A REINTERPRETATION OF THE KINETICS OF PYRUVATE CARBOXYLASE

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Pyruvate carboxylase (pyruvate:CO_2 ligase (ADP), EC 6.4.1.1) catalyses the reaction

$$\text{pyruvate} + CO_2 + MgATP^{2-} \rightarrow \text{oxaloacetate} + MgADP^{-} + Pi\ ;$$

the enzyme contains biotin and requires acetyl coenzyme A for activity. Keech and Barritt [1] investigated the effects of magnesium and ATP concentrations on the kinetics of the reaction, maintaining constant other factors which influence the reaction rate. They assumed that under their experimental conditions complex formation between Mg^{2+} and ATP^{4-} was complete. Table 1 shows the actual concentrations of Mg^{2+}, ATP^{4-}, and $MgATP^{2-}$ for points taken from their experimental curves. The calculations, which were done on the University of London

Table 1
Concentrations of Mg^{2+}, ATP^{4-}, and $MgATP^{2-}$ in the substrate mixtures used by Keech and Barritt [1]; the concentrations of Mg_{tot} and ATP_{tot} were taken from their figures.

	Mg_{tot} (M)	ATP_{tot} (M)	Mg^{2+} (M)	ATP^{4-} (M)	$MgATP^{2-}$ (M)
$Mg_{tot} = ATP_{tot}$	1.37×10^{-4}	1.37×10^{-4}	4.05×10^{-5}	2.99×10^{-5}	9.69×10^{-5}
	2.79×10^{-4}	2.79×10^{-4}	6.08×10^{-5}	4.48×10^{-5}	2.18×10^{-4}
	4.23×10^{-4}	4.23×10^{-4}	7.66×10^{-5}	5.65×10^{-5}	3.46×10^{-4}
	5.56×10^{-4}	5.56×10^{-4}	8.90×10^{-5}	6.56×10^{-5}	4.67×10^{-4}
	1.12×10^{-3}	1.12×10^{-3}	1.30×10^{-4}	9.55×10^{-5}	9.90×10^{-4}
	1.68×10^{-3}	1.68×10^{-3}	1.60×10^{-4}	1.18×10^{-4}	1.52×10^{-3}
$Mg_{tot} = ATP_{tot}$ + 1 mM	1.13×10^{-3}	1.34×10^{-4}	1.00×10^{-3}	1.65×10^{-6}	1.32×10^{-4}
	1.27×10^{-3}	2.69×10^{-4}	1.00×10^{-3}	3.29×10^{-6}	2.64×10^{-4}
	1.42×10^{-3}	4.16×10^{-4}	1.01×10^{-3}	5.08×10^{-6}	4.09×10^{-4}
	1.55×10^{-3}	5.54×10^{-4}	1.01×10^{-3}	6.74×10^{-6}	5.44×10^{-4}
	2.13×10^{-4}	1.13×10^{-3}	1.02×10^{-3}	1.36×10^{-5}	1.11×10^{-3}
$ATP_{tot} = Mg_{tot}$ + 0.4 mM	2.77×10^{-4}	6.77×10^{-4}	1.10×10^{-5}	3.03×10^{-4}	2.66×10^{-4}
	4.21×10^{-4}	8.21×10^{-4}	1.65×10^{-5}	3.07×10^{-4}	4.05×10^{-4}
	5.50×10^{-4}	9.50×10^{-4}	2.13×10^{-5}	3.11×10^{-4}	5.29×10^{-4}
	1.18×10^{-3}	1.58×10^{-3}	4.34×10^{-5}	3.27×10^{-4}	1.14×10^{-3}
	1.63×10^{-3}	2.03×10^{-3}	5.82×10^{-5}	3.38×10^{-4}	1.57×10^{-3}

'Atlas' computer, take into account complex formation between ATP^{4-} and Na^{+}; the extent of magnesium carbonate complex formation under their experimental conditions was negligible *.

Keech and Barritt's interpretation of their experimental data is invalid as it is based on the false assumption referred to above; however, it appears from their results that activation by magnesium ion or inhibition by ATP^{4-}, or a combination of both of these effects, must control the activity of pyruvate carboxylase. Eight models based on these alternatives have been examined in an attempt to elucidate the position. The models examined, with their corresponding rate equations, are as follows **:

(1) competitive inhibition of $MgATP^{2-}$ by ATP^{4-}

$$v = \frac{V[MgATP^{2-}]}{K_{mS}(1 + [ATP^{4-}]/K_i) + [MgATP^{2-}]};$$

(2) non-competitive inhibition by ATP^{4-}

$$v = \frac{V[MgATP^{2-}]}{(1 + [ATP^{4-}]/K_i)(K_{mS} + [MgATP^{2-}])};$$

(3) random order binding of Mg^{2+} and $MgATP^{2-}$, with no interaction between Mg^{2+} and $MgATP^{2-}$ binding sites

$$v = \frac{V[Mg^{2+}][MgATP^{2-}]}{(K_{mM} + [Mg^{2+}])(K_{mS} + [MgATP^{2-}])};$$

(4) as (3), but incorporating competitive inhibition of $MgATP^{2-}$ by ATP^{4-}

$$v = \frac{V[Mg^{2+}][MgATP^{2-}]}{(K_{mM} + [Mg^{2+}])(K_{mS}(1 + [ATP^{4-}]/K_i) + [MgATP^{2-}])};$$

(5) as (3), but incorporating non-competitive inhibition by ATP^{4-}

$$v = \frac{V[Mg^{2+}][MgATP^{2-}]}{(K_{mM} + [Mg^{2+}])(K_{mS} + [MgATP^{2-}])(1 + [ATP^{4-}]/K_i)};$$

(6) random order binding of Mg^{2+} and $MgATP^{2-}$, with interaction between Mg^{2+} and $MgATP^{2-}$ binding sites

$$v = \frac{V[Mg^{2+}][MgATP^{2-}]}{K_{mM}K_{mS}' + [Mg^{2+}]K_{mS}' + [MgATP^{2-}]K_{mM}K_{mS}'/K_{mS} + [Mg^{2+}][MgATP^{2-}]};$$

(7) compulsory order binding, with Mg^{2+} binding first

$$v = \frac{V[Mg^{2+}][MgATP^{2-}]}{K_{mM}K_{mS} + [Mg^{2+}]K_{mS} + [Mg^{2+}][MgATP^{2-}]};$$

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* Constants used in the calculations: final pK of ATP, 6.75 [2]; stability constant of $MgATP^{2-}$, 80,000 M^{-1} [3]; stability constant of $NaATP^{3-}$, 15 M^{-1} [3]. Other relevant conditions, as stipulated by Keech and Barritt [1]: pH 8.0; $[Na^{+}]$, 20 mM.

** Abbreviations: v, the reaction rate; V, the maximum velocity on the same scale; K_{mS}, the K_m for $MgATP^{2-}$; K_{mM}, the K_m for Mg^{2+}; K_{mS}', the K_m for $MgATP^{2-}$ if this is different from K_{mS} when Mg^{2+} binds first to the enzyme; K_i, the inhibitor-enzyme dissociation constant.

(8) compulsory order binding, with $MgATP^{2-}$ binding first

$$v = \frac{V[Mg^{2+}]\,[MgATP^{2-}]}{K_{mM}\,K_{mS} + [MgATP^{2-}]K_{mM} + [Mg^{2+}]\,[MgATP^{2-}]}\,.$$

This by no means exhausts the possibilities, but is sufficient for present purposes.

Using the concentrations of Mg^{2+}, ATP^{4-}, and $MgATP^{2-}$ given in table 1, the constants in each equation were adjusted by a computer programme to obtain the best fit to Keech and Barritt's experimental data. Models (1), (2), and (7) could not be made to fit the data. Model (3) gave a reasonable fit to the data with $V = 44$, $K_{mS} = 4.97 \times 10^{-4}$ M, and $K_{mM} = 7.84 \times 10^{-4}$ M; the continuous curves in fig. 1 were drawn using equation (3) with these values of the constants. Keech and Barritt's experimental points are superimposed. Model (4) gave no improvement over model (3), but model (5), which incorporates non-competitive inhibition by ATP^{4-}, did result in some improvement as indicated by the discontinuous curves in fig. 1. The constants used here were $V = 40$, $K_{mS} = 6.16 \times 10^{-4}$ M, $K_{mM} = 4.28 \times 10^{-4}$ M, and $K_i = 3.0 \times 10^{-4}$ M. Model (6) also resulted in some improvement over model (3), with constants $V = 82$, $K_{mS} = 2.28 \times 10^{-4}$ M, $K_{mM} = 2.75 \times 10^{-4}$ M, and $K_{mS} = 1.56 \times 10^{-3}$ M. Model (8) fitted the data, but much less convincingly than model (3). It is not surprising that models (5) and (6) fit the experimental data better than model (3) since they both involve 4 constants instead of 3.

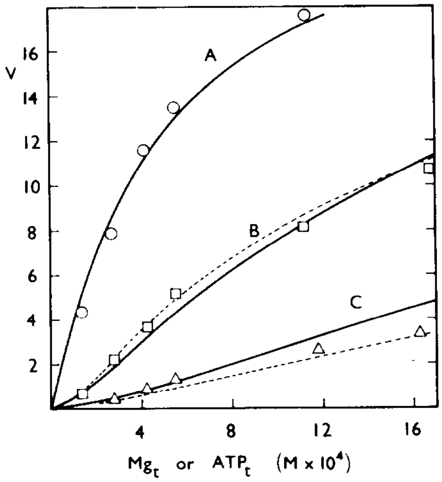

Fig. 1. The reaction velocity of pyruvate carboxylase as a function of total magnesium (Mg_t) and total ATP (ATP_t) concentrations. The continuous curves were drawn using the equation for model (3) in the text, and the discontinuous curves using the equation for model (5), each with the relevant set of values for the constants given in the text. Curve A is almost identical in the two cases. $[Mg^{2+}]$, $[ATP^{4-}]$, and $[MgATP^{2-}]$ were calculated from Mg_t and ATP_t. Curve A is for $Mg_t = ATP_t + 1$ mM; curve B is for $Mg_t = ATP_t$; curve C is for $ATP_t = Mg_t + 0.4$ mM. The left hand velocity scale is for curve A, and the right hand scale for curves B and C. The horizontal scale is for the lesser of Mg_t and ATP_t. Keech and Barritt's experimental points are superimposed.

It is clear from these results than both Mg^{2+} and $MgATP^{2-}$ are required for pyruvate carboxylase to have activity, and that binding of these two species probably occurs in a random order; the binding of one of these two species may affect the binding of the second. It is possible that ATP^{4-} may be a non-competitive inhibitor of pyruvate carboxylase.

As the K_m values for magnesium ion in the models which fit the experimental data are near the probable physiological magnesium ion concentration in tissues [4,5], the magnesium ion concentration must be considered as an important controlling factor in the reaction catalysed by pyruvate carboxylase.

References

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