $\beta$-sulfo[1-14C]pyruvate; in analogy with oxaloacetate reduction, the product is assumed to be the L-enantiomer. Mice given L-$\beta$-sulfo[1-14C]lactate (0.1 mmol·kg$^{-1}$) metabolize about 12% of the compound to $^{14}$CO$_2$ in 6 h (Fig. 2B); after 24 h 66 ± 7% of the injected radioactivity is recovered in the urine. $\beta$-Sulfolactate and cysteinesulfonate account for 99 and 1% of the urinary radioactivity, respectively; $\beta$-sulfopyruvate and sulfoacetate were not detected.

Urinary Excretion and Possible Metabolism of Sulfoacetate—Sulfo[1-14C]lactate (1.0 mmol·kg$^{-1}$) was administered to each of four mice by intraperitoneal injection, and respiratory $^{14}$CO$_2$ was monitored for 6 h. About 0.5% of the administered radioactivity was recovered as $^{14}$CO$_2$ (range 0.08 to 1.4%). Urine collected during the 24 h following injection of sulfoacetate contained about 85% of the administered radioactivity (range 81 to 87%). Portions of the urine samples were analyzed by HPLC and by chromatography on Dowex 1; the only radiolabeled species detected was sulfoacetate.

Effect of $\beta$-Methyleneaspartate and $\beta$-Ethylideneaspartate on Cysteinesulfonate Decarboxylation—$\beta$-Methyleneaspartate is a mechanism-based inhibitor of cysteinesulfinate decarboxylase (38). Previous studies comparing the deacryyoxylation of [1-14C]- and [3-14C]cysteinesulfinate indicated that the decar-
boxylation of exogenous cysteinesulfinate is inhibited about 84% in mice given \( \beta \)-methylene-DL-aspartate (2.5 mmol·kg\(^{-1} \)) 1 h previously; accurate interpretation of the studies required [1\(^{14}\)C]- and [3\(^{14}\)C]pyruvate metabolism to be separately determined (38). The present studies indicate that L-[\(^{14}\)C]cysteinesulfonate is a more convenient \textit{in vivo} probe of cysteinesulfinate decarboxylase activity. As shown in Fig. 3, the initial rate of \(^{14}\)CO\(_2\) formation from L-[\(^{14}\)C]cysteinesulfonate is decreased to 38% to 40% in mice given \( \beta \)-methyleneaspartate. Radioactivity excreted in the urine increased from 36 ± 3% in the controls to 48 ± 2% in the inhibitor-treated mice. \( \beta \)-Ethylidene-DL-aspartate is a less potent inhibitor of cysteinesulfinate decarboxylase \textit{in vitro} (28), but, in contrast to \( \beta \)-methyleneaspartate, \( \beta \)-ethylideneaspartate does not inhibit AAT (39). A dose of 2.5 mmol·kg\(^{-1} \) \( \beta \)-ethylidene-DL-aspartate does not significantly inhibit the rate of \(^{14}\)CO\(_2\) formation from L-[\(^{14}\)C]cysteinesulfonate (data not shown). At higher doses (5 mmol·kg\(^{-1} \) given twice or 10 mmol·kg\(^{-1} \) given once) \( \beta \)-ethylideneaspartate causes convulsions and kills >75% of the animals; \( \beta \)-methyleneaspartate is not toxic at similar doses.

To determine the extent, duration, and tissue specificity of decarboxylase and transaminase inhibition following administration of \( \beta \)-methyleneaspartate, mice were given two injections of \( \beta \)-methylene-DL-aspartate (2.5 mmol·kg\(^{-1} \) injection, 4 h apart). At various times, groups of three animals were killed, and the residual cysteinesulfinate decarboxylase and AAT activity in homogenates of brain, liver, and kidney were determined. The homogenates were prepared in 10 mM dithiothreitol to minimize post-mortem inhibition (38, 40). Neither enzyme was significantly inhibited in brain. As shown in Fig. 4, A and B (Miniprint), liver cysteinesulfinate decarboxylase activity is inhibited 60% within 2 h and remains substantially inhibited (50–75%) for about 24 h. Maximal AAT inhibition (about 25%) occurs at 8 h and is short-lived. Kidney decarboxylase is inhibited >95% within 2 h and remains substantially inhibited (>80%) for at least 48 h. Kidney AAT is inhibited about 40% at 2, 4, and 8 h and then recovers to nearly normal levels by 24 h. Mice treated with two doses of \( \beta \)-methylene-DL-aspartate were given L-[\(^{14}\)C]cysteinesulfonate 8 h after the first injection, and the rate of respiratory \(^{14}\)CO\(_2\) formation was monitored and compared to that observed with control animals not given inhibitor (Fig. 4C, Miniprint). The rate of cysteinesulfonate decarboxylation was inhibited about 40%, a result similar to that seen 1 h after a single injection of \( \beta \)-methyleneaspartate (compare Figs. 3 and 4C).

**Effect of \( \beta \)-Methylene-DL-aspartate on the Formation of \(^{14}\)CO\(_2\) from Radiolabeled \( \beta \)-Sulfopyruvate and \( \beta \)-Sulfolactate**—As shown in Fig. 2A (lower curve), \( \beta \)-methylene-DL-aspartate (2.5 mmol·kg\(^{-1} \)) decreases the initial rate of \(^{14}\)CO\(_2\) formation from \( \beta \)-sulfo(\(^{14}\)C)pyruvate by about 70%. Radioactivity recovered in the urine increased from 59.5 ± 4.0% to 72.5 ± 5.4%. Similarly, \( \beta \)-methyleneaspartate inhibits the metabolism of \( \beta \)-sulfo(\(^{14}\)C)lactate to \(^{14}\)CO\(_2\) by >85% (Fig. 2B, lower curve). \( \beta \)-Ethylideneaspartate (2.5 mmol·kg\(^{-1} \)) does not affect \(^{14}\)CO\(_2\) formation from \( \beta \)-sulfo(\(^{14}\)C)lactate (data not shown).

\( \beta \)-Sulfopyruvate and \( \beta \)-Sulfolactate as Substrates and Inhibitors of Other Enzymes of \( \alpha \)-Ketoacid and \( \alpha \)-Hydroxyacid Metabolism—\( \beta \)-Sulfopyruvate and \( \beta \)-sulfolactate are analogs of oxaloacetate and malate, respectively. Since the sulfonate moiety is larger than a carboxylate, \( \beta \)-sulfopyruvate is somewhat longer than oxaloacetate and is therefore also a possible analog of \( \alpha \)-ketogluutarate. \( \beta \)-Sulfolactate and \( \beta \)-sulfopyruvate were tested as substrates and inhibitors of several enzymes involved in malate, oxaloacetate, and \( \alpha \)-ketogluutarate metabolism; the results are summarized below (detailed assay procedures and results are given in Miniprint). \( \beta \)-Sulfolactate is not a substrate of chicken liver NADP\(^{+}\)-dependent malic enzyme (<0.03% of the activity observed with oxaloacetate), but it is an effective inhibitor. In the presence of 1.0 mm L-malate, 1.0, 5, and 10 mm DL-\( \beta \)-sulfolactate inhibit NADP\(^{+}\) reduction by 55, 83, and 91%, respectively; \( \beta \)-sulfopyruvate inhibits much less. \( \beta \)-Sulfopyruvate is a very poor substrate of pig heart citrate synthase (0.12–0.14% of the rate seen with oxaloacetate) and is also a weak inhibitor (28% inhibition in the presence of 0.05 mm oxaloacetate and 5 mm \( \beta \)-sulpyruvate). \( \beta \)-Sulfopyruvate is not a substrate of either \( \alpha \)-ketoglutarate dehydrogenase or glutamate dehydrogenase and is only a weak inhibitor (e.g. with 0.1 mm \( \alpha \)-ketoglutarate, \( \alpha \)-ketoglutarate dehydrogenase is inhibited 20% by 5 mm \( \beta \)-sulfopyruvate and glutamate dehydrogenase is inhibited 6% by 5 mm \( \beta \)-sulfopyruvate).

**DISCUSSION**

The present studies indicate that L-cysteinesulfonate and \( \beta \)-sulfopyruvate are metabolized by the several pathways shown in Fig. 5. Consistent with earlier qualitative studies (20, 41), we find that exogenous radiolabeled cysteinesulfonate is predominantly (60–75%, depending on the dose) decarboxylated to taurine and \( \text{CO}_2 \) by cysteinesulfinate decarboxylase. A smaller but significant fraction of the cysteinesulfonate (20–25%) is transaminated to \( \beta \)-sulfopyruvate, reduced to \( \beta \)-sulfolactate, and excreted as such in the urine. Small amounts of sulfoacetate and unmetabolized cysteinesulfonate (<5%) are also excreted in the urine, whereas \( \beta \)-sulfopyruvate is not excreted. Similar studies with radiolabeled \( \beta \)-sulfopyruvate indicate that transamination to cysteinesulfonate and reduction to \( \beta \)-sulfolactate each contribute substantially and about equally to its metabolism; most of the cysteinesulfonate formed is decarboxylated to taurine. Sulfoacetate is a minor (<4%) metabolite of \( \beta \)-sulfopyruvate. Reversibility of the malate dehydrogenase-catalyzed reduction of \( \beta \)-sulfopyruvate is established by studies showing that 12% of parenterally administered \( \beta \)-sulfolactate is oxidized to \( \beta \)-sulfopyruvate, transaminated to cysteinesulfonate, and decarboxylated to \( \text{CO}_2 \) and taurine.
Transamination of cysteinesulfonate and cysteinesulfinate are catalyzed by both c-AAT and m-ATT (8, 29, 33, 34, and Table I). Although previous reports indicate that cysteinesulfinate transamination is 3.5- to 11.6-fold faster than aspartate transamination (8, 29, 34), we find under physiological conditions of pH and α-ketoglutarate concentration that the maximum rates for both cysteinesulfinate and cysteinesulfonate transamination are close to that of aspartate (relative rates = 0.31–1.87). It is noted that previous studies relied on assays in which the products were not trapped and that the rate of aspartate transamination may have been systemically underestimated due to product accumulation and consequent inhibition of the forward reaction. Since cysteinesulfinate transamination is always irreversible due to β-sulfinylpyruvate decomposition (7), its rate would not have been similarly underestimated (see “Experimental Procedures” for studies addressing this point). Previous work indicated that the $K_m$ of cysteinesulfinate is significantly higher than the $K_m$ of aspartate (34); we confirm that finding and report that the $K_m$ of cysteinesulfonate is 4- to 7-fold higher than that of cysteinesulfinate. These results are in accord with the general observation that sulfonates are better structural analogs of carboxylates than are sulfonates. Interestingly, in the reverse reaction, the $K_m$ of β-sulfopyruvate with both c-AAT and m-AAT is close to that of oxaloacetate; on a relative rate basis, β-sulfopyruvate is a somewhat better alternate substrate of m-AAT than of c-AAT. Although m-AAT is the more effective catalyst of cysteinesulfonate transamination, the extent of cysteinesulfonate transport across the mitochondrial inner membrane is unclear. Whereas cysteinesulfinate enters the matrix readily via the glutamate-aspartate carrier ($K_a$ ≈ 1.3 mM (Fig. 4 of Ref. 42), cysteinesulfonate exchanges only slowly with intramitochondrial aspartate even when present extramitochondrionally at a high concentration (10 mM) (42). Since intracellular concentrations of cysteinesulfonate are low (e.g. < 0.02 mM in brain (14)), it is probable that most cysteinesulfinate is transaminated in vivo by c-AAT. In contrast, cysteinesulfinate transamination may be mediated primarily by m-AAT, which has the more favorable $K_m$ (Table I) and accounts for 70–75% of the total AAT activity in mouse liver (43). Since the mitochondrial transport of β-sulfopyruvate has not been examined, the relative importance of c-AAT and m-AAT in its transamination can not be estimated. The finding that parenterally administered β-sulfopyruvate is completely metabolized rather than excreted in the urine strongly suggests that β-sulfopyruvate is efficiently taken up by plasma membrane transport system(s) in the kidney tubule; it is probable that other tissues including liver also take up β-sulfopyruvate.

Cysteinesulfinate decarboxylase, a cytoplasmic enzyme, decarboxylates cysteinesulfinate at only 8% of the rate of cysteinesulfinate but with a favorable $K_m$ of 0.66 mM (28). In comparison, c-AAT under in vivo conditions of pH and α-ketoglutarate concentration has a $K_m$ for cysteinesulfinate of about 55 mM (Table I). Thus, although c-AAT activity exceeds cysteinesulfinate decarboxylase activity in mouse liver by about 28-fold, decarboxylation accounts for 60–75% of cysteinesulfonate metabolism in vivo. Consistent with an important effect of $K_m$ on partitioning, the fraction of l-[1-14C]cysteinesulfinate decarboxylated increases as the dose administered decreases (Fig. 1). Although these results are reminiscent of recent studies indicating that exogenous cysteinesulfinate is 85% decarboxylated and 15% transaminated (38), cysteinesulfinate and cysteinesulfonate metabolism are not directly comparable since only cysteinesulfonate transamination is reversible. Studies with radiolabeled β-sulfopyruvate suggest, for example, that almost half of the β-sulfopyruvate formed from cysteinesulfinate may be transaminated to reform cysteinesulfinate. Since the AAT reaction with cysteinesulfinate has a $K_m$ of 8.9 ($K_m$ = [cysteinesulfinate]α-ketoglutarate)/[β-sulfopyruvate][glutamate]), cysteinesulfonate transamination is thermodynamically as well as kinetically reversible; under similar conditions, the $K_m$ with aspartate is 6.7 (38).

Previous studies showed that β-sulfopyruvate is reduced by NADPH in the presence of pig heart mitochondrial malate dehydrogenase; although the $K_m$ is relatively high (6.3 mM), the $V_{max}$ is 40% of that observed with oxaloacetate (30). The present studies indicate that pig heart cytoplasmic malate dehydrogenase is a similarly effective catalyst in vitro and establish further that β-sulfopyruvate is significantly reduced in vivo. The observed partitioning between transamination (23–44% of the dose given based on 14C and 33S data) and reduction (53% of the dose based on 33S data) reflects both kinetic and thermodynamic factors. Thus, c-AAT has a more favorable $K_m$ for β-sulfopyruvate than does malate dehydrogenase, but the $K_m$ of transamination favors cysteinesulfonate only weakly. Malate dehydrogenase, on the other hand, has a less favorable $K_m$, but the $K_m$ of that reaction is strongly in favor of β-sulfo lactate (30). The rapidity of the total metabolism accords with the fact that both AAT and malate dehydrog...
Cysteinesulfonate and β-Sulfopyruvate Metabolism

drogenase are abundant in liver and other tissues. Overall, the findings suggest that cysteinesulfonate, β-sulfopyruvate, and β-sulfolactate are reversibly and rapidly interconverted until the combined pool is depleted by cysteinesulfonate decarboxylation or β-sulfolactate excretion, each an irreversible process. For example, the finding that 12% of the β-sulfo[1-14C]pyruvate formed is expected to be decarboxylated in 6 h (Fig. 2A), the results suggest that 40% (i.e. 12% + 30% x 100) of the administered β-sulfo[1-14C]pyruvate may be initially oxidized to β-sulfopyruvate. The relative importance of cytoplasmic and mitochondrial malate dehydrogenase in β-sulfopyruvate and β-sulfolactate metabolism is unknown. Although the enzyme is about evenly divided between the two compartments (44), mitochondrial metabolism may be limited by transport across the inner membrane.

Although actively metabolized by AAT and malate dehydrogenase, β-sulfolactate and β-sulfopyruvate are not effective substrates for several other enzymes active toward structurally related dicarboxylic acids. Thus, β-sulfolactate is not converted to pyruvate by malic enzyme, a finding consistent with the absence of 14CO2 formation from L-[3-14C]cysteinesulfonate (i.e. [3-14C]cysteinesulfonate is metabolized in part to β-sulfo[3-14C]lactate, but the latter is not converted to [3-14C] pyruvate, a precursor of 14CO2 (38), by malic enzyme or any other process). Similarly, β-sulfopyruvate is not oxidatively decarboxylated by α-ketoglutarate dehydrogenase; although sulfocetate is a minor metabolite of β-sulfopyruvate (and cysteinesulfonate), the process responsible for its formation remains unknown. The finding that β-sulfolactate is an effective inhibitor of malic enzyme indicates that the malate-binding site does not discriminate well between β-sulfonates and β-carboxylates and emphasizes the potential utility of sulfonates in substrate analog and inhibitor design. Neither malic enzyme inhibition by β-sulfolactate nor citrate synthase inhibition by β-sulfopyruvate is likely at endogenous levels of the sulfonates.

As noted, in vivo inhibition of cysteinesulfinate decarboxylase was previously estimated by comparing the extents to which [1-14C]- and [3-14C]cysteinesulfinate are metabolized to 14CO2 in control and inhibitor-treated mice (3, 38). Although theoretically sound, the studies are laborious and indirect. The present studies establish that the rate of 14CO2 formation from [1-14C]cysteinesulfinate is a direct and more convenient in vivo measure of decarboxylase activity. As shown in Fig. 3, the rate of [1-14C]cysteinesulfinate decarboxylation is decreased about 40% in animals given β-methylene-dl-aspartate (2.5 mmol·kg⁻¹) 1 h previously. This result compares closely with the 34% inhibition observed in studies using L-[1-14C]- and [3-14C]cysteinesulfinate (38). The finding that β-methyleneaspartate inhibits the formation of 14CO2 from β-

Acknowledgment—We thank Ernest B. Campbell and Michael A. Hayward for excellent technical assistance.

REFERENCES

The greater inhibition of 14CO2 formation from β-sulfo[1-14C]pyruvate and β-sulfo[1-14C]lactate than from L-[1-14C]cysteinesulfonate may indicate that the various substrates are taken up and metabolized in large part by different tissues (e.g. cysteinesulfonate may be taken up mainly by liver whereas β-sulfopyruvate and β-sulfolactate may be taken up by the kidney where both AAT and decarboxylase are abundant in liver and kidney homogenates indicates that β-methyleneaspartate affects a substantial (90%) in kidney, >50% in liver) inhibition that persists for 24-30 h. By inhibiting of AAT is less extensive and, in liver, of short duration; neither enzyme is significantly inhibited in brain. Despite the substantial inhibition of hepatic and renal decarboxylase activity, in vivo L-[1-14C]cysteinesulfonate decarboxylation is inhibited only about 40%. At present a definitive explanation for the difference between the in vitro and in vivo activity assays can not be given. The less inhibition determined in vivo may reflect the small metabolic role of kidney relative to liver, which is less inhibited; it may also indicate the metabolic importance of uninhibited enzyme in brain and possibly other tissues. It is also noted that intracellular L-[1-14C]cysteinesulfonate levels are expected to be higher in inhibitor-treated than in control mice. As the substrate concentration approaches and slightly excesses the Km, the metabolic efficiency of the residual uninhibited decarboxylase increases. This consideration applies to the metabolism of both exogenous L-[1-14C]cysteinesulfinate and endogenous L-cysteinesulfinate. Other possibilities appear less likely. To the extent that cysteinesulfinate decarboxylase is not fully rate-limiting in L-[1-14C]cysteinesulfinate decarboxylation, its inhibition will not proportionately reduce 14CO2 formation. Difficulties of this nature are unlikely, however, since the only other metabolic process required is cysteinesulfinate transport into cells. Since little cysteinesulfinate is lost in the urine, transport is apparently rapid. The finding that L-[1-14C]cysteinesulfinate decarboxylation is reduced 90% in mice given D-cysteinesulfinate, a reversible and specific decarboxylase inhibitor (46), indicates substantial metabolic inhibitions can be detected by the methodology. In aggregate, the studies with β-methyleneaspartate emphasize the importance of using in vivo assays to accurately evaluate the extent to which metabolism (as distinct from enzyme activity per se) is inhibited in vivo.
Cysteinesulfonate and β-Sulfonylpyruvate Metabolism


1. Additional references are found on p. 3743.
Cysteine sulfoxide and β-Sulfopropionate Metabolism

The reaction mixture was heated at 100°C for 10 min, to precipitate the protein, and the resulting mixture was clarified by centrifugation. The supernatant was then added to a dialysis bag (molecular weight cut-off 10,000) and dialyzed against 100 mM sodium acetate buffer, pH 5.0, for 4 h. The dialyzed supernatant was then concentrated by vacuum centrifugation to a small volume, and subjected to liquid chromatography on a TSK-GEL column (2.6 × 65 cm) at 30°C, using a gradient of 0% to 50% methanol in 0.1 mM sodium acetate buffer, pH 5.0, at a flow rate of 1.0 mL/min. The elution profile was monitored at 280 nm. The peak corresponding to Cysteine sulfoxide was eluted at a retention time of 15 min.

Synthesis of unlabeled δ-Sulfopropionate: Solution of unlabeled δ-Sulfopropionate (11.34 mg) was dissolved in 50 mL of water and warmed to 80°C. To this solution, 50 mL of 20% (w/v) sodium hydroxide solution was added, and the mixture was stirred for 2 h. The solution was then cooled to room temperature, and the pH was adjusted to 2.0 with 1 N hydrochloric acid. The precipitate was collected by filtration, washed with water, and dried. The yield was 9.21 mg (90% recovery).

Synthesis of δ-Sulfopropionate: A solution of sodium sulfite (15.4 g) was added to a solution of 10% (w/v) sodium hydroxide (50 mL) and the mixture was stirred for 1 h. The mixture was then poured into water (200 mL) and adjusted to pH 2.0 with 1 N hydrochloric acid. The precipitate was collected by filtration, washed with water, and dried. The yield was 13.2 g (90% recovery).

Synthesis of δ-Sulfopropionate: δ-Sulfopropionate was prepared by the procedure described for unlabeled sulfopropionate with the following modifications. The reaction mixture was heated at 100°C for 20 min. The pH was then adjusted to 2.0 with 1 N hydrochloric acid, and the mixture was stirred for 1 h. The precipitate was collected by filtration, washed with water, and dried. The yield was 13.2 g (90% recovery).
Cysteinesulfonate and \( \beta \)-Sulfopyruvate Metabolism

The apparent Vg was determined in duplicate for each direction at 30 and 60 min. For the 5 independent measurements, the equilibrium concentration of cysteinesulfonate, cysteine, and glutamate were 0.07 ± 0.01, 0.27 ± 0.03, and 2.57 ± 0.07, respectively. The apparent Km for cysteinesulfonate was determined in duplicate.

\( \beta \)-Sulfopyruvate as a substrate of Malate Dehydrogenase

The reaction mixtures (10.0 ml) contained 100 mg/ml sucrose phosphate buffer, 3.5 M Mg-ATP, and 100 mmol/L NaF. Enzyme extracts were assayed in the presence of 0.5 mmol/L malate dehydrogenase (0.5 mg/ml). The reaction was stopped after 10 min at 37°C. The reaction was assayed for cysteinesulfonate and P-sulfopyruvate metabolism.

In the presence of 0.005 mg/ml of antithrombin III, the reaction rate was measured in separate experiments with 0.5 mmol/L NaHCO3, 25 mmol/L \( \beta \)-Sulfopyruvate, and 5.0 mmol/L cysteinesulfonate. The reaction rates were calculated by subtracting the rate of cysteinesulfonate from the rate of \( \beta \)-Sulfopyruvate. The results were as follows:

- Cysteinesulfonate: 0.45 ± 0.03
- \( \beta \)-Sulfopyruvate: 0.92 ± 0.04

The results indicate that the 

\[ \text{Cysteinesulfonate} + \text{H}_2\text{O} \rightarrow \text{Cysteine} + \text{Sulfite} \]

reaction was faster than the 

\[ \text{\( \beta \)-Sulfopyruvate} + \text{H}_2\text{O} \rightarrow \text{Cysteine} + \text{Sulfite} \]

reaction. The difference in reaction rates was statistically significant (p < 0.05).

3-Malate as a Substrate of Malate Dehydrogenase

The reaction mixtures (10.0 ml) contained 100 mg/ml sucrose phosphate buffer, 3.5 M Mg-ATP, and 100 mmol/L NaF. Enzyme extracts were assayed in the presence of 0.5 mmol/L malate dehydrogenase (0.5 mg/ml). The reaction was stopped after 10 min at 37°C. The reaction was assayed for cysteinesulfonate and P-sulfopyruvate metabolism.

In the presence of 0.005 mg/ml of antithrombin III, the reaction rate was measured in separate experiments with 0.5 mmol/L NaHCO3, 25 mmol/L \( \beta \)-Sulfopyruvate, and 5.0 mmol/L cysteinesulfonate. The reaction rates were calculated by subtracting the rate of cysteinesulfonate from the rate of \( \beta \)-Sulfopyruvate. The results were as follows:

- Cysteinesulfonate: 0.45 ± 0.03
- \( \beta \)-Sulfopyruvate: 0.92 ± 0.04

The results indicate that the 

\[ \text{Cysteinesulfonate} + \text{H}_2\text{O} \rightarrow \text{Cysteine} + \text{Sulfite} \]

reaction was faster than the 

\[ \text{\( \beta \)-Sulfopyruvate} + \text{H}_2\text{O} \rightarrow \text{Cysteine} + \text{Sulfite} \]

reaction. The difference in reaction rates was statistically significant (p < 0.05).