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Oxoanionic or sulfur lone pair attack? The difference in reactivity of hydrogensulfite anion and neutral dimethylsulfite towards $[Bu_4N]_2[MoO_2\{S_2C_2(CN)_2\}_2]$ in the model reductive half reaction of sulfite oxidase

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pH dependent reactivity differences of dimethylsulfite towards the title complex 1 demonstrate the crucial need of oxo-anionic coordination of sulfite to the molybdenum centre of 1 in the model reductive half reaction of sulfite oxidase.

Sulfite oxidase catalyzes the physiologically vital oxidation of sulfite to sulfate.[1] It contains molybdenum associated with a molybdopterin dithiolene cofactor and a cytochrome *b*-type heme.[1,2] The two-electron oxidation of sulfite to sulfate occurs at the molybdenum site leading its reduction from Mo(VI) to Mo(IV) and the catalytic cycle is completed by two sequential one-electron oxidations of Mo(IV) to Mo(VI) *via* Mo(V) by cytochrome *c* through the cytochrome *b* site.[3]

The fundamental chemistry catalyzed by sulfite oxidase has been investigated using several model compounds possessing a $\{Mo^{VI}O_2\}$ moiety involving phosphines as model substrates.[4] $[Bu_4N]_2[MoO_2\{S_2C_2(CN)_2\}_2]$ **1** is shown to perform oxidation of HSO_3^- to HSO_4^- both in terms of saturation as well as anionic inhibition kinetics similar to the reductive half reaction of native sulfite oxidase.[5] The initial proposal of oxoanionic attack[5] of HSO_3^- on the Mo center of **1** has been questioned[6] based on the unique experimental results reported by Hille and Brody involving neutral dimethylsulfite as an alternate substrate of the native sulfite oxidase.[7] Nucleophilic attack on one of the Mo=O groups present in **1** by a lone pair of sulfur of HSO_3^-, similar to that of phosphorus in phosphines, have been suggested.[6] Hille and Brody have shown that the methylation of two oxoanions of sulfite reduces the affinity of dimethylsulfite for the active site of the native enzyme. Exhibition of saturation kinetics by dimethylsulfite implies the formation of a Michaelis complex which they interpreted in terms of possible electrostatic interaction between the active site and modified substrate.[7] It has also been reported that sulfite anion contributes 3.4 kcal mol^{-1}. towards the stablization of the E_{ox} S complex which is consistent with the direct coordination to Mo.† Direct oxoanionic coordination of sulfite to the Mo center has also been suggested by Rajagopalan and coworkers.[9] **1** has been shown to respond to saturation kinetics with typical anionic inhibition involving HSO_3^- as substrate.[5] In this communication we furnish proof for the importance of oxo-anionic coordination for such an enzymatic atom transfer reaction by presenting the pH dependent reactivity difference of dimethylsulfite towards **1** which is of relevance to its reaction with native protein.

In order to gain an insight into the reaction of **1** with reductants such as dimethylsulfite, PPh_3 or HSO_3^-; the reactivity of all these oxo-acceptors have separately been examined by following the absorbance change of **1** during the progress of the reaction. In the presence of an excess of dimethylsulfite, **1** in acetonitrile or in acetonitrile–water (pH, 5–7) did not show any change in its electronic spectrum suggesting the inability of dimethylsulfite to reduce **1** [Fig. 1(a)]. Interestingly, dimethylsulfite after standing at pH 8 for a few minutes was found to be capable to reduce **1**, the time-dependent progress of the reaction distinctly showed the formation of the reduced species, $[Bu_4N]_2[MoO\{S_2C_2(CN)_2\}_2]$ **2** [Fig. 1(b)].

The difference in reactivity of dimethylsulfite in the pH range 5–7 and 8 towards **1** was checked by its response to hydrolysis under similar conditions. Thus aqueous solutions of dimethylsulfite at different pH were tested with aqueous $BaCl_2$. In the pH range 5–7 there was no precipitation of $BaSO_3$ over a time period of hours but at pH 8 (and above) precipitation of $BaSO_3$ started to occur within minutes and its identity was confirmed by the standard $BaSO_4$ test.§ The reactions described above clearly demonstrate the inability of neutral dimethylsulfite to react with **1** suggesting the inability of the sulfur lone pair to initiate any redox reaction. Once the neutral dimethylsulfite is base hydrolysed (pH 8) to yield HSO_3^-, the reaction progresses smoothly [Fig. 1(b)].

Interestingly PPh_3 does reduce **1** to **2**; the time-dependent progress of the reaction is shown in Fig. 2.[6]

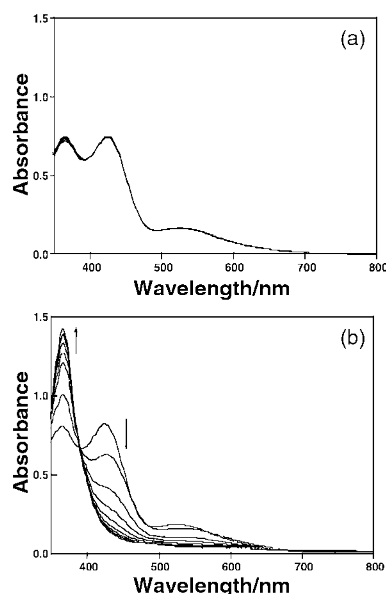

Fig. 1 (a) Spectral changes for the reaction between **1** (1×10^{-4} M) and dimethylsulfite (1.15×10^{-2} M) in acetonitrile. Spectra were taken for 150 min at intervals of 30 min. (b) Spectral changes for the reaction between **1** (1.075×10^{-4} M) and dimethylsulfite (3.4×10^{-2} M) at pH 8 in acetonitrile–water medium.‡ Spectra were taken for 14 min at intervals of 2 min.

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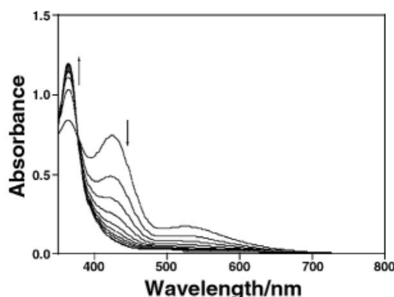

Fig. 2 Spectral changes for the reaction between **1** (1×10^{-4} M) and PPh_3 (5×10^{-3} M) in acetonitrile. Spectra were taken for 150 min at intervals of 15 min.¶

The difference in reactivity between HSO_3^- and PPh_3 should be noted in a sense that for all the reported reactions of model compounds including that of **1**, phosphines invariably respond to oxo abstraction by following second order kinetics (without the involvement of the Michaelis complex) whereas the unique reduction of **1** by HSO_3^- follows enzymatic kinetics (Michaelis complex) similar to that of the native sulfite oxidase.[5] Thus the reactivity of anionic HSO_3^- towards **1** is clearly dependent on the direct coordination of HSO_3^- to the Mo site as proposed earlier.[5] This also explains the observed competitive inhibiton by sulfate,[5] otherwise the lone pair attack of sulfur on the coordinated terminal oxo group and the direct binding of SO_4^{2-} to Mo would have resulted in mixed non-competitive inhibition. The heptacoordinated E_{ox} S type complex, {$[Bu_4N]_2[Mo(O)(O^-)\{S_2C_2(CN)_2\}_2\{O(OH)SO\}]$}, generated by the oxoanionic binding of HSO_3^- transforms the {$Mo(O)_2$} group to {$Mo^{VI}(O)(O^-)\{O(OH)SO\}$} moiety which in turn may lead to bidentate coordination of bisulfite to form {$Mo^{VI}(O)(\mu\text{-}O)_2(OH)S(O)$} for the product release step (Scheme 1). This reaction may thus be viewed as being similar to the reductive elimination reaction where the Mo(VI) containing heptacoordinated[11] E_{ox} S complex‖ changed to pentacoordinated Mo(IV) in **2** with the elimination of HSO_4^-.

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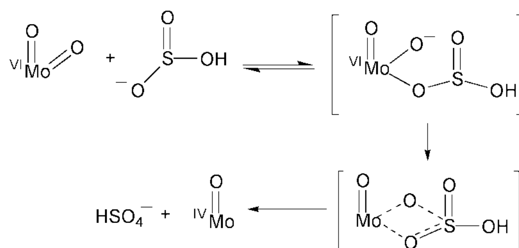

Scheme 1

Notes and references

† The possibility of direct binding of the oxoanion of HSO_3^- to Mo has not been considered in the calculation.[8]

‡ Aqueous dimethylsulfite solution was adjusted to pH 8 by sodium hydroxide and after 5 min its pH was lowered to ~6 by adding acetic acid. This was added to an acetonitrile solution of **1** to monitor the reaction.

§ Aqueous dimethylsulfite in the pH range 5–7 (adjusted by adding acetic acid) does not show any precipitation on addition of $BaCl_2$ over a time scale of hours but at pH 8 and above, precipitation started to occur within minutes. This precipitate readily dissolved in dilute HCl and on addition of Br_2 (aq) to this clear solution insoluble $BaSO_4$ was precipitated. At this point it is important to mention that the rate of hydrolysis of dimethylsulfite in water is 10^{-5} s^{-1} as quoted by Brody and Hille[7] from the work of Guthrie.[10] A careful inspection of the data mentioned in Guthrie's paper reveals that this actually refers to the hydrolysis of the methyl ester of methanesulfonic acid ($CH_3S(O)_2OCH_3$) for which the corresponding acid is CH_3SO_3H (methanesulfonic acid) with $pK_a = -1.92$. For dimethylsulfite ($CH_3OS(O)OCH_3$), the corresponding acid is sulfurous acid (HOS(O)OH) with pK_1 and pK_2 values = +1.90 and +7.25, respectively.

¶ A similar reaction using acetonitrile–water medium showed faster conversion of **1** to **2** compared to that in pure acetonitrile.

‖ Mono-oxoheptacoordinated Mo(VI) or dithiolene coordinated mono-oxoheptacoordinated W(VI) complexes in aqueous media have precedence in the literature.[12]

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