Citrate Synthase

Enzyme

2.3.3.1

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Reactants and Reaction

Citrate synthase is the first step in the oxidation of acetyl-CoA in the citric acid cycle. The reference chemical reaction, is

$$OAA^{2-} + ACCOA^{0} + H_2O \rightleftharpoons COAS^{-} + CIT^{3-} + 2H^{+}$$

where the abbreviations for the biochemical species are listed below. The biochemical reaction, involving biochemical reactants that are sums of species is

$$OAA + ACCOA \rightleftharpoons COASH + CIT$$

Table 1: Thermodynamic Parameter Values for Citrate Synthase. $\Delta_r G^o$ correspond to T = 298.15 K, 1 M reactants, I = 0.0 M, P = 1 atm; pK values correspond to T = 298.15 K, 1 M reactants, I = 0.10 M, P = 1 atm.

Reactant	Abbreviation	Reference species	$\Delta_f G^o$ (kJ/mol)	Ion-bound species	pK
water	H_2O	H_2O	-237.19	-	-
coenzyme A	COASH	$COAS^-$	0	$COASH^0$	8.09
acetyl-co-enzyme A	ACCOA	$ACCOA^0$	-188.52	-	-
oxaloacete	OAA	OAA^{2-}	-791.77	$MgOAA^0$	1.02
				$HOAA^-$	3.9
citrate	CIT	CIT^{3-}	-1157.36	$HCIT^{2-}$	5.67
				$MgCIT^-$	3.517
				$KCIT^{2-}$	0.60
adenosine triphosphate	ATP	ATP^{4-}		$HATP^{3-}$	6.71
				$MgATP^2$	4.28
				$KATP^{3-}$	1.00
adenosine diiphosphate	ADP	ADP^{3-}		$HADP^{2-}$	6.496
				$MgADP^-$	3.30
				$KADP^{2-}$	1.17
adenosine monophosphate	AMP	AMP^{2-}		$HAMP^-$	6.16
				$MgAMP^0$	1.74
				$KAMP^-$	1.00
succinyl-coenzyme A	SCOA	$SCOA^-$	-509.59	$HSCOA^0$	3.99

All values from [1].

Mechanism

The enzyme catalyzed reaction is assumed to proceed via the compulsory-ordered ternary mechanism, with dead-end binding of succinyl-CoA, ATP, ADP, and AMP. The mechanism involves four enzyme state transitions:

$$E + OAA^{2-} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} E \cdot OAA^{2-}$$

$$E \cdot OAA^{2-} + ACCOA^{0} \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} E \cdot OAA^{2-} \cdot ACCOA^{0}$$

$$E \cdot OAA^{2-} \cdot ACCOA^{0} \underset{k_{43}}{\overset{k_{34}}{\rightleftharpoons}} E \cdot CIT^{3-} + COAS^{-} + 2H^{+}$$

$$E \cdot CIT^{3-} \underset{k_{14}}{\overset{k_{41}}{\rightleftharpoons}} E + CIT^{3-}$$

where each state transition is assumed to proceed by mass action.

Equations

Thermodynamics

The standard Gibbs free energy is computed [2]:

$$\Delta_r G^0_{cits} = \Delta_f G^0_{COASH} + \Delta_f G^0_{CIT} - \Delta_f G^0_{OAA} - \Delta_f G^0_{ACCOA} - \Delta_f G^0_{H_2O} = 60.12\ kJ/mol,$$

where the basic thermodynamic data are listed in Table 1. The equilibrium constant for the reference biochemical reaction is computed from the standard Gibbs free energy

$$K^0_{eq,\text{cits}} = \frac{1}{h^2}\exp\left(-\frac{\Delta_r G^0_{\text{cits}}}{RT}\right),$$

where we have introduced the definition $h = 10^{-\text{pH}}$ and this equilibrium constant explicitly accounts for pH. Therefore $K^0_{eq,\text{cits}}$ represents the equilibrium ratio

$$[\text{COAS}^-][\text{CIT}^{3-}]/[\text{OAA}^{2-}][\text{ACCOA}^0]$$

at T = 298.15 K and I = 0.0 M.

The species concentrations are computed:

$$[\text{OAA}^{2-}] = [\text{OAA}]/P_{OAA}$$
$$[\text{ACCOA}^0] = [\text{ACCOA}]/P_{\text{ACCOA}}$$
$$[\text{CIT}^{3-}] = [\text{CIT}]/P_{\text{CIT}}$$
$$[\text{COAS}^-] = [\text{COASH}]/P_{\text{COASH}}$$
$$[\text{ATP}^{4-}] = [\text{ATP}]/P_{\text{ATP}}$$
$$[\text{ADP}^{3-}] = [\text{ADP}]/P_{\text{ADP}}$$
$$[\text{AMP}^{2-}] = [\text{AMP}]/P_{\text{AMP}}$$
$$[\text{SCOA}^-] = [\text{SCOA}]/P_{\text{SCOA}}$$

where the binding polynomials are expressed:

$$P_{OAA} = 1 + \frac{[\text{Mg}^{2+}]}{K_{Mg,OAA}}$$

$$P_{ACCOA} = 1$$

$$P_{CIT} = 1 + \frac{h}{K_{H,CIT}} + \frac{[\text{Mg}^{2+}]}{K_{Mg,CIT}} + \frac{[\text{K}^{+}]}{K_{K,CIT}}$$

$$P_{COASH} = 1 + \frac{h}{K_{H,COASH}}$$

$$P_{ATP} = 1 + \frac{h}{K_{H,ATP}} + \frac{[\text{Mg}^{2+}]}{K_{Mg,ATP}} + \frac{[\text{K}^{+}]}{K_{K,ATP}}$$

$$P_{ADP} = 1 + \frac{h}{K_{H,ADP}} + \frac{[\text{Mg}^{2+}]}{K_{Mg,ADP}} + \frac{[\text{K}^{+}]}{K_{K,ADP}}$$

$$P_{AMP} = 1 + \frac{h}{K_{H,AMP}} + \frac{[\text{Mg}^{2+}]}{K_{Mg,AMP}} + \frac{[\text{K}^{+}]}{K_{K,AMP}}$$

$$P_{SCOA} = 1 + \frac{h}{K_{H,COASH}}$$

Flux Expression

We use the variables *a*, *b*, *p*, and *q* to denote species concentrations: $a = [\text{OAA}^{2-}]$, $b = [\text{ACCOA}^0]$, $p = [\text{COAS}^-]$, $q = [\text{CIT}^{3-}]$.

The flux expression has the form:

$$J = \frac{n}{d}$$

where

$$n = \frac{V_m}{K_{eA}K_{mB}} \frac{\left(ab - \frac{pq}{K_{eq}}\right)}{\left(1 + \frac{h}{K_{iH}}\right)}$$

and

$$d = \left(1 + \frac{K_{mA}b}{K_{eA}K_{mB}} + \frac{K_{mQ}p}{K_{eQ}K_{mP}}\right)I_1 + \left(\frac{a}{K_{eA}} + \frac{K_{mQ}ap}{K_{eA}K_{mP}K_{eQ}} + \frac{K_{mA}pq}{K_{eA}^2 K_{mB}K'_{eq}}\right)I_2 +$$

$$\left(\left[\frac{1}{K_{eA}K_{mB}} - \frac{K_{mQ}K'_{eq}}{K_{eQ}^2 K_{mP}}\right]ab + \left[\frac{1}{K_{mP}K_{eQ}} - \frac{K_{mA}}{K_{eA}^2 K_{mB}K'_{eq}}\right]pq + \frac{K_{mQ}abp}{K_{eA}K_{eB}K_{mP}K_{eQ}} + \frac{K_{mA}bpq}{K_{eA}K_{mB}K_{eP}K_{eQ}}\right)I_3$$

$$\left(\frac{q}{K_{eQ}} + \frac{K_{mQ}K'_{eq}ab}{K_{eQ}^2 K_{mP}} + \frac{K_{mA}bq}{K_{eA}K_{mB}K_{eQ}}\right)I_4$$

and

$$K_{eP} = \frac{K^0_{eq,\text{cits}}K_{eA}K_{eB}}{K_{eQ}}.$$

The rate constant k_{43} is assumed to depend on pH according to the formula $k_{43} = (h/10^{-7})^2 k'_{43}$ where k'_{43} is independent of pH. Therefore the kinetic constant K_{mP} is defined to depend on pH as

$$K_{mP} = \left(\frac{10^{-7}}{h}\right)^2 K'_{mP}.$$

The inhibition factors I_1, I_2, I_3, and I_4, are computed:

$$I_1 = I_3 = I_4 = 1$$

$$I_2 = 1 + \frac{[\text{ATP}^{4-}]}{K_{iATP}} + \frac{[\text{ADP}^{3-}]}{K_{iADP}} + \frac{[\text{AMP}^{2-}]}{K_{iAMP}} + \frac{[\text{SCOA}^{-}]}{K_{iSCOA}}$$

simulating dead-end binding of adenine nucleotides and SCOA at state 2 of the catalytic cycle.

Parameter Values

Table 2: Kinetic Parameter Values for Citrate Synthase (some estimates not available)*

Parameter	Rat kidney 28°C (based on data from [2])	Rat liver 25°C (based on data from [3])	Bovine heart 21°C (based on data from [4])
V_{max} (μmol·min^{-1}·μg^{-1})	0.290	---	---
K_{mA} (μM)	5.79	1.33	1.00
K_{mB} (μM)	4.55	13.6	5.42
K'_{mP} (μM)	0.180	---	---
K_{mQ} (mM)	2.25	---	---
K_{eA} (μM)	3.09	1.96	1.50
K_{eB} (mM)	283	---	--
K_{eQ} (mM)	0.443	---	---
K_{iATP} (mM)	---	0.215	0.357
K_{iADP} (mM)	---	0.475	---
K_{iAMP} (mM)	---	1.97	---
K_{iSCOA} (μM)	---	---	53.8
K_{iH} (μM)	---	0.055	---

* These parameter estimates have been adjusted from the values in the original publication to be consistent with the thermodynamic property data estimated in Li et al. [1]. All model fits shown in Figures 1-5 of this document reflect these updated values for the thermodynamic data and the kinetic parameters.

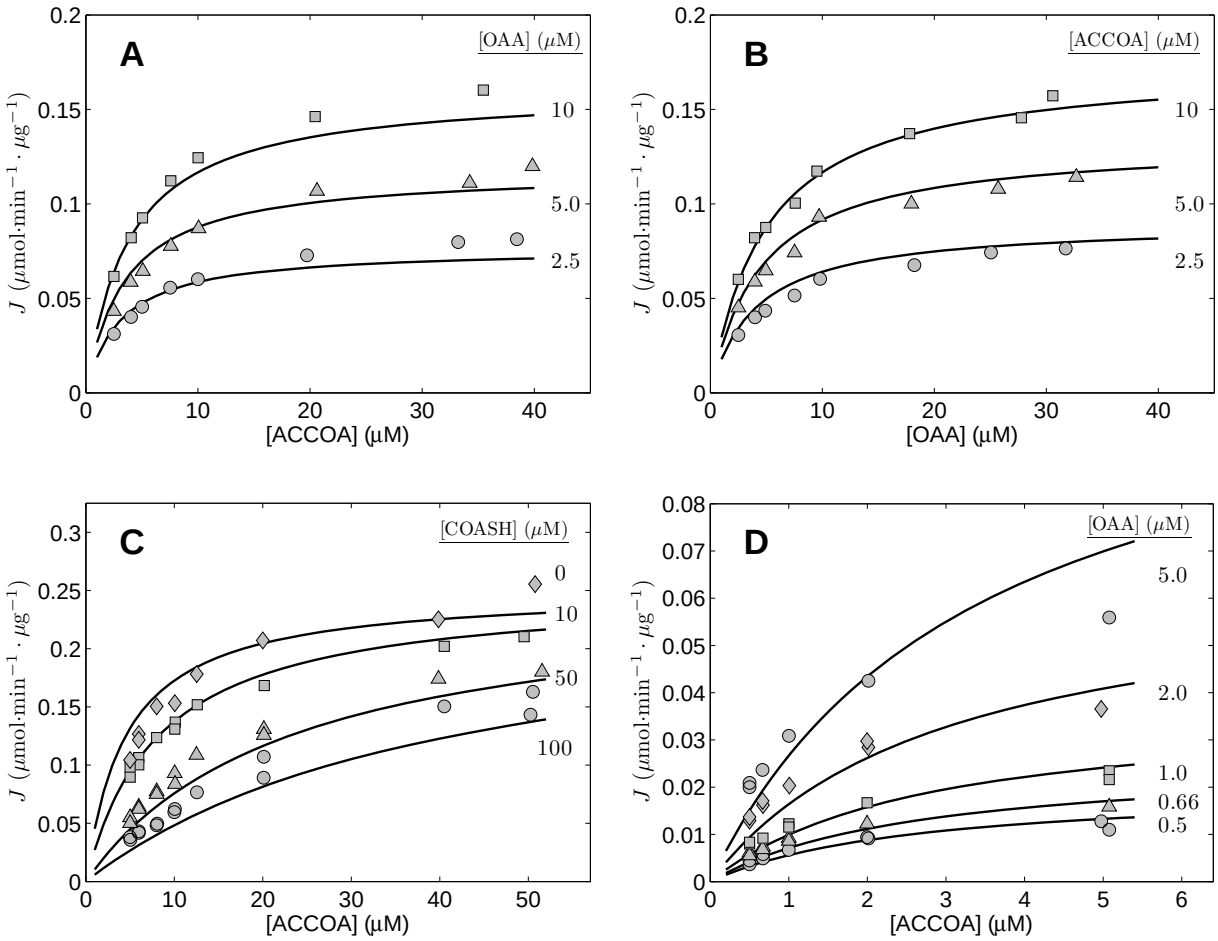

Figure 1: Fits to kinetic data from [3] on the forward operation of kidney enzyme. Measured flux as a function of substrate concentrations was obtained from Figures 2, 3, 6, 7, and 9 of [3]. Initial fluxes (μmol of COASH (or CIT) synthesized per minute per μg of enzyme) measured at the substrate concentrations indicated in the figures. For **A**, **B**, and **D**, the initial product (CIT and COASH) concentrations are zero. **C.** Flux measured with COASH added in various concentrations to investigate the kinetics of product inhibition. All data were obtained at pH = 8.1 at 28° C. Model fits are plotted as solid lines.

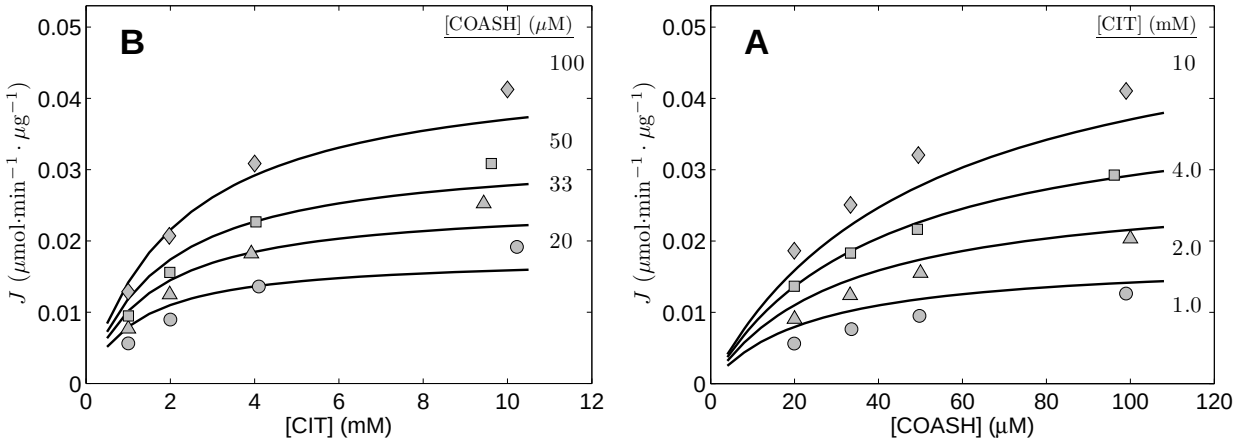

Figure 2: Fits to kinetic data from [3] on the reverse operation of kidney enzyme. Measured reverse flux as a function of concentrations of CIT and COASH was obtained from Figures 4 and 5 of [3]. Initial fluxes (µmol of COASH (or CIT) synthesized per minute per µg of enzyme) measured at the substrate concentrations indicated in the figures. All data were obtained at pH = 8.1 at 28° C. Model fits are plotted as solid lines.

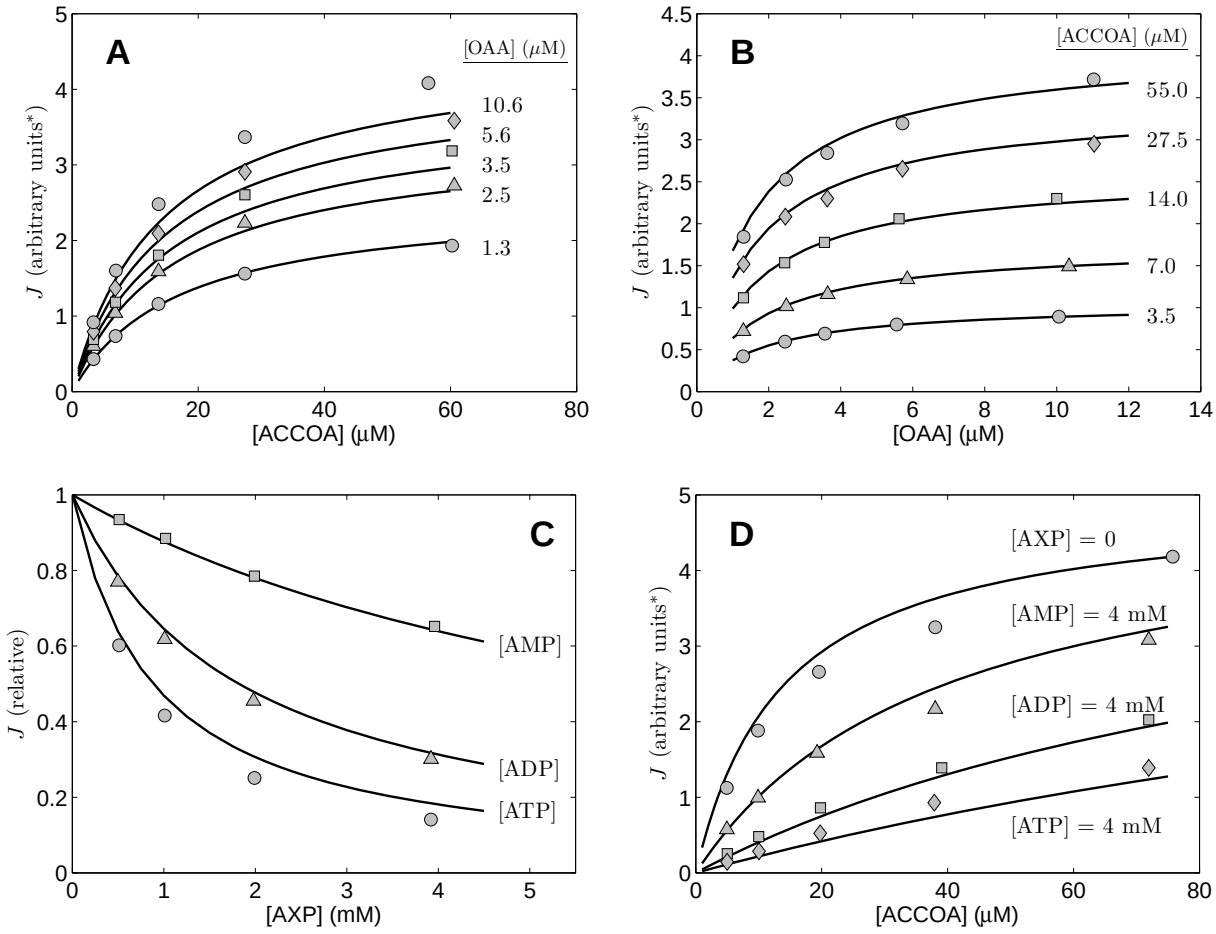

Figure 3: Fits to kinetic data from [4] on the forward operation of liver enzyme. Measured flux in arbitrary units was obtained from Figures 1,2,5, and 6 of [4]. For all cases the product (CIT and COASH) concentrations are zero and total substrate and inhibitor concentrations are indicated in the figure. **A** and **B** report data obtained with no inhibitors present. **C.** The relative activity (normalized to its maximum) of the enzyme is plotted as functions of [ATP], [ADP], and [AMP] measured at [ACCOA] = 11 μM and [OAA] = 1.9 μM. **D.** The measured flux is plotted as a function of [ACCOA] at [OAA] = 34 μM with ATP, ADP, and AMP present as indicated in the figure. All data were obtained at pH = 7.4 at 25° C. Model fits are plotted as solid lines.

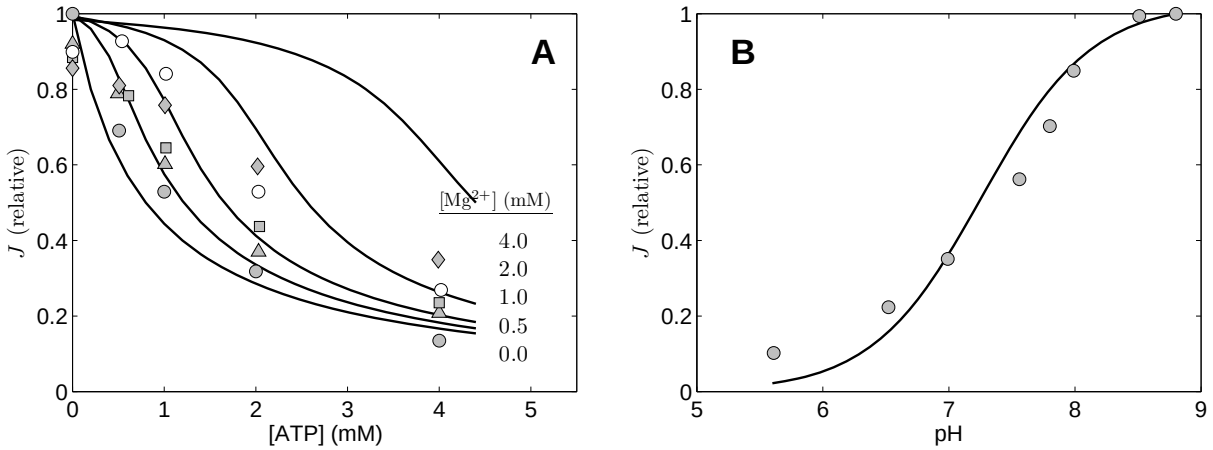

Figure 4: Impact of [Mg^{2+}] and pH on liver enzyme. Measured flux in arbitrary units was obtained from Figures 13 and 14 of [4]. **A.** The relative activity (normalized to its maximum) of the enzyme is plotted as functions of [ATP] at [Mg^{2+}] = 0 mM (shaded circles), 0.5 mM (shaded triangles), 1.0 mM (shaded squares), 2.0 mM (open circles), and 4.0 mM (diamonds). **B.** Relative activity is plotted as a function of pH. Substrate concentrations are [ACCOA] = 21 µM and [OAA] = 8.6 µM. All data were obtained at 25° C. pH is fixed a 7.4 for **A**. Model fits are plotted as solid lines.

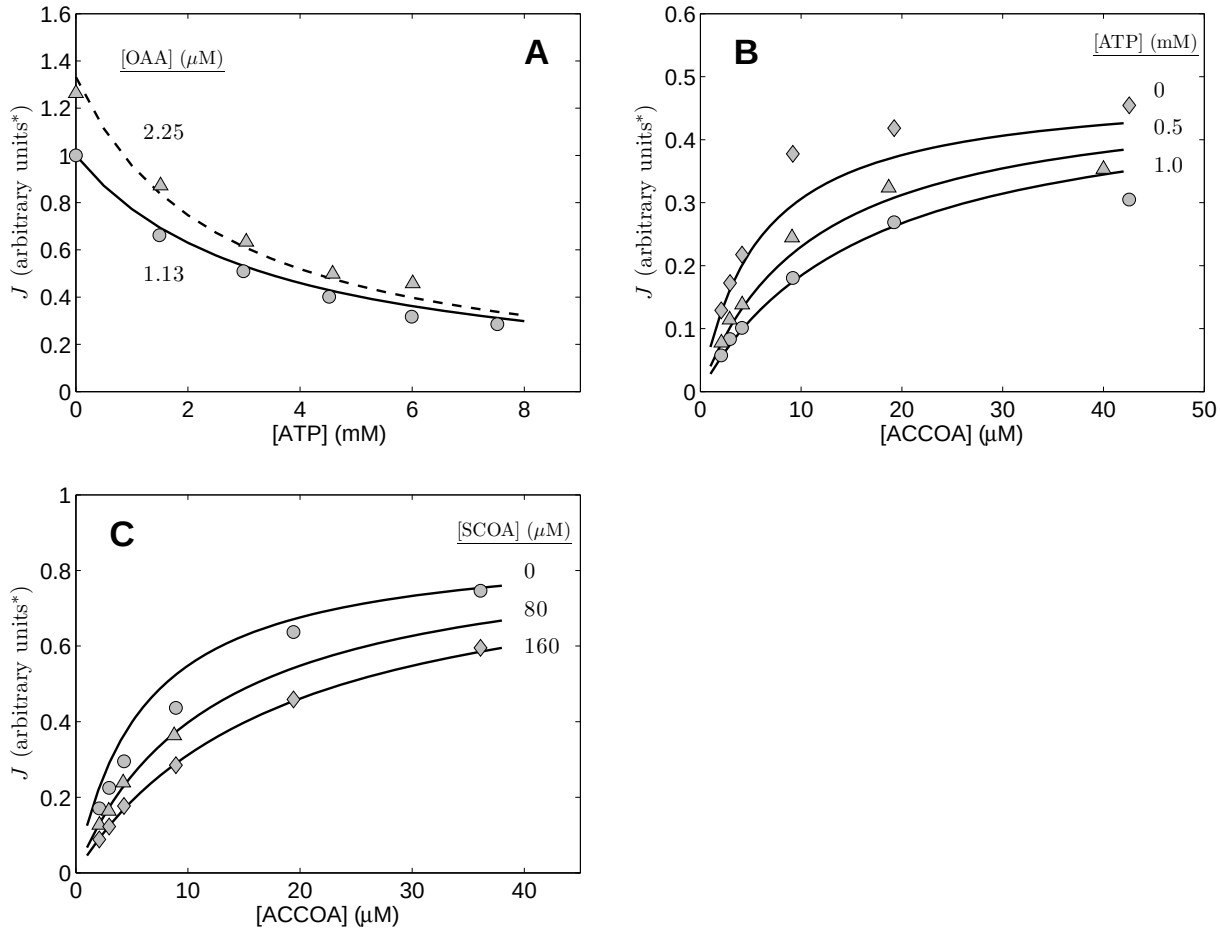

Figure 5: Inhibition of cardiac enzyme. Measured flux in arbitrary units was obtained from Figures 1 and 2 of [5]. **A.** Flux is plotted as a function inhibitor ATP concentration for [ACCOA] = 16 μM and [OAA] = 1.13 and 2.25 μM. **B.** Flux is plotted as a function of [ACCOA] at [OAA] = 5 μM at three different concentrations of ATP indicated in figure. **C.** Flux is plotted as a function of [ACCOA] at [OAA] = 3.1 μM at three different concentrations of SCOA indicated in figure. All data were obtained at pH = 7.4 at 21° C. Model fits are plotted as solid lines.

References

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3. Matsuoka, Y. and P.A. Srere, *Kinetic studies of citrate synthase from rat kidney and rat brain.* J Biol Chem, 1973. **248**(23): p. 8022-30.
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