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Oxo, Sulfido, and Tellurido Mo-enedithiolate Models for Xanthine Oxidase: Understanding the Basis of Enzyme Reactivity

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Knowledge of the electronic structure of molybdenum and tungsten enzymes[1,2] is becoming increasingly important in studies of these enzymes.[3,4] Recently, using first-principles[5] electronic structure calculations of a plausible active-site model[6] we have examined the reaction mechanism of xanthine oxidase—a widely distributed member of the molybdenum hydroxylase family of enzymes[4a]—with the substrate formamide,[7] Figure 1.

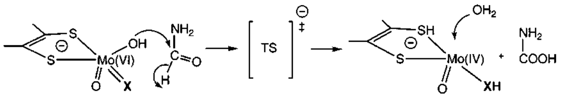

Figure 1. Formal reaction mechanism of the xanthine oxidase molybdopterin center with formamide.

The reaction is found to proceed via nucleophilic attack of a Mo(VI)-coordinated hydroxyl oxygen on substrate, with concomitant hydride transfer to the molybdenum sulfido ligand (X in Figure 1) to give a Mo(IV)·P species. Replacement of bound product with hydroxide from solvent yields $LMo^{IV}O(OH)(SH)$ (with L representing the enedithiolate ligand of the enzyme's molybdenum center) to complete the reaction.[8] The molybdenum center is initially in the +6 oxidation state, and carries a formal negative charge throughout the reaction.[7] Other formal charges, 0 and +1, were considered in preliminary studies with formamide and a related enzyme model and were found not to affect the qualitative features of the reaction model [Ilich and Hille, unpublished].

The electron-accepting Mo═S group plays a key role in the reaction, and indeed, the so-called "desulfo" form of the enzyme, in which the Mo═S group has been replaced by a second Mo═O group, is catalytically inert. Higher members of the VIA group of elements have been known to replace sulfur in proteins, and selenocysteine in particular is found in the active site of at least one molybdenum-containing enzyme.[9] Tellurium substitution has been achieved in other proteins,[10] but no tellurido congeners of any molybdenum- or tungsten-containing enzyme have been reported to date. The oxygen congener of xanthine oxidase can be obtained by removal of the sulfido ligand upon reaction with cyanide[11] and in CO dehydrogenase apparently represents the natural form of the active site.[12] Here we examine the electronic structures of both O and Te congeners of an active site and compare their properties with the native S-containing congener to establish the mechanistic determinants for reaction with formamide. The transition states (first-order saddle points) for the three congeners were calculated using quadratic search method, performed at the UMP2/LanL2DZ level of theory while selected structures of the interacting molecular pairs immediately preceding and succeeding the transition states were optimized at the UMP4(SDTQ) level of theory. Attempts to use hybrid Hartree−Fock/density functional theory methods, specifically the Becke three-parameter exchange and the Lee, Parr, and Young correlation functional, repeatedly failed to yield a transition state.

Transition-state structures for the O and Te congeners, along with that seen for the native S congener for comparison, are shown in Figure 2. All three yielded the same equatorial geometry with a nearly planar X−Mo−O(H)···C (substrate) parallelogram (the dihedral angle ranges from 1.1° in the native, sulfido form to 3.8° in the oxo form).[13] The substrate hydrogen is offset from the plane by 9° (sulfido) to 18° (oxo). As expected, the largest differences are found in the Mo−X bond lengths: 1.98 Å in the O congener to 2.38 Å in the S[8] and 2.59 Å in the Te. Interestingly, the extent of Mo−X bond elongation in the transition state follows the opposite trend: the Mo−Te bond extends by only 2%, to 2.65 Å in the transition state; the corresponding values for the S and O congeners are 4 and 9%, respectively. The O congener thus requires a significantly larger relative deformation in forming the transition state, and the energy barrier to the transition state is thus highest for the oxo form (and lowest for the tellurido form), Figure 3.

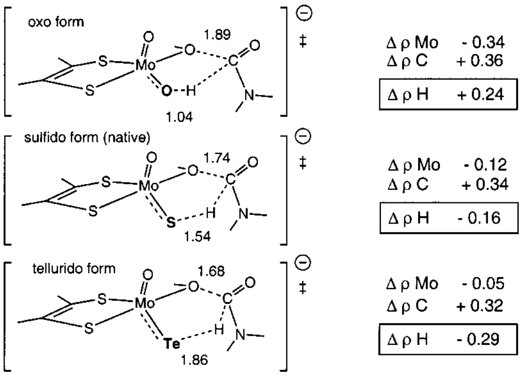

Figure 2. The geometries [Å] and natural electron density changes [q_e/bohr^3] (in comparison to reactants) for the three transitions states.

The differences in chemical properties of the three congeners lie in the details of the bond lengths and electron density distributions in the three transitions states. For example, the line of the nucleophilic attack of the molybdenum-coordinated hydroxide oxygen on the substrate carbon atom, 1.74 Å in the native, sulfido, and 1.68 Å in the tellurido form, is expected to increase to 1.89 Å in the oxo form. The elongation predicted for the oxo form corresponds to a 22−25% weaker Coulombic interaction between the Mo−OH oxygen and the carbonyl carbon of substrate and suggests an earlier, higher-energy transition state for the O congener relative to the S (or Te) congener. On the other hand, the large formamide C−H distance, 1.78 Å, and the short (Mo═O)- -H distance, 1.04 Å, in the Te congener suggest a late, lower-energy transition state. The origin of this difference can be understood by considering the change in electron density on the hydrogen being transferred from substrate to the molybdenum X-ligand: it is increased in the native, sulfido form (as well as in the tellurido form) but decreased in the case of the oxo Mo−dithiolene complex with formamide. This

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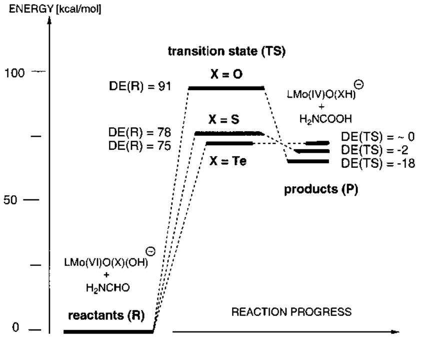

Figure 3. The approximate energy profile for the reaction of three Mo-enedithiolate congeners (oxo, sulfido, and tellurido) with formamide in vacuo. Transition-state energies are given relative to a pair of relaxed, infinitely separated formamide and Mo-enedithiolate anion; the energies of the “primary products” (hydrogen carbamate and nonhydrated molybdenum center) are given with respect to the corresponding transition-state energies.

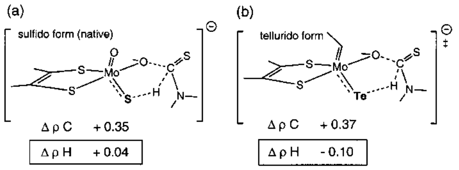

Figure 4. The changes in the natural electron densities (in comparison to reactants) on the thioformamide C−H centers in complex with sulfido Mo-enedithiolate (a) and in the transition state with the Te congener (b).

suggests that the O congener, while likely to engage in initial nucleophilic attack of the Mo−OH, is not suited to complete the subsequent hydride transfer. The loss of activity in the “desulfo” form of xanthine oxidase[11] corroborates this conclusion.

By contrast to the oxo congener, the transition state for the tellurido congener is similar to that for the native, sulfido form and accommodates an even greater electron density on the hydrogen being transferred from substrate. The data suggest that a tellurido form of the enzyme should be at least as effective catalytically as the native sulfido form. Catalytic differences between the S and Te congeners could be discerned in a reaction with a poor substrate, and our electronic structure calculations suggest thioformamide [Ilich and Hille, 1999, unpublished]. The sulfido congener and thioformamide are expected to form a weakly interacting molecular pair that oscillates around an intermediate geometry without reaching a true transition state. Analysis of the electron density of this complex shows a lower degree of the C═S orbital overlap and decreased polarization of the thioformamide C−H bond, Figure 4a, in comparison to the same bond in formamide, Figure 2. As a result, no negatively charged hydrogen species is transferred to the Mo(VI) complex and no hydroxylation of the substrate occurs; thioformamide is thus expected to inhibit native xanthine oxidase, Figure 4a. A qualitatively different picture emerges from the simulation of the reaction of thioformamide with the Te congener: a true transition state forms with increased (though slightly) electron density on the substrate hydrogen in comparison to the reactant complex, Figure 4b. The Te congener presumably induces sufficient polarization of the thioformamide C−H bond to result in effective hydride transfer and subsequent hydroxylation of the substrate.

Our results indicate that replacement of the native sulfido ligand in the active-site model with an oxo or tellurido group results in species with significantly different reactivities. Specifically, the transition state seen with the O congener has (1) reduced electron density on the hydrogen being transferred, (2) a shorter O−H bond, (2) a longer nascent O−C bond of product, and (4) a higher energy barrier. These factors combine to compromise reactivity in the “desulfo” form of the enzyme where S has been replaced by O.[5a,11] Our results suggest that a Te congener of xanthine oxidase should exhibit catalytic activity. In particular, the increase in electron density on the hydrogen being transferred to the Mo═Te group and the increased ability of tellurium to accept reducing equivalents due to its larger orbital set[14] provide a basis for increased reactivity, although the extended Mo═Te bond (in comparison to the Mo═S bond of native enzyme) and the slightly different geometry of the transition state in the case of the Te congener (Figure 2) may compromise reactivity toward substrates larger than formamide (e.g., hypoxanthine and xanthine). The tellurido congener may have lower activity toward purine and pteridine substrates for steric reasons, but greater activity toward a wide range of smaller aldehyde, ketone, and amide substrates.[15] Efforts to prepare the tellurido form of xanthine oxidase are presently under way to test this prediction.

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Note Added after ASAP: The version posted ASAP, 5/23/2002, was incorrect; the corrected version was posted 5/24/2002.

Supporting Information Available: Three tables of graphic and text material, containing the Hartree−Fock and MP2 energies and the Cartesian coordinates for the transition states of the oxo Mo-enedithiolate with formamide, sulfido Mo-enedithiolate with formamide, and tellurido Mo-enedithiolate with formamide (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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