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COMMENT

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

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Recently, Gauld and co-workers convincingly showed that the mechanism of methionine sulfoxide reductase B (MsrB) passes through a sulfonium cation[1] similarly to that shown for MsrA.[2,3] In their DFT study, three chemical models of the MsrB were considered to provide insights into the role of various residues in the chemistry and substrate specificity. For most of them, their role had already been demonstrated by site-directed mutagenesis associated with kinetic studies.[4] The small model presented in their study was used only to consider the inherent chemistry of key species and intermediates. The two other models also included active site residues based on the crystal structure of the MsrB of *Neisseria gonorrhoeae* and the kinetic properties of mutants of the active site residues. These two models confirm the enzymatic studies that showed the essential role of His480.[4] In both models, two protonated residues, i.e., His477 and Arg466, form hydrogen bonds with the sulfurane's −OH oxygen via water molecules. For the medium model, the preferred proton donor is Arg466, which leads to formation of the sulfonium cation, while His477 is the preferred donor in the large model. In this context, site-directed mutagenesis studies, which were carried out by Neiers et al., showed that, in contrast to that observed with the H477A mutant, substituting Arg466 with Ala has no significant effect on the catalytic efficiency of the reductase step,[5] similar to that already observed when substituting Arg493 with Leu. Therefore, the kinetic data obtained with R466A mutant strongly supports an absence of role for Arg466 in formation of the sulfonium and sulfenic acid intermediates in MsrBs.

Moreover, Gauld and co-workers showed that with the model used, the preferred route from a thermodynamic point of view is a direct nucleophilic attack of the recycling Cys440 at the $S_{Cys495}$ center of the sulfonium ion. Thus, formation of the disulfide bond Cys440−Cys495 does not pass through a sulfenic acid intermediate. Such a result contradicts the kinetic data obtained with the MsrB from *Neisseria meningitidis*, which showed that formation of the disulfide bond is preceded by formation of a sulfenic acid intermediate. Indeed, a sulfenic acid intermediate was shown to be formed on the catalytic Cys of the MsrB when the recycling Cys was mutated,[6] and the rate of formation of methionine is similar in the presence and absence of the recycling Cys.[7] Therefore, the discrepancy between the experimental and theoretical data suggests that the model used in the DFT study is not totally representative of the active site of MsrB.

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