Published in final edited form as:

Biochem Soc Trans. 2008 December ; 36(Pt 6): 1129–1133. doi:10.1042/BST0361129.

Structures and reaction pathways of the molybdenum centres of sulfite-oxidizing enzymes by pulsed EPR spectroscopy

John H. Enemark¹, Andrei V. Astashkin, and Arnold M. Raitsimring

Department of Chemistry, University of Arizona, 1306 E. University Blvd, Tucson, AZ 85721-0041, U.S.A.

Abstract

SOEs (sulfite-oxidizing enzymes) are physiologically vital and occur in all forms of life. During the catalytic cycle, the five-co-ordinate square pyramidal oxo-molybdenum active site passes through the Mo(V) state, and intimate details of the structure can be obtained from variable frequency pulsed EPR spectroscopy through the hyperfine and nuclear quadrupole interactions of nearby magnetic nuclei. By employing variable spectrometer operational frequencies, it is possible to optimize the measurement conditions for difficult quadrupolar nuclei of interest (e.g. ¹⁷O, ³³S, ³⁵Cl and ³⁷Cl) and to simplify the interpretation of the spectra. Isotopically labelled model Mo(V) compounds provide further insight into the electronic and geometric structures and chemical reactions of the enzymes. Recently, *blocked* forms of SOEs having co-ordinated sulfate, the reaction product, were detected using ³³S (*I* = 3/2) labelling. This blocking of product release is a possible contributor to fatal human sulfite oxidase deficiency in young children.

Keywords

molybdenum centre; pulsed EPR spectroscopy; sulfite-oxidizing enzyme (SOE)

Introduction

SOEs (sulfite-oxidizing enzymes) catalyse the oxidation of sulfite to sulfate as the terminal step in the metabolism of sulfur-containing amino acids, and they are physiologically vital for all forms of life. The crystal structures of SOEs [1-3] from vertebrates, plants and bacteria show nearly identical, approximately square-pyramidal, five-coordinate geometries of their molybdenum centres in the fully oxidized Mo(VI) state, with axial and equatorial oxoligands, and three equatorial sulfur ligands (two from the pyranopterindithiolate cofactor and one from a cysteinyl side chain). In the first step of the proposed catalytic cycle [4] (Figure 1, **A**→**B**), the equatorial oxo ligand reacts with sulfite to form an enzyme-product complex, in which sulfate is co-ordinated to Mo(IV). Replacement of the sulfate ligand by water or hydroxide (Figure 1, **B**→**C**) and subsequent one-electron oxidations [Mo(IV/V/VI)] and concomitant deprotonations (Figure 1, **C**→**D**→**A**) return the molybdenum centre to the fully oxidized resting state. The paramagnetic Mo(V) state (Figure 1, **D**) is relatively stable, and EPR spectroscopy has provided a convenient method of directly investigating the structures of various Mo(V) species as a function of pH, anions in the medium, organism, method of generation of the Mo (V) state and enzyme mutations.

¹To whom correspondence should be addressed (email jenemark@u.arizona.edu)..

Historically, Bray and co-workers [5-7] first classified vertebrate SOEs into three forms based upon the appearance of their CW (continuous wave) EPR spectra: the high-pH form (*hpH*), generated at pH ≥ 9 ; the low-pH form (*lpH*) generated at pH ≤ 7.5 (depending on the concentration of Cl⁻ in the buffer); and the phosphate-inhibited form (*Pi*) that was observed at low pH in the presence of phosphate. The CW EPR spectra provided a convenient phenomenological classification of SOEs, but the spectral resolution was too low to define the structural details of the Mo(V) centres generated under different conditions or, in some cases (e.g. for the *hpH* and *Pi* SOE), even to help identify the nature of the exchangeable equatorial ligand. Detailed structural investigations of the Mo(V) centres of SOEs require higher-resolution techniques, and ESEEM (electron spin-echo envelope modulation) and ENDOR (electron nuclear double resonance) spectroscopies, supplemented by the extensive isotopic labelling and site-directed mutagenesis, have become the methods of choice for such investigations.

In recent years, pulsed EPR experiments have revealed a wealth of information about the structural details for the 'original' three forms of SOE introduced by Bray [8]. Additional investigations have demonstrated that, under certain conditions, reduction with sulfite leads to formation of a Mo(V) centre in which the reaction product (sulfate) remains co-ordinated. This new form was called *blocked* (Figure 1) because it was hypothesized that the sulfate ligand was prevented from leaving the active site (i.e. blocked) by some steric hindrance [9].

The *blocked* form (Figure 1) was first identified for plant SOE from *Arabidopsis thaliana* (At-SO) by observing the ³³S-ESEEM in samples prepared with ³³S-labelled sulfite at low pH [9]. A similar method was used later to detect *blocked* forms for some of the mutants of human sulfite oxidase (hSO) [10]. In addition, the pulsed EPR results for the R160Q mutant of hSO [10] supported the suggestion that, in some of these *blocked* forms, the Mo(V) centre can be six-co-ordinate, with an additional (axial) ligand *trans* to the oxo ligand [11].

Within the EPR spectroscopic framework, the structural information about the SOEs is obtained from the hyperfine interaction (*hfi*) and nuclear quadrupole interaction (*nqi*) parameters of magnetic nuclei in the vicinity of the Mo(V) centre. The complexity of estimating the *hfi* and *nqi* parameters depends upon the type of magnetic nucleus. For example, the detection of ¹H and ²H and analysis of their spectra are rather straightforward. On the other hand, the pulsed EPR investigations of ¹⁷O, ³³S, ³⁵Cl and ³⁷Cl are currently in an embryonic stage, and many results are from our laboratory. In an Mo(V) site, these quadrupolar nuclei often have a combination of spectroscopic parameters that is unfavourable for easy detection and/or spectral interpretation. To enable their successful investigation, the level of theory should allow qualitative estimates of the *hfi* and *nqi* parameters to be obtained directly from the spectra. For detecting these quadrupolar nuclei, it is of a paramount importance that the experiments be performed at appropriate operational microwave frequencies, ν_{mw} , specially selected to optimize the detection and/or the analysis of the spectra for the nucleus of interest. Below we present some recent applications of variable-frequency ESEEM spectroscopy to the study of such difficult nuclei in the vicinity of the Mo(V) centres of SOEs.

Axial oxo-¹⁷O ligand

There are two oxygen ligands at the Mo(V) centre (see **D** in Figure 1), one of which is the oxo ligand, and the other one is the oxygen of an equatorial OH ligand. The main oxygen isotope (¹⁶O, 99.76%) has spin $I = 0$, and, to be observable by EPR, oxygen has to be enriched in ¹⁷O ($I = 5/2$). Early CW EPR experiments in ¹⁷O-enriched water revealed the hyperfine splittings as large as ~35 MHz that were assigned to the equatorial OH ligand [6,12]. More recent pulsed EPR investigations in our laboratory have shown that the axial oxo ligand also exchanges in H₂¹⁷O [13]. The *hfi* of the oxo-¹⁷O was found to be substantially smaller, which is why it was

not resolved by CW EPR. Importantly, the pulsed techniques also allowed determination of the nqi , which cannot, in principle, be measured directly by CW EPR.

The simple theory behind these measurements is as follows. When the hfi and nqi are weak compared with the nuclear Zeeman interaction, the frequencies of the nuclear transitions between the spin projections m_I and $m_I + 1$ ($m_I \equiv \langle I_Z \rangle$) within the α and β electron spin manifolds [14,15] are:

$$\nu_{\alpha\beta} \approx \nu_I \pm \frac{1}{2} (a_{iso} + T_{ZZ}) + \frac{T_{ZX}^2 + T_{ZY}^2}{8\nu_I} + \tilde{Q} (2m_I + 1)$$

where ν_I is the nuclear Zeeman frequency, a_{iso} is the isotropic hfi constant, T_{ij} are the components of the anisotropic hfi tensor and $\tilde{Q} \propto e^2 Qq/h$ is the nqi -related parameter. The first-order hfi term, $a_{iso} + T_{ZZ}$, can be measured as $\nu_\alpha(m_I) - \nu_\beta(m_I)$. Although the nqi splits each of the fundamental frequencies (ν_α and ν_β) into $2I$ different components, these splittings are usually impossible to detect in one-dimensional experiments because of the broadening of the fundamental lines induced by the anisotropic hfi . Fortunately, however, in addition to the fundamental lines, the spectra obtained by some of the ESEEM techniques also contain a sum combination line at the frequency $\nu_\sigma = \nu_\alpha + \nu_\beta$. For a nucleus of spin I , in the limit of weak hfi and nqi , the sum combination line splits into a multiplet of $2I$ lines with the frequencies:

$$\nu_\sigma(m_I) \approx 2\nu_I + \frac{T_{ZX}^2 + T_{ZY}^2}{4\nu_I} + 2\tilde{Q} (2m_I + 1)$$

The hfi term causes the second-order frequency shifts that are useful for estimating the anisotropic hfi , but may be detrimental for the nqi estimates. However, it can be made arbitrarily small by increasing ν_I through an increase in the resonance magnetic field, B_0 , concomitant to the increase in the microwave operational frequency. The splitting between any two neighbouring ν_σ lines is determined only by the nqi :

$$\Delta\nu_\sigma = |\nu_\sigma(m_I + 1) - \nu_\sigma(m_I)| \approx 4\tilde{Q}$$

Examples of spectra of axial ^{17}O in model Mo(V) complexes and SOEs obtained from various organisms and mutants, detected by the τ -integrated four-pulse ESEEM technique, are shown in Figure 2. The ^{17}O hfi obtained from the positions of the fundamental lines is 5-6 MHz for all varieties of SOE and model systems examined. Evidently, this ^{17}O hfi value is characteristic of an oxo group ($\text{Mo} \equiv \text{O}$ entity). The nuclear quadrupole coupling constant, $e^2 Qq/h$, determined from the splitting between the components of the ν_σ quintet was under 1.5 MHz for a five-co-ordinate model compound (Figure 2, trace 1) and for all SOEs investigated (Figure 2, traces 3-5) except the R160Q mutant of hSO (trace 6, Figure 2). The similar values of $e^2 Qq/h$ imply that no drastic changes in the geometry of the Mo(V) centre occur among organisms and with varying pH.

In the R160Q mutant of hSO, the mutation of the active-site Arg¹⁶⁰ [located on the side of the Mo(V) centre opposite to the oxo-ligand] to a glutamine residue has resulted in the dramatic increase of the oxo- ^{17}O $e^2 Qq/h$ to approx. 5 MHz (Figure 2, trace 6) [10]. We hypothesized that this increase in the nqi could result from co-ordination of the active-site Glu¹⁶⁰ to the Mo(V) centre *trans* to the oxo-ligand [10]. This hypothesis is in line with the earlier EXAFS results [11], and is supported by the observation of significant increase in the oxo- ^{17}O nqi in a model six-co-ordinate Mo(V) complex (Figure 2, trace 2) as compared with the five-coordinate one (~3 MHz compared with 1.45 MHz) [10,14]. From these results, it appears that the oxo- ^{17}O nqi provides a simple and dependable means to follow the co-ordination changes of the Mo(V) site of SOEs.

Detection of a nearby Cl⁻ in *lpH* SOE

On the basis of the pH-dependence of the CW EPR of vertebrate SOEs in the presence of different concentrations of Cl⁻, Bray et al. [7] proposed that Cl⁻ should be an integral part of the Mo(V) active centre in the *lpH* SOE. However, they did not succeed in confirming its existence directly. Recently, the quest to find this form was continued [16], but, despite considerable effort, these recent CW EPR experiments still do not unambiguously demonstrate coordination of Cl⁻ to Mo(V).

The way to clearly prove co-ordination of Cl⁻ to Mo(V) is by ESEEM spectroscopy. Indeed, the experiments on the *lpH* chicken SO (sulfite oxidase) with the natural abundance of the Cl isotopes and on samples enriched in ³⁵Cl and ³⁷Cl (both $I = 3/2$) have shown unequivocally the presence of Cl⁻ in vicinity of the Mo(V) centre. From the *hfi* and *nqi* parameters ($a_{\text{iso}} \sim 4\text{--}5$ MHz; $T_{\perp} \sim 0.2$ MHz; $e^2Qq/h \sim 3\text{--}4$ MHz for ³⁵Cl), it is likely that this Cl⁻ is weakly co-ordinated to the Mo(V) centre. From comparison with the model oxomolybdenum complexes with equatorial chloro ligands [17] and from the available general information on the structure of the Mo(V) centres of SOEs, it is clear that the co-ordination position of this weak chloro ligand should be axial, *trans* to the oxo ligand [18].

No ³⁵Cl/³⁷Cl ESEEM was detected for *hpH* SOE or for the *blocked* forms of SOE. On the other hand, *lpH* SOE prepared without added Cl⁻ still shows Cl ESEEM with the same amplitude as *lpH* samples containing ~ 100 mM Cl⁻. These ESEEM results suggest a Cl⁻-binding site of relatively high affinity. Cl⁻ may play a physiological role in fine tuning the pK_a of the OH ligand of the Mo(V) centre, which is observed to shift by $\sim 1\text{--}1.3$ pK_a units for *lpH* SOE with added Cl⁻ [7,16]. Using the analysis of the effect of a unit charge on the pK_a of a titratable group [19,20], gives an estimate of $\sim 4\text{--}4.5 \text{ \AA}$ ($1 \text{ \AA} = 0.1 \text{ nm}$) for the distance from the Cl⁻ ion to the OH ligand.

Using the ³³S labelling to identify the *blocked* forms

In contrast with vertebrate SOEs, the Mo(V) centre of At-SO did not reveal the presence of nearby exchangeable protons upon reduction with sulfite at low pH [21]. Therefore we proposed that the At-SO protein at low pH adopts a 'closed' conformation that forces the sulfate to remain co-ordinated to Mo(V) (see Figure 1), and the corresponding form of SOE was called *blocked*. To verify this hypothesis, At-SO was reduced with sulfite labelled with ³³S ($I = 3/2$). As an example, in Figure 3(a), the field-integrated primary ESEEM spectrum of [³³S]At-SO is compared with that obtained with the natural abundance of sulfur isotopes (94.93% ³²S, $I = 0$). Figure 3(b) shows the HYSCORE (hyperfine sublevel correlation) spectrum for [³³S]At-SO obtained at the low-field (*gz*) turning point of the EPR spectrum. The measurements were performed at $\nu_{\text{mw}} \sim 29$ GHz which was estimated to be about the optimum microwave frequency for observing the co-ordinated sulfate [9].

The spectra of Figure 3 unambiguously demonstrate the co-ordination of sulfate to Mo(V). However, the evaluation of the spectroscopic parameters from these spectra was not straightforward because the ³³S *nqi* was much stronger than the *hfi* and the Zeeman interaction. Such a case has been little analysed in ESEEM spectroscopy. To overcome this problem, we derived analytical expressions which allowed us to qualitatively interpret the ESEEM spectra and to estimate the interaction parameters from them [9,10]. In the present paper, we show some simplified results that are useful for qualitative understanding of the primary ESEEM and HYSCORE spectra.

A strong *nqi* of ³³S separates its nuclear states into two degenerate pairs (doublets) with $m_I = \pm 1/2$ and $m_I = \pm 3/2$. The degeneracy is then removed by the nuclear Zeeman and hyperfine interactions. In the two-pulse ESEEM spectrum of Figure 3(a), the feature of interest is the

peak at ~18 MHz that belongs to one of the inter-doublet transitions ($| \pm 1/2 \rangle \leftrightarrow | \pm 3/2 \rangle$) of ^{33}S within the electron spin manifold where the Zeeman and hyperfine interactions approximately cancel each other. The frequency of this inter-doublet transition is:

$$\nu_{\text{id}} \sim e^2 Qq/2h$$

and we can easily estimate the quadrupole coupling constant as $e^2 Qq/h \approx 36$ MHz.

In the HYSCORE spectrum of Figure 3(b), the major off-diagonal cross-peaks at (3,11) MHz belong to $|1/2\rangle \leftrightarrow |-1/2\rangle$ transitions of ^{33}S . The frequencies of these transitions, ν_α and ν_β , are equal to:

$$\nu_{\alpha\beta} \approx c_{\text{hq}} (\nu_1 \pm A/2)$$

where c_{hq} is a numerical factor that depends on the orientation of the magnetic field vector, $\mathbf{B}_0$, relative to the nqi principal axes frame. The main qualitative difference with the $I=1/2$ system is that the pairs of HYSCORE cross-peaks are symmetrical with respect to $\nu_c = (\nu_\alpha + \nu_\beta)/2$ instead of ν_1 .

Using these results, one can estimate the hfi constant as $A = 2\nu_1 \cdot (\nu_\beta - \nu_\alpha)/(\nu_\beta + \nu_\alpha)$. This estimate can then be refined by using numerical simulations of the HYSCORE spectra. Using this approach for $[^{33}\text{S}]\text{At-SO}$ has resulted in $a_{\text{iso}} = 3.3$ MHz; the anisotropic hfi tensor in the principal axes system $(T_{11}, T_{22}, T_{33}) = (1.3, 1.5, -2.8)$ MHz; $e^2 Qq/h = 40$ MHz and the nqi asymmetry parameter was found to be close to zero, $\eta < 0.3$ (near-axial nqi tensor).

Further investigations have shown that the *blocked* form is not unique to At-SO: such forms have also been found for the R160Q and Y343F mutants of hSO [10], and recently, for the R55Q mutant of bacterial succinate dehydrogenase (analogous to R160Q hSO) (A.V. Atashkin, T. Rapson, U. Kappler and J.H. Enemark, unpublished work). For R160Q, the *blocked* form was present in significant quantities at physiological pH. Since this form represents a dead end in the catalytic cycle of Figure 1, this form may be a contributing factor in the lethality of the R160Q mutation in children.

Conclusions

The results presented above demonstrate the potential of high-resolution pulsed EPR techniques, particularly ESEEM spectroscopy of difficult quadrupolar nuclei, for structural investigations. The most important aspects of such investigations are the ability to freely adjust the microwave operational frequency to the needs of the particular spin system (in order to optimize the observation conditions for a particular magnetic nucleus and/or facilitate the interpretation of the ESEEM spectra) and selective isotopic labelling to make the nucleus of interest observable by ESEEM (e.g. ^{17}O compared with ^{16}O) or simply modify its spectroscopic parameters (e.g. ^{35}Cl compared with ^{37}Cl). The results presented here demonstrate applications to the Mo(V) centre of SOEs, but the same methods can be used for paramagnetic active sites of other types of metalloenzymes.

Acknowledgements

We gratefully acknowledge support for this research by the NIH (National Institutes of Health) (GM-37773 to J.H.E.) and by grants from the NSF (National Science Foundation) (DBI-0139459, DBI-9604939, BIR-9224431) and the NIH (S10RR020959) for development of the pulsed EPR facility. We thank Dr K. Johnson-Winters and Dr E.L. Klein for helpful discussions.

Abbreviations used

At-SO, *Arabidopsis thaliana* sulfite oxidase

CW, continuous wave
 ESEEM, electron spin-echo envelope modulation
 hfi, hyperfine interaction
 hpH, high-pH form
 hSO, human sulfite oxidase
 HYSCORE, hyperfine sublevel correlation
 lpH, low-pH form
 nqi, nuclear quadrupole interaction
 Pi, phosphate-inhibited form
 SO, sulfite oxidase
 SOE, sulfite-oxidizing enzyme

References

1. Kisker C, Schindelin H, Pacheco A, Wehbi W, Garrett RM, Rajagopalan KV, Enemark JH, Rees DC. Molecular basis of sulfite oxidase deficiency from the structure of sulfite oxidase. *Cell* 1997;91:973–983. [PubMed: 9428520]
2. Schrader N, Fischer K, Theis K, Mendel RR, Schwarz G, Kisker C. The crystal structure of plant sulfite oxidase provides insights into sulfite oxidation in plants and animals. *Structure* 2003;11:1251–1263. [PubMed: 14527393]
3. Kappler U, Bailey S. Molecular basis of intramolecular electron transfer in sulfite-oxidizing enzymes is revealed by high resolution structure of a heterodimeric complex of the catalytic molybdopterin subunit and a c-type cytochrome subunit. *J. Biol. Chem* 2005;280:24999–25007. [PubMed: 15863498]
4. Hille R. The mononuclear molybdenum enzymes. *Chem. Rev* 1996;96:2757–2816. [PubMed: 11848841]
5. Lamy MT, Gutteridge S, Bray RC. Electron paramagnetic resonance parameters of molybdenum(V) in sulphite oxidase from chicken liver. *Biochem. J* 1980;185:397–403. [PubMed: 6249254]
6. Gutteridge S, Lamy MT, Bray RC. The nature of the phosphate inhibitor complex of sulphite oxidase from electron paramagnetic resonance studies using oxygen-17. *Biochem. J* 1980;191:285–288. [PubMed: 6258584]
7. Bray RC, Gutteridge S, Lamy MT, Wilkinson T. Equilibria amongst different molybdenum(V)-containing species from sulphite oxidase: evidence for a halide ligand of molybdenum in the low pH species. *Biochem. J* 1983;211:227–236. [PubMed: 6307274]
8. Enemark JH, Astashkin AV, Raitsimring AM. Investigation of the coordination structures of the molybdenum(V) sites of sulfite oxidizing enzymes by pulsed EPR spectroscopy. *Dalton Trans* 2006:3501–3514. [PubMed: 16855750]
9. Astashkin AV, Johnson-Winters K, Klein EL, Byrne RS, Hille R, Raitsimring AM, Enemark JH. Direct demonstration of the presence of coordinated sulfate in the reaction pathway of *Arabidopsis thaliana* sulfite oxidase using ³³S labeling and ESEEM spectroscopy. *J. Am. Chem. Soc* 2007;129:14800–14810. [PubMed: 17983221]
10. Astashkin AV, Johnson-Winters K, Klein EL, Feng C, Wilson HL, Rajagopalan KV, Raitsimring AM, Enemark JH. Structural studies of the molybdenum center of the pathogenic R160Q mutant of human sulfite oxidase by pulsed EPR spectroscopy and ¹⁷O and ³³S labeling. *J. Am. Chem. Soc* 2008;130:8471–8480. [PubMed: 18529001]
11. Doonan CJ, Wilson HL, Rajagopalan KV, Garrett RM, Bennett B, Prince RC, George GN. Modified active site coordination in a clinical mutant of sulfite oxidase. *J. Am. Chem. Soc* 2008;130:6298–6305.
12. Cramer SP, Johnson JL, Rajagopalan KV, Sorrell TN. Observation of ¹⁷O effects on Mo^V EPR spectra in sulfite oxidase, xanthine dehydrogenase, and MoO(SC₆H₅)₄[−]. *Biochem. Biophys. Res. Commun* 1979;91:434–439. [PubMed: 229850]
13. Astashkin AV, Feng C, Raitsimring AM, Enemark JH. ¹⁷O ESEEM evidence for exchange of the axial oxo ligand in the molybdenum center of the high pH form of sulfite oxidase. *J. Am. Chem. Soc* 2005;127:502–503. [PubMed: 15643856]

14. Astashkin AV, Neese F, Raitsimring AM, Cooney JJA, Bultman E, Enemark JH. Pulsed EPR investigations of systems modeling molybdenum enzymes: hyperfine and quadrupole parameters of oxo- ^{17}O in $[\text{Mo}^{17}\text{O}(\text{SPh})_4]^-$. *J. Am. Chem. Soc.* 2005;127:16713–16722. [PubMed: 16305262]
15. Raitsimring AM, Astashkin AV, Baute D, Goldfarb D, Caravan P. W-band ^{17}O pulsed electron nuclear double resonance study of gadolinium complexes with water. *J. Phys. Chem. A* 2004;108:7318–7323.
16. Doonan CJ, Wilson HL, Bennett B, Prince RC, Rajagopalan KV, George GN. Mo^{V} electron paramagnetic resonance of sulfite oxidase revisited: the low-pH chloride signal. *Inorg. Chem* 2008;47:2033–2038. [PubMed: 18271529]
17. Astashkin AV, Klein EL, Enemark JH. Toward modeling the high chloride, low pH form of sulfite oxidase: K_a -band ESEEM of equatorial chloro ligands in oxomolybdenum(V) complexes. *J. Inorg. Biochem* 2007;101:1623–1629. [PubMed: 17644181]
18. Klein EL, Astashkin AV, Enemark JH. High resolution pulsed EPR and ^{17}O -NMR studies of molybdoenzyme model complexes. *J. Biol. Inorg. Chem* 2007;12(Suppl 1):S80.
19. Tanford C, Roxby R. Interpretation of protein titration curves: application to lysozyme. *Biochemistry* 1972;1972:2192–2198. [PubMed: 5027621]
20. Bashford D, Karplus M. Multiple-site titration curves of proteins: an analysis of exact and approximate methods for their calculation. *J. Phys. Chem* 1991;95:9556–9561.
21. Astashkin AV, Hood BL, Feng C, Hille R, Mendel RR, Raitsimring AM, Enemark JH. Structures of the $\text{Mo}(\text{V})$ forms of sulfite oxidase from *Arabidopsis thaliana* by pulsed EPR spectroscopy. *Biochemistry* 2005;44:13274–13281. [PubMed: 16201753]

Figure 1. Catalytic cycle of SOEs
 The *blocked* form represents a catalytic dead end.

Figure 2. Cosine Fourier transformation spectra of K_a -band (~ 29 GHz) τ integrated four-pulse ESEEM obtained at the low-field EPR turning point (gz). Traces 1-6 correspond to five-co-ordinate $[\text{Mo}^{17}\text{O}(\text{SPh}_4)]^-$ [14], six-co-ordinate $(\text{dtttd})\text{Mo}^{17}\text{O}({}^{17}\text{Otms})$ [10], *hpH* chicken SO [8], *lpH* chicken SO, *hpH* Y236F SDH and *blocked* R160Q hSO [10] respectively.

Figure 3. K_a -band (~29 GHz) ESEEM spectra of $[^{33}\text{S}]\text{At-SO}$

(a) Amplitude Fourier transformation spectra of the field-integrated two-pulse ESEEM of $[^{33}\text{S}]\text{At-SO}$ (upper trace) and $[^{32}\text{S}]\text{At-SO}$ (lower trace). (b) HYSCORE spectrum of $[^{33}\text{S}]\text{At-SO}$ obtained at the low-field EPR turning point (g_z)[9].