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Mechanism of the Cysteine Sulfenic Acid *O*—Sulfenylation of 1,3-Cyclohexanedione

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Abstract: The density functionals B3LYP, B3PW91, M062X, and CAM-B3LYP with the 6-311+G(d,p) basis set predict the cysteine sulfenic acid *O*—sulfenylation of the *s-cis*-ketoenol tautomer of 1,3-cyclohexanedione proceeds through a cyclic 14-membered transition state structure containing three water molecules.

Protein sulfenylation is a post-translational modification of emerging importance in eukaryotes and 1,3-cyclohexanedione (**1**, Figure SI-1 in the Supplementary Information) and 5,5-dimethyl-1,3-cyclohexanedione (dimedone, **2**) based chemoselective probes represent important tools for its detection.[1] Generally, the covalent modification of the cysteine sulfenic acid is considered to take place through the *C*-sulfenylation on carbon 2 of the 1,3-cyclic diketones (**1**, **2**, Figure 1) to form sulfides (thioethers).[1-3] In this manuscript, the investigation of an alternative reaction that leads to sulfenic ester formation through *O*-sulfenylation is presented and supported (Figure 1).

1b R = R = H
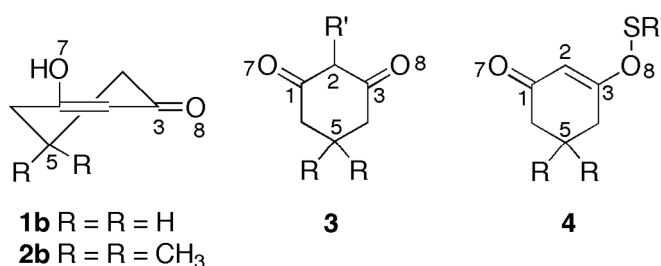**2b** R = R = CH$_3$
3
4

Figure 1. Enol tautomers of 1,3-cyclohexanedione (**1b**) and 5,5-dimethyl-1,3-cyclohexanedione (**2b**) and the *C*-sulfenylated (**3**) and *O*-sulfenylated (**4**) products from R'SOH.

The presence of the conjugated system in cyclic 1,3-diketones suggest that *O*-sulfenylation to form unstable sulfenate esters (**4**, Figure 1) is reasonable.[2] It is known

that electrophilic sulfenyl sulfur reacts with nucleophiles such as alkenes, enamines, and enols. Owing to the vital importance and significance of the chemoselectivity of 1,3-cyclohexanedione (**1**) and 5,5-dimethyl-1,3-cyclohexanedione (**2**) in detecting protein sulfenic acids, it is important to understand the fundamental mechanisms and products of the sulfenylation reactions. We are using computational quantum chemistry (see Supplementary Information) to investigate the fundamental chemistry, properties, and structures of sulfenic acids and their reactions with (**1**) and (**2**) in order to acquire data and information to assist in elucidating the nature of the chemistry of protein thiols and protein sulfenic acids.[2,4,5]

Amino acids exist as zwitterions in aqueous solution and in the crystalline state and in the neutral (nonionized) forms in the gas phase. In the gas phase, the lowest energy conformer of cysteine sulfenic acid (Cys-SOH, **5**, Figure 2) is stabilized by strong intramolecular hydrogen bonds, dispersive and steric interactions, and electrostatic, exchange, and hyperconjugative electronic effects owing in part to the polar functional groups (amino, carbonyl, hydroxyl, hydroxysulfanyl) which possess acceptor and donor properties.[6] One of the objectives of this study is to utilize specific cysteine---water complexes that maintain the conformation and nonbonding interactions of the water free lowest energy conformer of cysteine sulfenic acid (**5**, Figure 2).[2]
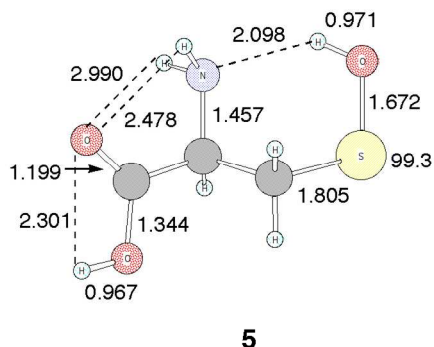

5

Figure 2. M062X/6-311+G(d,p) geometry optimized structure of the sulfenyl tautomer of cysteine sulfenic acid (Cys-SOH, **5**).

The energy barriers for the *O*-sulfenylation reaction of cysteine sulfenic acid (**5**) with the *s-cis*-ketoenol (**1b**) tautomer through cyclic transition states **TS**H_2O (10-membered ring, boat or sofa conformation), **TS**2H_2O (12-membered ring, boat or sofa conformation), and **TS**3H_2O (14-membered ring, sofa conformation, τ = C1—C2—C3—C4 = -1.3°; C1—C6—C4—C3 = -0.8°; C3—C2-C1—C6 = 0.4°) are shown in Table SI-2 in the Supplementary Information. It is seen that the inclusion of water molecules in the transition state structures lowers the activation barrier. The inclusion of water molecules in the transition state structures helps to relieve strain, helps to stabilize charges, and helps to set up a system to facilitate proton transfer from O7 in *s-cis*-ketoenol (**1b**) through a hydrogen-bonded network of water molecules in a Grotthuss-type mechanism.[7] The geometry-optimized structure of transition state **TS**3H_2O is shown in Figure 3 and the structures of transition states **TS**H_2O and **TS**2H_2O are given in Figure SI-2 in the Supplementary Information. The geometry-optimized structure of the *O*-sulfenylation product (**6**) is given in Figure 3. Sulfenate esters are known to be unstable and sulfenate anions, which are also unstable, may be oxidized sulfinate anions and may disproportionate to the corresponding sulfinate and thiolate anions.[8]

The cysteine sulfenic acid (**5**) *O*—sulfenylation of the *s-cis*-ketoenol tautomer (**1b**) of 1,3-cyclohexanedione (**1**) to afford the sulfenate ester (**6**) proceeds through a cyclic 14-membered transition state structure containing three water molecules (**TS**3H_2O, Figure 3). The half-chair conformation of *s-cis*-ketoenol (**1b**) becomes a sofa conformation (τ = C1—C2—C3—C4 = -1.3°; C1—C6—C4—C3 = -0.8°; C3—C2-C1—C6 = 0.4°) along the reaction path from ground state to transition state **TS**3H_2O. B3LYP, B3PW91, M062x, and CAM-B3LYP predict the energy barrier for the *O*-sulfenylation reaction of the three-water complex of cysteine sulfenic acid (**5**---3H_2O) with the *s-cis*-ketoenol (**1b**) to be 7.4, 5.3, 6.6, and 7.1 kcal/mol, respectively. The small energy barrier of only 2 kcal/mol between the two waters and three waters transition states may be due to entropic and other environmental effects. Larger energy barriers are predicted for transition state structures involving one water molecule (**TS**H_2O (10-membered ring) and two waters (**TS**2H_2O, 12-membered ring, Figure SI-2 in the Supporting Information).
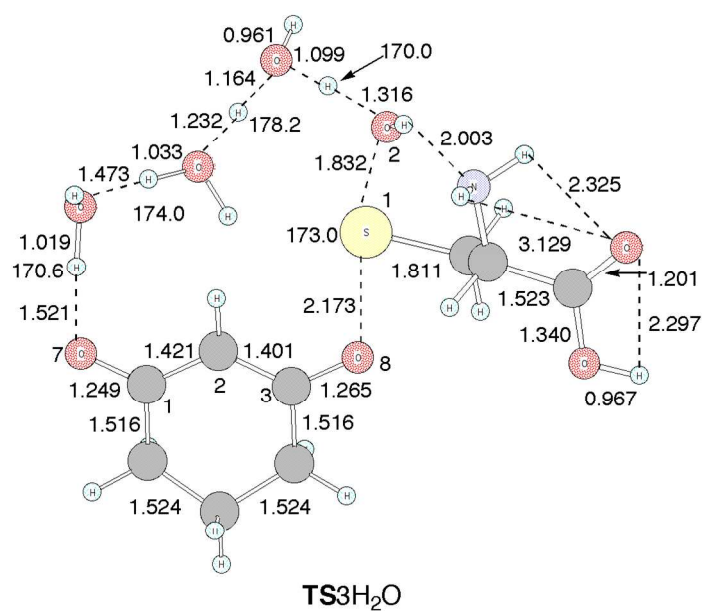
TS3H$_2$O

Figure 3. M062X/6-311+G(d,p) geometry optimized structure of the three waters transition state (**TS**3H$_2$O) for the cysteine sulfenic acid (Cys-SOH, **5**) *O*-sulfenylation of the *s-cis*-ketoenol tautomer (**1b**) of 1,3-cyclohexanedione (**1**).
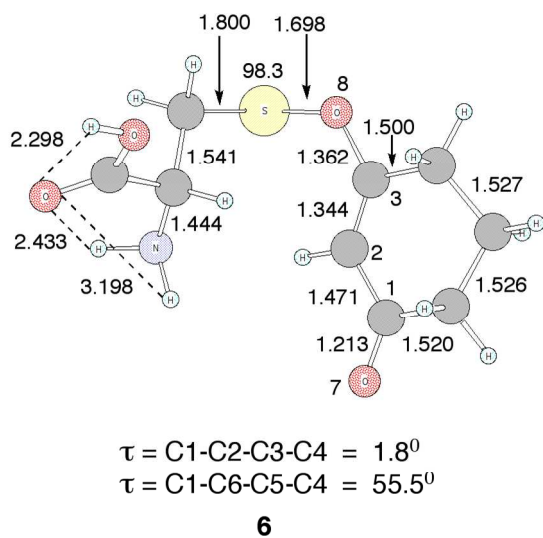

$\tau = C1\text{-}C2\text{-}C3\text{-}C4 = 1.8^0$
$\tau = C1\text{-}C6\text{-}C5\text{-}C4 = 55.5^0$

6

Figure 4. M062X/6-311+G(d,p) geometry optimized structure of sulfenate ester (**6**).

4

The mechanism for the formation of the *O*-sulfenylated product (**6**) involves attack of O8 in the enol or enolate form of the *s-cis*-ketoenol tautomer (**1b**) on the sulfur atom in cysteine sulfenic acid (**5**). The inclusion of water molecules assists in orienting the structure of the transition state in order to facilitate the proton transfer process. There is electron delocalization involving O7—C1—C2—C2—O8 in transition state **TS**3H$_2$O as evidenced by the equal C1—C2 and C2—C3 bond lengths and by the similar C1—O7 and C3—O8 bond lengths. In contrast, the C1—O7, C1—C2, C2—C3, and C3—O8 bond lengths in the half-chair ground state conformation of *s-cis*-ketoenol (**1b**) are 1.355 Å, 1.350Å, 1.464 Å and 1.221 Å, respectively.

Conclusions

The results of this computational quantum chemistry study are consistent with the half-chair *s-cis*-ketoenol tautomer (**1b**) of 1,3-cyclohexanedione (**1**) reacting with the three water complex of the lowest energy conformer of cysteine sulfenic acid (**5**---3H$_2$O) through a cyclic 14-membered transition state structure **TS**3H$_2$O to afford the sulfenate ester (**6**).

Geometry optimized structures and relative energies of the five low energy conformers of 1,3-cyclohexanedione, energy barriers for the water assisted transition states, and computational quantum chemistry methods.

Notes and References

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1. (a) V. Gupta and K. S. Carroll, *Biochim. Biophys. Acta* 2014, **1840**, 847-875. (b) C. E. Paulsen and K. S. Carroll, *Chem. Rev.* 2013, **113**, 4633-4679. (c) M. L. Conte and K. S. Carroll, Jakob, U.; Reichmann, D., Eds., Springer Netherlands 2013, Chapter 1, pp 1-41. (d) L. B. Poole, K. J. Nelson and P. A. Karplus, Jakob, U.; Reichmann, D., Eds., Springer Netherlands, 2013, Chapter 4, pp 85-118. (e) C. Klomsiri, K. J. Nelson, E. Bechtold, L. Soito, L. C. Johnson, W. T. Lowther, S.-E. Ryu, S. B. King, C. M. Furdui, and L. B. Poole, *Methods in Enzymology*, 2010, **473**, 77-94.

2. F. Freeman, I. T. Adesina, J. L. La, J. Y. Lee and A. A. Poplawski, *J. Phys. Chem. B* 2013, **117**, 16000-16012.

3. G. Wagner, G. *Tetrahedron* 2013, **69**, 7243-7252.

4. Gaussian 09, Revision A.1, Gaussian, Inc., Pittsburgh PA, 2003. The complete reference citation is in the Supplementary Information.

5. Spartan, Wavefunction, Inc., Irvine, CA 92612. The complete reference citation is in the Supplementary Information.

6. (a) J. J. Wilke, M. C. Lind, H. F. Schaefer III, A. G. Császár, and W. D. Allen, *J. Chem. Theory Comput.* **2009**, *5*, 1511-1523. (b) A. Fernández-Ramos, E. Cabaleiro-Lago, J. M. Hermida-Ramón, E. Martınez-Nuñez, and A. Peña-Gallego, *J. Mol. Struct. Theochem* **2000**, *498*, 191-200. (c) J. Sadlej, J. Cz. Dobrowolski, J. E. Rode, and M. H. Jamróz, *J. Phys. Chem. A* **2007**, *111*, 10703-10711.

7. (a) C. J. T. de Grotthuss, *Ann. Chim.* (Paris) **1806**, *58*, 54-73. (b) S. Cukierman, *Biochimica Biophysica Acta* **2006**, *1757*, 876-885. (c) O. Markovitch, H. Chen, S. Izvekov, F. Paesani, A. Voth, and N. Agmon, *J. Phys. Chem. B* **2008**, *112*, 9456-9466.

8. (a) M. C. Aversa, P. Bonaccorsi, D. Madec, G. Prestat, and G. Poli in *Innovative Catalysis in Organic Synthesis: Oxidation, Hydrogenation, and C—X Bond Forming Reactions,* Andersson, P. G., Editor, Wiley-VCH Verlag, 2012, Chapter 3 pp 47-76. (b) J. S. O'Donnell and A.L. Schwan, A. L. *J. Sulfur Chem.* **2004**, *25*, 183-211.