Thioredoxin-dependent Redox-sensing Molecular Switches in Hydrogen Sulfide and/or Polysulfides Producing Enzyme, 3-Mercaptopyruvate Sulfurtransferase

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Abstract

The rat 3-mercaptopyruvate sulfurtransferase (MST), a multifunctional enzyme, has redox-sensing molecular switches, a catalytic Cys247 and two cysteines, Cys154 and Cys263, on the outer surface of the enzyme. These switches are reduced or oxidized according to the redox state of their surrounding environment and require a redox active cysteine in thioredoxin (Trx) to interact with MST. Recently, MST has been demonstrated to be involved in the production of possible signaling molecules such as hydrogen sulfide, polysulfides, and/or sulfur oxides. However, the relationship between the production of signaling molecule(s) and action of these redox-sensing molecular switches has not been clarified, and a precise investigation of this relationship is underway.

Background

3-Mercaptopyruvate sulfurtransferase (MST, EC 2.8.1.2) is an enzymes involved in cysteine catabolism [1]. MST is present in various tissues and exists in both the mitochondria and cytosol [2,3]. Its activity ratio differs depending on the tissue [2,3]. MST catalyzes transsulfuration of a sulfur-donor substrate to a sulfur-acceptor substrate, and prefers 3-mercaptopyruvate to thiosulfate as sulfur-donor substrate. In cyanide detoxification, MST uses cyanide as a sulfur-acceptor substrate to produce thiocyanate [2]. When the sulfur-acceptor substrate is a thiol, persulfide is created, and hydrogen sulfide is generated by the reaction between persulfide and thiol [4]. Recently, it was reported that MST is involved in the production of hydrogen sulfide[4-7], polysulfides [7], and/or sulfur oxides [8]. Polysulfides are more effective as signaling molecules compared to hydrogen sulfide [9]. In humans, a congenital lack of MST causes mercaptolactate-cysteine disulfiduria [10,11], and the patients with this condition often presented with mental retardation [10,12]. Recently, MST-knockout mice were produced as animal models of the human congenital disease and displayed an increase of anxiety-like behavior [13]. Oxidation suppresses cysteine degradation and reduction promotes it. MST activity is controlled by the redox state via redox-sensing molecular switches, which are exposed cysteines. Rat MST (RnMST) has five cysteines, Cys64, Cys154, Cys247, Cys254, and Cys263. Cys64 and Cys254 are buried cysteines, while the other cysteines are exposed [14]. Cys247 is a catalytic cysteine, and Cys154 and Cys263 are positioned at the surface of the enzyme; these cysteines function as redox-sensing molecular switches. Cys154 is a unique residue in RnMST. Furthermore, thioredoxins (Trxs) have redox-active cysteines. Escherichia coli Trx (EcTrx) has two redox active cysteines, one of which interacts with RnMST [15].

Properties of intermolecular switches of RnMST

Cys154 and Cys263, which are positioned the surface of RnMST, form dimers via disulfide bonds [15]. Activities of wild-type and C64S (Cys64 is replaced with Ser) and C254S mutant RnMSTs were significantly increased after treatment with reduced EcTrx or EcTrx with a reducing system (Escherichia coli Trx reductase and NADPH) [15]. However, activities of C154S, C263S, and C154S/C263S (Cys64 and Cys263 are replaced with Ser) mutant RnMSTs were not significantly increased by reduction [15]. These findings suggest that Cys154 and Cys263 are Trx-dependent redox-sensing molecular switches for regulation of MST activity, probably via the induction of conformational changes [15].
Properties of intramolecular switch of \textit{RnMST}

The catalytic cysteine of \textit{RnMST} (Cys\textsuperscript{247}) is easily oxidized by hydrogen peroxide, which cause the inhibition or inactivation of \textit{RnMST} [16]. During oxidation, Cys\textsuperscript{247} was sulfenated, sulfinated, and finally sulfonated by hydrogen peroxide (Figure 1). The sulfenated Cys\textsuperscript{247} was reduced and MST activation was restored by dithiothreitol (DTT), reduced EcTrx, or EcTrx with the reducing system [16]. However, reduced glutathione or glutathione with its reducing system (glutathione reductase and NADPH) could not restore the \textit{RnMST} activity [16]. Moreover, sulenate formation at Cys\textsuperscript{247} was confirmed by Trx peroxidase activity on addition of \textit{RnMST} to the mixture of hydrogen peroxide and Trx with the reducing system [16].

Interaction between \textit{RnMST} and \textit{EcTrx}

EcTrx has two redox active cysteines, Cys\textsuperscript{32} and Cys\textsuperscript{35}. DTT-treated \textit{RnMST} was activated by the reduced C35S EcTrx mutant and not by the reduced C32S EcTrx mutant [15]. Further, HPLC analysis showed that \textit{RnMST} formed a complex with C35S EcTrx and not with C32S EcTrx [15]. These results indicate that the Cys\textsuperscript{32} of EcTrx attacks and cleaves the intermolecular disulfide bond between dimeric \textit{RnMST} molecules and forms a complex with EcTrx. Then, Cys\textsuperscript{35} attacks and cleaves the disulfide bond between \textit{RnMST} and EcTrx (Figure 2) [15]. In the case of plants, a recent study on mouse-ear cress sulfurtransferases and mouse-ear cress Trxs confirmed that both redox-active cysteines of Trx were necessary for the interaction between MSTs and Trxs [16].

Do hydrogen sulfide and polysulfides interact with redox-sensing switches as reductants?

MST produces hydrogen sulfide and polysulfides in the catalytic process [5-7]. Oxidative stress inhibits MST activity, resulting in a decrease in the production of hydrogen sulfide and polysulfides. However, there is no report on the effect of hydrogen sulfide and polysulfides on MST activity via interaction with the thioredoxin-dependent redox-sensing switches. The low redox potential of sulenate at a Cys\textsuperscript{247} catalytic site in an inactive MST is reduced by the addition of thioredoxin (E\textsuperscript{0} = −270 mV), but not by the addition of glutathione (E\textsuperscript{0} = −240 mV [17,18]. Therefore, hydrogen sulfide (E\textsuperscript{0} = −140 mV [19]) may not reduce the cysteine-sulfenate group. Conversely, polysulfides (E\textsuperscript{0} > −260 mV [19,20]) could reduce the sulfenate and convert the inactive MST to an active form. Thioredoxin effectively reduces inactive dimeric MST to an active monomeric form by reducing one of the intersubunit disulfide bridges that link the two subunits, \textit{i.e.}, the reduction of the disulfide bridges between Cys\textsuperscript{154} and Cys\textsuperscript{154}, Cys\textsuperscript{263} and Cys263, or Cys\textsuperscript{154} and Cys\textsuperscript{263} results in the formation of an active form of MST [15]. Therefore, polysulfides other than hydrogen sulfide may also cleave these intersubunit disulfide linkages.

![Figure 1](image1.png) The redox potential of Cys\textsuperscript{247} of \textit{RnMST}.

![Figure 2](image2.png) The reaction between \textit{RnMST} and EcTrx.
References


