The Role of Mitochondrial Matrix Enzymes in the Metabolism and Toxicity of Cysteine Conjugates*

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The submitochondrial localization and identity of enzymes which metabolize cysteine conjugates were investigated. Glutamine transaminase K was purified from rat kidney mitochondrial soluble fraction and was shown to be a cysteine conjugate β-lyase. The purified mitochondrial enzyme is similar to the cytosolic glutamine transaminase K whose β-lyase activity with S-(1,2-dichlorovinyl)-L-cysteine (DCVC) is regulated by concurrent transamination (Stevens, J. L., Robbins, J. D., and Byrd, R. A. (1986) J. Biol. Chem. 261, 15529-15537). However, β-lyase activity in whole mitochondria is largely independent of regulation by cosubstrates for transamination suggesting that factors present in mitochondria are able to support the β-lyase activity in the absence of added α-keto acid. Fractionation of mitochondria results in a loss of the independent β-lyase activity. However, the majority of the β-lyase activity can be recovered in the matrix if it is stimulated by the addition of α-keto-γ-methylbutyrate.

The data suggest that the regulation of β-elimination by the competing transamination pathway is different for each substrate and that multiple β-lyases may exist in rat kidney. S-(2-Benzothiazolyl)-L-cysteine (BTC) is a poor substrate for purified glutamine transaminase K from mitochondria and cytosol, but BTC is as active as DCVC in a crude mitochondrial matrix suggesting that other enzymes may be present. In contrast to DCVC, with BTC as substrate, the β-lyase activity of the purified enzyme and enzyme(s) in the mitochondrial matrix is largely α-keto acid-independent. The existence of multiple enzymes is also supported by the observation that α-keto acids which are not substrates for purified glutamine transaminase K from mitochondria and cytosol do stimulate β-lyase activity in the mitochondrial matrix fraction.

Mitoplasts were found to be sensitive to DCVC toxicity consistent with the matrix localization of β-lyase activity. The possible role in cysteine conjugate toxicity of matrix enzyme regulation by α-keto acids is discussed.

Classically, mercapturate biosynthesis has been considered primarily a detoxication system (1, 2). However, recent studies have shown that the mercapturic acid pathway produces toxic species from a variety of drugs and xenobiotics (3-5). The toxicity of some mercapturic acid pathway intermediates depends on their degradation to cysteine conjugates which are subsequently activated to toxic species by cysteine conjugate β-lyase, a pyridoxal phosphate (PLP) 1-dependent enzyme (Equation 1 (3-19)). This mechanism was first proposed by Schultze and co-workers (6, 7) who suggested that S-(1,2-dichlorovinyl)-L-cysteine (DCVC) is metabolized by β-elimination (Equation 1) to a reactive thiol which binds covalently to cellular macromolecules. In recent studies this mechanism has been proposed to account for DCVC toxicity in several models (4, 5, 8, 9, 14, 15), and a similar mechanism has been suggested for the toxicity of other cysteine conjugates (4, 5, 12, 14, 18).

\[ \text{NH}_2\text{CH(COOH)}\text{CH}_3\text{SR} \rightarrow \text{CH}_3\text{(C=O)COOH} + \text{NH}_3 + \text{RSH} \quad (1) \]

Stoppard and Parker (8-10) first demonstrated in vivo and in vitro that mitochondria might be the cellular target for DCVC. Recent studies of Jones et al. (12, 13) with S-(1,2,3,4,4-pentachlorobutadienyl)glutathione and Lash et al. (14, 15) with S-(1,2-dichlorovinyl)glutathione (DCVG) and DCVC showed that mitochondria are a target in isolated rat kidney cells. With both S-(1,2,3,4,4-pentachlorobutadienyl)glutathione and DCVG, toxicity is blocked by inhibition of γ-glutamyltransferase with AT125 or anthglutin or inhibition of cysteine conjugate β-lyase by aminooxyacetic acid (AOA) (12-15). Their data also indicate that perturbation of mitochondrial calcium sequestration may be an early event related to toxicity.

However, the available data do not explain a number of questions regarding the mechanism of toxicity and the organ specificity. For example, both DCVG and DCVC are nephrotoxins (11, 16) suggesting that events subsequent to degradative conversion of DCVG by γ-glutamyltransferase and cysteinylglycine dipeptidase could be involved in the organ specificity. Furthermore, within the kidney the proximal tubule is the target and within the proximal tubule itself the pars recta (S3) segment is most sensitive (11, 18). Nash et al. (18) showed that the binding of radiolabel from hexachloro[14C]butadiene was localized to S3 indicating that more conjugate might be activated there. A more complete understanding of conjugate delivery to the target tissue and the biochemical regulation of their activation in target cells and organelles may explain the factors which regulate the mechanism and specificity for toxicity. In this regard, the study of the segment-specific toxicity of cysteine conjugates may be a useful tool in invest-

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The abbreviations used are: PLP, pyridoxal phosphate; MTA, α-keto-γ-methylbutyrate; DCVC, S-(1,2-dichlorovinyl)-L-cysteine; DCVG, S-(1,2-dichlorovinyl)glutathione; DMO, S-(1,2-dichlorovinyl)-3-mercapto-2-oxopropionic acid; BTC, S-(2-benzothiazolyl)-L-cysteine; TPA, trifluoroacetic acid; PMF, pyridoxamine phosphate; mGTP, mitochondrial glutamine transaminase K; eGTP, cytosolic glutamine transaminase K; AOA, aminoxyacetic acid.

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tigating the biochemistry underlying heterogeneity in renal function.

We have been characterizing the cysteine conjugate β-lyase(s) from rat kidney (17, 19) and have made two observations which may be important in understanding the biochemical basis for cysteine conjugate toxicity. First, a tissue-specific cytosolic transaminase, glutamine transaminase K, has been purified from rat kidney and shown to be a β-lyase. Second, cytosolic glutamine transaminase K catalyzes transamination as well as β-elimination, and DCVC partitions equally between the two pathways. The transamination reaction regulates the rate of β-elimination when an inadequate supply of cosubstrate, i.e. an α-keto acid, is present because the enzyme will accumulate in the pyridoxamine (PMP) form after half-transamination. DCVC cannot form a Schiff base with PMP enzyme, and β-elimination is inhibited. L-Amino acid oxidase (L-α-hydroxycarboxylic oxidase (22)) cooperates with cytosolic glutamine transaminase K to support β-elimination by directly oxidizing DCVC to DMOP which serves as a source of α-keto acid (17).

Rat kidney contains a mitochondrial counterpart of cytosolic glutamine transaminase K (20) which is located in the matrix (14) but whose properties as a β-lyase have not been studied. In this study, we characterize the sub mitochondrial localization of enzymes which metabolize DCVC and characterized the regulation of mitochondrial enzymes by transamination. The role of toxicity of β-lyases from different mitochondrial compartments is investigated with particular attention to the participation of mitochondrial glutamine transaminase K. Previous fractionation experiments by Lash et al. (14) suggested that mitochondrial β-lyase is localized predominantly in the outer membrane and that mitochondrial glutamine transaminase K is not the mitochondrial β-lyase. Our results differ from those of Lash et al. (14), and the differences are discussed. The possibility that fractionation experiments could be complicated by compartmentalization of factors which support the β-elimination reaction is considered.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

**Metabolism of DCVC and the Covalent Binding of Reactive Species in Mitochondria and Mitoplasts**—Initially, we investigated the metabolism of [14C]DCVC in mitochondria and mitoplasts in the presence and absence of added α-keto-γ-methylbutyrate (MTB) (Table 1). Without MTB added to the incubations, activity decreased upon removal of the mitochondrial outer membrane (mitoplasts). However, when MTB was added to the mitoplasts, the specific activity was comparable to that found in mitochondria. β-Lyase activity in mitochondria was stimulated only 60% by MTB while in mitoplasts the activity increased 4-fold (Table 1). The difference in stimulation was due to a loss of activity in the mitoplasts when no MTB was added to the incubations. It appeared that factors which support the β-lyase activity are lost when the outer membrane is removed with digitonin, and those factors can be replaced by MTB.

The [14C]DCVC assay measures both β-elimination and transamination but does not distinguish between the two pathways (17). However, β-elimination from [35S]DCVC yields a reactive 35S-labeled cleavage fragment which covalently binds to macromolecules (5–7). Experiments with rat kidney amino acid oxidase, which converts DCVC exclusively to DMOP (17), the transamination product of DCVC, showed that DMOP is not reactive and does not bind to macromolecules (see “Experimental Procedures”). Therefore, the binding of 35S label is a measure of the β-elimination pathway and not transamination. When we measured the binding of 35S label from [35S]DCVC to trichloroacetic acid-insoluble material in mitochondria and mitoplasts, there was little difference in the MTB stimulation of binding, ~30% in both cases, or the specific activity (Table 1). Therefore, though total 14C-metabolites are increased in mitoplasts when MTB is added, the difference is due primarily to an accumulation of [14C]DMOP with little change in the amount of DCVC metabolized by β-elimination. The mechanism of the differential stimulation of metabolism and binding will be addressed further under “Discussion.”

AOA blocks the toxicity of DCVC in mitochondria (14) and other model systems (5, 12–15). AOA inhibited both the binding of 35S label and the metabolism of DCVC. The component of AOA-insensitive binding (Table 1) was somewhat higher than the value for metabolism. This is probably due to a degree of nonenzymatic breakdown of [35S]DCVC to reactive species.

**Submitochondrial Localization of Glutamine Transaminase K and β-Lyase**—Since mitoplasts did appear to retain β-lyase activity we investigated the submitochondrial distribution of the activity and compared it to the distribution of mitochondrial glutamine transaminase K (Table 2). The majority of the β-lyase activity with DCVC and the mitochondrial glutamine transaminase K activity is located in the matrix. However, in the absence of MTB, only 42% of the DCVC activity was recovered in all fractions, and only 21% was in the matrix. When MTB was included in the assays, recovery increased to 120% with 71% recovered in the matrix. The matrix DCVC activity was much more dependent on added MTB than the activity in whole mitochondria. Some of the β-lyase (31%) and mitochondrial glutamine transaminase K (24%) activities were recovered in the intermembrane space fraction suggesting that some of the mitoplasts ruptured during removal of outer membrane with digitonin. The results indicated that both cysteine conjugate β-lyase activity and mitochondrial glutamine transaminase K activity were located in the matrix fraction and that when mitochondria are fractionated, factors are lost that support the β-lyase activity. These factors can be replaced by the addition of MTB. Repeated attempts to stimulate the activity by recombining the various fractions were unsuccessful (data not shown).

**Purification of Glutamine Transaminase K and Characterization of Its Role as a β-Lyase**—Since cytosolic glutamine transaminase K and mitochondrial glutamine transaminase K have similar substrate specificity and physical properties (20, 21), it seemed that the matrix activity might be due to mitochondrial glutamine transaminase. If this were true, then the stimulation of matrix β-lyase activity by α-keto acids should have a structure-activity relationship similar to that seen with cytosolic glutamine transaminase K (17, 20, 21), i.e. a predilection for hydrophobic α-keto acids. When a variety of α-keto acids was added to the matrix fraction, phenylpyruvate and MTB were most stimulatory, but two of the hydrophilic α-keto acids (α-keto glutarate and oxaloacetate), which are inactive with cytosolic glutamine transaminase K (see Table V in Ref. 17), also stimulated the matrix activity substantially (Table 3). When MTB was increased from 0.5 to 1.0 mM there was no increase in the stimulation suggesting that 0.5 mM MTB was a saturating concentration. However,
Effect of \( \alpha \)-keto-\( \gamma \)-methyloxybutyrate on \( \beta \)-lyase activity in mitochondria and mitoplasts

Metabolism was determined with \([\text{C}]\)DCVC and binding with \([\text{S}]\)DCVC as described under "Experimental Procedures." Since the error in the metabolism and binding data was greater between experiments than within an experiment, the specific activities shown are from triplicate determinations on one preparation of mitoplasts or mitochondria (\(X \pm S.D.\) and are representative of experiments on three separate preparations. However, the ratio (+MTB/−MTB) is the mean ± S.D. of the ratios calculated from three separate preparations (\(n = 3\)). AOA (0.2 mM) controls were not subtracted from the data prior to calculating the ratio (+MTB/−MTB; see text). This concentration of AOA is sufficient to inhibit purified enzyme (17) and mitochondrial metabolism (14) >98%; therefore, the difference between the activity +AOA and −AOA represents the PLP enzyme component of binding and metabolism.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Metabolism</th>
<th>Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+MTB/−MTB</td>
<td>+MTB/−MTB</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>14.0 ± 0.8</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>7.6 ± 0.3</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Mitoplasts</td>
<td>8.0 ± 1.7</td>
<td>4.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>9.5 ± 0.5</td>
<td>1.3 ± 0.2</td>
</tr>
</tbody>
</table>

 Localization of \( \beta \)-lyase and glutamine transaminase K activity in mitochondria subfractions

Mitochondria were fractionated by the method of Greenawalt (25). Glutamine transaminase K was assayed by the method of Cooper and Meister (20), and DCVC metabolism (nmol/10 min/mg) was assayed with the extraction procedure and 1 mM DCVC in the presence and absence of 0.5 mM MTB for 10 min at 37°C (see "Experimental Procedures"). The specific activities shown are in the presence of 0.5 mM MTB. The percent recovery of DCVC metabolism is expressed with regard to the appropriate mitochondrial value with or without MTB. Therefore, although the mitochondria are shown as 100% in both cases, the value in the absence of MTB is 2-fold less than in the presence of MTB (Table 1). Typically 250–400 mg of protein were used for one fractionation experiment, and the data are the mean ± S.D. for four independent fractionations from pooled kidneys of four to eight rats.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Activity with DCVC</th>
<th>Glutamine transaminase K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>+MTB/−MTB</td>
<td>+MTB/−MTB</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>28 ± 5</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>Outer membrane</td>
<td>9 ± 1</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>Inner membrane</td>
<td>7 ± 2</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>Matrix</td>
<td>98 ± 30</td>
<td>71 ± 15</td>
</tr>
<tr>
<td>Intermembrane space</td>
<td>4 ± 9</td>
<td>31 ± 15</td>
</tr>
</tbody>
</table>

Stimulation of DCVC metabolism in mitochondrial matrix

Mitochondrial matrix was prepared from 12 rat kidneys as described by Greenawalt (26) and stored at −70°C until used. Metabolism was determined with DCVC (1 mM) using the extraction assay and an incubation time of 10 min at 37°C (see "Experimental Procedures"). The data are the X ± S.D. from triplicate determinations on one preparation of matrix and are representative of experiments from two preparations. Error within an experiment was much less than error between experiments; therefore, one experiment is shown. Experiment 2: no addition = 84 nmol/10 min/mg; 0.5 mM MTB = 133 nmol/10 min/mg; 0.5 mM MTB + 0.5 mM \( \alpha \)-ketoglutarate = 48 nmol/10 min/mg; 0.5 mM MTB + 0.5 mM oxaloacetate = 200 nmol/10 min/mg.

Comparison of Mitochondrial and Cytosolic Glutamine Transaminase K—When the purified rat kidney mitochondrial enzyme was compared to cytosolic glutamine transaminase K (17) it was found to be similar in several ways. First, like cytosolic glutamine transaminase K, mitochondrial glutamine transaminase K was completely inhibited by 0.1 mM hydroxylamine or aminoxyacetic acid and 50% by potassium cyanide (data not shown), three inhibitors of PLP-dependent enzymes. Second, the \( \beta \)-lyase activity was markedly stimulated by the addition of hydrophobic \( \alpha \)-keto acids (i.e. MTB and phenylpyruvate) but not by hydrophilic \( \alpha \)-keto acids (i.e.
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TABLE 4
Copurification of cysteine conjugate β-lyase and glutamine transaminase K from rat kidney mitochondria

Purification was done as previously described (17). Glutamine transaminase K activity (transaminase, TRA) was assayed by the method of Cooper and Meister (20), and the cysteine conjugate β-lyase (CBL) activity with [4C]DCVC was assayed in the presence of 0.5 mM MTB using the extraction assay as described under “Experimental Procedures.” The ratio of the glutamine transaminase to cysteine conjugate β-lyase is shown (TRA/CBL). Protein values were rounded off to the nearest mg, but the recoveries and specific activities were calculated from the protein data expressed to the nearest 0.1 mg.

<table>
<thead>
<tr>
<th>Step</th>
<th>Protein</th>
<th>β-Lyase</th>
<th>Transaminase</th>
<th>Ratio, TRA/CBL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>μmol/10 min</td>
<td>μmol/10 min</td>
<td>μmol/10 min</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>4490</td>
<td>0.01</td>
<td>44.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Supernatant</td>
<td>1157</td>
<td>0.03</td>
<td>34.7</td>
<td>0.29</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>822</td>
<td>0.05</td>
<td>41.1</td>
<td>0.29</td>
</tr>
<tr>
<td>DEAE</td>
<td>118</td>
<td>0.15</td>
<td>19.5</td>
<td>0.95</td>
</tr>
<tr>
<td>Hydroxylapatite</td>
<td>7</td>
<td>2.2</td>
<td>14.5</td>
<td>10.1</td>
</tr>
<tr>
<td>Sephacryl S-300</td>
<td>2</td>
<td>2.9</td>
<td>6.1</td>
<td>12.0</td>
</tr>
<tr>
<td>Q-300</td>
<td>1</td>
<td>3.9</td>
<td>3.3</td>
<td>20.7</td>
</tr>
</tbody>
</table>

TABLE 7
Comparison of BTC and DCVC activity

Mitochondrial matrix was prepared by the method of Greenswalt (26) as described under “Experimental Procedures” and stored at −70 °C until use. Purified mitochondrial and cytosolic glutamine transaminase K (GTK) were prepared as described under “Experimental Procedures.” BTC (2 mM) and DCVC (2 mM) were incubated under the standard assay conditions in the presence (+) or absence (−) of 0.5 mM MTB. The data are the mean ± the range from two separate experiments using one matrix preparation from 12 rats kidneys and one purified mitochondrial or cytosolic glutamine transaminase K preparation.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>MTB</th>
<th>BTC</th>
<th>DCVC</th>
<th>Ratio, BTC/DCVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial matrix</td>
<td>+</td>
<td>70 ± 10 mg</td>
<td>71 ± 24</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>Mitochondrial GTK</td>
<td>+</td>
<td>322 ± 57 mg</td>
<td>3960 ± 460</td>
<td>550 ± 550</td>
</tr>
<tr>
<td>Cytosolic GTK</td>
<td>+</td>
<td>757 ± 160 mg</td>
<td>9865 ± 1888</td>
<td>163 ± 163</td>
</tr>
</tbody>
</table>

oxaloacetate and α-ketoglutarate) (Table 5). The dependence on MTB showed a similar behavior to cytosolic glutamine transaminase K, i.e. an increasing stimulation of DCVC activity up to about 1000 μM MTB after which the activity decreased (Fig. 1). Third, the enzyme was stimulated by the addition of purified rat kidney amino acid oxidase (Fig. 2). Finally, by Ouchterlony double diffusion analysis, using affinity-purified antibody raised against the cytosolic glutamine transaminase K, no differences between the two proteins were detected (Fig. 3). The two forms were not separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown). There was a difference in the Vmax values for DCVC with cytosolic (22 μmol/10 min/mg (17)) and mitochondrial glutamine transaminase K (4 μmol/10 min/mg), but the Km values were similar, 0.3 (17) and 0.48 mM, respectively. The only detectable difference between the cytosolic and mitochondrial glutamine transaminase K was their separation by elution from hydroxylapatite at 3.4 and 4.3 mmoles, respectively. These data show that the purified mitochondrial glutamine transaminase K is very similar to cytosolic glutamine transaminase K, as reported by Cooper and Meister (20, 21) and that the β-lyase characteristics of the two enzymes are similar.

The observation that the purified enzyme and the matrix activity are stimulated by the addition of α-keto acids indicated that the matrix mitochondrial glutamine transaminase K may catalyze both transamination and β-elimination reactions as has been described for the cytosolic glutamine transaminase K (17). If this were true, then DMOP should be a product. When the 14C-metabolites from incubations containing purified enzyme, MTB, and [4C]DCVC were analyzed by HPLC, both DMOP and pyruvate were detected in a ratio of 1:1 (Table 6). Therefore, the lack of activity of mitochondrial matrix β-lyase may be due to the absence of α-keto acids to complete the transamination reaction as suggested for cytosolic glutamine transaminase K (17). More importantly, this mechanism accounts for the loss of β-lyase activity when mitochondria are fractionated.

Comparison of BTC and DCVC Activities—BTC is a commonly used substrate for the measurement of β-lyase activity, and the activity of β-lyase with this substrate is similar to that exhibited with DCVC in mitochondrial matrix (25). In three preparations, 107 ± 9% (X ± S.D.) of the BTC activity was recovered in the matrix fraction without MTB added to the incubations (n = 3). Less than 7% of the activity was recovered in the other fractions. We compared the activity of DCVC to that of BTC in the mitochondrial matrix and with purified mitochondrial and cytosolic glutamine transaminase K (Table 7). The activity with BTC in the matrix fraction was not dependent on the presence of MTB. With the purified enzymes, BTC had about 10% of the activity seen with DCVC, and the activity was stimulated only 2-fold by MTB. In contrast, MTB stimulated DCVC activity 7-fold and 60-fold with purified mitochondrial and cytosolic glutamine transaminase K, respectively. In the absence of MTB, the β-lyase activity was very low, and accurate measurement of metabolism was difficult as indicated by the large range of the data.

The Role of Mitochondrial Matrix Enzymes in the Toxicity of DCVC—Our data (Table 2) led us to conclude that mitochondrial matrix enzymes have β-lyase activity with DCVC, and these mitochondrial enzyme(s) are retained in mitoplasts. Therefore, we investigated the role of matrix metabolism in the toxicity of DCVC by comparing the effect of DCVC on succinate-stimulated respiration in mitochondria and mitoplasts (Fig. 4). When the time course of DCVC toxicity was determined, DCVC inhibited both state 3 and state 4 respiration in mitochondria and mitoplasts. Therefore, we concluded that mitochondrial matrix enzymes can metabolize...
DCVC to toxic species which inhibit mitochondrial respiration.

**DISCUSSION**

Cysteine conjugate \(\beta\)-lyase activity is a property of cellular PLP-dependent enzymes (17, 32). One of these enzymes is cytosolic glutamine transaminase K, a kidney enzyme which catalyzes both \(\beta\)-elimination and transamination with DCVC as substrate. Because both reaction are catalyzed by one enzyme, the pathways interact through a complex regulatory scheme in which the accumulation of the PLP cofactor as PMP inhibits the flow of DCVC through the \(\beta\)-elimination pathway (see Ref. 17 for discussion). An appreciation of this scheme is important in understanding cysteine conjugate toxicity. If cellular cofactors for transamination are limiting, enzyme could accumulate as PMP enzyme causing \(\beta\)-lyase activity and toxicity to decrease. Elfarra et al. (33) have suggested that MTB increases DCVC toxicity and BTC metabolism in mitochondria. However, since metabolism of DCVC and \(^{35}\)S binding were not measured, it is not clear that \(\beta\)-elimination of DCVC increased in the presence of MTB.

The results presented here suggest that enzymes located in the matrix fraction are responsible for the metabolism of DCVC in whole mitochondria. When mitochondria are fractionated using the digitonin method (26), the matrix enzymes become less active unless a source of \(\alpha\)-keto acid is added to the incubation. However, intact mitochondria retain factors which support the \(\beta\)-lyase activity with DCVC. Presumably, these unknown factors provide the necessary \(\alpha\)-keto acid to complete the transamination reaction and return PMP enzyme to PLP enzyme. The data point toward two possible identities for these unknown factors. The first is L-amino acid oxidase which, when added to incubations, stimulates cytosolic (17) and mitochondrial glutamine transaminase K (Fig. 2) by providing an external source of DMOP (17). The excess DMOP serves as an amino acceptor for PMP enzyme. L-Amino acid oxidase is concentrated in peroxisomes (22) which do not separate well from the mitochondrial fraction (34, 35), particularly in kidney (34). Damage to the peroxisomal membrane during tissue homogenization probably accounts for the purification of this enzyme from the cytosolic compartment (17, 22, 34, 35). The second possible source of \(\alpha\)-keto acids are those normally present in mitochondria as Kreb's cycle intermediates. Both oxaloacetate and \(\alpha\)-ketoglutarate, as well as their precursors aspartate and glutamate, are normal components of mitochondria (36), and both of these \(\alpha\)-keto acids stimulate metabolism in mitochondrial matrix (Table 3). During removal of the outer membrane with digitonin, it is possible that these small molecules are lost from the mitoplasts resulting in an apparent loss of enzyme.

At first glance the binding and metabolism data in Table 1 appear paradoxical. How can metabolism decrease in mitoplasts if binding of \(^{35}\)S label does not change to the same extent? The explanation lies in the recycling of DMOP to DCVC by the PMP enzyme. With the cytosolic enzyme (see Table V in Ref. 17) we showed that at limiting MTB concentrations the DMOP:pyruvate ratio decreased to 1:4 while at higher MTB concentrations the ratio was 1:3:1. The ratio changes because the DMOP pool decreased at limiting MTB concentrations while the amount of pyruvate, formed via the \(\beta\)-elimination pathway, remained constant. This is similar to the relationship between the \(^1\)C extraction and the \(^{35}\)S binding data in Table 1 since covalent binding reflects only the \(\beta\)-elimination pathway while \(^1\)C extraction represents total metabolism (see "Experimental Procedures" for further discussion). Therefore, the pool of DMOP is consumed during

![Fig. 4. Time course of DCVC toxicity in rat kidney mitochondria and mitoplasts.](image)
a complex formed between PLP and a substrate which does not have a good leaving group on the β-carbon. Leaving group character could play a significant role in toxicity by determining the ratio of β-elimination and transamination and the ability of cofactors to regulate the rate of metabolism. However, this must remain speculative until leaving group character is correlated with the metabolism and toxicity of the conjugates.

Our results differ from those of Lash et al. (14) who recovered most of the β-lyase activity with BTC and DCVC in the outer membranes and <2% of either activity in the matrix fraction. Several differences are worthy of consideration. α-Keto acids were not added in their assays, and other differences include the strain of rat, the specific activities reported (see "Experimental Procedures" for specifics for the β-lyase assay), and the amount of digitonin used in the fractionation. In our studies, concentrations of digitonin higher than 0.12 mg/mg mitochondrial protein resulted in uncoupling of State 4 respiration, and State 4 respiration is less sensitive to DCVC (Fig. 4). The differences in the specific activity might relate to differences in the assay methods or the method of protein determination (31, 37). It is likely that different mitoplast preparations show differences in the retention of mitochondrial factors which support the β-lyase activity; therefore, several differences in the methods and model might account for the differences in the data.

In conclusion, our results suggest that metabolism of DCVC in mitochondria is regulated by the availability of α-keto acid. It is conceivable that differences in enzyme concentration or activity in the different segments could account for differences in the toxicity within the proximal tubule. Though glutamine transaminase K activity is highest in the cortex (38), the localization of β-lyase enzymes within the proximal tubule has not been determined. Other factors such as transport and the sensitivity of the tissue must also be considered. Elucidation of the biochemical factors which control the segment specific toxicity of DCVC may lead to a better understanding of biochemical heterogeneity in the proximal tubule and its relationship to renal function as well as nephrotoxicity.

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Cysteine Conjugate Toxicity

Supplementary Material To: The Role of Mitochondrial Matrix Enzymes in the Metabolism and Toxicity of Cysteine Conjugates

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Table 6. Distribution of Products from the Metabolism of DCCD by the Purified Mitochondrial B-1-4

<table>
<thead>
<tr>
<th>Product Formed</th>
<th>PMT</th>
<th>Substrate Metabolized</th>
<th>Product Formed</th>
<th>PMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>10 ± 0.3</td>
<td>DCCD</td>
<td>Pyruvate</td>
<td>10 ± 0.3</td>
</tr>
<tr>
<td>L-3-Histidine-L-cysteine (L-3-HCL)</td>
<td>17.2 ± 0.3</td>
<td>L-3-Histidine-L-cysteine (L-3-HCL)</td>
<td>L-3-Histidine-L-cysteine (L-3-HCL)</td>
<td>17.2 ± 0.3</td>
</tr>
</tbody>
</table>

Figure 1. Stimulation of purified mitochondrial cysteine lyase. Assay by MTB. A. MOL. 10 min/ml. B. log MOL. MTB. C. AAO (log ng).

Figure 2. Stimulation of DCCD metabolism by rat kidney, L-cysteine oxidase. Purified mitochondrial B-1-4 (10 µg) was incubated with [14C]DCCD and assayed as described (17). Activity was measured using 1 µM [14C]DCCD and incubations were carried out for 10 min at 37°C.

Table 5. Structure Activity Relationship for the Stimulation of DCCD Metabolism by Purified Mitochondrial Glutathione Transamine K.

<table>
<thead>
<tr>
<th>Activity &amp; Structure</th>
<th>MOL. MTB/10 min</th>
<th>MOL. MTB/10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Soluble Acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a- Keto-3-Methylbutyrate</td>
<td>3.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Phenylpyruvate</td>
<td>9.4</td>
<td>9.4</td>
</tr>
<tr>
<td>3-Hydroxybutyrate</td>
<td>9.4</td>
<td>9.4</td>
</tr>
<tr>
<td>Salicylate</td>
<td>9.4</td>
<td>9.4</td>
</tr>
<tr>
<td>Glutathione</td>
<td>9.4</td>
<td>9.4</td>
</tr>
</tbody>
</table>

Figure 3. Glutathione double diffusion analysis of purified cysteine and glutathione transamine K. Wells 1, 2 and 3 (cysteine oxidase) contained 5 µg of purified cysteine oxidase transamine K and wells 1, 2 and 3 (cysteine oxidase) contained 5 µg of purified glutathione transamine K. Well 1 in the upper right quadrant contained 5 µg of purified cysteine oxidase transamine K and 5 µg of cysteine oxidase transamine K. Well 2 in the lower left quadrant contained 5 µg of purified glutathione transamine K and 5 µg of cysteine oxidase transamine K. Well 3 in the lower right quadrant contained 5 µg of purified cysteine oxidase transamine K and 5 µg of glutathione transamine K.