Supplemental material

APPENDIX

Formulation of the 11-state model - Enzyme states associated with rapid-equilibrium protonation and deprotonation steps can be lumped together as follows (see Fig. A1):

$$\begin{aligned}
&E_{26m} = E_2 + E_{6m}\,,\\
&E_{35f} = E_3 + E_{5f}\,,\\
&E_{14m} = E_{1m} + E_{4m}\,,\\
&E_{14f} = E_{1f} + E_{4f}\,. \qquad \text{(Eq. A1)}
\end{aligned}$$

The resulting 7-state model is illustrated in Fig. A1.

In order to account for proton binding steps in this kinetic model, the following factors are defined which are used to translate the kinetic constants in the scheme of Fig. 2A to those of Fig. A1:

$$\begin{aligned}
&f_{26} = \frac{1}{1 + h/K_{26}}\,,\\
&k'_{21} = f_{26}k_{21}\,,\ k'_{23} = f_{26}k_{23}\,,\ k'_{6m6} = \left(1 - f_{26}\right)k_{6m6}\,,\\
&f_{35} = \frac{1}{1 + h/K_{35}}\,,\\
&k'_{31} = f_{35}k_{31}\,,\ k'_{32} = f_{35}k_{32}\,,\ k'_{5f5} = \left(1 - f_{25}\right)k_{5f5}\,,\\
&f_{14m} = \frac{1}{1 + h/K_{14m}}\,,\\
&k'_{12} = f_{14m}k_{12}\,,\ k'_{4m4} = \left(1 - f_{14m}\right)k_{4m4}\,,\\
&f_{14f} = \frac{1}{1 + h/K_{14f}}\,,\\
&k'_{13} = f_{14f}k_{13}\,,\ k'_{4f4} = \left(1 - f_{14f}\right)k_{4f4}\,. \qquad \text{(Eq. A2)}
\end{aligned}$$

At steady-state, the net production of malate is equal to the net consumption of fumarate:

$$\begin{aligned}
&E_6k_{64} - E_4mk_{46} + E_2k_{21} - E_{1m}mk_{12} =\\
&\quad E_4fk_{45} - E_5k_{54} + E_{1f}fk_{13} - E_3k_{31}. \qquad \text{(Eq. A3)}
\end{aligned}$$

Using the simplified formulation described above, one can easily derive the steady-state flux expression by applying the King-Altman (KA) procedure to the reduced seven-state mechanism of Fig. A1.

The KA procedure consists in using the directional diagrams associated with each state in the enzyme mechanism. There are 48 possible KA patterns, and $48 \times 7 = 336$ directional diagrams (48 for each of the 7 states) associated with this mechanism. Each directional diagram is associated with a product of the pseudo-first order rate constants corresponding to arrows in the directional diagram. The relative steady-state concentration of each enzyme state E_{i} is thus proportional to the sum of the 48 terms associated with the 48 directional diagrams, associated with state i. This sum is Σ_i and E_i is computed

$$E_\mathrm{i} = E_0 \frac{\Sigma_\mathrm{i}}{\Sigma}, \qquad \text{(Eq. A4)}$$

where Σ is the sum over all 7 sets of 48 terms for all states, and E_0 is the total enzyme concentration. The computer package KAPattern (1) is used to generate the functions for the seven functions Σ_i.

In order to account for possible dead-end competitive binding of inhibitors to any of the 11 individual enzyme states E_i, Σ can be expressed as a sum of 11 terms associated with the 11 states in the full reaction scheme of Fig. 2A:

$$\begin{aligned}\Sigma = {} & f_{14m}\Sigma_{14m}I_{1m} + f_{14f}\Sigma_{14f}I_{1f} + f_{26}\Sigma_{26m}I_2 \\ & + f_{35}\Sigma_{35f}I_3 + \Sigma_4 I_4 + (1-f_{14m})\Sigma_{4m}I_{4m} \\ & + (1-f_{14f})\Sigma_{4f}I_{4f} + \Sigma_5 I_5 + (1-f_{35})\Sigma_{5f}I_{5f} \\ & + \Sigma_6 I_6 + (1-f_{26})\Sigma_{6m}I_{6m}.\end{aligned} \qquad \text{(Eq. A5)}$$

Equations 19 and 22 are used to account for inhibition and activation respectively, and the net quasi-steady-state flux of the reaction is computed from equation 24.

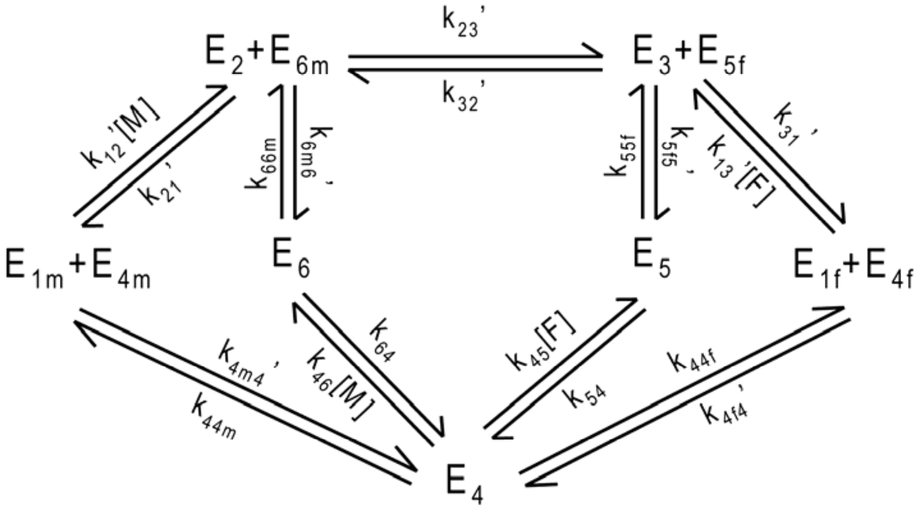

Fig. A-1. Reduced seven-state mechanism used in the King-Altman procedure. Enzyme states in rapid equilibrium (proton binding-release) are lumped together and some of the rate constants are weighted by a factor to account for associated proton binding/release.

Relationships between reference species concentrations and reactant concentrations - In solutions of ionic strength of approximately 0.1 M, a hydrogen ion dissociates from the protonated specie *i* with an acid-base pK, where pK is defined as $-\log_{10} K_{\mathrm{H,i}}$. Values for fumarate, malate and inorganic phosphate are given in Table 1 of the paper. The equilibrium expressions are:

$$K_{H,FUM} = 10^{-pK_{H,FUM}} = \frac{[FUM^{2-}][H^+]}{[HFUM^-]},$$

$$K_{H,MAL} = 10^{-pK_{H,MAL}} = \frac{[MAL^{2-}][H^+]}{[HMAL^-]}, \text{`}$$

$$K_{H,Pi} = 10^{-pK_{H,Pi}} = \frac{[HPO_4{}^{2-}][H^+]}{[NaH_2PO_4{}^-]}. \qquad \text{(Eq. A6)}$$

In the case of sodium dissociation, the equilibrium expressions become:

$$K_{Na,MAL} = 10^{-pK_{Na,MAL}} = \frac{[MAL^{2-}][Na^+]}{[NaMAL^-]},$$

$$K_{Na,Pi} = 10^{-pK_{Na,Pi}} = \frac{[HPO_4{}^{2-}][Na^+]}{[Na_2HPO_4{}^-]}. \qquad \text{(Eq. A7)}$$

If we denote total fumarate, malate and phosphate concentrations [FUM], [MAL] and [Pi] respectively, and assume that the system is in equilibrium, then

$$\begin{aligned}
[FUM] &= [FUM^{2-}] + [HFUM^-] \\
&= [FUM^{2-}] + \frac{[FUM^{2-}][H^+]}{K_{H,FUM}} \\
&= [FUM^{2-}]\left(1 + \frac{[H^+]}{K_{H,FUM}}\right) \\
&= [FUM^{2-}]P_{FUM},
\end{aligned} \qquad \text{(Eq. A8)}$$

$$\begin{aligned}
[MAL] &= [MAL^{2-}] + [HMAL^-] + [NaMAL^-] \\
&= [MAL^{2-}] + \frac{[MAL^{2-}][H^+]}{K_{H,MAL}} + \frac{[MAL^{2-}][Na^+]}{K_{Na,FUM}} \\
&= [MAL^{2-}]\left(1 + \frac{[H^+]}{K_{H,MAL}} + \frac{[Na^+]}{K_{Na,MAL}}\right) \\
&= [MAL^{2-}]P_{MAL},
\end{aligned} \qquad \text{(Eq. A9)}$$

$$\begin{aligned}
[Pi] &= [HPO_4{}^{2-}] + [NaH_2PO_4{}^-] + [Na_2HPO_4{}^-] \\
&= [HPO_4{}^{2-}] + \frac{[HPO_4{}^{2-}][H^+]}{K_{H,Pi}} + \frac{[HPO_4{}^{2-}][Na^+]}{K_{Na,Pi}} \\
&= [HPO_4{}^{2-}]\left(1 + \frac{[H^+]}{K_{H,Pi}} + \frac{[Na^+]}{K_{Na,Pi}}\right) \\
&= [HPO_4{}^{2-}]P_{Pi},
\end{aligned} \qquad \text{(Eq. A10)}$$

where P_{FUM}, P_{MAL} and P_{Pi} are defined as the binding polynomials, which describe the relationships between reference species concentrations and reactant concentrations.

REFERENCES

1. Qi, F., Dash, R. K., Han, Y., and Beard, D. A. (2009) *BMC Bioinformatics* **10**, 238-