Inorganica Chimica Acta, 193 (1992) 125–128

Inorganica
Chimica Acta
LETTER

Acceleration by Fe(II) of thiomolybdate formation from aqueous molybdate and sulfide. A simplified synthesis of $[\mathrm{Fe(MoS_4)_2}]^{3-}$

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(Received November 12, 1991; revised February 7, 1992)

An Mo/Fe/S cluster is known to exist at the active site of the nitrogenase MoFe protein, which is isolated from organisms where molybdate is the only known biological uptake and transport form of molybdenum [1, 2]. Mo in the MoFe protein apparently contains up to three sulfido ligands in its coordination sphere [3], but the pathway of molybdate incorporation into the active site cluster is unknown. We have recently shown that thiols dramatically accelerate formation of $[\mathrm{MoO}_{4-x}\mathrm{S}_x]^{2-}$ ($x=1$–4, hereafter referred to as $\mathrm{S_1}$–$\mathrm{S_4}$)** from aqueous molybdate and sulfide at basic pH [4]. However, despite numerous reports of mixed-metal clusters resulting from reactions of divalent heterometal ions, M(II), with $\mathrm{S_2}$, $\mathrm{S_3}$ or $\mathrm{S_4}$ [5, 6], the effects of M(II) on the reaction of aqueous molybdate and sulfide have never been clearly distinguished from the effects of $\mathrm{H^+}$. In the present study we show that Fe(II) in the presence of thiols can further accelerate formation of $\mathrm{S_1}$–$\mathrm{S_4}$ from aqueous molybdate and sulfide at pH 9. This acceleration provides a simpler route to the previously characterized 'linear' trinuclear cluster, $[\mathrm{Fe(MoS_4)_2}]^{3-}$ ($\mathrm{FS_4}$) [7, 8].

Experimental

Electronic absorption spectra were obtained at room temperature using 0.5-mm pathlength cylindrical quartz cuvettes fitted with rubber septa, and the samples therein were maintained under an Ar atmosphere. Concentrations of $\mathrm{S_1}$–$\mathrm{S_4}$ and $\mathrm{FS_4}$ in the reaction mixtures were calculated from absorption spectra using either our own or published extinction coefficients [4, 9] and a set of simultaneous equations (see 'Supplementary material').

Solvents and reagents were of the highest purity commercially available and were used without further purification. All manipulations were carried out at room temperature under a purified Ar atmosphere in either Schlenk-type glassware or septum-capped vials attached to a vacuum manifold. Stainless steel tubing and gastight syringes were used to transfer reagents and samples. Aqueous solutions were prepared from distilled, deionized water. Unless otherwise specified the buffer was 0.3 M TAPS/KOH pH 9.05. Elemental analyses were performed by either the Department of Inorganic and Metallo-Organic Chemistry, University of Milan or by the Pascher Mikroanalitisches Laaboratorium, Bonn, FRG. $\mathrm{(NH_4)_2[MoO_2S_2]}$ was prepared by a previously described method [9]. Buffered stock solutions of 2–3 M sodium sulfide were prepared and standardized as described previously [4].

$\mathrm{(Et_4N)_3[Fe(MoS_4)_2]\cdot H_2O}$

The following compounds were dissolved successively in 250 ml of buffer: 1.21 g (5.00 mmol) sodium molybdate; 2.85 ml (200 mmol) 2-mercaptoethanol; 1.96 g (5.00 mmol) ferrous ammonium sulfate hexahydrate. After at least 30 min of stirring this mixture, sodium sulfide was added from a stock solution to give a final sulfide concentration of 200 mM. After 2–3 h of stirring, the reaction mixture was filtered through a Celite pad and 4.62 g (12.5 mmol) of $\mathrm{Bu_4NI}$ was added to the filtrate. (Tests had shown that $\mathrm{Et_4NCl}$ failed to precipitate the desired complex.) After overnight incubation at 0 °C, the resulting fine black precipitate was collected by filtration onto a Celite pad, washed successively with water, ethanol and ethyl ether, then dissolved in 50 ml of acetonitrile. The resulting solution was made 0.5 M in $\mathrm{Et_4NCl}$ and 50 ml of ethyl ether was added. After overnight incubation at -20 °C, the resulting purplish black solid was collected by filtration and washed successively with ethyl ether, ethanol, water and ethyl ether and then dried *in vacuo*. Exchange of quaternary ammonium salt was quantitative as judged from ^{1}H NMR spectra of the product, which also indicated the absence of any thiol. The elemental analysis was consistent with one water of hydration. *Anal.* Calc. for

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**Abbreviations used: $\mathrm{S_1}$, $[\mathrm{MoO_3S}]^{2-}$; $\mathrm{S_2}$, $[\mathrm{MoO_2S_2}]^{2-}$; $\mathrm{S_3}$, $[\mathrm{MoOS_3}]^{3-}$; $\mathrm{S_4}$, $[\mathrm{MoS_4}]^{2-}$; $\mathrm{FS_4}$, $[\mathrm{Fe(MoS_4)_2}]^{3-}$; TAPS, sodium 3-[[tris(hydroxymethyl)methyl]amino]propane-sulfonate; 2-ME, 2-mercaptoethanol; $\mathrm{Et_4N}$, tetraethylammonium cation; $\mathrm{Bu_4N}$, tetra-n-butylammonium cation.

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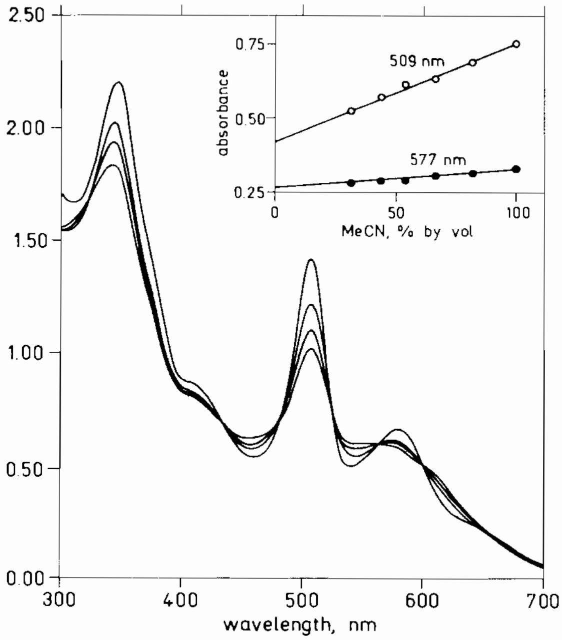

Fig. 1. Solvent-induced modifications in the electronic absorption spectrum of $(Et_4N)_3[Fe(MoS_4)_2]\cdot H_2O$. All operations were performed anaerobically. The solid compound was dissolved in acetonitrile and, after recording a spectrum, the solution was progressively diluted with water. From top to bottom at 509 nm the spectra correspond to 100, 66, 44 and 31 vol.% acetonitrile. Spectra have been corrected for dilution. Inset shows the variation in absorbance at two selected wavelengths as a function of volume percentage of acetonitrile.

$C_{24}H_{62}N_3FeMo_2OS_8$: C, 31.57; H, 6.84; N, 4.60. Found: C, 31.44; H, 6.41; N, 4.55%. Yield 2.07 g (45%).

Results and discussion

The method described here for the synthesis of $(Et_4N)_3[Fe(MoS_4)_2]\cdot H_2O$ uses simpler reagents and results in higher yields than previously reported for the corresponding anhydrous compound [7, 8]. The electronic absorption (Fig. 1) and EPR spectra (see 'Supplementary material') of $(Et_4N)_3[Fe(MoS_4)_2]\cdot H_2O$ closely resemble those published for the anhydrous compound, which were reported to be highly solvent dependent [7, 8]. The spectra in Fig. 1 were obtained in acetonitrile/water mixtures because the compound was found to be sparingly soluble in water. The published extinction coefficients in acetonitrile [7, 8] were extrapolated to pure water, as shown in the inset to Fig. 1, for use in the studies described below*.

We have previously shown that, in solutions of aqueous molybdate, sulfide and thiol at pH 9, the major molybdenum species after a few minutes of reaction are S_3 and S_4, whose concentrations can be conveniently monitored and quantitated spectrophotometrically [4]. Figure 2 shows the effects of Fe(II) in such mixtures. The buildup of S_4 relative to S_3, indicated by the upward and downward arrows at 470 and 395 nm, respectively, occurred increasingly rapidly as the Fe(II) concentration was increased. Figure 2 also shows that, as the Fe(II) concentration was increased, additional features developed in the absorption spectra at ≈ 580, 500 and 340 nm. Comparison with the spectra in Fig. 1 shows that these features are due to FS_4. The synthesis of this complex described in 'Experimental' used essentially the same conditions as those for Fig. 2, except with equimolar iron and molybdenum. From absorption spectral time courses of reactions such as those shown in Fig. 2 and in our previous paper [4], the time courses for formation of S_3, S_4 and FS_4 were calculated. Figure 3 shows the results of such calculations using sets of spectral data obtained either in the presence or absence of iron. The concentration time courses show that in the absence of iron nearly all of the starting molybdate has been converted to S_3 after 30 min, and the remaining reaction involves only $S_3 \rightarrow S_4$ conversion. In the presence of 0.5 mm Fe(II) the same statement applies after

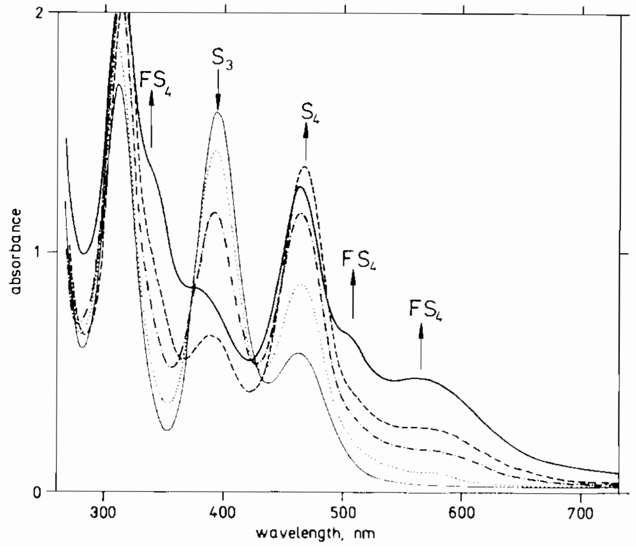

Fig. 2. Absorption spectra of buffered (0.3 M TAPS pH 9.0) aqueous solutions containing 4 mM molybdate, 200 mM 2-mercaptoethanol, 100 mM sodium sulfide and the following millimolar concentrations of ferrous ammonium sulfate (from bottom to top at 550 nm): 0, 0.2, 0.5, 1 and 2. Spectra were recorded 180 min after addition of sulfide to an otherwise complete reaction mixture. Wavelengths for absorption maxima of $[MoOS_3]^{2-}$ (S_3), $[MoS_4]^{2-}$ (S_4) and $[Fe(MoS_4)_2]^{3-}$ (FS_4) are indicated by arrows. Direction of the arrow indicates increase (upward) or decrease (downward) in concentration with added iron.

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*Significant hydrolytic decomposition of FS_4 is inconsistent with the several isosbestic points evident in Fig. 1.

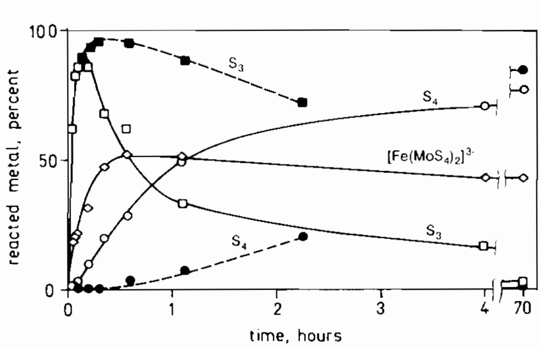

Fig. 3. Concentration time courses for formation of $[MoOS_3]^{2-}$ (S_3), $[MoS_4]^{2-}$ (S_4) and $[Fe(MoS_4)_2]^{3-}$ (FS_4). Spectra of buffered (0.3 M TAPS pH 9.0) aqueous solutions containing 4 mM sodium molybdate and 400 mM 2-mercaptoethanol in the absence (full symbols) or presence (open symbols) of 0.5 mM ferrous ammonium sulfate were recorded at the times indicated by the data points after addition of sodium sulfide to a concentration of 100 mM. Concentrations of S_3 (squares) and S_4 (circles) were calculated from absorbance data and a set of simultaneous equations. The concentrations of S_3 and S_4 are plotted as percent of total molybdenum concentration, whereas the concentration of FS_4 is plotted as percent of total iron concentration.

about 10 min. Similarly, after 1 h in the absence of iron, less than 10% of the molybdate has been converted to S_4, whereas, in the presence of 0.5 mM Fe(II), >50% of the molybdate has been converted to S_4. Note that FS_4 is plotted as percent of total Fe (0.5 mM) and never accumulates to more than about 6% of the total Mo (4 mM) under the conditions of Fig. 3.

Delineation of the role of Fe(II) in accelerating the reaction of aqueous molybdate with sulfide is complicated by two factors. First, excess thiol must be present in order to avoid precipitation of iron sulfides. In the presence of the large excess of 2-ME at pH 9 used here, the majority of Fe(II) will be complexed as $[Fe(SCH_2CH_2OH)_4]^{2-}$ [10, 11], and, therefore, an iron–thiolate complex could be the reactive species. Second, in the presence of excess thiol and sulfide, the product distribution is highly dependent on the Fe(II)/molybdate mole ratio. When this mole ratio is in the range of 0–0.5, the major product is S_4. At equimolar Fe(II) and molybdate, the major product is FS_4. At Fe(II)/molybdate mole ratios >2, the major product is the tetrairon 'cubane' cluster $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ [12]. Furthermore, starting with preformed S_4 and excess Fe(II) and 2-ME at pH 9, the 'double-cubane' cluster, $[Mo_2Fe_6S_8(SCH_2CH_2OH)_9]^{3-}$ forms rapidly and in high yield [12]. Thus, the presence or absence of thiol, the Fe/Mo mole ratio and the form of $[MoO_{4-x}S_x]^{2-}$ presented to iron all greatly affect the product distributions in aqueous Mo/Fe/S chemistry. These effects must be taken into account when considering pathways of biological Mo/Fe/S cluster assembly.

A previous study conducted at pH 12 *without* thiol showed that Fe(II) accelerates the hydrolysis of preformed S_2, S_3 and S_4 and that the hydrolysis products could be trapped as the 'linear' tetranuclear complex, $[(O_2Mo(\mu\text{-}S)_2Fe(\mu\text{-}S)_2Fe(\mu\text{-}S)_2MoO_2]^{4-}$ [13]. We have verified spectrophotometrically that this same product is obtained in high yield at pH 9 upon mixing ferrous ammonium sulfate and $(NH_4)_2[MoO_2S_2]$ in a 1:5 Fe(II):S_2 mole ration [14]. Prior to its incorporation into the 'linear' tetranuclear complex, Fe(II) may play the same role as does H^+ in accelerating hydrolyses of S_2–S_4 [15]. Thus, under the appropriate conditions, Fe(II) can be demonstrated to accelerate both formation and hydrolysis of S_2–S_4. However, complexation by sulfide, S_2 or S_4 competes with and to a large extent masks the catalytic role of iron.

Supplementary material

EPR spectra of $(Et_4N)_3[Fe(MoS_4)_2]\cdot H_2O$ and a listing of extinction coefficients and sets of simultaneous equations used to calculate concentrations of $[MoO_{4-x}S_x]^{2-}$ ($x=1$–4) and $[Fe(MoS_4)_2]^{3-}$ from absorbance data (5 pages) may be obtained upon request from author D.M.K.

Acknowledgement

This research was supported by a NATO Collaborative Research Grant (D.M.K.) and M.U.R.S.T., Rome, Italy, National Research Program 'Enzymatic Biotechnologies' (F.B.).

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