Supporting Information

Characterization of chloride-depleted human sulfite oxidase by EPR spectroscopy: experimental evidence for the role of anions in product release

Asha Rajapakshe, Kayunta Johnson-Winters, Anna R. Nordstrom, Kimberly Meyers, Safia Emesh, Andrei V. Astashkin, [*] *and John H. Enemark* [*]

Department of Chemistry and Biochemistry, University of Arizona, Tucson, Arizona 85721

[*] *Phone: 520-621-2245, Fax: 520-626-8065 E mail: jenemark@u.arizona.edu*

[*] *Phone: 520-621-9968, Fax: 520-626-8065 Email: andrei@u.arizona.edu*

List of mutants of hSO that exhibit the *blocked* species and convert to the *lpH* form with the addition of NaCl.

1. W226A
2. W226F
3. W338A
4. W338F
5. Y273A
6. Y273F
7. H337R
8. H337F
9. P105A
10. P111A
11. P118A
12. P105A/P111A
13. ΔK108V109A110
14. ΔK108V109A110T112
15. ΔK108V109A110T112V113

Biochemical and biophysical characterization of mutants 1-8 [Rajapakshe, A., Johnson-Winters, K., Nordstrom, A. R., Meyers, K., Emesh, S., Astashkin, A. V., Enemark, J. H.; unpublished experiments], and 9-15 [S1]

^{33}S HYSCORE spectra of the *blocked* form of *wt* hSO

Figure S1 shows ^{33}S hyperfine correlation (HYSCORE) spectra obtained at the EPR turning points. The relevant theory explaining the features of the HYSCORE spectra for ^{33}S under the condition of a strong nuclear quadrupole interaction (*nqi*) was derived earlier [S2]. The peak at about (18,18) MHz in these spectra corresponds to the "interdoublet transition" between the doublet states $|\pm 1/2\rangle$ and $|\pm 3/2\rangle$ separated in energy by the strong *nqi* (similar to Kramers doublets in EPR, where the zero-field separation is effected by the crystal field interaction). From the frequency of this transtion, ν_{id}, one can estimate the nuclear quadrupole coupling constant:

$$e^2Qq/h \approx 2\,\nu_{id} \approx 36 \text{ MHz}$$

similar to that estimated elsewhere for the *blocked* forms of plant SO [S2] and R160Q mutant of hSO [S3].

The features of the $|1/2\rangle \leftrightarrow |\pm 1/2\rangle$ transitions form antidiagonal ridges at all of the EPR turning points. While the ridges are expected for the intermediate turning point, g_Y, where a range of orientations contribute to the spectrum, the spectra at the low-field (g_Z) and high-field (g_X) turning points correspond to a single-crystal-like situation. The observation of extended HYSCORE features for these turning points indicates a statistical distribution of orientations of the sulfate ligand (specifically, the dihedral angle between the plane of d_{xy} orbital of the Mo(V) ion and the Mo-O-S(O_3) plane).

From the positions of the off-diagonal maxima ((4.1, 11.1) MHz at g_X and (4.3, 8.5) MHz at g_Z and g_Y) the respective *hfi* constants can be estimated using the expressions derived in ref. [S2], which can be reduced to

$$A = 2\nu_I|\nu_\alpha - \nu_\beta|/(\nu_\alpha + \nu_\beta)$$

This results in (A_X,A_Y,A_Z) = (3.2,2.3,2.3) MHz, from which the isotropic *hfi* constant a_{iso} = 2.6 MHz and anisotropic *hfi* tensor components (T_X,T_Y,T_Z) = (0.6,-0.3,-0.3) MHz can be estimated. These parameters are in qualitative agreement with those found earlier for plant SO [S2] and R160Q mutant of hSO [S3].

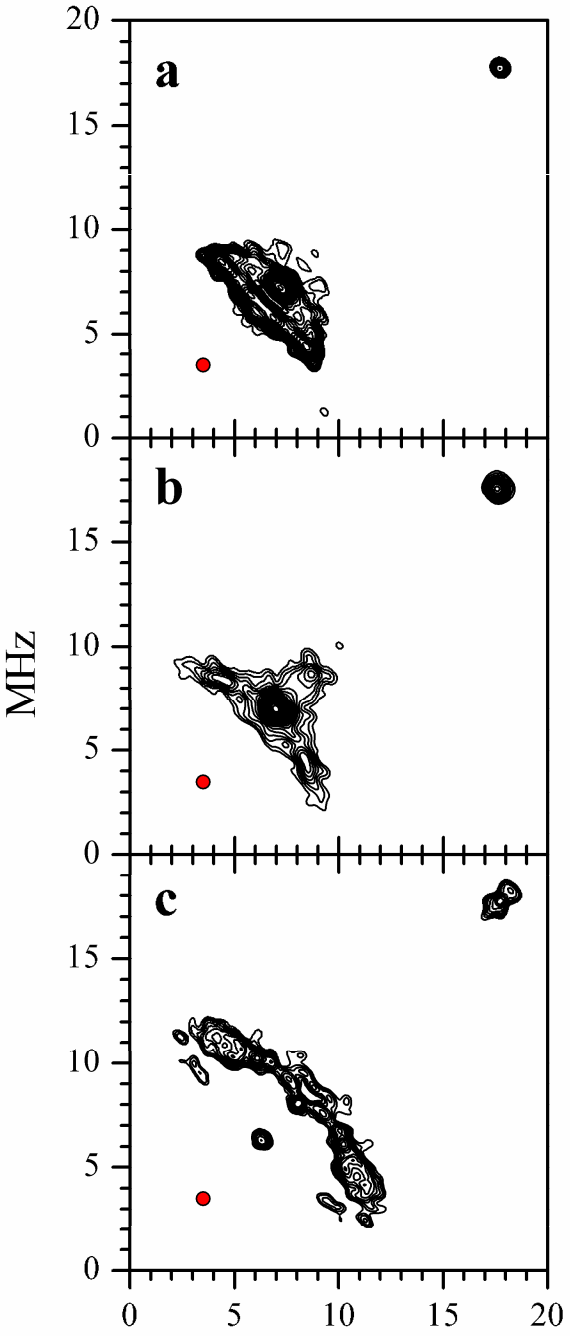


Figure S1. Panels a, b and c show the ^{33}S HYSCORE spectra obtained, respectively, at g_Z, g_Y and g_X EPR turning points of wt hSO enriched with ^{33}S-enriched sulfite at pH 5.8. Experimental conditions: mw frequency, 29.51 GHz, B_o = 1060.5 mT (g_Z), 1074.1 mT (g_Y) and 1079 mT (g_X); mw pulses, 10, 10, 16 and 10 ns. Each spectrum represents a sum of the spectra obtained at the time intervals between the first two mw pulses τ = 150, 180, 210 and 240 ns; temperature, 77K. The red dot indicates the ^{33}S Zeeman frequency: $\nu_I \approx 3.5$ MHz in each spectrum.

Kinetic analysis of the effect of added chloride on *wt* hSO

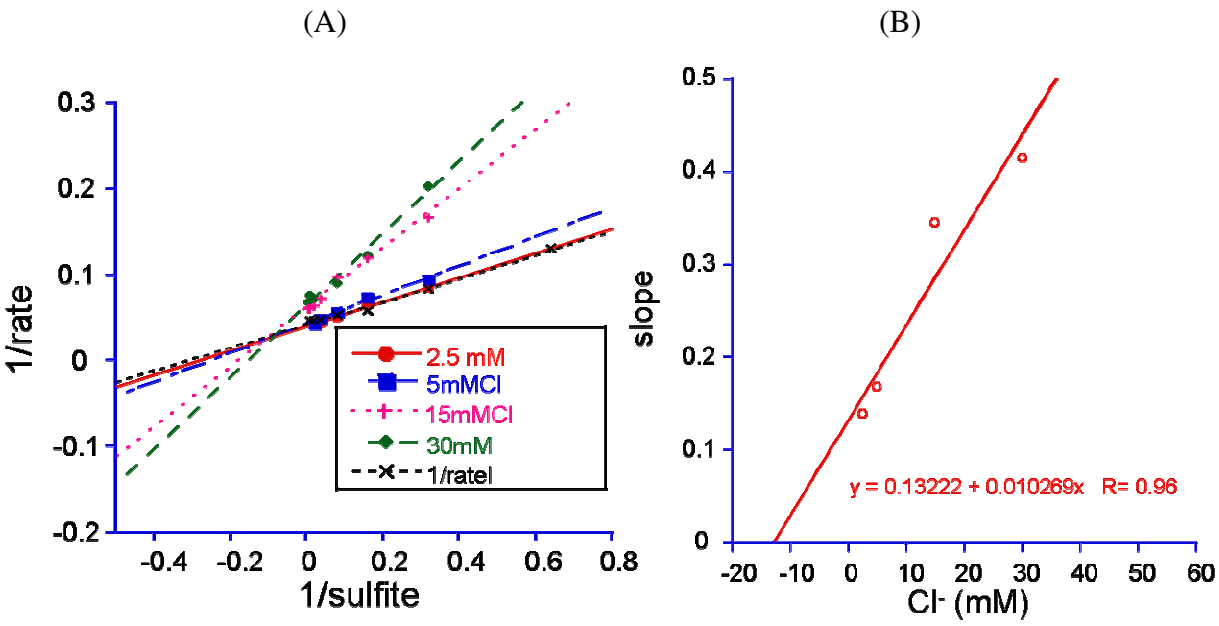
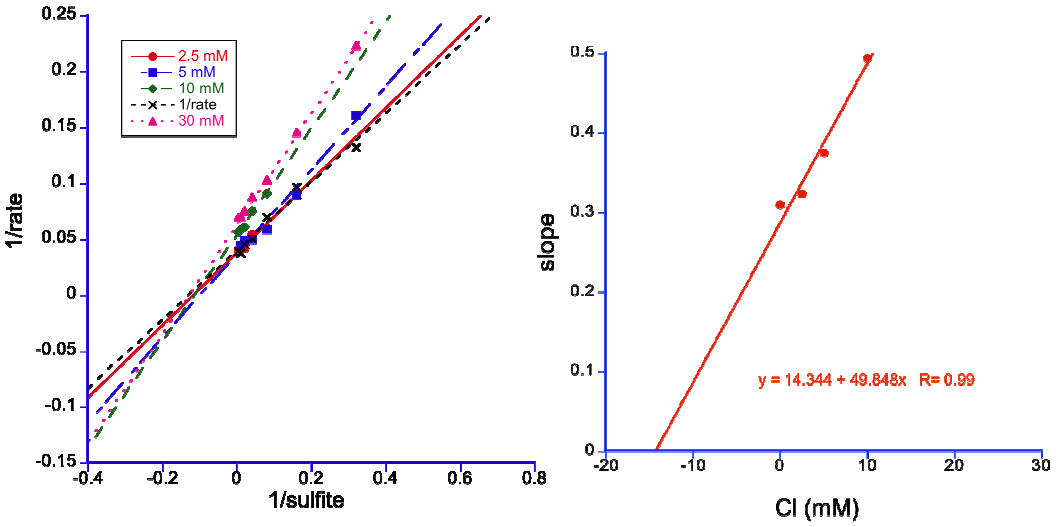


Figure S2. (A) Inhibition pattern of added chloride (2.5, 5, 15, 0, 30 mM) with sulfite as the variable substrate and the concentration of cytochrome *c* fixed at 400 µM at pH 5.8 in 100 mM Bis Tris acetate buffer. The lines drawn in the double reciprocal plot were calculated from its fits to the general rate equation. (B) The slopes of the lines in (A) were used in the replot.



(A) (B)

Figure S3. (A) Inhibition pattern of added chloride (2.5, 5, 10, 0, 30 mM) with sulfite as the variable substrate and the concentration of cytochrome *c* fixed at 400 µM at pH 8.0 in 20 mM Tris acetate buffer. The lines drawn in the double reciprocal plot were calculated from its fits to the general rate equation. (B) The slopes of the lines in (A) were used in the replot. At concentrations higher than 10 mM added chloride, the enzyme becomes saturated by chloride and (A) exhibits a limiting slope. Therefore, the slopes above 10 mM added chloride were not included in the slope replot in (B).

References

S1. Johnson-Winters, K.; Nordstrom, A. R.; Emesh, S.; Astashkin, A. V.; Rajapakshe, A.; Berry, R. E.; Tollin, G.; Enemark, J. H. (2010) Effects of Interdomain Tether Length and Flexibility on the Kinetics of Intramolecular Electron Transfer in Human Sulfite Oxidase *Biochemistry* , 49(6), 1290-1296.

S2. Astashkin, A. V., Johnson-Winters, K., Klein, E. L., Byrne, R. S., Hille, R., Raitsimring, A. M., and Enemark, J. H. (2007) 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. 129*, 14800-14810.

S3. Astashkin, A. V., Johnson-Winters, K., Klein, E. L., Feng, C., Wilson, H. L., Rajagopalan, K. V., Raitsimring, A. M., and Enemark, J. H. (2008) Structural Studies of the Molybdenum Center of the Pathogenic R160Q Mutant of Human Sulfite Oxidase by Pulsed EPR Spectroscopy and ^{17}O and ^{33}S Labeling. *J. Am. Chem. Soc. 130*, 8471-8480.