

Supporting information for

Azide as a probe of proton transfer reactions
in photosynthetic oxygen evolution[‡]

Ian B. Cooper and Bridgette A. Barry*

School of Chemistry and Biochemistry and the Petit Institute for Bioengineering and
Bioscience, Georgia Institute of Technology, Atlanta, GA 30332

**To whom correspondence should be addressed: bridgette.barry@chemistry.gatech.edu;*

Phone: 404-385-6085; Fax: 404-894-2295.

[‡]Supported by NSF MCB 03-55421

Figure S1. Michaelis-Menten plots, considering chloride as the substrate, and showing the effect of azide inhibition on PSII oxygen evolution activity.

PSII was treated with sulfate in order to deplete chloride, and the PSII sample was then treated with 0 (red data points), 0.25 (orange data points), 0.38 (green data points), 0.50 (light blue data points), 0.75 (dark blue data points), or 1.0 (purple data points), 1.8 (black data points), and 2.5 (brown data points; pH 7.5 only) mM N_3^- . In (A), the assay conditions were taken from reference (38) and employed 400 mM sucrose, 50 mM MES-NaOH, pH 6.3, and 2 mM recrystallized paraphenylbenzoquinone. In (B), the assay conditions were 400 mM sucrose, 50 mM HEPES-NaOH, pH 7.5, and 2 mM recrystallized paraphenylbenzoquinone. The concentrations of chloride and azide were adjusted by addition from 1 M NaCl and 100 mM NaN_3 stock solutions, respectively, in the appropriate assay buffer. Error bars represent one standard deviation.

Double reciprocal plots (Fig. 1) showed a family of lines that intersected (approximately) to the left of the y axis. This result suggests that azide is a non-competitive (or mixed) inhibitor of oxygen evolution. In Figure S1, fits to the data, using the Michealis-Menten equation for mixed inhibition,

$$v_0 = \frac{(V_{\max} [Cl^-])}{K_m \left(1 + \frac{[N_3^-]}{K_i}\right) + [Cl^-] \left(1 + \frac{[N_3^-]}{K_i'}\right)}$$

confirm this suggestion and are shown superimposed on the data points (0-1.8 mM azide) as the color-coded solid lines. In the equation above, K_i is the azide inhibition constant for the competitive site and K_i' is the azide inhibition constant for the uncompetitive site.

A simultaneous fit to the data in Fig. S1A was employed to derive kinetic constants at pH 6.3. Similarly, a simultaneous fit to the data in Fig. S1B was employed to derive kinetic constants at pH 7.5. The 2.5 mM azide points, which exhibit non-linearity in double-reciprocal plots (Fig. 1), were not used in the fits.

At pH 6.3, the K_m for chloride was derived as 0.7 mM, the V_{\max} was derived as 360 $\mu\text{mol O}_2$ (mg chl-h) $^{-1}$, the K_i for azide binding to the competitive site was derived as 0.3 mM, and the K_i' for the uncompetitive site was derived as 2 mM. At pH 7.5, the K_m for chloride was derived as 3 mM, the V_{\max} was derived as 330 $\mu\text{mol O}_2$ (mg chl-h) $^{-1}$, the K_i for azide binding to the competitive site was derived as 1 mM, and the K_i' for the uncompetitive site was derived as 8 mM.

Figure S1.

