



# Rate and equilibrium constants of O<sub>2</sub>-binding and O<sub>2</sub> release: “The forward and reverse steps for the T<sub>state</sub> → R<sub>state</sub> change for human Hb<sub>4</sub>/BPG, under standard conditions”

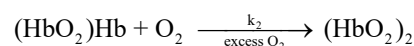
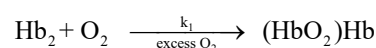
Francis Knowles\*, Samantha Doyle, Douglas Magde

Department of Chemistry and Biochemistry, University of California, San Diego, USA

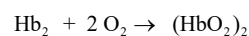
## APPENDIX

Consecutive equivalent first order reactions

Consider a scheme of two consecutive pseudo-first-order reactions with stoichiometry given by



The hypothetical macromolecule, Hb<sub>2</sub>, is defined to be dimeric, each constituent monomer containing a heme moiety capable of reversibly binding a molecule of either O<sub>2</sub> or CO. The dimeric model is intended to be a simplified model for human hemoglobin. In the presence of excess O<sub>2</sub>, (HbO<sub>2</sub>)Hb is produced as an unstable intermediate in the overall reaction given by



We can write the following rate equations, understanding that the rate constants, k<sub>1</sub> and k<sub>2</sub>, are pseudo first-order rate constants, the concentration of O<sub>2</sub> being much greater than the concentration of Hb<sub>2</sub>.

$$\frac{d[\text{Hb}_2]}{dt} = -k_1 [\text{Hb}_2] \equiv -k_1' [\text{O}_2] [\text{Hb}_2]$$

$$\frac{d[(\text{HbO}_2)\text{Hb}]}{dt} = k_1 [\text{Hb}_2] - k_2 [(\text{HbO}_2)\text{Hb}] \equiv k_1' [\text{O}_2] [\text{Hb}_2] - k_2' [\text{O}_2] [(\text{HbO}_2)\text{Hb}]$$

$$\frac{d[(\text{HbO}_2)_2]}{dt} = k_2 [(\text{HbO}_2)\text{Hb}] \equiv k_2' [\text{O}_2] [(\text{HbO}_2)\text{Hb}]$$

The first of these three equations is solved by the method for a first-order equation. The equation of state for [Hb<sub>2</sub>] is

$$[\text{Hb}_2] = [\text{Hb}_2]_0 \exp(-k_1 t)$$

Substituting the value for [Hb<sub>2</sub>] into the second rate equation and rearranging, we obtain a first order linear differential equation.

$$\frac{d[(\text{HbO}_2)\text{Hb}]}{dt} + k_2 [(\text{HbO}_2)\text{Hb}] = k_1 [\text{Hb}_2]_0 \exp(-k_1 t)$$

If [(HbO<sub>2</sub>)Hb]<sub>0</sub>=0, the solution for [(HbO<sub>2</sub>)Hb] is

$$[(\text{HbO}_2)\text{Hb}] = [\text{Hb}_2]_0 \left( \frac{k_1}{k_2 - k_1} \right) (\exp(-k_1 t) - \exp(-k_2 t))$$

The solution for [(HbO<sub>2</sub>)<sub>2</sub>] can be obtained directly from the mass conservation equations.

$$[\text{Hb}_2]_0 = [\text{Hb}_2] + [(\text{HbO}_2)\text{Hb}] + [(\text{HbO}_2)_2]$$

$$[(\text{HbO}_2)_2] = [\text{Hb}_2]_0 - [\text{Hb}_2] - [(\text{HbO}_2)\text{Hb}]$$

Substituting the equations of state for [Hb<sub>2</sub>] and [(HbO<sub>2</sub>)Hb] into the second mass conservation equation one obtains the equation of state for [(HbO<sub>2</sub>)<sub>2</sub>].

$$[(\text{HbO}_2)_2] = [\text{Hb}_2]_0 (1 - \exp(-k_1 t) - \left( \frac{k_1}{k_2 - k_1} \right) (\exp(-k_1 t) - \exp(-k_2 t)))$$

With the equations of state presented above, it is possible to simulate data for each of the concentrations of the reactants in the conversion of Hb<sub>2</sub> to (HbO<sub>2</sub>)<sub>2</sub>.

Special considerations for models of human hemoglobin

In the case of reaction of a dimeric hemoglobin molecule with, for example, O<sub>2</sub>, it would be normal to start with a solution free of O<sub>2</sub>. In this case, then, the intermediate, (HbO<sub>2</sub>)Hb, and the end product, (HbO<sub>2</sub>)<sub>2</sub>, are absent at t=0. The progress curve monitored at an appropriate wavelength in the O<sub>2</sub> difference spectrum. Such monitoring records the time dependence of all intermediates, simultaneously. The time course obtained by spectroscopic procedures, then, is directly proportional to fractional saturation of Hb<sub>2</sub> with O<sub>2</sub>. The equation of state for fractional saturation of

**Correspondence to:** Francis Knowles, Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, USA, E-mail: fknowles@ucsd.edu

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Hb<sub>2</sub> binding sites with O<sub>2</sub> is

$$F = \frac{[(\text{HbO}_2)\text{Hb}] + 2 [(\text{HbO}_2)_2]}{2 [\text{Hb}_2]_0}$$

A rate law based on fractional saturation is readily obtained by substituting the individual rate laws for (HbO<sub>2</sub>)Hb and (HbO<sub>2</sub>)<sub>2</sub> presented above.

$$F = \frac{[\text{Hb}_2]_0 (2 \exp(-k_2 t) - 2 \exp(-k_1 t)) + 2 [\text{Hb}_2]_0 (1 + \exp(-k_1 t) - 2 \exp(-k_2 t))}{2[\text{Hb}_2]_0}$$

Factoring out common terms and combining similar quantities, the rate law reduces to a first order equation.

$$F = 1 - \exp(-k_2 t)$$