SUPPORTING INFORMATION

Probing Oxygen Activation Sites in Two Flavoprotein Oxidases Using Chloride as an Oxygen Surrogate[†‡]

Phaneeswara-Rao Kommoju[§], Zhi-wei Chen[¶], Robert C. Bruckner[§], F. Scott Mathews*[‖], and Marilyn Schuman Jorns*[§]

Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102

Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, St. Louis, MO 63104

Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110

*To whom correspondence and requests for reprints should be addressed. M.S.J.: Phone: (215) 762-7495; FAX: (215) 762-4452; E-mail: marilyn.jorns@drexelmed.edu. F.S.M.: Phone (314) 362-1080; FAX: (314) 362-7183; E-mail: mathews@biochem.wustl.edu.

Running Title: Probing Oxygen Activation Sites in Flavoprotein Oxidases

Table S1 Summary of data collection and refinement for GOX

	Form A	Form B
Data collection		
Wavelength (Å)	0.9	0.9
Space group	$P3_121$	$C222_1$
No. molecules per asymmetric unit	1	1
Res. Range (last shell) (Å)	40-1.3	40-1.2
No. observations	1056927	1039740
Unique observations	132764	179408
Completeness (last shell) (%)	99.2(98.7)	97.9(97.8)
R_{merge}[a] (last shell) (%)	5.6(32.1)	6.6(28.9)
$I/\sigma(I)$[b] (last shell)	29.9(3.6)	23.1(4.2)
Refinement		
Resolution (Å)	40-1.3	40-1.2
$\|F\|/\sigma(\|F\|)$	>0	>0
R_{cryst}[c]	0.180	0.178
R_{free}[d]	0.198	0.188
Reflections (working/test)	125987/6736	169694/8838
Protein atoms	4452	4452
Water molecules	1010	1296
FAD atoms	53	53
Cl^- ions	1	1
Rmsd bond lengths[e] (Å)	0.014	0.012
Rmsd angles[e] (°)	1.6	1.6
Rms ΔB ($Å^2$) (mm/ms/ss)[f]	1.28/1.53/2.33	1.01/1.36/2.12
<B> protein ($Å^2$)	13.7	12.2
<B> water molecules ($Å^2$)	33.9	35.7
<B> FAD ($Å^2$)	9.0	8.1
<B> Cl^- ions ($Å^2$)	20.4	18.9
Ramachandran plot (%)		
Allowed region	99.8	99.8
Generously allowed region	0.2	0.2
Disallowed region	0.	0.0

[a] $R_{merge} = \sum_h\sum_i \vdash I(h)-I_i(h) \dashv\!\Leftrightarrow / \sum_h\sum_i I_i(h)$, where $I_i(h)$ and $I(h)$ are the ith and mean measurements of reflection h. [b] $I/\sigma(I)$ is the average signal to noise ratio for merged reflection intensities. [c] $R = \sum_h \vdash F_o-F_c \dashv / \sum_h \vdash F_o \dashv$, where F_o and F_c are the observed and calculated structure factor amplitudes of reflection h. [d] R_{free} is the test reflection data set, about 5 % selected randomly for cross validation during crystallographic refinement. [e] Root-mean-squared deviation (Rmsd) from ideal bond lengths and angles and Rmsd in B-factors of bonded atoms. [f] mm, main chain to main chain; ms, main chain to side chain, ss, side chain to side chain.

Table S2 Effect of pH on the stability of the complex formed with GOX and chloride[a]

pH	K_d (mM)
3.0	6.2 ± 0.1
3.5	8.7 ± 0.2
4.0	19.0 ± 0.3
4.5	45 ± 1
5.0	137 ± 6
5.5	460 ± 20
5.75	630 ± 30
6.0	830 ± 40 (900 ± 80)
6.25	1130 ± 40
6.5	1640 ± 70
7.0	1900 ± 200

[a]Titrations were conducted in 50 mM potassium citrate (pH 3.0 to 6.0) or 50 mM potassium phosphate (pH 6.0 to 7.0) buffer at 25 ºC. Two titrations were performed at pH 6.0. The value shown in parentheses was obtained in phosphate buffer.