BBA - Bioenergetics 1859 (2018) 19–27

Contents lists available at ScienceDirect

BBA - Bioenergetics

journal homepage: www.elsevier.com/locate/bbabio

The central active site arginine in sulfite oxidizing enzymes alters kinetic properties by controlling electron transfer and redox interactions

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ABSTRACT

A central conserved arginine, first identified as a clinical mutation leading to sulfite oxidase deficiency, is essential for catalytic competency of sulfite oxidizing molybdoenzymes, but the molecular basis for its effects on turnover and substrate affinity have not been fully elucidated.

We have used a bacterial sulfite dehydrogenase, SorT, which lacks an internal heme group, but transfers electrons to an external, electron accepting cytochrome, SorU, to investigate the molecular functions of this arginine residue (Arg78). Assay of the SorT Mo centre catalytic competency in the absence of SorU showed that substitutions in the central arginine (R78Q, R78K and R78M mutations) only moderately altered SorT catalytic properties, except for R78M which caused significant reduction in SorT activity. The substitutions also altered the Mo-centre redox potentials ($Mo^{VI/V}$ potential lowered by ca. 60–80 mV). However, all Arg78 mutations significantly impaired the ability of SorT to transfer electrons to SorU, where activities were reduced 17 to 46-fold compared to $SorT^{WT}$, precluding determination of kinetic parameters. This was accompanied by the observation of conformational changes in both the introduced Gln and Lys residues in the crystal structure of the enzymes. Taking into account data collected by others on related SOE mutations we propose that the formation and maintenance of an electron transfer complex between the Mo centre and electron accepting heme groups is the main function of the central arginine, and that the reduced turnover and increases in $K_{Msulfite}$ are caused by the inefficient operation of the oxidative half reaction of the catalytic cycle in enzymes carrying these mutations.

1. Introduction

Sulfite is a natural by-product of various metabolic processes including the degradation of sulfur-containing amino acids, and can cause damage to DNA, proteins and lipids, all of which become modified and functionally impaired [1–3]. Sulfite–oxidizing molybdoenzymes (SOEs) play a major role in protecting cells from sulfite-induced oxidative damage, and thus they are vital for maintaining cell function and integrity in all forms of life (Fig. 1) [1,3].

The catalytic competency of SOEs is determined by the amino acids surrounding the Mo active site, where a conserved arginine residue (Fig. 1) is involved in tuning the potential of the redox centres and influences electron transfer between the Mo and other redox centres such as cytochromes [4–8]. By hydrogen bonding to the catalytic oxo-group of the Mo centre, this residue is thought to be essential for substrate binding [7,9] (Fig. 1) and has also been proposed to affect the conformation of residues in the substrate access channel [10]. However, the molecular details of the function of this residue have never been fully elucidated, and here we provide new insights into the molecular function of this central, and highly conserved residue.

Most of the available data on mutations of this central arginine residue come from human and chicken sulfite oxidases (HSO, CSO) (Figs. 1 & S1). The arginine to glutamine (R160Q) mutation was first identified in a five year old girl presenting with sulfite oxidase deficiency and has received significant attention due to its drastic effect on HSO catalysis [8]. Specifically, $K_{Msulfite}$ increased by a factor of 1000 from 1.7 μM to 1.7 mM (pH 8.5) while enzyme turnover was reduced by 85%, rendering the resulting enzyme unable to function in its cellular

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http://dx.doi.org/10.1016/j.bbabio.2017.10.001
Received 12 July 2017; Received in revised form 29 September 2017; Accepted 2 October 2017
Available online 03 October 2017
0005-2728/

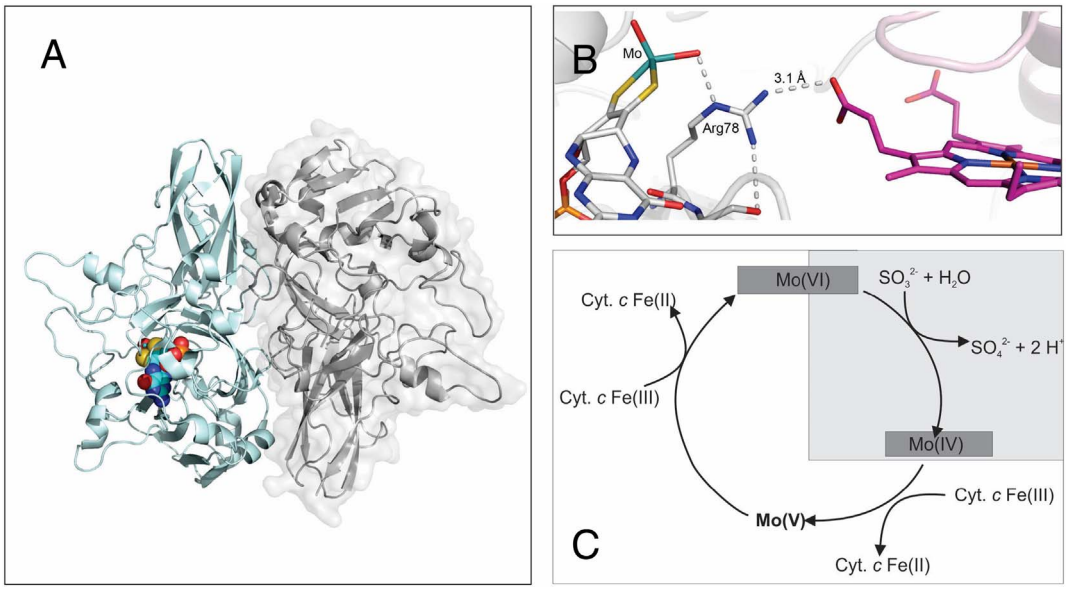

Fig. 1. Structural and kinetic properties of the SorT sulfite dehydrogenase. Panel A: 3D structure of the $SorT^{WT}$ homodimer. Panel B: Interactions between the redox centres of $SorT^{WT}$-SorU, heme group – pink, Mo - green. Panel C: Proposed reaction mechanism of the Mo-only $SorT^{WT}$ enzyme.

environment [8,10]. The equivalent R138Q mutation in the highly homologous CSO showed similar kinetic alterations, with a $K_{Msulfite}$ of 250 μM for $rCSO^{R138Q}$ (WT: 8 μM) and a k_{cat} of 3.4 s^{-1} (WT: 73.3 s^{-1}, pH 8.5) [10]. The same study also determined the kinetic parameters of recombinant HSO^{R160Q} where they found a k_{cat} of 3.3 s^{-1} and a $K_{Msulfite}$ of 0.86 mM (pH 8.5), in good agreement with the original data. Both recombinant enzymes showed a reduction in the rate of intramolecular electron transfer between the heme *b* and Mo redox centres, which is an essential step in the catalytic cycle (Fig. S1) [11]. These two domains are connected by a flexible loop and are thought to undergo repositioning during catalysis. Properties of an HSO^{R160Q} equivalent substitution were also reported for a bacterial SOE, the SorAB sulfite dehydrogenase (SDH) [7,12]. This enzyme differs structurally from the vertebrate enzymes in that it is a permanent, but non-covalently-linked, complex of a Mo- and a heme–containing subunit, while the heme *b* domain of HSO/CSO is mobile and requires repositioning during catalysis [13]. As expected, $SorAB^{R55Q}$ showed an increased $K_{Msulfite}$ (~3500 times), and k_{cat} was reduced by ~70% [6,14].

Currently, the structure of CSO^{R138Q} is the only available crystal structure of an SOE carrying this substitution. It shows the Gln138 residue hydrogen bonding to the equatorial oxo-group of the Mo centre in the same way observed for Arg138, but due to the shorter side chain length and different molecular structure, the mutation created a small cavity in the active site structure and caused changes in the electrostatic environment [10].

These changes were thought to be the cause of a significant structural change in the conformation of Arg450 (CSO numbering), a residue located in the substrate access channel close to the enzyme surface. In the CSO^{R138Q} crystal structure, Arg450 was observed in a conformation folded toward the Mo-active site, leading to a reduction in the positive surface charge around the active site entrance and the partial exposure of the negatively charged residue Asp321 [10]. This conformational change also caused a narrowing of the substrate access channel, suggestive of a 'gating' function for the Arg_{450} residue. In the CSO^{WT} structure, Arg450 pointed towards the active site when sulfate was present, but away from the active site in the resting enzyme, leading to a maximal opening of the substrate access channel.

EPR studies then revealed that at low pH, the HSO^{R160Q} Mo centre exists in a so-called 'blocked' form, that is catalytically inactive and has a sulfate molecule bound to the one electron reduced Mo(V) centre, which could explain the reduced catalytic competence of the enzyme [15]. This 'blocked', sulfate-bound EPR signal at low pH (~6 and below) was also observed for the bacterial SorAB enzyme [14]. Although some early EXAFS data suggested coordination of the Glu160 sidechain to Mo as a sixth ligand, a combination of DFT calculations and EPR spectroscopy later confirmed that all EPR active species of HSO^{R160Q} were five coordinate, as seen in the CSO^{R138Q} crystal structure [10].

The kinetic data available for SOEs with mutations in the central arginine residue so far agree well across enzymes with different quarternary structures, but have all been collected for SOEs that contain either a heme *b* or heme *c* redox centre in addition to the Mo cofactor. In contrast, there are no data available for a novel and emerging group of bacterial SOEs that lack heme cofactors, such as the recently described SorT sulfite dehydrogenase from *Sinorhizobium meliloti* [4,16]. There are also open questions, including how this mutation leads to the striking reduction of catalytic efficiency observed in SOEs, and whether substrate channel gating plays a role in the activity of the enzymes.

Here we report the structural and spectroscopic effects of active site mutations targeting the HSO^{R160}-equivalent residue Arg78 and its impact on the activity of SorT, which is a representative of the most common type of bacterial SDH [2,16]. SorT is a homodimer of Mo-pyranopterindithiolene (PPT) binding subunits oriented in a head-to-tail configuration (Figs. 1 & S1), and interacts with a small *c*-type cytochrome, SorU, as its natural electron acceptor [4,17]. The reaction cycle of SorT is less complex than that for heme-containing SOEs as it does not require intramolecular electron transfer events to occur. However, docking of SorT with SorU is still necessary for successful sulfite oxidation. This makes this system an ideal model for isolating the reductive half reaction (sulfite oxidation by SorT) from the oxidative half reaction (SorT oxidation by SorU) and investigating the effects of the active site arginine on SOE catalysis.

2. Materials and methods

2.1. Cloning and mutagenesis

The SorT R78Q, R78K and R78M variants were generated essentially as previously described [18] using the pProex-SorT plasmid [17] and the following mutagenesis primers:

SMsorT_R78QF