Adaptation to Earth's atmosphere under oxidizing conditions: Directed molecular evolution of an enzyme

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Mercaptopyruvate sulfurtransferase (MST, [EC.2.8.1.2]) is a rhodanese family, which catalyzes the transsulfuration from mercaptopyruvate to pyruvate in cysteine catabolism. MST is widely distributed in prokaryotes and eukaryotes, and in mammalians, the same molecules are found in cytosol and mitochondria. It is considered that a primitive rhodanese molecule (TST, [EC.2.8.1.1]) is a precursor of a rhodanese family, and duplication and repeated stochastic mutageneses evoluted the molecule.

Rat MST has a monomer (active form)-dimer (inactive form) equilibrium, and the dimer is formed via an intersubunit disulfide bond by oxidation of exposed cysteine residues on the surface of two subunits (Nagahara and Sawada, 2006, Curr Med Chem, 13: 1219-1230; Nagahara *et al*., 2007, J Biol Chem, 282: 1561-1569). This disulfide bond serves as a thioredoxin-specific molecular switch: Under oxidizing conditions, a dimer is formed, and MST is converted to an inactive form, resulting in decline of cysteine catabolism, and increase of cellular reducing molecules. Then thioredoxin-specific reduction of the disulfide bond dissociates a dimer to be an active form, monomer. Furthermore a catalytic site cysteine is conserved in a rhodanese family. In rat MST, the cysteine is easily oxidized to form a low redox potential sulfenate, resulting in loss of the activity. Thioredoxin can uniquely restore the activity (Nagahara and Katayama, 2005, J Biol Chem, 14: 34569-34576). Thus MST serves as a antioxidant protein, and maintaines cellular redox homeostasis.

Prokaryotic MSTs do not contain the cysteine residue (a part of the molecular switch), and on the other hand, the cysteine residue is conserved in eukaryotes (a part of fungi, insects, birds, and mammals except plants and fishes). The molecular switch is not observed in life (prokaryotes and plants), which was emerged and differentiated in atmosphere under reducing conditions. On the other hand, the molecular switch is conserved in life (a part of fungi, insects, birds, and mammals), which was evoluted in atmosphere under oxidizing conditions. As fishes, aquatic life, are less exposed to danger of oxidative stress, the inherited amino acids may be conserved. It is noteworthy that all three bases of a codon for the cysteine residue of the molecular switch are substituted. After this event, although a single base replacement repeatedly occurred, the cysteine residue was conserved. These findings strongly suggest that replacement of an inherited amino acid to the cysteine residue is a directed molecular evolution in oxidizing atmosphere of Earth. As a result, MST acquired diversity of MST functions such as a redox-regulation and a defense against oxidative stress.