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Cross-talk Between (Hydrogen)Sulfite and Metalloproteins: Impact on Human Health

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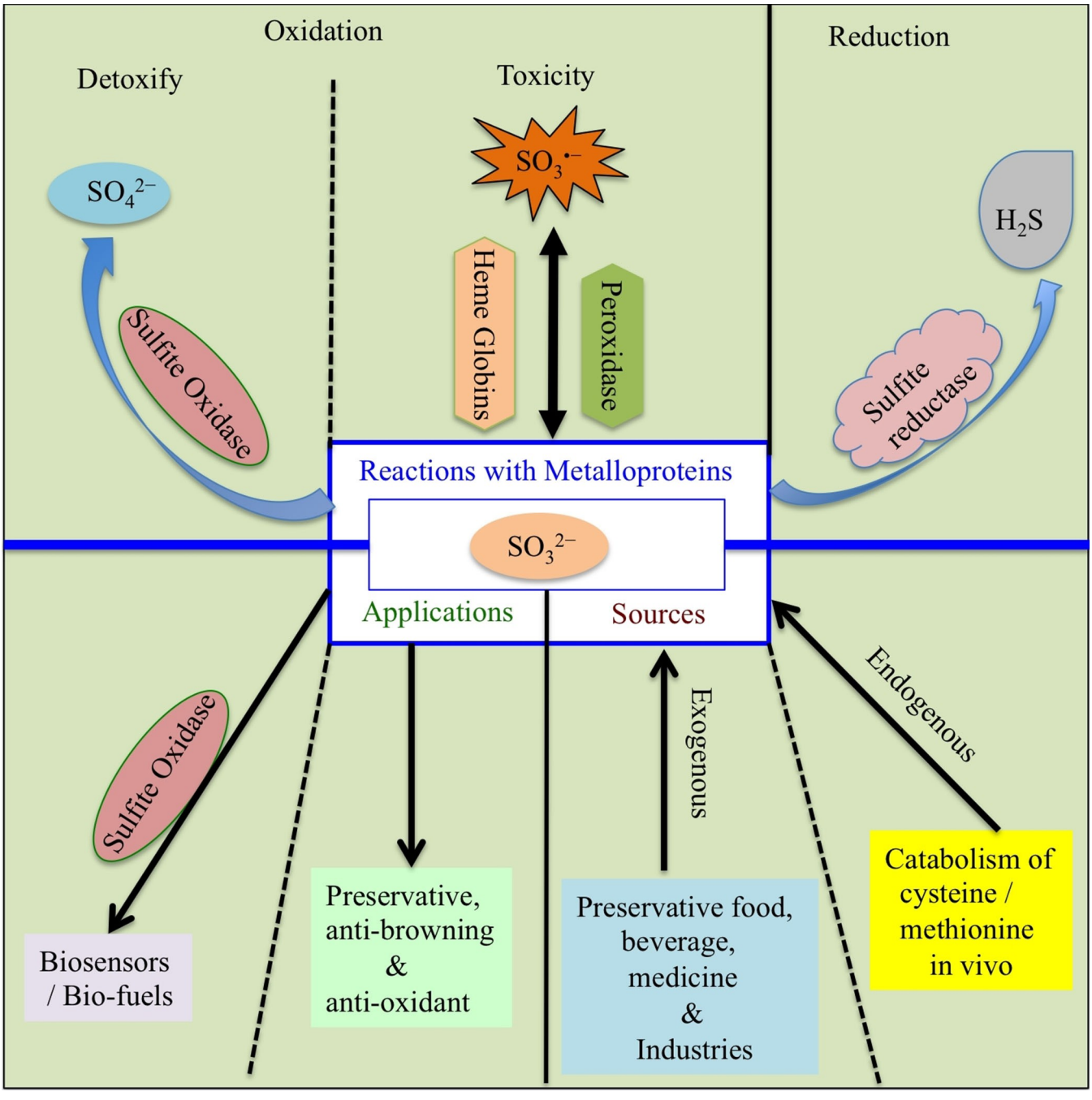
Dedicated to Professor José J. G. Moura on the occasion of his 70th birthday



Abstract: Sulfite is a potent toxic substance causing harm to multi-organ in human. Despite toxicity, it is widely used as preservative, anti-browning and anti-oxidant in foods, beverages, and pharmaceuticals, which cause easy admission of sulfite in human. Sulfite is also produced endogenously during the catabolism of cysteine and methionine. In vivo, the serum sulfite level at physiological range is strictly maintained by a molybdenum dependent sulfite oxidase (SO), which catalyzes sulfite to sulfate oxidation via a two-electron oxidation pathway. The loss of SO activity causes high serum sulfite level that fosters several diseases, including asthma, neurological dysfunction, birth defects, and heart diseases. The cytotoxicity of (bi)sulfite is implicated as sulfite radicals, which are generated by mainly heme-peroxidases via a one-electron oxidation pathway. On the other hand, the toxic sulfite radicals are neutralized to sulfite by heme-globins. The enzymatic reduction of sulfite to sulfide is catalyzed by sulfite reductase, which contains an unusual metal cofactor, siroheme-[4Fe4S]-cluster. Overall, the interaction of sulfite with various metalloproteins in vivo is a close relation with human health. Therefore, this review describes the metabolic conversion of (bi)sulfite to sulfate, sulfite radical or sulfide via oxidation or reduction pathways by various metalloproteins (specially SOs, peroxidases, heme-globins, and sulfite reductases), and the potential applications of sulfite in biosensors/biofuel cells, anti-browning, and advance oxidation process.

1. Introduction

Overproduction of many air pollutants by human activities and/or nature is putting a serious impact on human health. Among them, sulfur dioxide (SO_2) is a major air pollutant that causes several diseases, including asthma, heart diseases, allergic reactions, neurological dysfunction and birth defects.[1–4] Despite toxicity, its hydrated forms, sulfite (SO_3^{2-}) and hydrogen sulfite (HSO_3^-, also known as bisulfite) are widely used as preservative, anti-browning, and anti-oxidants in many foods, beverages and medicines.[5,6] So, exogenous sulfite can be easily ingested in human body by consumption of sulfite containing foods or inhalation of SO_2 gas. Overdose of sulfite is detrimental to human body, suggesting a significant correlation between serum sulfite level and human health. Hence, the United States Food and Drug Administration (FDA) has decided that labeling is required if foods or beverages contain more than 10 ppm (125 μM) concentration of (bi)sulfite.[7]

Sulfur dioxide is also produced endogenously during the normal catabolism of sulfur-containing amino acids.[8–11] It readily dissolves in serum and generates hydrated forms, (bi)sulfite. The physiological amount of serum (bi)sulfite (0–10 μmol) has a protective role to be found in human, such as antioxidant, anti-hypertension, anti-inflammatory, vasorelaxation, inhibition of vascular smooth muscle, regulation of the cardiovascular structure, and regulation of the cardiac channel function,[12–15] whereas high serum (bi)sulfite level has negative consequence in human health.[1–4,11,16–18] Therefore, serum (bi)sulfite level in human body[19] is strictly maintained by a mitochondrial enzyme, sulfite oxidase (SO), which detoxifies excess sulfite to sulfate in a two-electron oxidation pathway.[20] The deficiency of SO in human causes accumulation of excess sulfite in plasma,[21,22] leading to cellular toxicity.[17,18,23–25]

The cytotoxicity of (bi)sulfite is mediated by sulfite derived radical, namely oxy-sulfur anion radicals (OSRs), which are sulfur trioxide ($SO_3^{\bullet-}$), peroxymonosulfate ($SO_5^{\bullet-}$), and sulfate ($SO_4^{\bullet-}$) anion radicals.[25,26] OSRs are relatively strong oxidants,[26] which quickly oxidize many biomolecules such as proteins and DNA.[26–28] OSRs, one-electron oxidized products of sulfite, are generated by either cellular free transition metal ions (Fe, Cu, and Co),[29] or certain heme-peroxidases, such as human eosinophil peroxidase (EPO),[30] human myeloperoxidase (MPO),[31] prostaglandin H synthetase (PHS),[32] and horseradish peroxidase (HRP).[33] Interestingly, sulfite radical is also neutralized to sulfite by heme-proteins, including hemoglobin (Hb), myoglobin (Mb), neuroglobin (Ngb) and flbohemglobin (flboHb).[34]

Sulfite is an intermediate species in the sulfur cycle. Therefore, it is not only oxidized by SO but also reduced to sulfide by sulfite reductase, which contains an unusual metal-cofactor, namely shiroheme-[4Fe4S] cluster, found in many organism such as assimilatory and dissimilatory sulfate reducing bacteria (SRB).[35,36] Interestingly, SRB are found in human gut, which can participate in H_2S homeostasis in human.[37]

This manuscript reviews the role of different enzymes in the conversion of sulfite, where both oxidation to sulfate and reduction to sulfide, and formation of radicals through one-electron transfers are considered. There is some novelty in this approach, as often sulfite oxidation and reduction are topics of specific review papers focusing on only one of these two processes, both of which are research areas with considerable scope. In addition, it also describes the applications of SO in sulfite biosensors,[38] and biofuel cells,[39] the inhibition role of sulfite to polyphenol oxidase activity, which is responsible for browning in foods and vegetables,[40] and oxidative role of toxic sulfate radical in degradation of micro-pollutants in water bodies.[41]

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2. General Chemistry of SO_2

Sulfur dioxide, a colorless toxic gas, is readily soluble in water (94 mg mL^{-1}, 25 °C) to yield two hydrated forms, bisulfite (HSO_3^-) and sulfite (SO_3^{2-}) in 1:3 molar ratio (Scheme 1).[42,43] Under physiological pH ~7.4, two ionized forms are readily interconvertible, thus difficult to isolate one species from other.[16,44] Therefore, physiological effects of SO_2 are attributed by both hydrated forms, HSO_3^- and SO_3^{2-}.

Sulfite is a relatively strong nucleophile that can attack on numerous cellular components, leading to cellular toxicity.[16] Indeed, the nucleophilic addition reaction of SO_3^{2-} to cysteine or S–S bridges in biomolecules to yield a covalent modified unit, cysteine-S-sulfate (also known as S-sulfocysteine) resulting in impairment of protein structure and function.[45] Both cysteine and S–S bridges in biomolecules are essential for many biological processes, including metal binding, redox hemostasis, maintaining the tertiary structure, which directly associate with human health and diseases.[45,46] Indeed, sulfur containing a lone pair in sulfite attacks to the disulfide bond in thioredoxins to open the S–S-bridge, thus yielding a S-sulfocysteine derivative that leads to a unfolded protein structure.[46,47] The (bi)sulfite can be regenerated from S-sulfocysteine by a biological reducing agent such as NADPH or glutathione.[26,48] Therefore, S-sulfocysteine may act as a reservoir for sulfite in vivo.[49] Furthermore, (bi)sulfite acts as a substrate for many metalloenzymes[20] where sulfite can directly interact at metal site of metalloproteins and regulates the protein function due to its strong nucleophilic and Lewis-base character.[32]

Besides nucleophilicity, (bi)sulfite has another important role in redox chemistry, which is very complex in a biological system. Oxidation state of S-atom in SO_3^{2-} is +IV and reduction potential of SO_4^{2-}/SO_3^{2-} couple is −515 mV vs. NHE, indicating that sulfite is a strong reducing agent,[26] and thus, it is readily oxidized to SO_4^{2-} via two-electron oxidation pathway. Oxidation of sulfite is also more prone to one-electron oxidation, generating sulfur-centered radical intermediate, sulfur trioxide anion ($SO_3^{\bullet-}$). Once $SO_3^{\bullet-}$ is formed, it quickly reacts with molecular oxygen to generate another oxygen-centered radical, peroxymonosulfate anion radical ($SO_5^{\bullet-}$), which further reacts with another molecule of SO_3^{2-} or undergoes self-reaction to yield an ultimate reactive oxygen-centered sulfate anion radical ($SO_4^{\bullet-}$).[26,50] The reduction redox potentials of all radical anions are 0.76 V vs. NHE for $E(SO_3^{\bullet-}/SO_3^{2-})$, 1.75 V vs. NHE for $E(HSO_5^{\bullet-}/SO_4^{2-})$ and 2.43–3.08 V vs. NHE for $E(SO_4^{\bullet-}/SO_4^{2-})$.[26,51] Compared to hydroxyl radical ($HO^{\bullet}$, E = 1.8–2.7 V vs. NHE and $t_{1/2}$ = 1 μs,),[52,53] $SO_4^{\bullet-}$ is a stronger oxidant, as well as shows relatively longer half-life ($t_{1/2}$ = 30–40 μs).[26,50] Therefore, $SO_4^{\bullet-}$ is a chemically strong reactive species, which readily oxidizes biomolecules, including proteins, DNA, and lipids.[26–28]

$$SO_{2(g)} + H_2O_{(l)} \rightleftharpoons H_2SO_{3(aq)} \overset{pK_{s1}=1.9}{\rightleftharpoons} HSO_3^-{}_{(aq)} + H^+_{(aq)} \overset{pK_{s2}=7.2}{\rightleftharpoons} SO_3^{2-}{}_{(aq)} + 2H^+_{(aq)}$$

Scheme 1. Hydration of SO_2 and dissociation of H_2SO_3 in water.

Sulfur has variable oxidation states within range −II to +VI, in inorganic and organic compounds, including −II (sulfide; S^{2-} and reduced organic sulfur, cysteine), 0 (sulfur; S), +II (thiosulfate; $S_2O_3^{2-}$), +IV (SO_3^{2-} and sulfinic acid; RSO_2H) and +VI (SO_4^{2-} and sulfonic acid; RSO_3H). Sulfate is the major bioavailable species in nature, cycling between sulfate and sulfide through the Intermediate sulfur species, S, $S_2O_3^{2-}$ and SO_3^{2-} (Figure 1). So, multiple forms of sulfur species are the basis of the biogeochemical sulfur cycle. All living organisms participate in the biological sulfur cycle, but a certain classes of bacteria such as sulfate reducing bacteria (SRB) and sulfide oxidizing bacteria (SOB) use large amount of sulfur as energy source.[55] The assimilation of sulfur into organic sulfur compounds including cysteine and methionine is one of the vital metabolic reactions in biological sulfur cycle.[55] This reaction requires the reduction of sulfate to sulfide, which is the precursor of cysteine and methionine. The cysteine is decomposed to sulfide (see section 3), maintaining the sulfur cycle. Many enzymatic systems involve in this biological sulfur cycle through oxidation, reduction and disproportion pathways.[55] In this sulfur cycle, the intermediate sulfite species is converted to either 2e$^-$ oxidized species, sulfate[20] or 6e$^-$ reduced species sulfide.[36] Due to strong nucleophilic and reducing power ($E^0(SO_4^{2-}/SO_3^{2-}) = -515$ mV), sulfite is extensively used in food industries and pharmaceuticals.[5,6,54] In vivo, reactive sulfite radicals are likely to oxidize biomolecules, resulting in the progression of several diseases.[26–28]

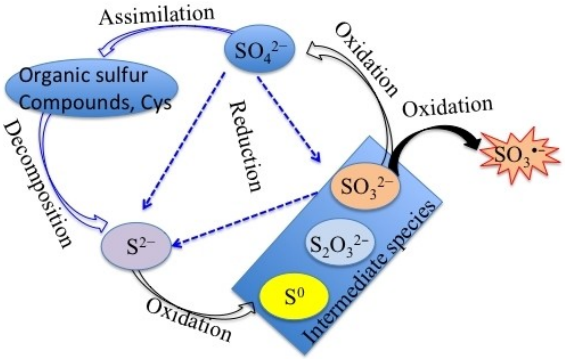

Figure 1. Biological sulfur cycle with various forms of sulfur.

Dr. Biplab K. Maiti received his doctorate from the Indian Institute of Technology Kanpur (IITK), India (2008). He had a first postdoctoral position at Friedrich Schiller Universität Jena, Germany, and second postdoctoral position at Faculdade de Ciencas e Technologia Universidade Nova de Lisboa, Portugal. He has been appointed as an Assistant Professor at the Department of Chemistry, National Institute of Technology Sikkim, India from 2019. The main field of research is the role of metals in Biology.

3. Production of Bi(sulfite) in vivo

The production of bi(sulfite) is proceeded during normal catabolism of sulfur-containing amino acids (cysteine, and methionine) in cytosol and mitochondria by enzymatic or non-enzymatic pathways as shown in Figure 2.[8–11,56] In mammalian cells, the first step of cysteine catabolism is the oxidative transformation of thiol to sulfinic acid upon addition of O_2 to the S of cysteine. This reaction is proceeded by a non-heme iron dioxygenase, namely cysteine-dioxygenase (CDO).[57,58] Then, the sulfinic acid is converted to β-sulfinylpyruvate by pyridoxal phosphate (PLP)-dependent aspartate aminotransferase (AAT), which is considered as a workhorse of sulfur metabolism. Finally, β-sulfinylpyruvate spontaneously decomposes to yield pyruvate and SO_2 gas that is readily hydrated in plasma to yield (bi)sulfite.[59,60]

Hydrogen sulfide gas (H_2S) is another metabolite of cysteine, which is generated in vivo by three distinct enzymes including cystathionine γ-lyase (CSE) or cystathionine (CBS), cysteine aminotransferase (CAT; also known as AAT) and mercaptopyruvate sulfurtransferase (MPST) (Figure 2).[10,61,62] The L-cysteine is converted to pyruvate and H_2S by Pyridoxal 5′-phosphate (PLP)-dependent CSE/CBS and/or PLP-independent MPST (a zinc-dependent enzyme).[10,61,62] In the final step, the SO_2 is generated from the oxidation of H_2S, which is mediated by either NADPH oxidase or putative sulfide oxidase (SDO) coupled with putative membrane-bound sulfide:quinone oxidoreductase (SQR) or thiosulfate reductase (TST).[8,63] Sulfide is converted to persulfide by SQR and then SDO oxidizes persulfide to sulfite.[8,64] In alternative way, TST catalyzes the reaction of persulfide to thiosulfate ($Na_2S_2O_3$), which is finally converted to SO_2 by glutathione (GSH).[49]

It is noticed that in healthy people, total serum SO_3^{2-} content is ranging from 0 to 10 μmol L^{-1},[19,65] but patients with asthma, dyspnea, and respiratory arrest have elevated serum SO_3^{2-} level (10 μM to ~2 mM).[66,67] So, endogenous SO_3^{2-} level is strictly maintained at physiological range, otherwise increases the degree of diseases.[11,17,18,23–25] In human, a vital mitochondrial enzyme, SO is responsible for maintaining the serum sulfite level at physiological range, and facilitating oxidation of sulfite to sulfate, which is excreted in urine.[20]

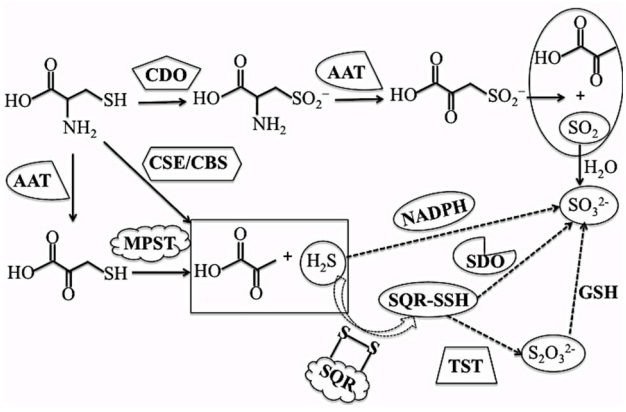

Figure 2. The formation of (bi)sulfite by possible pathways in vivo. Aspartate aminotransferase (AAT); cysteine-dioxygenase (CDO); cystathionine γ-lyase (CSE); cystathionine (CBS); mercaptopyruvate sulfurtransferase (MPST); sulfide:quinone oxidoreductase (SQR); thiosulfate sulfurtransferase (TST); sulfide oxidase (SDO); NADPH oxidase; and glutathione (GSH).

4. Oxidation of Sulfite

4.1. Two-electron oxidation of sulfite to sulfate

4.1.1. Sulfite oxidase

Sulfite oxidase (SO) is a molybdenum dependent metalloenzyme that oxidatively detoxifies the toxic SO_3^{2-} to nontoxic SO_4^{2-}, coupled with subsequent reduction of two equivalents of ferricytochrome-c, (cyt-c)ox, in the final step of catabolism of sulfur-containing amino acid (Eq. (1)).[20,68–70]

$$SO_3^{2-} + H_2O + 2(\text{cyt } c)_{ox} \xrightarrow{SO} SO_4^{2-} + 2e^- + 2(\text{cyt } c)_{red} \quad (1)$$

SO is found in almost all living organisms including animals, bacteria and plants.[20,68–70] Animal SO and bacterial sulfite dehydrogenase (SDH) both contain two redox centers, a molybdopterin cofactor (Moco as a catalytic center) and a heme containing domain (heme as an electron acceptor), whereas plant SO contains only Moco (Figure 3). However, no crystal structure of native human SO (hSO) is reported yet. Therefore, presently, structure of chicken SO (cSO) is used as a structural template for scrutinizing the hSO. The crystal structure of native cSO reveals that cSO is a homodimer (α_2) and each subunit is composed of three domains: a) N-terminal domain harboring b5-type heme (HD), b) C-terminal domain harboring Moco (MD) and c) a central domain containing a flexible polypeptide tether (P_{84}DEAPAAPDAQDP$_{96}$), which connects HD and MD domains, generating a large distance between them (Mo-to-Fe distance = ~30 Å) (Figure 3A).[20,70–73] Unlike vertebrate, the bacterial SDH is a heterodimer ($\alpha\beta$), and two redox centers, Moco and heme are located in separated subunits, α and β respectively. These two redox partners are locked by a strong interaction between two subunits, resulting in the reduction of Mo-to-Fe distance to ~16 Å (Figure 3B).[74] In spite of the overall structural differences, the coordination geometries of Mo-center in Moco are almost identical in all SOs. The oxidized form of Mo^{VI} in Moco is bonded with five ligands, which are a sulfur atom from protein-derived cysteine residue, two sulfur atoms from a unique pyranopterin, and two terminal oxygen atoms.[20,69]

The catalytic cycle of SO involves in two main events: oxidation of sulfite, and relay of electrons from the Moco to Cyt c via heme. In the first step of catalytic cycle, the fully oxidized form of the catalytic center of SO, Moco (Mo^{VI}) catalyzes the two-electron oxidation of sulfite to sulfate with concomitant two-electron reduction of Mo^{VI} to Mo^{IV} (Figure 4).[20,68–70,75] The reoxidation of the Mo^{IV} to Mo^{VI}, is proceeded by two sequential one-electron intramolecular electron transfer (IET) steps. The first IET step involves in $Mo^{IV} \rightarrow Fe^{III}$ and generates a Mo^{V}/Fe^{II}

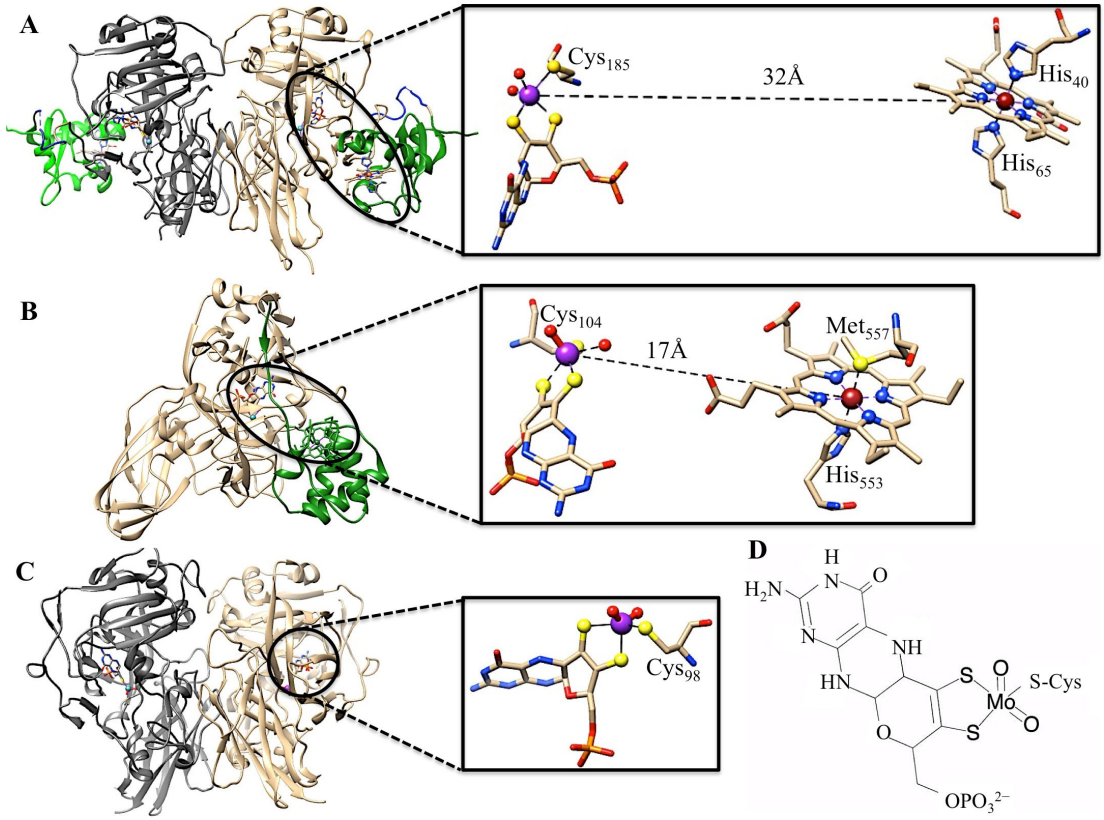

Figure 3. Crystal structure of SOs: A) cSO from chicken liver; PDB 1SOX; comprising N-terminal domain (green color), C-terminal domain (grey color) and a central domain (blue color) in a monomer, B) bacteria sulfite dehydrogenase (SDH) from S. novella; PDB; 2BPB comprising N-terminal domain (green color), and C-terminal domain (grey color), and (C) plant SO from A. thaliana; PDB; 1OGP). Highlighted heme and Moco-domains. Brown ball; Fe, purple ball; Mo, yellow ball; S, red; O, blue; N, orange; P and grey; C. (D) Schematic diagram of Moco in SO.

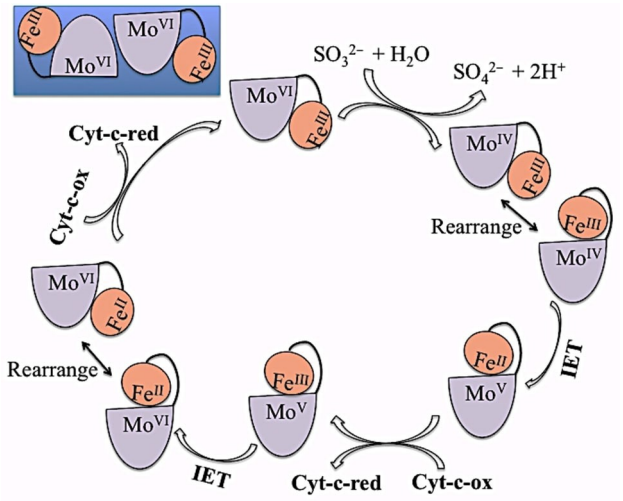

Figure 4. Proposed catalytic reaction mechanism of animal SO with sulfite.

state wherein Fe^{II} transfers one electron to one equivalent biological electron acceptor, ferricytochrome c ($(cyt\text{-}c)_{ox}$), generating a Mo^{V}/Fe^{III} intermediate. The second IET step involves in $Mo^{V} \rightarrow Fe^{III}$ and generates a Mo^{VI}/Fe^{II} state, which reduces another one equivalent $(cyt\text{-}c)_{ox}$, and subsequently regenerates a fully oxidized Mo^{VI}/Fe^{III} state of SO (Figure 4).[20,68–70,75]

The conversion of sulfite to sulfate is occurred by the directly reacting of sulfite substrate to the $Mo^{VI}O_2$ unit in Moco, resulting that one O-atom of MoO_2 unit is transferred to sulfite to yield a Mo bound sulfate product, and then water molecule displaces the sulfate ion from the Mo-sulfate complex. The diverse interaction modes of sulfite ion with MoO_2 unit are present. Therefore, the mechanism of this reaction is suggested by three possible different pathways based on experimental and computational studies: (a) lone pair of the sulfur atom of sulfite ion directly attacks to the equatorial O-atom of MoO_2 unit, yielding sulfate (S→OMo mechanism),[76] (b) the one of O-atoms of sulfite ion first binds to the Mo-center prior to react with the oxo group of MoO_2 (O→Mo mechanism),[77] and (c) sulfite ion may directly coordinate to Mo-center by the sulfur atom prior to react with the oxo group of MoO_2 (S→Mo mechanism).[78]

The apparent IET rate constant (K_{Et}) values for wild type cSO, hSO and SDH are $1318 \pm 28\ s^{-1}$ at pH 7.4,[79] 491 ± 11 at pH 7.5[79] (or $411 \pm 15\ s^{-1}$ at pH 6),[80] and $122 \pm 5\ s^{-1}$ at pH 6[79,81] respectively. Among them, vertebrates show a rapid IET between the Moco and heme-domain, despite a large distance between them (~32 Å).[72] The rapid IET in vertebrates is possible when flexible loop alters protein conformation, result-

ing in the reduction of distance between Moco and Fe-domain, facilitating the IET rate.[82,83] Indeed, Modeling and docking studies indicate that the distance between Moco and Fe-domain is lower of 12–19 Å during catalytic process that ensures fast IET.[82]

4.1.2. Mutation of SO

The impairment or deficiency of SO is an inborn error of sulfur metabolism in human that is characterized by severe neurological dysfunction, birth defects, dislocation of ocular lenses, mental retardation, and early childhood death.[23–25] This inborn error arises from either mutations of SO protein (so-called isolated SO deficiency (ISOD)) or inability to correct synthesis the pyranopterindithiolate cofactor (so-called Moco deficiency (MoCD)) in vivo, resulting in the accumulation of excess toxic sulfite in serum.[84–86] Both ISOD and Moco deficiencies have been reported in several patients.[87–91] In 1967, Mudd et al., first reported the ISOD and then it is known as a rare autosomal recessive disorder in human.[92]

Several studied have reported that metabolic disorder is caused by pathogenic variants in SO encoded SUOX gene.[87–92] These variants associate with ISOD and MoCD, leading to the impairment of active site and dimerization of SO, resulting in the disruption of IET from Moco to heme-center.[87,89,93] Several mutations are identified in hSO with ISOD.[87,88,94] Indeed, $G_{473}D$ (glycine to aspartic acid) and $A_{208}D$ (alanine to aspartic acid) (equivalent G_{451} and A_{186} in cSO) mutations are identified in hSO with ISOD.[71,89] Both A_{186} and G_{451} localize in the vicinity of Moco and distances from the Mo atom are ~3.8 Å° and ~12 Å° respectively. So, A_{186} participates in sulfite binding, whereas G_{451} participates in dimerization of protein. Therefore, the IET rate of mutated hSO ($G_{473}D$ and $A_{208}D$) is three orders magnitude slower than the wild type, indicating deleterious impact on patient with SO deficiency.[89] In addition, $G_{362}S$ (glycine to serine) mutation has been found in a patient with ISOD, which is vicinity to the Moco-C4′-phosphate.[95] The IET rate of $G_{362}S$ mutated SO is significantly slower (~90-fold) than WT SO in vitro.[95] Furthermore, $R_{160}Q$ (arginine to glutamine) variant in hSO has been identified clinically in a SO-deficient patient exhibiting neurological abnormalities, which is close to the catalytic site and positively charged Agr_{160} strongly attracts the negatively charged sulfite molecule at the catalytic site, resulting in a significantly decreased the k_{cat} value (16 to 2.4 s^{-1}) and increased the K_m value (17 μM to 1.7 mM).[87] Therefore, patient-derived fibroblasts isolated R160Q SO shows significantly lower catalytic activity compared to native SO, suggesting that this mutation gene has no functional SO in that patient resulting in high amount of sulfite in urine of patient.[87]

4.2. One-electron oxidation of sulfite to sulfite radical

4.2.1. Peroxidase

Besides the two-electron enzymatic oxidation of SO_3^{2-} to SO_4^{2-}, the one-electron oxidation of SO_3^{2-} to $SO_3^{\bullet-}$ radical is also a competitive redox process of sulfite in vivo, and it is mediated by a particular family of metalloenzymes, heme-peroxidase, such as human eosinophil peroxidase (EPO),[30] human myeloperoxidase (MPO),[31] prostaglandin H synthetase (PHS),[32] and horseradish peroxidase (HRP).[33]

The human peroxidases, MPO and EPO, are abundant heme-proteins in neutrophils and eosinophils respectively. Both peroxidases utilize halide and H_2O_2 for the synthesis of cytotoxic oxidants, hypohalous acids[96–98] that participate in host defense against infection, but over activation of these enzymes lead to a variety of inflammatory diseases.[30,97,98] Both neutrophils and eosinophils are the specialized and important forms of white blood cells, and their primary functions are host defense against infection, and pathogenesis. But elevated levels of neutrophils and eosinophils associate with asthma, and allergic inflammatory disorders.[97–100] The stimulation of neutrophils and eosinophils causes respiratory burst, releasing abundant MPO and EPO, which produce the toxic reactive oxygen species (ROS) by consuming O_2.[101,102] So, human peroxidases play a dual role: beneficial as well as detrimental to multi-organ in human. Therefore, research area of mammalian heme-peroxidases is a considerable interest.

Mammalian heme-peroxidases are independent from bacteria, fungal and plants due to substantial differences in their amino acid sequences, overall structures and prosthetic groups.[103] Human MPO is a cationic homo-dimeric (α_2) glycoprotein with molecular mass of 146 kDa, possessing two identical subunits (73 kDa), which are connected by a single disulfide bond (Figure 5A). Each subunit is composed of two polypeptide chains, a light chain (14.5 kDa), and a heavy chain

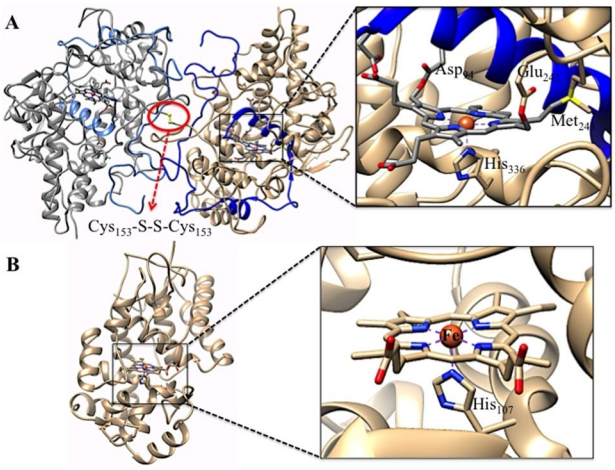

Figure 5. A) Human Myeloperoxidase (PDB: 1MHL) composed of light chain (blue) and heavy chain (grey) in monomer, and B) horseradish peroxidase (PDB: 1HCH). The active site and protoporphyrin-IX covalently bonded amino acid residues are highlighted.

(58.5 kDa).[104–106] The heme group in MPO is a derivative of protoporphyrin-IX, which is covalently linked with carboxyl groups derived from Asp_{94} in the light chain and Glu_{242} in the heavy chain. In addition, protoporphyrin-IX is covalently bonded with sulfur atom of Met_{243} in heavy chain, giving a sulfonium ion linkage. Due to covalent linkage, prosthetic group is distorted from planar conformation.[106–108] Other human peroxidase, EPO is an unknown 3D structure yet, but it shares 70% amino acid homology with MPO.[109] Human EPO is a cationic monomeric glycoprotein, comprising of a light peptide chain with molecular mass of 11.9 kDa and a heavy peptide chain with molecular mass of 57.9 kDa.[104–106] Like MPO, EPO harbors a heme-prosthetic group, which is also covalently bonded to the polypeptide backbone by carboxyl groups of Glu_{241} and Asp_{93} residues.[105,106] The Fe-center in protoporphyrin-IX coordinates a proximal protein derived His residue, which is conserved in all members of peroxidase family. In contrary to human peroxidase, the heme-prosthetic group in other peroxidase such as horseradish peroxidase (HRP) is not covalently bonded to the polypeptide backbone (Figure 5B).[110] Due to this structural difference in their active sites, the oxidative abilities of these enzymes are also different.

Primarily, both MPO and EPO oxidize the physiological substance in presence of H_2O_2 via two-electron oxidation pathway.[30,31,103,111] The porphyrin-Fe^{III}-MPO reacts with H_2O_2 to generate a two-electron oxidized species, compound-I (oxy-ferryl radical; porphyrin$^{\bullet}$–Fe^{IV}=O), but it is converted back to native state by halides or two sequential one-electron reduction pathway via an intermediate, compound-II (oxy-ferryl; porphyrin-Fe^{IV}=O). Therefore, both compound-I and compound-II can catalyze the one-electron oxidation of substrates to a number of radical species (Figure 6).[30,31,111]

EPO/MPO can use (bi)sulfite as an one-electron donor substrate in the catalytic cycle wherein MPO/EPO-compound-I and MPO/EPO-compound-II intermediates are generated.[67,112] Both MPO/EPO-compound-I and MPO/EPO-compound-II can oxidize SO_3^{2-} to $SO_3^{\bullet-}$ through one-electron oxidation process (Figure 6).[32,113] Once $SO_3^{\bullet-}$ radical is formed, it is rapidly transformed to more reactive $SO_4^{\bullet-}$ in presence of molecular oxygen.[26–28] One electron oxidation of sulfite may be also metabolically activated by a number of other heme-enzymes including HRP[33] and prostaglandin-H-synthetase[32], as well as non-heme enzymes including xanthine oxidase,[114,115] and copper-zincsuperoxide dismutase (Cu,ZnSOD).[116] Horseradish peroxidase and prostaglandin H synthetase also generate sulfite radical via $1e^-$ oxidation process and reaction proceeds in the same catalytic cycle of MPO/EPO.[32,33,117–119]

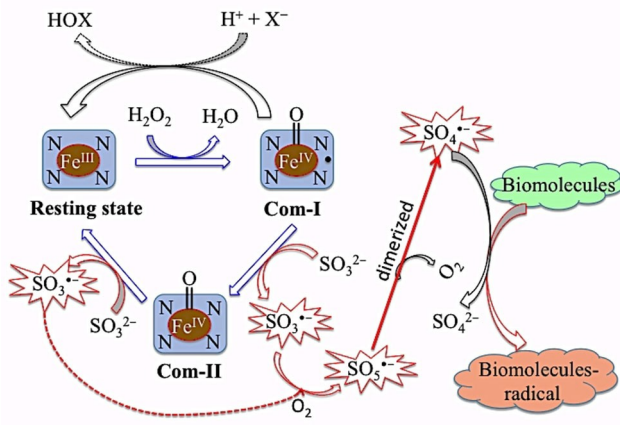

Figure 6. Proposed mechanism of sulfite derived radical formation by peroxidase (MPO and EPO). The same catalytic reaction of PHS, and HRP.

Xanthine oxidase (XO) is a molybdenum-dependent enzyme, and in human, it is functioning in the oxidation of hypoxanthine and xanthine to ultimate uric acid utilizing molecular oxygen as a co-substrate.[20] The active site of XO is characterized by a Moco, in which Mo-center is surrounded by one hydroxo (–OH), one terminal oxo (=O), one terminal sulfido (=S), and two sulfur ligands derived from pterin group.[120,121] XO also involves in several human diseases due to the formation of uric acid and reactive oxygen species (H_2O_2 and $O_2^{\bullet-}$). Interestingly, XO derived $O_2^{\bullet-}$ generates $SO_3^{\bullet-}$ from the oxidation of sulfite.[115] It is also reported that the XO has capable to synthesis $SO_3^{\bullet-}$ radical during the oxidation of SO_3^{2-} in presence of H_2O_2.[114]

Another radical mechanism of one-electron oxidation of sulfite is proceeded by Cu–Zn-superoxide-dismutase [Cu,Zn-SOD]. In vivo, primary function of SOD is the disproportionation of superoxide radicals ($O_2^{\bullet-}$) to O_2 and H_2O_2, thereby protecting the cell from the toxic products of aerobic respiration.[122] In [Cu,ZnSOD], Zn^{II} ion plays as structural role, whereas Cu^{II} plays as catalytic role and both metals are connected by a His residue.[123] It is also able to oxidize sulfite to sulfite radical like peroxidase enzymes.[116] The proposed mechanism is that Cu^{II} site of [Cu,ZnSOD] catalyzes one electron oxidation of SO_3^{2-} to $SO_3^{\bullet-}$.[116]

The cytotoxicity of SO_3^{2-} has been implicated as a free radical intermediate.[25] The formation of highly reactive sulfite-derived radicals in the enzymatic system can further proceed to initiate the radical chain reaction, generated biomolecule radicals, such as most abundant plasma protein radical, human serum albumin (HSA) radical, which may be responsible for the tissue injury in SO_3^{2-} prompted allergic reactions.[30,116] Clinical manifestations indicate that sulfite causes deterioration of human health. The toxicity of sulfite has been studied on human normal cells. Indeed, human hepatocytes L02 cells are incubated with sodium sulfite in different concentration to evaluate the sulfite toxicity at cellular level. This result shows that damage of L02 cell is more with the high dose of sulfite and long treatment time.[124] These results suggest that the probability of (bi)sulfite free radical metabolism and consequent proteins/cells damage in vivo.

4.3. Detoxification of sulfite radical to sulfite

4.2.1. Heme-globins

Sulfite-derived free radicals have been suggested as sulfite toxicity in cells,[26] and it can be scavenged by heme-globins in cells.[34] For instance, saccharomyces cerevisiae, known as

baker's yeast produces sulfite in the cell.[125,126] The cellular sulfite level is assumed to be elevated due to lack of mitochondrial SO.[20,127] Surprisingly, no sulfite radicals are observed in yeast. Therefore, the detoxification of sulfite-derived free radicals is expected by yeast flavohemoglobin (flavoHb or YHb) in cells.[128] A large number of heme-globins, including hemoglobin (Hb), myoglobin (Mb), neuroglobin and flavoHb are investigated to understand the ability of detoxification of sulfite radicals.[34]

The flavoHbs, a heme protein, found in various microorganisms serves as a protective enzyme against nitrosative stress.[129] Indeed, yeast flavoHb is a monomeric protein with molecular mass of 45 kDa, comprising a heme-b and a flavin adenine dinucleotide (FAD) domains.[130] The active site of YHb is characterized by a five coordinated Fe^{II}-protoporphyrin-IX, wherein 5th coordination site of Fe is coordinated by a proximal His residue. Interestingly, no distal His residue is present in YHb like human hemoglobin (Hb) and myoglobin (Mb) (Figure 7).[130]

Hb, a tetrameric protein, found in human red blood cells, serves as an O_2 transporter from the lungs to tissues, whereas Mb, a monomeric protein stores O_2 and supplies O_2 to the mitochondria for oxidative phosphorylation.[131,132] Both proteins have identical active site, possessing a five-coordinated Fe-protoporphyrin-IX, wherein 5th position of Fe is bonded by a conserved proximal His residue. Both Hb and Mb contain an uncoordinated distal His residue (Figure 7).[133] Besides Hb and Mb, neuroglobin (Ngb), a third heme protein in human, is predominately expressed in brain, which also reversibly binds O_2 and protects the neurons from hypoxia.[134–136] Ngb is an analogous of Mb due to its monomeric structure with molecular mass of ~17 kDa and high O_2 affinity like Mb.[136] The active site of Ngb is a six-coordinated Fe^{II}-protoporphyrin-IX, wherein both proximal and distal sites of Fe are coordinated by two His residues unlike Mb and Hb (Figure 7).[137,138] So, six-coordinated Fe-globins are a complex event for O_2 binding compared to five-coordinated Fe-globins. The oxygenation of Ngb is involved in the dissociation of six-coordinate to yield a transient five coordinate species, which allows for O_2 binding.[138,139] The association and dissociation rate constants (k_{+H} and k_{-H}) are independent of ligand as well acid-base.[137–140]

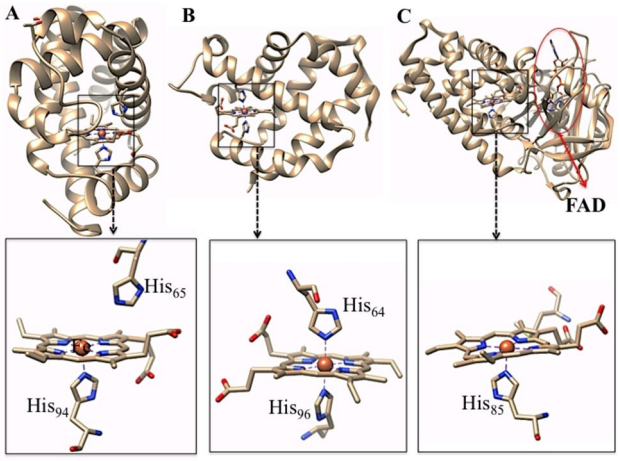

Figure 7. Crystal structures of human Mb (PDB; 1TES) (A), Ngb (PDB; 1OJ6) (B), and YHb (PDB; 4G1V) (C).

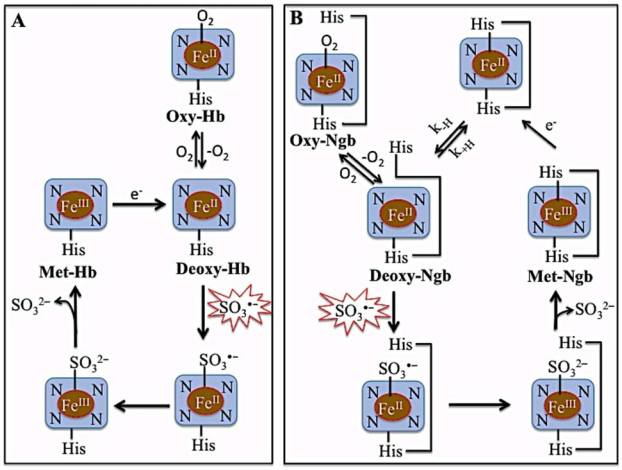

Figure 8. Probable mechanism of detoxification of sulfite radical by five-coordinated heme-globins (A) and six-coordinated heme-globins (B).

Besides native role, deoxy form of Fe^{II}-globin plays as a sulfite radical scavenger (Figure 8). The 5-coordinated Fe^{II}-globin can coordinate $SO_3^{\bullet-}$ at the distal site wherein O_2 binds reversibly. The reduction of $SO_3^{\bullet-}$ to SO_3^{2-} is mediated by Fe^{II}-globin and subsequently Fe^{II}-globin is oxidized to Fe^{III}-globin. The 6-coordinated Fe^{II}-globin first dissociates the distal His residue generating a 5-coordinated Fe^{II}-globin intermediate, which can bind $SO_3^{\bullet-}$ and reduce $SO_3^{\bullet-}$ to SO_3^{2-}. The reaction rate constants of sulfite radical neutralization are 38 $M^{-1}s^{-1}$ for myoglobin, 120 $M^{-1}s^{-1}$ for hemoglobin, 2,600 $M^{-1}s^{-1}$ for neuroglobin, and $>7{,}500\times10^6$ $M^{-1}s^{-1}$ for flavohemoglobin. These rates are highly correlated with their redox potentials.[34] The redox potentials of $SO_3^{\bullet-}/SO_3^{2-}$, met-HbA/HbA, met-Mb/Mb, met-Ngb/Ngb, and met-flavoHb/flavoHb are +0.73 V,[141] +0.144 V,[142] +0.046 V,[143] −0.129 V,[137] and −0.125 V[144] respectively. Despite the same redox potentials of Ngb and flavoHbs, the reaction rate of flavoHbs is 4–8-fold greater than Ngb.[34] This may be presence of 6th coordinated distal His residue that is a competitor with sulfite binding at Fe^{II}–Ngb.[137,139]

5. Reduction of Sulfite to Sulfide

5.2. Sulfite reductase

The reduction of sulfite to sulfide is a vital step in the biogeochemical sulfur cycle, which is driven by a class of metalloenzyme, namely sulfite reductase (SiR).[145–148] These enzymes are widespread in bacteria, plants and fungi. It falls into two major classes: (1) assimilatory sulfite reductases (aSiRs) that reduce the sulfite to sulfide with small quantity for the synthesis of sulfur-containing bio-constituents such as cysteine, and (2) dissimilatory sulfite reductases (dSiRs) that reduce the

sulfite to sulfide in the respiratory pathway, producing large quantity of sulfide.[145,149–151] Both classes share a unique and an unusual assembly of two metal-cofactors in their active sites, namely siroheme and [4Fe-4S] clusters, covalently connected by a cysteine residue, which serves as a ligand to the [4Fe-4S] as well as proximal ligand to the siroheme (Figure 9). However, siroheme binds substrate at distal site, whereas cubane [4Fe-4S] cluster acts as an electron pump, which facilitates e^- transfer to distally bound substrate of siroheme, enabling the six electron reduction of sulfite to sulfide.[35] The crystal structure of aSiR from *E. coli* is a complex hemoflavo-protein, composed of two subunits, α and β in a $\alpha_8\beta_4$ arrangement, containing siroheme coupled with cubane-[4Fe4S] cluster in the active site (Figure 9A).[35] The crystal structure of dSiR from *D. vulgaris* is a heteromultimers (Figure 9B), composed of α-, β-, and γ-proteins (corresponding to DsrA, DsrB, and DsrC subunits), containing

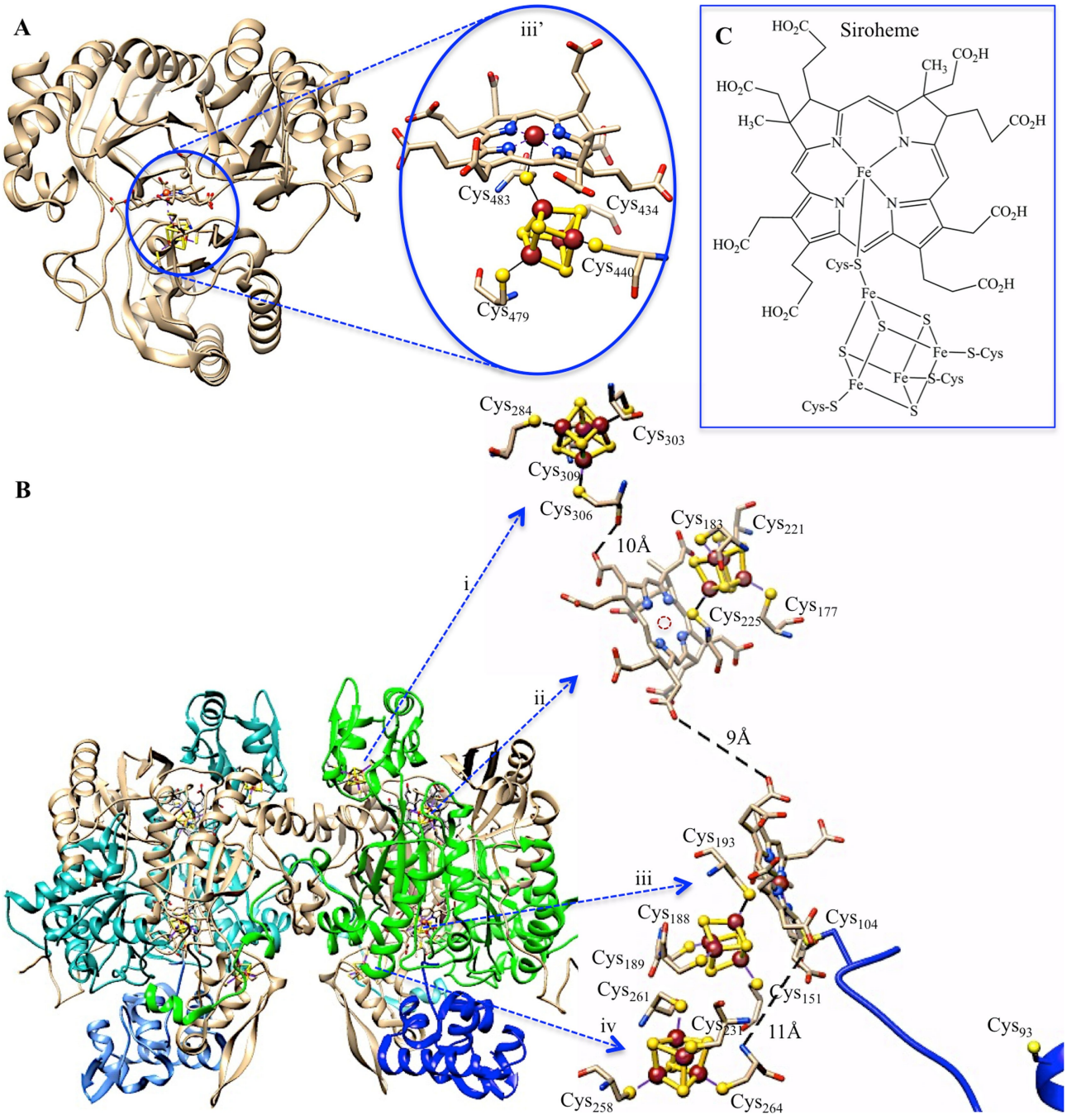

Figure 9. Structure of assimilatory sulfite reductase from E. coli (PDB ID: 1aop) (A) and dissimilatory sulfite reductases from D. vulgaris Hildenborough (PDB ID: 2v4j) (B). The α (DsrA; green/cyano), β (DsrB; grey/grey) and γ (DsrC; blue/light blue) for dimer of αβγ dissimilatory sulfite reductase. Sirohemes (iii and iii'), sirohydrochlorins (ii, no Fe (red dotted circle)) and [4Fe-4S] clusters (i & iv distal and ii & iii proximal) are highlighted. Brown ball; Fe, yellow ball; S, and blue ball; N. (C) Schematic diagram of siroheme bound [4Fe-4S] cluster.

two sirohemes, two sirohydrochlorins (metal free), four proximal covalently associated iron–sulfur clusters and four remote [4Fe-4S] clusters, which are located in only DsrA and DsrB subunits. DsrC subunit contains a highly conserved penultimate cysteine residue, Cys_{104} at C-terminal arm, which covalently binds the siroheme and participates in catalytic cycle. During catalytic reaction, Cys_{104} interacts with another Cys_{93} residue in DsrC and plays with intra-disulfide bond formation (Figure 9).[36,152]

Despite their same catalytic site, the product profile of dSiR is different from aSir. The aSiR produces sulfide only, while dSiR produces sulfide (S^{2-}) along with byproducts, thiosulfate ($S_2O_3^{2-}$) and trithionate ($S_3O_6^{2-}$), suggesting the involvement of DsrC in the catalytic cycle of the dSiR.[36,149,153,154] However, both a/dSiRs catalyze the six-electron reduction of SO_3^{2-} to S^{2-} (Figure 10).[152–155] The reduction of sulfite to sulfide is proceeded via two possible pathways: 1) six-electron reduction involving in three successive $2H^+/2e^-$ steps combined with sulfite in the catalytic cycle yielding only H_2S, and 2) sulfite reduction involving in a more complex process with formation of a mixture of products, S^{2-}, $S_2O_3^{2-}$ and $S_3O_6^{2-}$ (Figure 10).

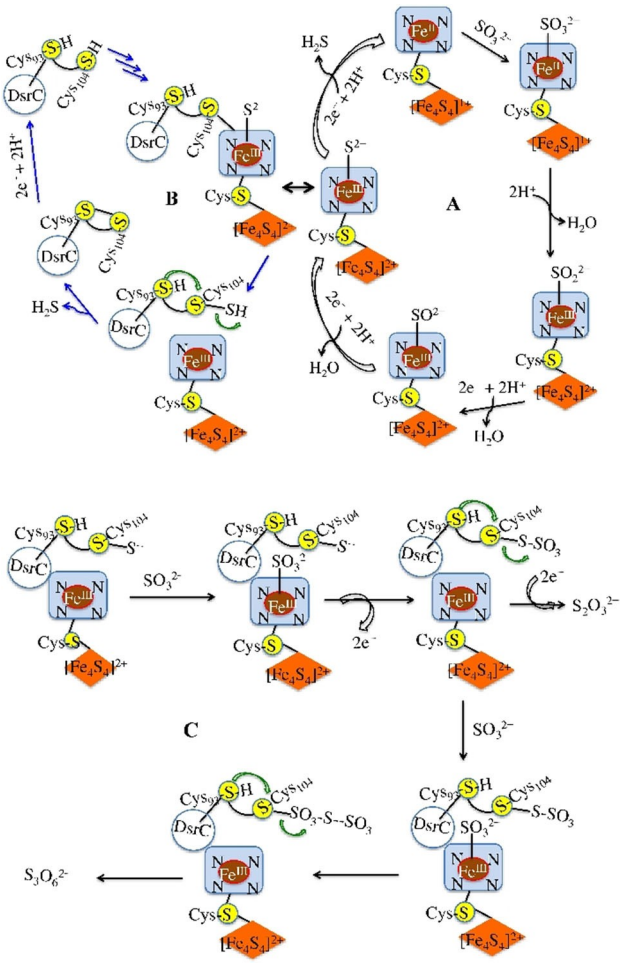

Figure 10. Proposed mechanism of sulfite reduction by sulfide reductases, aSiR and dSiR. A) Sirohemes-[4Fe-4S] catalyzes sulfite to sulfide. B) DsrC involves in the formation of S^{2-}. C) Production of sulfide, thiosulfate and trithionate by dSiR.

In the first step of the catalytic cycle, the reduced $[4Fe4S]^+$-siroheme-Fe^{II} binds the sulfite at the distal site of siroheme to produce $[4Fe4S]^+$-siroheme-Fe^{II}–SO_3^{2-} complex (Figure 10A), in which bound SO_3^{2-} is reduced by $2H^+/2e^-$ to produce one molecule of H_2O and oxidized $[4Fe4S]^{2+}$-siroheme-Fe^{III}–SO_2^{2-} intermediate. Then bonded SO_2^{2-} is reduced by $2H^+/2e^-$ to produce one molecule of H_2O and $[4Fe4S]^{2+}$-siroheme-Fe^{III}–SO^{2-} intermediate, which further proceeds two electron reduction coupled with hydration to yield $[4Fe4S]^{2+}$-siroheme-Fe^{III}–S^{2-} complex. In the final step, $[4Fe4S]^{2+}$-siroheme-Fe^{III}–S^{2-} releases H_2S and regenerates the reduced $[4Fe4S]^+$-siroheme-Fe^{II} for next catalytic cycle upon addition of $2H^+/2e^-$ (Figure 10A).[152–155] In alternative way, H_2S can be released from $[4Fe4S]^{2+}$-siroheme-Fe^{III}–S^{2-} by the involvement of DsrC (Figure 10B).[36] The bound S^{2-} in $[4Fe4S]^{2+}$-siroheme-Fe^{III}–S^{2-} attacks at S of Cys_{104} in DsrC generating a DsrC-persulfide intermediate. Then sulfide is displaced from DsrC-persulfide intermediate by the formation of intramolecular disulfide bond between Cys_{104} and Cys_{93} in DsrC. The intramolecular disulfide bond is reduced by $2H^+/2e^-$, regenerating reduced DsrC for next catalytic cycle (Figure 10B).[152–157] Prior to release the sulfide from DsrC-persulfide intermediate, $[4Fe4S]^{2+}$-siroheme-Fe^{III} may bind another SO_3^{2-} molecules and then DsrC-persulfide intermediate interacts with bound SO_3^{2-} to generate DsrC-$S_2O_3^{2-}$ intermediate, which releases the $S_2O_3^{2-}$ by intramolecular disulfide bond formation between Cys_{104} and Cys_{93} in DsrC. Furthermore, prior to release the $S_2O_3^{2-}$ from DsrC-$S_2O_3^{2-}$ intermediate, it can binds one more sulfite and proceeds to same catalytic pathway to yield $S_3O_6^{2-}$, suggesting that dSiR produces sulfide along with byproducts (Figure 10C).[36,149,153,154]

Interestingly, sulfate reducing bacteria (SRB), a most common genera, *Desulfovibrio* sp. are present in human digestive tract,[156,157] which participates in the sulfur cycling of human intestine.[10,61,62] SRB utilize inorganic sulfate as a terminal electron acceptor for conversion of energy, with the concomitant formation of H_2S.[158] In the dissimilatory pathway, the conversion of sulfate to sulfide proceeds in three steps. The first step starts with activation of sulfate by ATP to yield adenosine 5′-phosphosulfate (APS) and this reaction is catalyzed by ATP sulfurylase.[159] Next step is the reduction of APS to sulfite by APS reductase (adenylylsulfate reductase).[160] The final step finishes with the reduction of sulfite to sulfide by sulfite reductase.[147,148] So foods, beverages and drugs containing sulfites play an important role in human sulfur metabolism. Thus, both human intestinal cell and SRB regulate H_2S homeostasis, and therefore, low-level H_2S is beneficial for healthy tissues but high level H_2S contributes to colonic pathology, such as inflammatory diseases,[158,161] which may associate with dysfunction of many metalloenzymes such as, cytochrome c oxidase, heme-globins and zinc-dependent metalloenzymes by the interaction of S^{2-} with metallo-cofactor in metalloenzymes.[162,163]

6. Applications

6.1. Sulfite oxidase: biosensor/biofuel cells

In SO, the MD (Moco domain) catalyzes the oxidation of sulfite to sulfate, while HD (heme-domain) relays the electrons from Mo-center to other natural redox partner, cyt-c, restoring the resting state of SO.[20] This electron relay mechanism can be assumed to be involved in the unmediated (where electrode acts as an electron acceptor) or mediated (where natural or artificial redox mediator acts as an electron acceptor) electron transfer to electrode. Based on this concept, SO coupled cyt-c systems have been explored for biosensors and fuel cells.[7,39,164–167]

The alternative electrochemical approach is direct oxidation of sulfite that is relatively simple, but lacks the selectivity due to high working potential.[168] Therefore, the enzymes-based bio-electrochemical approach is suggested, where working potential is lower than sulfite or other species in solution. To achieve this, SOs have been immobilized on variety of surface modified electrodes, including pyrolytic graphite electrodes,[169,170] thiols modified gold electrodes,[171] silver electrodes,[172] and nanoparticles,[167,173,174] that facilitate the electron transfer between SO and electrode surface, developing the sulfite biosensors[38] or sulfite-based biofuel cells.[39,175]

The direct electrochemistry of SO has been extensively studied.[172,176] However, Elliott et al., first reported the direct bioelectrocatalytic activity of immobilized cSO at pyrolytic graphite electrodes or thiol-modified gold electrodes where catalytic rate was significantly lower than native SO with cyt-c.[170] Animal SOs have two subunits, Moco and Fe-domain, which are connected by a flexible loop and alter conformations during electron transfer between two domains. This may be reason that the immobilized SO reduces the rapid conformational change during catalysis, leading to decrease the electron transfer from Moco to Fe-domain. Zeng et al. have reported that the hSO alters the conformational even in the immobilized on electrode, but IET rate of hSO is slower than in solution, contributing lower activity of hSO upon immobilization.[176] When immobilized bacteria SDH at pyrolytic graphite electrode is employed in direct electrochemistry study, it retains the native catalytic activity.[169,177] This result suggests that the electron transfers from Moco to heme-domain in SDH without repositioning of these domains.

The cyt-c acts as a natural electron acceptor of native SO. Therefore, several reports on sulfite biosensor have been developed by fabrication of cyt-c (as a mediator or electron acceptor) with SO on electrode to enhance the electrochemical activity of SO.[7,164] The main advantage of this arrangement is the possibility to generate a fully electro-active multilayer, where cyt-c can well communicate between SO (Moco and heme) and electrode through ET. The electrons flow from substrate (sulfite) to electrode in a series of steps, which are followed as substrate to Moco, Moco to heme b5 (intra-molecular), heme b5 to cyt-c (inter-molecular) and finally cyt-c to electrode (interfacial).[178] Indeed, two proteins, hSO and cyt-c are co-immobilized on the gold electrode to generate fully

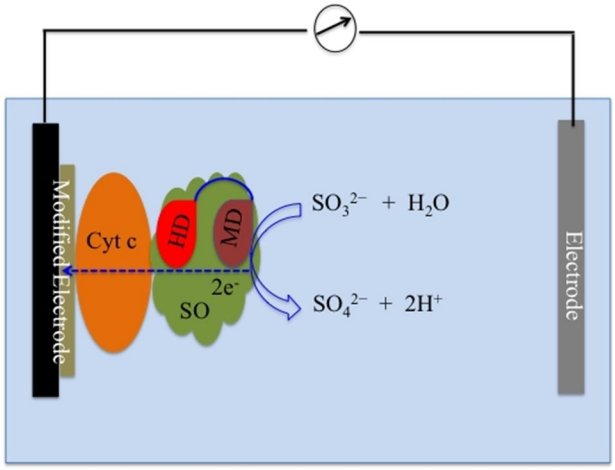

Figure 11. Cartoon represents the electrochemical sulfite sensors. HD; heme domain, MD; Moco-domain and SO; sulfite oxidase.

electro-active multilayer, which shows comparable catalytic rate to native in solution (Figure 11).[164]

Another sulfite biosensor has been developed by co-immobilization of two proteins, bacterial SO and cyt-c on thiol modified Au-electrode.[7] This sulfite biosensor shows sulfite sensitivity with a linear range of 1–6 μM.[7] The sulfite biosensor of unmediated hSO is relatively lower sensitivity, but when hSO is immobilized on indium tin oxide without mediator, the sensor shows fast response as well as high sensitivity of sulfite with a linear range of 0.5–2 mM.[38] This assembly is of general interest to develop efficient enzymatic biofuel cells (EBFCs).[39] Zeng et al., have reported a direct electron transfer (DET)-based sulfite/O_2 biofuel cell by combination of the hSO/PEI/thiol/AuNP/Au modified anode and MvBO/AuNPs/Au modified cathode (PEI: poly(ethyleneimine); MvBO: Myrothecium verrucaria bilirubin oxidase; AuNP: gold nano-particle).[175] This cell achieves the maximum power densities of 8 $\mu W\,cm^{-2}$ and an open circuit voltage of 0.57 V. However, the overall cell performance is still limited. Improvements of the bio-device are required to overcome these limitations. Recently, Tang et al. have developed a DET-based sulfite/O_2 biofuel cell composed of graphene-based hSO bioanode and Pt cathode[39] with maximum power density of $61 \pm 6\ \mu W\,cm^{-2}$ and open circuit voltage of 0.64 ± 0.01 V at 30 °C. This is the highest value compared to previously reported value. So, the EBFC may be a great potential electrical device for harvesting electrical energy from sulfite containing foods, wastewater and beverages.

6.2. Polyphenol oxidases: Anti-browning

Enzymatic browning is a common and serious problem in the food industry. Polyphenol oxidases (PPOs) are responsible for brown-pigment formation, causing adverse effects on color, flavor, taste and nutritional properties of fruits and vegetables.[179] PPOs are a class of type-II Cu-enzymes that catalyze the oxidation of several phenolic substrates to reactive

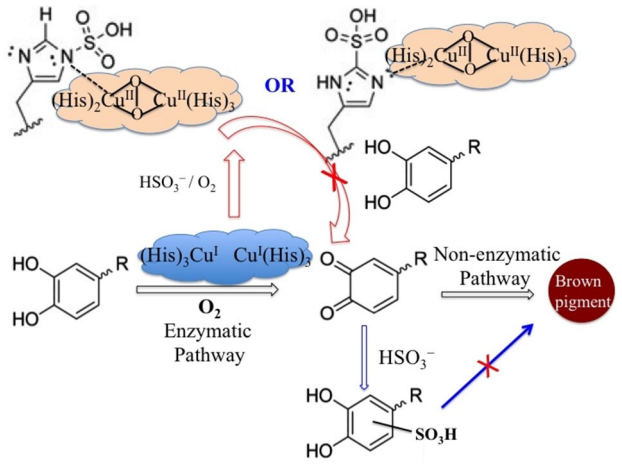

Figure 12. Possible mechanism of inactivation of tyrosinase by sulfite.

intermediates, *o*-quinones, which is further proceeded by non-enzymatic pathway to yield brown polymer, known as melanin (Figure 12).[180,181] As enzymatic browning involves in both enzymatic and non-enzymatic pathways, the browning can be halted by either inactivation of PPO or inhibition of o-quinone formation. One of the most common anti-browning agents is sulfite, which exhibits both anti-browning effects (Figure 12).[40,182] PPO catalyzes the oxidation of o-quinol derivative to a *o*-quinone derivative, which is susceptible to nucleophilic attack by sulfite to yield sulfoquinolic derivative, resulting in inhibition of the browning process.[183]

The inactivation of PPO may be a covalent modification between histidine and sulfite.[40] The catalytic site of the PPO possesses two copper ions, each of which is bonded by three histidine residues.[184] Copper can catalyze covalent modification of His-Cys derivative that is found in PPOs.[185] This result suggests that other histidine residues in the active site can be modified by nucleophilic addition of sulfite to produce sulfite-His unit, thereby inhibiting the PPO activity (Figure 12).[40]

6.3. Environmental application of sulfite radical

Micro-pollutants (MPs) are often discharged into the environment, particularly water bodies, which seriously threaten human health as well as the ecosystem due to poor degradation of MPs.[186,187] A major group of MPs is antibiotics, which are growing concerns due to extensive use for the treatment of bacterial infection in human and increasing the weight growth of animals worldwide.[188,189] Therefore, an urgent technology is required that efficiently degrades the MPs in water-bodies. One of the efficient technologies is advanced oxidation process (AOP).[190] The oxy-sulfur radical is a powerful oxidant, that can significantly degrade the MPs[191,192] due to its high reduction potential (E = 2.5–3.1 V vs. NHE), as well as longer half-life ($t_{1/2}$ = 30–40 μs) compared to $HO^•$ ($HO^•$, E = 1.8–2.7 V and $t_{1/2}$ = 1 μs,).[52,53] In AOP, (bi)sulfite has been extensively employed as a source of $SO_4^{•-}$ for removing MPs from water.[193] Several metalloproteins have ability to activate the sulfite to produce the $SO_4^{•-}$,[32,33] although, still free transition metal ions are extensively employed to generate the $SO_4^{•-}$ for the degradation of MPs in water treatment.[193] Fe^{II}/sulfite system has been used to degrade various MPs such as herbicide,[194] endocrine disruptor,[195] and pharmaceutical.[194] Indeed, sulfamethoxazole (SMX; m/z = 254), a broad-spectrum antibiotic, belongs to a sulfonamide drug family,[189,196,197] which is markedly decomposed in water by Fe^{III}/Na_2SO_3/persulfate system to generate variety of organic fragments with m/z at 126, 101, 90, and 59.[41]

7. Conclusions and Perspectives

This review describes the role of various metalloenzymes in the metabolic conversion of sulfite to sulfate, sulfite radical and sulfide via oxidation or reduction pathways. The SO detoxifies the toxic sulfite to sulfate but impairment of SO activity causes accumulation of excess sulfite in serum, attributing to cytotoxicity of (bi)sulfite. A class of heme-peroxidases initiates the one electron oxidation of sulfite to sulfite radical, which enables oxidative damage of biomolecules. Indeed, human serum albumin (HSA), a most abundant plasma protein, has a close biological relationship with (bi)sulfite, enabling the sulfite-induced HSA radicals formation, resulting in the progression of various diseases states via sulfur metabolism pathway.[198] Interestingly, albumin-containing metal binding ATCUN motif (amino terminal Cu and Ni binding motif)[199] has no report to the formation of sulfite radical from sulfite, but designed metal-ATCUN has ability to generate sulfite radical at physiological condition in vitro.[200] Therefore, mechanism of synergistic effect of HSA and bi(sulfite), in vivo, still is obscure.

Interestingly, sulfite is detoxified by SO, which is a competitive to MPO-derived sulfite radical. In these two competitive pathways, to investigate the toxicity of sulfite, a rodent is used as a human model because activity of SO in rodents is significantly higher than in human SO. The study demonstrates that mice with the SO deficiency treated with (bi)sulfite show higher sulfite-derived protein radical formation than mice with normal SO.[28] Therefore, SO should be counted as a vital enzyme while studying the sulfite toxicity on humans.

The reduction of sulfite to sulfide by diSR is also an important part to regulate the sulfide homeostasis in human intestine cells. The imbalance of sulfide level can attack many metalloproteins, resulting in the dysfunction of their native activity. So, in the cellular system, (bi)sulfite can interact with various metalloproteins that have not been yet properly elucidated. There is a strong drive to explore the relationship between sulfite and metalloproteins in vivo, which is likely to attract attention to overcome the critical challenges encountered in the sulfur metabolism pathway.

Recently, SO is a general interest in the development of biosensors/biofuel-cells. Various cells have been reported, but the efficiencies of cells are not yet to reach our demand. Therefore, SO/nanoparticles (or SO/photosensitizer) assembly on modified electrodes may be a good approach that may

develop an efficient solar-driven and/or a photo-electrochemically biosensor/biofuel-cell.[201]

Sulfite is widely used as food additives and anti-browning, but the mechanism of sulfite with foods is not clear yet. Recent study has reported that (bi)sulfite cannot significantly alter the properties of vitamin B_{12} (cyano-cobalamin) in food, but a small amount of sulfite-cobalamin complex is formed, indicating the interaction between sulfite and cobalt in B_{12}.[202]

Recently, (bi)sulfite is growing attention as a precursor of $SO_4^{\bullet-}$ radical for expelling MPs from water. However, metal/(bi)sulfite system has limitation to produce the $SO_4^{\bullet-}$ in water, but a class of metalloproteins has ability to produce $SO_4^{\bullet-}$ from sulfite in water. Therefore, metalloprotein derived $SO_4^{\bullet-}$ radical may hold great promise for the water treatment in future.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: biosensor-biofuel cells · (hydrogen)sulfite · peroxidase · siroheme-[4Fe-4S] · sulfite-oxidase

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