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Invited Review

Mercaptopyruvate sulfurtransferase as a defense against cyanide toxication: Molecular properties and mode of detoxification

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Summary. In cyanide poisoning, metalloproteins and carbonyl groups containing proteins are the main target molecules of nucleophilic attack by cyanide. To defend against this attack, cyanide is metabolized to less toxic thiocyanate via transsulfuration. This reaction is catalyzed by rhodanese and mercaptopyruvate sulfurtransferase (MST). Rhodanese is a well characterized mitochondrial enzyme. On the other hand, little was known about MST because it was unstable and difficult to purify. We first purified MST to homogeneity and cloned MST cDNA from rat liver to characterize MST. We also found that MST was an evolutionarily related enzyme of rhodanese. MST and rhodanese are widely distributed in rat tissues, and the kidney and liver prominently contain these enzymes. Immunohistochemical study revealed that MST is mainly distributed in proximal tubular epithelial cells in the kidney, pericentral hepatocytes in the liver, the perinuclear area of myocardial cells in the heart, and glial cells in the brain, and immunoelectron microscopical study concluded that MST was distributed in both cytoplasm and mitochondria, so that MST first detoxifies cyanide in cytoplasm and the cyanide which escapes from catalysis due to MST enters mitochondria. MST then detoxifies cyanide again in cooperation with rhodanese in mitochondria. Tissues other than the liver and kidney are more susceptible to cyanide toxicity because they contain less MST and rhodanese. Even in the same tissue, sensitivity to cyanide toxicity may differ according to the kind of cell. It is determined by a balance between the amount of proteins to be attacked and that of enzymes to defend.

Key words: Mercaptopyruvate sulfurtransferase, Rhodanese, Cyanide poisoning, Tissue distribution, Subcellular distribution, Immunohistochemistry, Immunoelectron microscopy

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Introduction

Cyanides have been used for various purposes in industry. The lethal oral dose of potassium cyanide is about 200 mg in an adult (Ellenhorn and Barceloux, 1988). In acute cyanide toxicosis, respiratory disturbance is a characteristic symptom. In chronic toxicosis, enlargement of the thyroid gland without dysfunction is occasionally observed. It is noticed that environmental pollution by cyanides causes sublethal damage to the central nervous and cardiovascular systems of wild animals, such as migratory birds (Ma and Pritsos, 1997). Cyanide attacks some metals belonging to metalloproteins such as heme iron of cytochrome c oxidase in mitochondria. In this case, electron transport is inhibited in the respiratory chain, and consequently the production of ATP is disturbed in cells. Depletion of ATP then induces apoptosis of the cells (Lin et al., 1998).

In a healthy person, a small amount of cyanide compounds is detected in plasma at the level of 4 ng/ml (Ellenhorn and Barceloux, 1988). These are exogenous metabolites taken from foods such as cyanogenic plants or smoking, or endogenous metabolite of vitamin B12 (cyanocobalamine). It is surprising that animals can detoxify cyanide to a certain extent. In man, intravenous cyanide was found to be eliminated at the rate of about 17 μg/kg body weight a minute (McNamara, 1976). Metabolic detoxification of cyanide is performed by sulfuration as in the following reaction:

$CN^- + S \rightarrow SCN^-$. Thiocyanate as a metabolite is much less toxic and is excreted mainly in urine. This reaction is catalyzed by rhodanese (EC2.8.1.1, thiosulfate sulfurtransferase) and mercaptopyruvate sulfurtransferase (EC2.8.1.2, MST). It is possible that herbivorous animals have acquired a defense mechanism against various cyanides contained in grass by using these two enzymes (Aminlari et al., 1989). But it is obvious that cyanide is not a natural substrate of these enzymes from the results of kinetic studies for MST and rhodanese (Nagahara et al., 1995).

Rhodanese was found in rat liver by Lang (1933)

and first purified from bovine liver by Sörbo (1953). This enzyme is widely distributed in prokaryotes and eukaryotes (Ludewig and Chanutin, 1950; Sörbo, 1951; deDuve et al., 1955). Recombinant rhodaneses from various animal tissues (Miller et al., 1991, 1992; Trevino et al., 1995; Dooley et al., 1995; Nagahara et al., 1995) were overexpressed in *Escherichia coli* and well characterized. Bovine liver enzyme was crystallized (Sörbo, 1953; Horowitz and DeToma, 1970) and its tertiary structure was determined (Ploegman et al., 1978a,b, 1979).

MST was found in rat liver by Meister (1953) and Wood and Fiedler (1953), but little was known about MST previously, because MST was unstable and difficult to purify. This enzyme is also distributed widely in prokaryotes and eukaryotes (Meister, 1953, Wood and Fiedler, 1953; Jarabak and Westley, 1978). The enzyme was recently purified to homogeneity for the first time (Nagahara et al., 1995) and cDNA was cloned from rat liver (Nagahara and Nishino, 1996b). Recombinant enzyme was overexpressed in *Escherichia coli* and characterized (Nagahara and Nishino, 1996b). We also found that MST was evolutionarily related to rhodanese. In fact, we succeeded in the conversion of the kinetic properties of rhodanese to MST (Nagahara et al., 1995) and from MST to rhodanese (Nagahara and Nishino, 1996b) by replacement of some amino acid residues with site-directed mutagenesis for each wild type enzyme.

As to intracellular distribution of enzymes in the cell, rhodanese is localized only in mitochondria (deDuve et al., 1955; Taniguchi and Kimura, 1974; Koj et al., 1975; Kuo et al., 1983; Nagahara et al., 1998). On the other hand, MST is distributed both in cytosol and mitochondria (Ludewig and Chanutin, 1950; Sörbo, 1951; deDuve et al., 1955; Taniguchi and Kimura, 1974; Koj et al., 1975; Devlin et al., 1989; Nagahara et al., 1998). In this review, we introduce molecular properties of MST in comparison with those of rhodanese. A role of MST in cyanide detoxification is also proposed, based on the tissue and subcellular distribution patterns of the enzyme.

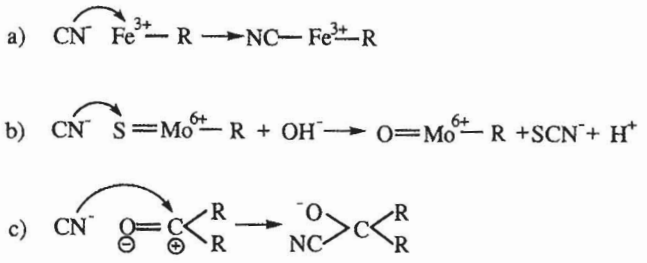

Fig. 1. Nucleophilic attack reactions by cyanide. **a.** Cyanide attacks oxidized form of iron (Fe^{3+}) belonging to a prosthetic group of metalloprotein such as heme *a* of cytochrome c oxidase or metheme and forms a cyano-iron complex. **b.** Cyanide attacks a sulfur atom bound to molybdenum (VI) of xanthine dehydrogenase and forms a cyano-molybdenum (IV) complex as an intermediate. Then the cyanide is spontaneously replaced with an oxygen atom to form an oxygen-molybdenum (VI) complex (Nishino, 1985). **c.** Cyanide attacks a carbonyl group of a protein to form cyanohydrin.

Both MST and rhodanese defend metalloproteins against cyanide toxicity

Cyanide is a nucleophile and readily attacks electrophiles such as metals belonging to prosthetic groups in metalloproteins or the carbonyl groups of proteins (Fig. 1). These metals serve as ligands of oxygen or sulfur atoms and control electron transport in each protein. The cyano-metal complex inhibits binding of an oxygen atom because the binding affinity of cyanide to the metal is extremely high (Fig. 1a). Alternatively, cyanide replaces a sulfur atom on a metal with an oxygen atom (Fig. 1b). A carbonyl group spontaneously reacts with cyanide to form cyanohydrin (Fig. 1c). As a result, many metalloproteins and carbonyl group-containing proteins are attacked and their functions are disturbed, so that metalloproteins and carbonyl group-containing proteins are targets of attack in cyanide poisoning.

While cyanide is sulfurated, the thiocyanate formed cannot attack any metalloproteins, so that the sulfuration reaction is identical to the detoxification of cyanides. This reaction is catalyzed by MST or rhodanese. Historically these two enzymes have been found in rat liver, and are able to convert cyanide to thiocyanate (Lang, 1933; Meister, 1953; Wood and Fiedler, 1953; Kun and Fanshier, 1959). Therefore, these enzymes are defensive factors in cyanide poisoning. When the body is exposed to cyanide, all tissues are not similarly affected. Sensitivity of the tissue to cyanide toxicity may be determined by a balance between the amounts of metalloproteins to be attacked and amounts of the defending enzymes in the tissue.

Structure of MST resembles that of rhodanese

Part of a sequence consisting of MST and rhodanese genes in human chromosome 22 has been recently determined (HSE146D10, Hunt, 1998). The genomic structure in which MST and rhodanese genes are arranged in tandem is shown in Fig. 2. MST gene spans

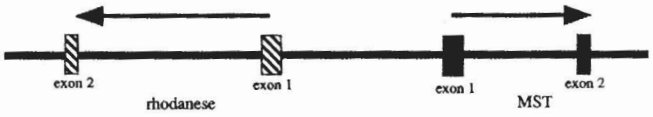

Fig. 2. A schema of genomic organization of MST and rhodanese genes in human chromosome 22. A part of a genomic DNA sequence was directly submitted by Hunt (1998), (HSE146D10). Rhodanese complement gene (7.8 kbp) and MST gene (5.6 kbp) are arranged in tandem on chromosome 22. The spacer between MST and rhodanese genes loci is about 5.5 kbp. The rhodanese gene contains two exons, exon 1 (595 bp) and exon 2 (299 bp). The intron between the two exons spans about 5.5 kbp. The MST gene also contains two exons, exon 1 (595 bp) and exon 2 (299 bp). The intron between the two exons spans about 4.4 kbp. Arrow: direction for transcription; Shadowed box: exon of rhodanese; closed box: exon of MST.

5.6 kbp of DNA and consists of two exons. On the other hand, the rhodanese gene, which spans 7.8 kbp of DNA and consists of two exons, is complementarily arranged. Sequence identity in cDNA and the deduced amino acid sequence between human MST (Pallini et al., 1991) and rhodanese (Arita et al., 1997) are 67% and 59% respectively. These findings strongly suggest that MST and rhodanese are evolutionarily related enzymes.

As for rat enzymes, rat liver MST consists of 295 amino acid residues with a calculated molecular mass of 32,808 Da (Nagahara and Nishino, 1996b). On the other hand, rat liver rhodanese is a 33,275 Da protein with 296 amino acid residues (Weiland and Dooley, 1991). Identity in cDNA and the deduced amino acid sequence are 65 and 60%, respectively between rat liver MST and rhodanese (Fig. 3).

Furthermore, the tertiary structure of rat MST was estimated with QUANTA/CHARMm (Molecular Simulations Inc.), based on the data for bovine rhodanese obtained by x-ray crystallography (Ploegman et al., 1978a,b, 1979) (Fig.4). The estimated tertiary structure of MST consists of two domains (N- and C-domains) which are almost identical in molecular size. A catalytic site, Cys 247, is located at the bottom of a shallow cavity in the C-terminal domain. The orifice of the cavity opens toward the interdomain space. When MST catalyzes mercaptopyruvate, an "induced fit", meaning a structural change must occur in consideration of the molecular size of mercaptopyruvate in relation to the depth of the cavity in the active center.

But some amino acid residues around the active center of MST are different from those of rhodanese

Primary structures of MST have been determined in man (deduced from liver MST cDNA, which was reported as a human rhodanese, Pallini et al., 1991) and rat (deduced from liver cDNA, Nagahara et al., 1995). Arg187 (#1 in Fig. 3), Arg196 (#2), Cys247 (#3), Gly248 and Ser249 of rat MST are conserved in two MSTs. Lys249 (#4) in rhodanese is replaced by Ser249 in MST. Arg196 (#2) is a unique amino acid residue and no corresponding residue is found in rhodanese. Arg187(#1) (Arg186 in rhodanese) and Cys247 (#3) are conserved between MST and rhodanese. Kinetic studies on mutant MST enzymes with site-directed mutagenesis

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MST    1:AAPQLFRALV SAQWVAEALK SPRASQPLKL LDASWYLPKL GRDARREFEE RHIPGAAFFD
             * **** *  * **    *       *   ****** *    * ** *  * ** *** ***
TST    1:VHQVLYRALV STKWLAESIR SGKVGPSLRV LDASWYSPGT -RQARKEYQE RHVPGASFFD

MST   61:IDRCSDHTSP YDHMLPSATH FADYAGSLGV SAATHVVIYD GSDQGLYSAP RVWWMFRAFG
          *  * * *** *  **** * * ** * **  *  **** ** * * *   ** ******* **
TST   60:IEECRDTTSP YEMMLPSEAH FGDYVGNLGI SNDTHVVVYD GDDLGSFYAP RVWWMFRVFG

MST  121:HHSVSLLDGG FRYWLSQNLP ISSGKSPSEP AEFCAQLDPS FIKTHEDILE NLDARRFQVV
          *  ** * ** ** **    *   *  *  ** * * * *  *   ** *  ** **   *** *
TST  200:HRTVSVLNGG FRNWLKEGHP VTSEPSRPEP AVFKATLNRS LLKTYEQVLE NLQSKRFQLV

              #1        #2
MST  181:DARAAGRFQG TQPEPRD--G IEPGHIPGSV NIPFTEFLTS EGLEKSPEEI QRLFQEKKVD
         * ** **  * ***** *  *    *** *** * **  ***   * ******     ** ****
TST  180:DSRAQGRYLG TQPEP-DAVG LDSGHIRGSV NVPFMNFLTE DGFEKSPEEL RAIFQDKKVD
              #1
                #3
MST  239:LSKPLVATCG SGVTACHVVL GAFLCGKPDV PVYDGSWVEW YMRAQPEHVI SQG-RGKTL
         ** ** ***   ******  *  * *******  ****** **   ** **    ***  **
TST  239:LSQPLIATCR KGVTACHIAL AAYLCGKPDV AVYDGSWSEW FRRAPPETRV SQGKSGKA
                #3 #4
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Fig. 3. Comparison of primary structure of rat MST with that of rhodanese. MST: deduced primary structure from rat liver MST (Nagahara and Nishino, 1996b); TST: deduced primary structure from rat liver rhodanese, revised form (Weiland and Dooley, 1991); #1: Arginine 187 in MST and Arginine 186 in rhodanese; #2: Arginine 196; #3: Cysteine 247; #4: Lysine 249; *: identical amino acid residue.

suggest that Arg187 (#1) and Arg196 (#2) are binding sites of mercaptopyruvate (Fig. 5) (Nagahara et al., 1995; Nagahara and Nishino, 1996b). The fact that the α-keto group is necessary for a donor substrate (Porter and Baskin, 1995) supports our proposal that Arg187 (#1) interacts with a carbonyl group of mercaptopyruvate. The estimated tertiary structure around the active site of rat MST (Nagahara and Nishino, 1996a) also justifies our proposal of binding sites of mercaptopyruvate. Cys247 is a catalytic site and forms persulfide as an intermediate during catalysis as shown in Fig. 5.

Fig. 4. Estimated tertiary structure of rat liver MST. The three-dimensional structure of rat liver MST is estimated by computer simulation analysis, based on the data from bovine liver rhodanese by x-ray crystallography (Ploegman et al., 1978a,b). Cys247: catalytic site cysteine 247.

Primary structures of rhodanese in human (deduced from liver cDNA of rhodanase, Arita et al., 1997), rat (deduced from liver cDNA, Weiland and Dooley, 1991), hamster (deduced from liver cDNA, Trevino et al., 1995), mouse (deduced from liver cDNA, Dooley et al., 1995), cattle (purified liver protein, Miller et al., 1992) and chicken (purified liver protein, Kohanski and Heinrikson, 1990) have been determined. Arg186 (#1), Cys247 (#3), Arg248 and Lys249 (#4) of rat rhodanese are conserved in all rhodaneses. The roles of Arg186 (#1), Cys247 (#3) and Lys 249 (#4) in rat or their corresponding residues in bovine were investigated precisely with site-directed mutagenesis or chemical modification of the amino acid residues described above (Cannella et al., 1975; Weng et al., 1978; Luo and Horowitz, 1994; Nagahara et al., 1995; Gliubich et al., 1996). These results showed clearly that Arg 186 (#1) and Lys249 (#4) are binding sites of thiosulfate and that

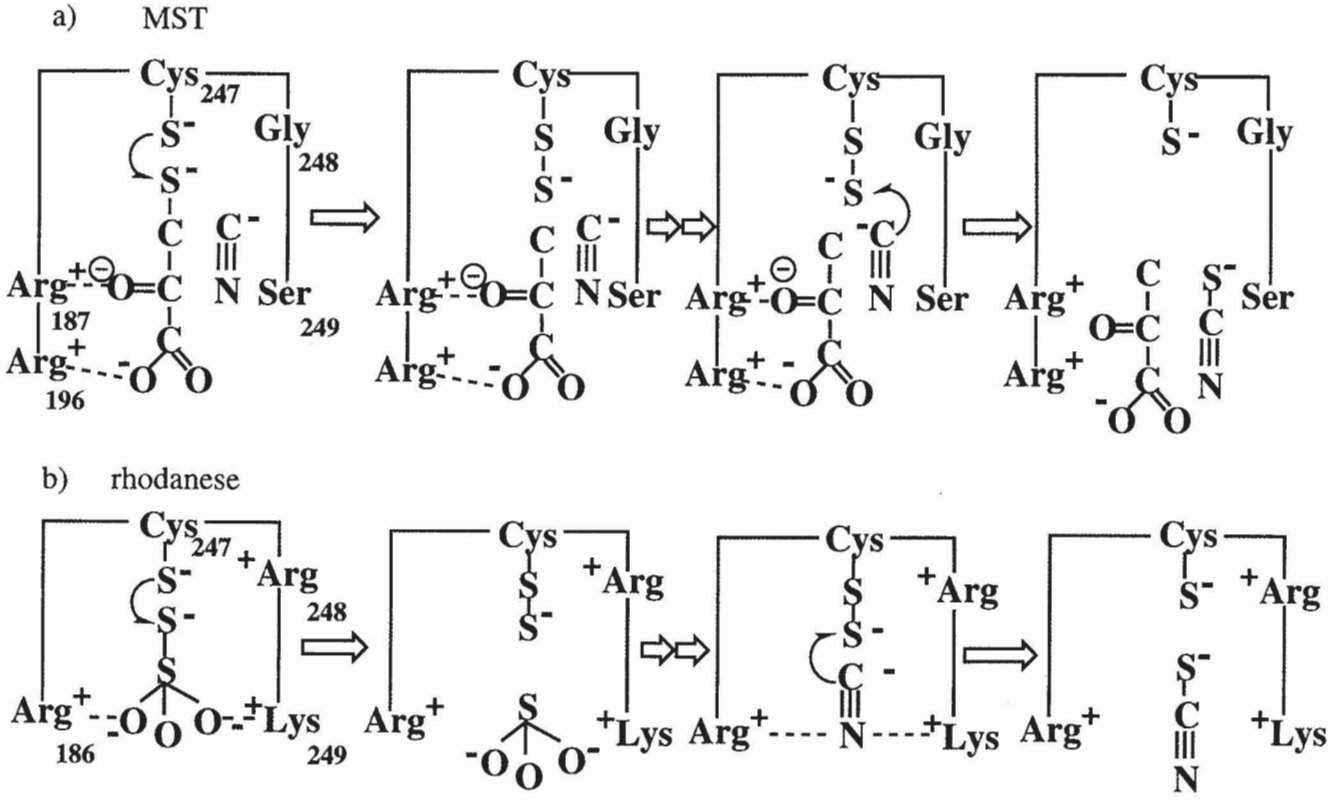


Fig. 5. Proposed mechanism of cyanide detoxification by MST and rhodanese. **a.** The schema shows a proposed detoxification process by MST with mercaptopyruvate as a donor substrate. It is probable that MST follows a sequential kinetic pattern. **b.** The schema shows a proposed detoxification process by rhodanese with thiosulfate as a donor substrate. Rhodanase follows a double displacement mechanism. Each enzyme forms persulfide as an intermediate and the outer sulfur is transferred to a carbon atom of cyanide to form thiocyanate.

Cys247 (#3) is a catalytic site.

Further, each kinetical property was reciprocally converted from MST to rhodanese, and from rhodanese to MST by replacement of the unique amino acid residues with site-directed mutagenesis (Table 1), (Nagahara et al., 1995; Nagahara and Nishino, 1996b). We concluded that sequences around the catalytic site cysteine, Cys-Arg-Lys and Cys-Gly-Ser (Figs. 3, 5) are unique for rhodanese and MST, respectively. Another arginine (Arg186) placed at the orifice of the entrance to an active center is also a unique amino acid residue for MST (Figs. 3, 5).

Cyanide can be an acceptor substrate for MST and rhodanese

Kinetic studies have been performed on MST (Jarabak and Westley, 1978; Nagahara and Nishino,

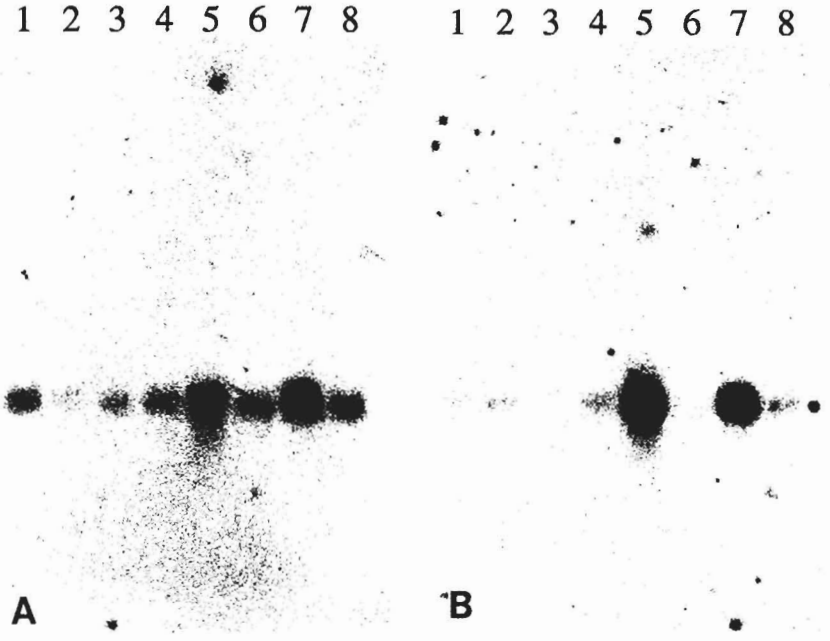


Fig. 6. Northern blot analyses for MST and rhodanese in various rat tissues. **A.** Northern blot analysis is performed with P^{32}-labeled probe for rat MST. A 375-bp probe was synthesized by polymerase chain reaction (PCR) with 5' sense primer (GAATTCGACCCCGCCTTCATCAAG; 475-492 bp of rat liver MST cDNA with EcoRI restriction site) and 3' antisense primer (AAGCTTCATGTACCAC TCCACCCA; 826-843 bp with Hind III site) and rat liver single-stranded cDNA as a template. The probe was labeled with [α-P^{32}] dCTP by using a random primed DNA labeling kit (Boehringer Mannheim). For hybridization, a rat multiple tissue northern blot (Clonthch) was used. Each lane on the filter contains 2 μg of poly(A)$^+$ RNA from various rat tissues. To confirm mRNA content in each lane, control Northern blot study was also performed using a probe for actin (data not shown). **B.** For northern blot analysis of rat rhodanese, a 323-bp probe was synthesized with PCR by using 5' sense primer and 3' antisense primer and rat liver single-stranded cDNA as a template (Nagahara et al., 1995). Procedures in labeling of the probe and hybridization are the same as those for MST. 1: heart; 2: brain; 3: spleen; 4: lung; 5: liver; 6: muscle; 7: kidney; 8: testis.

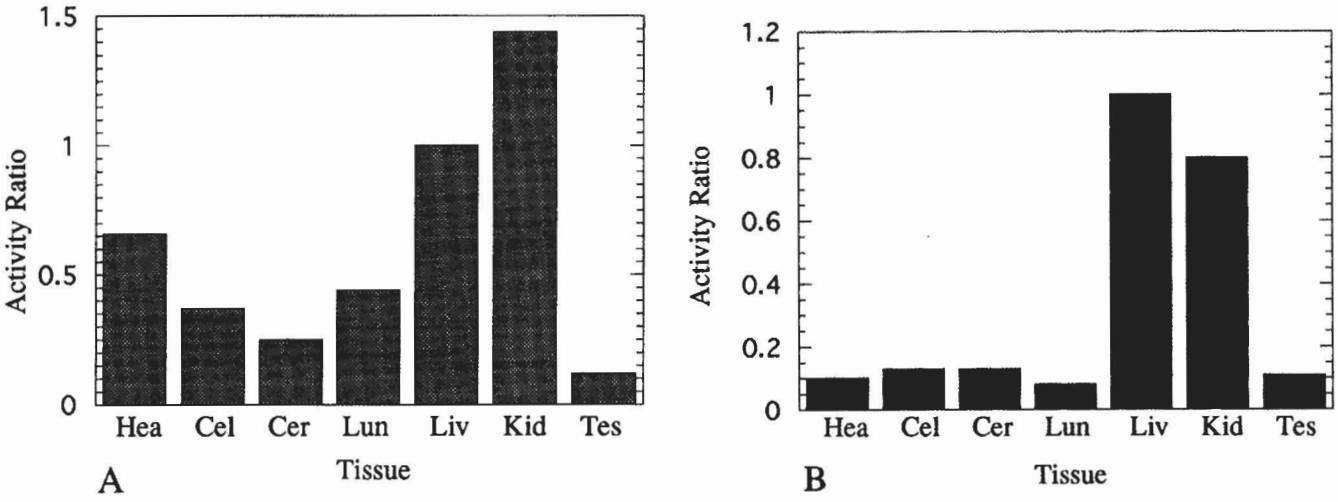


Fig. 7. Ratio of specific activity of enzyme in rat tissues. **A.** MST activity ratio. **B.** Rhodanese activity ratio. Each rat tissue homogenate was obtained according to the method of Nagahara et al. (1998). Then each enzyme activity was measured by the rate of pyruvate formation by a modification of the method of Vachek and Wood (1972) for MST (Nagahara et al., 1995), and by the rate of thiocyanate formation by a modification of the method of Sörbo (1953) for rhodanese (Nagahara et al., 1995). The protein concentration was determined with a Coomassie protein assay kit (Pierce) with crystalline bovine serum albumin as the standard. Each activity ratio was calculated as specific activity of each tissue/that of the liver. Hea: heart; Cel: cerebellum; Cer: cerebrum; Lun: lung; Liv: liver; Kid: kidney; Tes: testis.

1996b) and on rhodanese (Westley and Heyse, 1971; Schlesinger and Westley, 1974). In a kinetic study of MST, double reciprocal plots of velocity versus the mercaptopyruvate concentration show a mixed inhibition pattern indicating a sequential mechanism. In a kinetic study of rhodanese, double reciprocal plots of velocity versus thiosulfate concentration show an uncompetitive pattern indicating a ping pong mechanism (Westley and Heyse, 1971; Schlesinger and Westley, 1974). But these reaction mechanisms cannot be determined only by kinetic studies, the reaction intermediates should be confirmed by X-ray christallography.

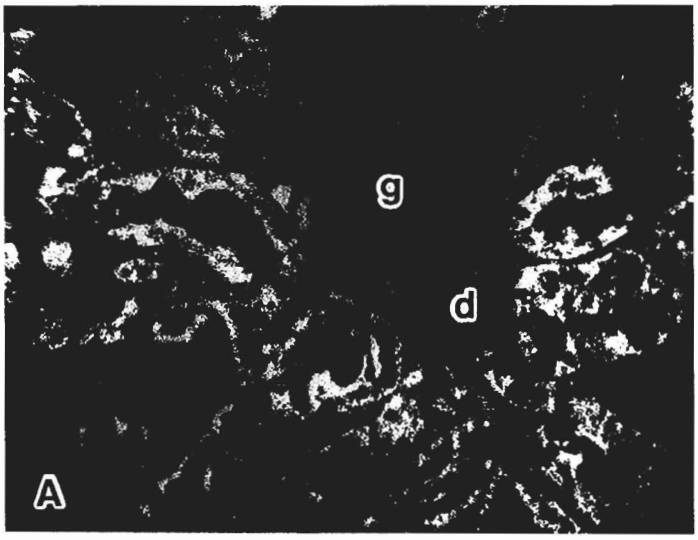
MST and rhodanese can use various sulfur-containing compounds as substrates. In MST, sulfite (Sörbo, 1957b), sulfinate (Sörbo, 1957b) and mercaptoethanol (Vachek and Wood, 1972) can be acceptor substrates. In rhodanese, thiosulfonate (Sörbo, 1953) and persulfides (Sörbo, 1960; Villarejo and Westley, 1963) can be donor substrates, and, on the other hand, sulfite (Sörbo, 1957a), sulfinates (Sörbo, 1957a) and various thiol compounds (Koj, 1968) can be acceptor substrates.

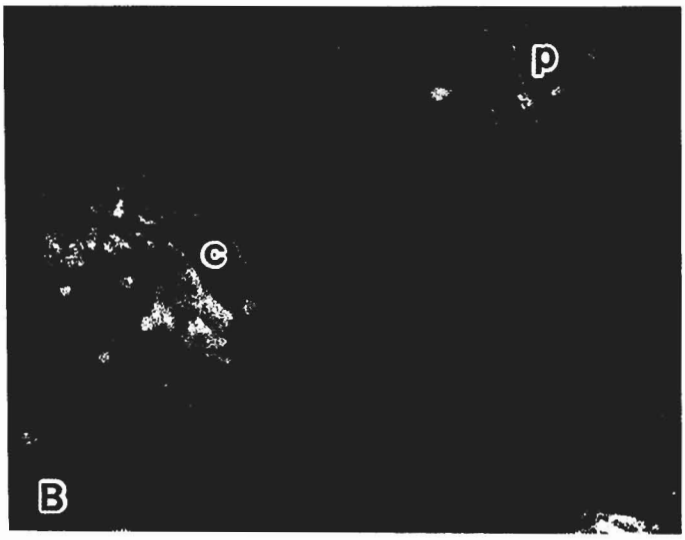
It is interesting that cyanide can be an acceptor substrate for MST (Jaraback and Westley, 1980) and rhodanese (Sörbo, 1953). Proposed mechanisms of these two enzymes in cyanide detoxification are shown in Fig.5. But because double reciprocal plots of velocity versus potassium cyanide concentration do not show a

Table 1. Effect of replacement of amino acid residues on kinetic properties.

ENZYME	REPLACEMENT	kcat/Km	
		MST[b]	Rhodanase[c]
Rhodanase			
	Arg248→Gly	increase	decrease
	Lys249→Ser	increase	decrease
	Arg248 & Lys 249→Gly & Ser[a]	increase	decrease
MST			
	Arg196→Gly	decrease	increase
	Ser249→Lys	decrease	increase

[a]: replacement of two amino acid residues; [b]: apparent kcat for MST activity/apparent Km for mercaptopyruvate; [c]: apparent kcat for rhodanase activity/apparent Km for thiosulfate (apparent Km for thiosulfate as a substrate using a constant concentration of KCN at 60mM).





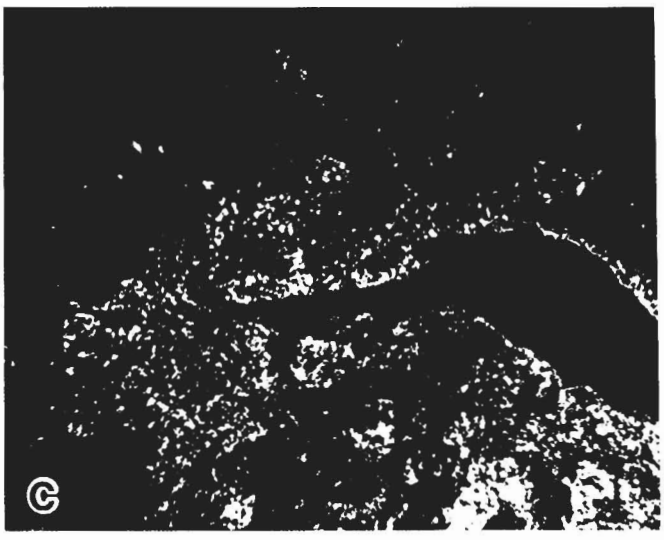


Fig. 8. Immunohistochemistry for MST localization in rat tissues. **A.** In the kidney, proximal tubular epithelial cells are stained. Distal tubular epithelial cells (d) and glomerular cells (g) are weakly stained or negative. x 360. **B.** In the liver, staining is stronger in hepatocytes around the central vein (c) than those around the portal area (p). x 120. **C.** Hepatocytes around the central vein show strong stain. x 360. Preparation and characteristics of anti-rat MST polyclonal antibody were described previously (Nagahara et al., 1995, 1998). Fluorescein isothiocyanate-conjugated gbat anti-rabbit immunoglobulin (Cappel) was used as a secondary antibody. Further, tetramethyl rhodamine isothiocyanate-conjugated phalloidin (Sigma) was used for staining of F-actin. Stained sections were observed by confocal laser fluorescence microscopy. The procedure was described in a previous paper. (Nagahara et al., 1998).

straight line in our study (data not shown), it appears that cyanide is not a natural substrate.

Tissue distribution pattern of MST is different from that of rhodanese

MST and rhodanese are widely distributed in animal tissues (Kuo et al., 1983; Ubuka et al., 1985; Nagahara et al., 1998; Aminlari et al., 1989 for MST; Sörbo, 1951; Koj and Frendo, 1962; Aminlari et al., 1989; Nagahara et al., 1998 for rhodanese). The results of northern blot analyses for MST and rhodanese in rat tisues are shown in Fig. 6. The kidney, liver and heart contain abundant MST mRNA. Other tissues contain fewer mRNA. On the other hand, rhodanese mRNA is also detected predominantly in the liver and kidney, but only a small amount of mRNA is detected in other tissues. MST or rhodanese mRNA content correlates to the activity of

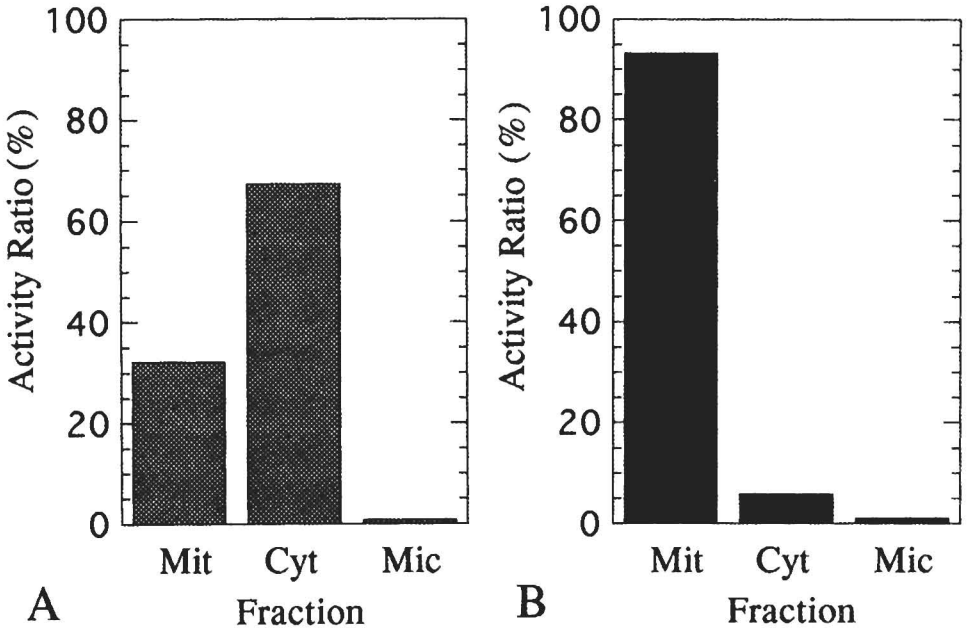


Fig. 9. Subcellular distribution of MST and rhodanese in rat liver. **A.** MST activity ratio of subcellular fractions. **B.** Rhodanese activity ratio of subcellular fractions. After preparation of the subcellular fractions (Nagahara et al., 1998), activities of MST and rhodanese were measured in each fraction. Each activity ratio was calculated as total activity of each fraction/total tissue activity. Mit: mitochondrial fraction; Cyt: cytosolic fraction; Mic: microsomal fraction.

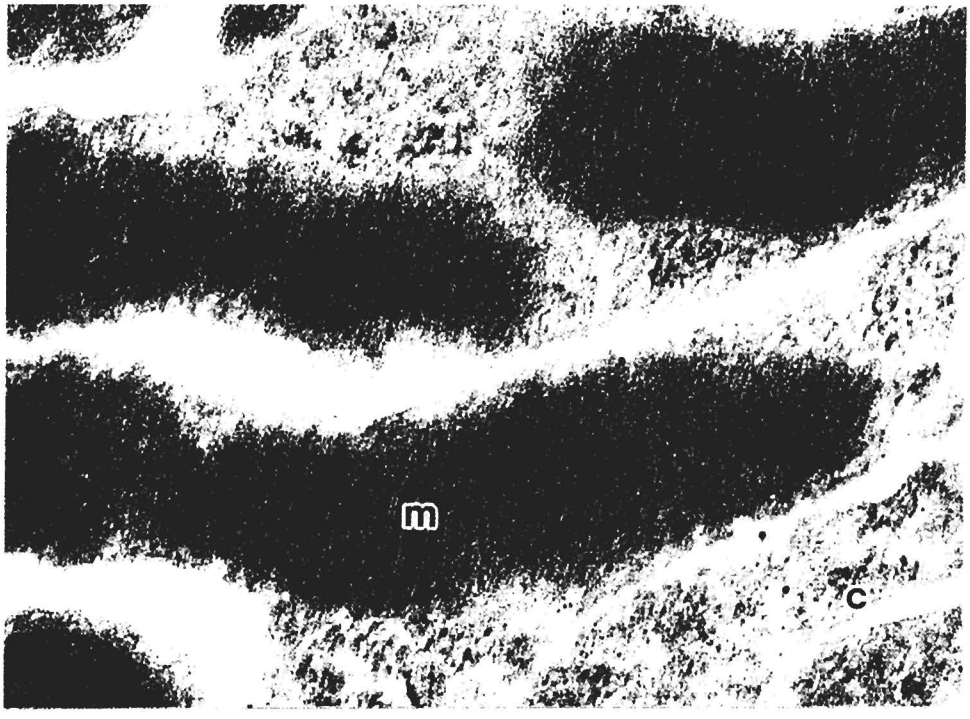


Fig. 10. Subcellular localization of MST in rat kidney. Immunoelectron microscopic study was performed by using anti-MST antibody and 10-nm gold-conjugated protein A (British BioCell International) for labeling of the antibody. The procedure was described previously (Nagahara et al., 1998). c: cytoplasm; m: mitochondria. x 20,000

MST or rhodanese in each tissue. Furthermore, western blot analyses of rat MST (Nagahara et al., 1998) revealed that tissue MST content correlates with MST activity (Fig. 7). It is concluded that the ratio MST contents/rhodanese contents varies in each tissue.

An immunohistochemical study with anti-MST polyclonal antibody (Nagahara et al., 1995) for the distribution of rat MST is shown in Fig. 8. In the kidney, which shows signs of the highest specific activity, the enzyme is distributed mainly in proximal tubular epithelial cells (Fig. 8A). In the liver, MST is localized mainly in pericentral hepatocytes (Fig. 8B,C). In the brain, MST is distributed mainly in glial cells (Nagahara et al., 1998). In the heart, myocardial cells contain MST predominantly in the perinuclear area (Nagahara et al., 1998). In the lung, MST is distributed diffusely in bronchiolar epithelial cells (Nagahara et al., 1998). On the other hand, localization of rhodanese in bovine liver, lung and kidney had the same pattern as that of MST (Sylvester and Sander, 1990), but, it is possible that MST had cross-reacted with the antibody. It is interesting that red blood cells also contain abundant MST and rhodanese but their biological functions have not been clarified.

Pattern of subcellular distribution of MST is also different from that of rhodanese

As shown in Fig. 9A, MST activity is detected in both cytoplasm and mitochondria. The cytosolic fraction of MST activity is about twice that of mitochondria. This is consistent with the results of western blot analysis of the subcellular fraction of the liver (Nagahara et al., 1998). Immunoelectron microscopic study (Fig. 10) confirms that MST is localized both in cytoplasm and mitochondria (Nagahara et al., 1998). The results of previous studies on subcellular distribution of MST (deDuve et al., 1955; Taniguchi and Kimura, 1974; Koj et al., 1975; Kuo et al., 1983) are consistent with our results. Because no other MST has been found during the purification from rat liver (Nagahara et al., 1995) and the cDNA cloning (Nagahara and Nishino, 1996b), the same molecule may be distributed in cytoplasm and mitochondria. The mechanism to determine the ratio of MST distribution in cytoplasm and mitochondria has not been clarified.

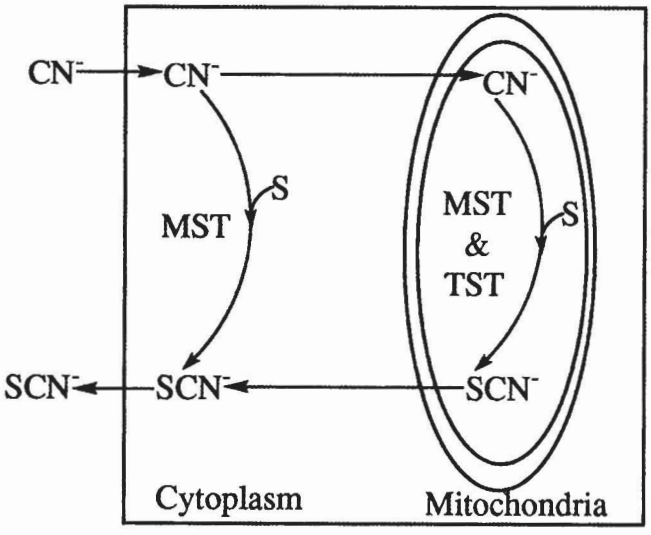


Fig. 11. Metabolic detoxification of cyanide in the cell. Details are given in the text. TST: rhodanese.

On the other hand, rhodanese is distributed only in mitochondria (Fig. 9B) (Ludewig and Chanutin, 1950; Sörbo, 1951; deDuve et al., 1955; Taniguchi and Kimura, 1974; Koj et al., 1975; Devlin et al., 1989; Nagahara et al., 1998). A targeting signal for mitochondria of rat rhodanese was elucidated by Hammem et al. (1994) and Waltner et al. (1996). This signal forms an amphipathic α-helix and the sequence, at least from Lys13 to Gly22, is necessary for mitochondrial import. After the enzyme reaches the mitochondrial matrix, this signal is not cleaved. The repetitive sequence $(Gly\text{-}Lys\text{-}X)^2$ at the C terminus of bovine rhodanese was proposed to be a signal for retention in the mitochondrial matrix (Miller et al., 1991). MST is estimated to form an α-helical structure near the N-terminal region. This α-helical of MST is considered to be a signal for mitochondrial import. The difference in the number of basic amino acid residues in the N-terminal region or posttranslational modification such as phosphorylation/dephosphorylation may cause the difference between MST and rhodanese in subcellular distribution.

MST works as a part of a defense against cyanide toxicity in both cytoplasm and mitochondria

When cyanide enters cytoplasm, cytosolic MST rapidly catalyzes a sulfuration reaction (Fig. 11). The remaining cyanide which escapes detoxification may enter organelles. The mitochondrion is considered to be a critical organelle in cyanide poisoning, because the function of cytochrome c oxidase in the respiratory chain is disturbed by cyanide, and consequently ATP production is disturbed. To avoid formation of a cyano-iron complex in cytochrome c oxidase, mitochondrial MST and rhodanese detoxify cyanide together.

Because the liver and kidney contain abundant MST and rhodanese, they can withstand cyanide poisoning. But the heart, brain and lung contain a much smaller quantity of these enzymes in spite of the abundant blood flow, making these tissues liable to be attacked by cyanide. In fact, severe disorders of the central nervous system and cardiovascular system are observed in cyanide poisoning. In tissues other than the liver and kidney, it appears from our results on the tissue distribution of MST and rhodanese that MST mainly defends against cyanide toxicity.

The increase in transcription of MST and rhodanese increases resistance to cyanide toxicity, but these enzymes have been considered to be house keeping. Ma

and Pritsos (1997) reported recently that activities of MST and rhodanese were increased to a level of about 133% and 157%, respectively in mallard duck brain by cyanide exposure. The tissue-specific mechanism of trans-criptional regulation and posttranslational modification of these enzymes has not been elucidated. Further, other defensive factors in cooperation with these two enzymes have to be considered. Cystathionine γ-lyase (EC 4.4.1.1) (Szczepkowski and Wood, 1967; Wood, 1980; Stipanuk et al., 1990; Porter et al., 1996) and thiosulfate reductase (Westley et al., 1983) were proposed to participate in detoxification of cyanide. But these enzymes have not been well characterized.

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