Derivation of modified peroxidase kinetic scheme

The result is an equation that can be interpreted within the framework of the Michealis-Menten kinetics, but is not the same as the M-M in its form.

The peroxidase rate scheme is given as:
This scheme is also known as the ping-pong mechanism.

$$DHP + H_2O_2 \xrightarrow{k_1} CmpI + H_2O$$

$$CmpI + AH \xrightarrow{k_2} CmpII + A\cdot$$

$$CmpII + AH \xrightarrow{k_3} DHP + A\cdot$$

First we write rate equations for each component in the peroxidase rate scheme. We have defined A· as P, the product, and DHP as the ferric form of the enzyme in the rate equations below.

$$\frac{d[DHP]}{dt} = -k_1[DHP][H_2O_2]$$

$$\frac{d[CmpI]}{dt} = k_1[DHP][H_2O_2] - k_2[CmpI][AH]$$

$$\frac{d[CmpII]}{dt} = k_2[CmpI][AH] - k_3[CmpII][AH]$$

$$\frac{d[P]}{dt} = (k_2[CmpI] + k_3[CmpII])[AH]$$

Next we apply the steady state approximation to both the CmpI and CmpII intermediates.

$$0 \approx k_1[DHP][H_2O_2] - k_2[CmpI][AH]$$

$$k_1[DHP][H_2O_2] \approx k_2[CmpI][AH]$$

and

$$0 \approx k_2[CmpI][AH] - k_3[CmpII][AH]$$

$$k_2[CmpI][AH] \approx k_3[CmpII][AH]$$

Thus,

$$[CmpI] \approx \frac{k_1[DHP][H_2O_2]}{k_2[AH]} \quad [CmpII] \approx \frac{k_1[DHP][H_2O_2]}{k_3[AH]}$$

Substituting the equations for CmpI and CmpII back into the rate equation for product formation, we have,

$$v_o \approx \frac{d[P]}{dt} = (k_2[CmpI] + k_3[CmpII])[AH]$$

$$v_o \approx \left(k_2\left[\frac{k_1[DHP][H_2O_2]}{k_2[AH]}\right] + k_3\left[\frac{k_1[DHP][H_2O_2]}{k_3[AH]}\right]\right)[AH]$$

$$v_o \approx 2k_1[DHP][H_2O_2]$$

For the peroxidase scheme presented above,

$$[E]_o = [DHP] + [CmpI] + [CmpII]$$

Substituting the equations for CmpI and CmpII into the above expression, we have,

$$[E]_o = [DHP] + \frac{k_1[DHP][H_2O_2]}{k_2[AH]} + \frac{k_1[DHP][H_2O_2]}{k_3[AH]}$$

$$[E]_o = [DHP]\left(1 + \frac{k_1[H_2O_2]}{k_2[AH]} + \frac{k_1[H_2O_2]}{k_3[AH]}\right)$$

$$[E]_o = [DHP]\left(1 + \frac{k_1[H_2O_2]}{[AH]}\left(\frac{1}{k_2} + \frac{1}{k_3}\right)\right)$$

For traditional Michaelis Menton kinetics,

$$\frac{v_o}{[E]_o} = \frac{k_2[S]}{\frac{k_{-1} + k_2}{k_1} + [S]}$$

$$v_o = \frac{k_2[E]_o[S]}{\frac{k_{-1} + k_2}{k_1} + [S]}$$

$$v_o = \frac{V_{max}[S]}{K_M + [S]}$$

$$V_{max} = k_2[E]_o \qquad K_M = \frac{k_{-1} + k_2}{k_1}$$

Mapping the peroxidase rate scheme onto the Michaelis Menton equation yields,

$$\frac{v_o}{[E]_o} = \frac{k_1[DHP][H_2O_2]}{[DHP]\left(1 + \frac{k_1[H_2O_2]}{[AH]}\left(\frac{1}{k_2} + \frac{1}{k_3}\right)\right)}$$

$$\frac{v_o}{[E]_o} = \frac{k_1[H_2O_2]}{\left(1 + \frac{k_1[H_2O_2]}{[AH]}\left(\frac{1}{k_2} + \frac{1}{k_3}\right)\right)}$$

$$\frac{v_o}{[E]_o} = \frac{k_1[H_2O_2]}{\left(1 + \frac{k_1[H_2O_2]}{[AH]}\left(\frac{1}{k_2} + \frac{1}{k_3}\right)\right)}$$

$$\frac{v_o}{[E]_o} = \frac{1}{\left(\frac{1}{k_1[H_2O_2]} + \frac{1}{[AH]}\left(\frac{1}{k_2} + \frac{1}{k_3}\right)\right)}$$

$$\frac{v_o}{[E]_o} = \frac{1}{\left(\frac{[AH]}{k_1[H_2O_2][AH]} + \frac{k_1[H_2O_2]}{k_1[H_2O_2][AH]}\left(\frac{1}{k_2} + \frac{1}{k_3}\right)\right)}$$

$$\frac{v_o}{[E]_o} = \frac{1}{\left(\frac{[AH] + k_1[H_2O_2]}{k_1[H_2O_2][AH]}\left(\frac{1}{k_2} + \frac{1}{k_3}\right)\right)}$$

$$\frac{v_o}{[E]_o} = \frac{k_1[H_2O_2][AH]}{\left([AH] + k_1[H_2O_2]\left(\frac{1}{k_2} + \frac{1}{k_3}\right)\right)}$$

Rearranging,

$$v_o = \frac{k_1[H_2O_2][E]_o[AH]}{\left(\left(\frac{1}{k_2} + \frac{1}{k_3}\right)k_1[H_2O_2] + [AH]\right)}$$

By analogy to the traditional Michaelis Menton equation,

$$v_o = \frac{k_1[H_2O_2][E]_o[AH]}{\left(\left(\frac{1}{k_2} + \frac{1}{k_3}\right) k_1[H_2O_2] + [AH]\right)}$$

$$V_{max} = k_1[H_2O_2][E]_o \quad \text{and} \quad K_M = \left(\frac{1}{k_2} + \frac{1}{k_3}\right) k_1[H_2O_2]$$

Thus,

$$v_o = \frac{V_{max}[AH]}{(K_M + [AH])}$$

Here, [AH] is equivalent to [XAOH] from the main text of the manuscript.