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COMMENT

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Reply to the "Comment on 'A Sulfonium Cation Intermediate in the Mechanism of Methionine Sulfoxide Reductase B: A DFT Study'"

Jesse J. Robinet, Hisham. M. Dokainish, David J. Paterson, and James W. Gauld*

Department of Chemistry and Biochemistry, University of Windsor, Windsor, Ontario N9B 3P4, Canada

Neiers at al.[1] have commented on our computational investigation[2] of the catalytic mechanism of *Neisseria gonorrhoeae* MsrB. In particular, they acknowledged that the formation of a sulfonium cation along the catalytic pathway was convincingly[1] shown. Incidentally, they noted that our subsequent preferred pathway for formation of the Cys440-S−S-Cys495 disulfide bond was not in agreement with experimental kinetic studies of Olry et al.[3] on a wild-type and a C65S mutant *Neisseria meningitidis* MsrB. Specifically, Olry et al.[3] concluded from their studies that a sulfenic acid intermediate is formed prior to the above disulfide bond. Consequently, Neiers et al.[1] conclude that our chemical model incompletely represents the active site.

In our DFT-based cluster approach, those residues that were known or thought to be mechanistically important were explicitly included in the chemical model. As is common, we also included "second-shell" residues that may be important in, for example, charge stabilization on active site residues. A select number of atoms were held fixed at their X-ray crystal structure positions to maintain the integrity of the cluster (PDB 1L1D; see ref 2 for details). The rest of the surrounding environment was then implicitly modeled via the use of a polarizable continuum model (PCM). This type of computational model has been widely applied to enzymatic systems and has been found to often be reliable and accurate with errors in calculated relative energies amounting to 12 kJ mol^{-1} for systems of first- and second-row heavy atoms.[4]

Using such an approach, for our largest and most complete cluster model (Scheme 5)[2] the catalytic mechanism of *Neisseria gonorrhoeae* MsrB was shown to initially lead to formation of a sulfonium cation via proton transfers from both His480 and His477 to the substrates sulfoxide oxygen. The overall barrier for this process was calculated to be just 17.1 kJ mol^{-1} (Figure 3).[2] In contrast, the commonly suggested[5] alternate process involving initial formation of a sulfurane followed by a 1,3-sigmatropic rearrangement to give a sulfenic acid intermediate was found to be considerably higher in energy according to our present models.

It is noted that in our largest and most complete cluster model (Scheme 5),[2] the water and R-groups of residues (Ser438, Ser444, Arg493, and Asp484) located immediately around the thiol of Cys440, as observed in the X-ray crystal structure (PDB 1L1D), were included. We examined two possible pathways by which the sulfonium cation may react to give the final product: (i) in two steps via a Cys495-SOH intermediate (i.e., stepwise) and (ii) via direct attack of Cys440-S$^-$ at the sulfonium cation's Cys495-S center (i.e., concerted). For the stepwise path, we found that the barrier to formation of the sulfenic acid and its subsequent reaction to give the final product were almost the same, at just 54.6 and 58.3 kJ mol^{-1}, with respect to the sulfonium cation intermediate (Figure 9).[2] For the alternate concerted path the barrier was just 23.8 kJ mol^{-1} (Figure 3),[2] and thus, we concluded that it was preferred. Importantly, however, both pathways were found to be enzymatically feasible and, furthermore, have reaction barriers that differ by only 30 kJ mol^{-1} within the model used.

Experimentally it has been found that methionine sulfoxide reductases (Msr's) exhibit a remarkable degree of variability with respect to their active site cysteine residues. For example, the distance between the two sulfur atoms, one belonging to the recycling cysteine and the other to the catalytic cysteine, is 6.83 Å in an X-ray crystal structure of a *Mycobacteria tuberculosis* MsrA-protein-bound methionine complex (PDB 1NWA).[6] In contrast, they are just 3.29 Å apart in an X-ray crystal structure of a *Neisseria gonorrhoeae* MsrB-substrate analogue complex (PDB 1L1D).[7] Furthermore, the environments of the thiols of the recycling cysteine's differ. In the former, it includes the R-groups of an aspartyl, tyrosyl and histidyl residues, while in the latter, it contains the R-groups of two serinyl and an arginyl and a backbone carbonyl. In addition, some Msr's contain selenocysteine,[8] while an archaebacterial MsrB[9] (PDB 2K8D) has been found to only contain a catalytic cysteine. Given this variability, the catalytic pathway and mechanism of MsrB's, and Msr's in general, behoove both computational and experimental investigations to examine in further detail the role and influence of protein environment on the stability and reactivity of relevant sulfur species along the pathway. Our computational investigation[2] is the first comprehensive study to use such methods to examine plausible overall pathways for the catalytic mechanisms of MsrB's. As such, it provides a starting point for further detailed computational investigations on this fascinating class of enzymes.

AUTHOR INFORMATION

Corresponding Author

*E-mail: gauld@uwindsor.ca.

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