Rate and Equilibrium Constants

| | cytochrome $c$ conformation | | | | |
|---|---|---|---|---|---|
| | oxidized | | | reduced | alkaline |
| monoclonal antibody | $k_1{}^a$ (M$^{-1}$ s$^{-1}$) | $k_{-1}{}^b$ (s$^{-1}$) | $K_a{}^c$ (M$^{-1}$) | $k_1{}^a$ (M$^{-1}$ s$^{-1}$) | $k_1{}^a$ (M$^{-1}$ s$^{-1}$) |
| 2B5 | $(6.5 \pm 1.0) \times 10^5$ | $\sim 8 \times 10^{-5}$ | $\sim 9 \times 10^9$ | $(5.2 \pm 0.5) \times 10^5$ | $(4.2 \pm 0.5) \times 10^5$ |
| 5F8 | $(1.5 \pm 0.2) \times 10^6$ | $(1.0 \pm 0.6) \times 10^{-4}$ | $1.5 \times 10^{10}$ | $(1.5 \pm 0.4) \times 10^6$ | $(1.5 \pm 0.3) \times 10^6$ |

[a] Averages of bimolecular association rate constants for binding to different conformations of cytochrome $c$. For the 2B5 MAb, 33 values are averaged for oxidized cytochrome $c$, 12 values are averaged for reduced cytochrome $c$, and 15 values are averaged for binding to alkaline cytochrome $c$. For 5F8 MAb, 46 values are averaged for oxidized cytochrome $c$, 14 values are averaged for reduced cytochrome $c$, and 24 values are averaged for binding to alkaline cytochrome $c$. Measurements are made at 20 °C. Errors are standard deviations of separate measurements of the association rate constants. [b] The dissociation rate constants were measured using an ELISA procedure (see Materials and Methods). These were carried out at room temperature ($\sim 23$ °C), 0.1 M sodium phosphate, pH 7.0. For MAb 5F8, the errors in $k_{-1}$ are estimated as the standard deviations of separate measurements. Errors are not given for MAb 2B5 since there were only two successful measurements of the dissociation rate. [c] Equilibrium association constants for monoclonal antibody binding to oxidized cytochrome $c$ have been estimated assuming that $K_a = k_1/k_{-1}$. Note that $k_1$ was measured at 20 °C while $k_{-1}$ was measured at room temperature ($\sim 23$ °C).