METAL IONS AND ENZYME EQUILIBRIA: A MATHEMATICAL TREATMENT

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Where one or more of the substrates of a reversible enzyme catalysed reaction can form complexes with metal ions, the apparent equilibrium constant of the reaction is markedly affected by the metal ion concentration. In particular this has been observed for aconitase (aconitate hydratase, EC 4.1.2.3) [1,2], creatine kinase (ATP: creatine phosphotransferase, EC 2.7.3.2) [3], and adenylate kinase (ATP: AMP phosphotransferase, EC 2.7.4.3) [4]. Askonas [10] derived equations for a general case to show how the equilibrium constant changes with the concentration of both free metal ion and total metal. A method for deriving similar equations, relating the apparent equilibrium constant to the metal ion concentration, the stability constants of substrate-metal complexes and the apparent equilibrium constant at zero metal ion concentration, and which takes pH into account, is herewith presented. The validity of the method is confirmed by experimental measurement.

The method for deriving the equation for magnesium and the aconitase equilibrium is as follows. The apparent equilibrium constant is taken as the total-citrate/total-isocitrate concentration ratio, and, without making an assumption about the true substrate species, it is assumed that $[C^{3-}]/[I^{3-}]$ * remains constant at equilibrium as $[Mg^{2+}]$ is varied. At physiological pH and zero $[Mg^{2+}]$

$$C_t = [C^{3-}] + [HC^{2-}] = [C^{3-}]\,(1 + [H^+]\,K_c) = [C^{3-}]\,X\,,$$

putting $X = (1 + [H^+]\,K_c)$. With $[Mg^{2+}]$ low, MgHC will not be a significant species, and so

$$C_t = [C^{3-}] + [HC^{2-}] + [MgC^-] = [C^{3-}]\,(1 + [H^+]\,K_c + [Mg^{2+}]\,Ks_c) = [C^{3-}]\,X'\,,$$

where $X' = (1 + [H^+]\,K_c + [Mg^{2+}]\,Ks_c)$. The corresponding equations for isocitrate, using the relevant constants, are

$$I_t = [I^{3-}]\,Y \quad \text{and} \quad I_t = [I^{3-}]\,Y'\,.$$

Now $K(app) = C_t/I_t$ so that $K(app)_o = \dfrac{[C^{3-}]\,X}{[I^{3-}]\,Y}$

and

* Abbreviations: K(app), apparent equilibrium constant; $K(app)_o$, apparent equilibrium constant at zero $[Mg^{2+}]$; C_t, I_t, ATP_t, ADP_t, AMP_t, Cr_t, CP_t, total concentrations of citrate, isocitrate, ATP, ADP, AMP, creatine, and creatine phosphate; C^{3-}, I^{3-}, HC^{2-}, $MgATP^{2-}$, etc., represent the citrate tri-anion, isocitrate tri-anion, citrate di-anion, the complex between Mg^{2+} and ATP^{4-} etc.; Ks_c, Ks_i, Ks_{atp}, Ks_{cp}, etc., are the stability constants of the complexes between magnesium and the indicated substrates (fully ionised); K_c, K_{atp}, etc., are the reciprocals of the last acid dissociation constants of the indicated substrates e.g., $K_c = [HC^{2-}]/[H^+][C^{3-}]$.

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$$K(app) = \frac{[C^{3-}]X'}{[I^{3-}]Y'} = K(app)_o \frac{YX'}{XY'}.$$

This expression is independent of the total concentrations of magnesium and substrates.

For the adenylate kinase equilibrium,

$$K(app) = \frac{ATP_t \cdot AMP_t}{ADP_t^2}$$

and without making assumptions about true substrate species it is assumed that $[ATP^{4-}][AMP^{2-}]/[ADP^{3-}]^2$ is constant at equilibrium irrespective of total substrate and magnesium concentrations. In the absence of magnesium

$$ATP_t = [ATP^{4-}](1 + [H^+]K_{atp}) = [ATP^{4-}]R$$

and in the presence of magnesium

$$ATP_t = [ATP^{4-}](1 + [H^+]K_{atp} + [Mg^{2+}]Ks_{atp}) = [ATP^{4-}]R'.$$

Likewise, using the relevant constants

$$ADP_t = [ADP^{3-}]P \quad \text{or} \quad ADP_t = [ADP^{3-}]P'$$

and

$$AMP_t = [AMP^{2-}]Q \quad \text{or} \quad AMP_t = [AMP^{2-}]Q'$$

in the absence or presence of magnesium; then in the same way as for the aconitase equilibrium

$$K(app) = K(app)_o \frac{R'Q'PP}{P'P'RQ}.$$

With creatine kinase, as an alternative to using $K(app)_o$, the true equilibrium constant has been used; this is defined by Morrison and White [3] as

$$K_T = \frac{[MgADP^-][CP]}{[MgATP^{2-}][Cr]}.$$

Now

$$K(app) = \frac{ADP_t \cdot CP_t}{ATP_t \cdot Cr_t}$$

from which, using the methods outlined above, it can be deduced that

$$K(app) = K_T \cdot \frac{Ks_{atp}P'Z'}{Ks_{adp}R'} \quad \text{where} \quad Z' = 1 + [Mg^{2+}]Ks_{cp}.$$

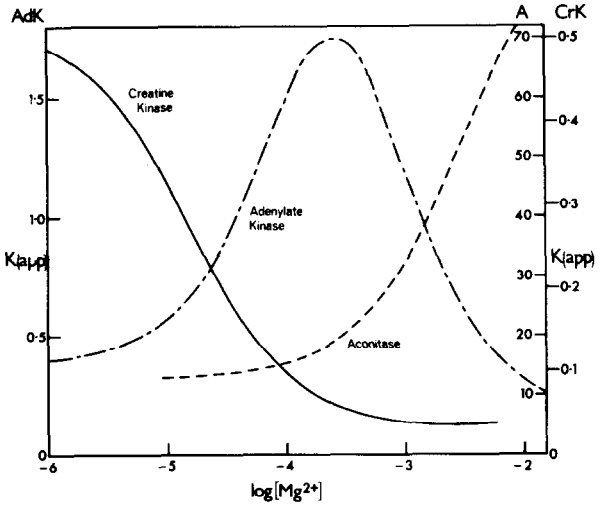

Fig. 1. Variation of K(app) with $[Mg^{2+}]$ for adenylate kinase (AdK) aconitase (A(and creatine kinase (CrK). The curves are drawn from equations derived in the text.

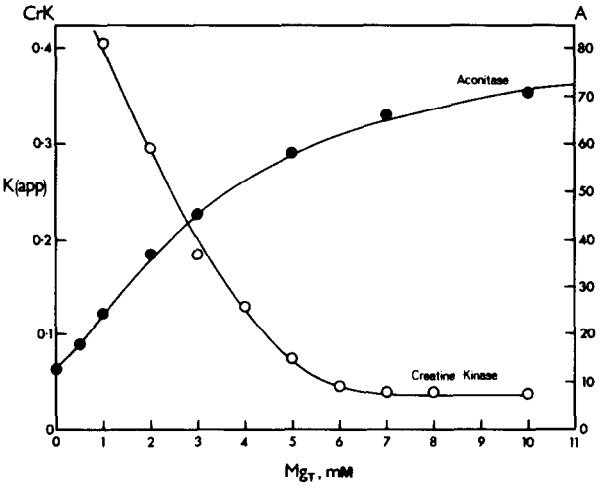

Fig. 2. Theoretical curves of the variation of K(app) with Mg_t for creatine kinase and aconitase, with experimental points superimposed.

By using suitable values for the constants, and experimentally determined values of $K(app)_o$ or K_T, the variation of the apparent equilibrium constant for each of these equilibria can be plotted against magnesium ion concentration (fig. 1) *. These curves allow $[Mg^{2+}]$ to be determined from experimental values of K(app). The variation of the apparent equilibrium constants with magnesium ion concentration is so profound that it is unlikely to have escaped useful application during the course of evolution.

The above equations can be modified so that the total magnesium concentration can be calculated for given total substrate concentrations and a given magnesium ion concentration. The validity of the treatments can thus be confirmed by a direct comparison with experimental results. The approach is illustrated with the modification developed for creatine kinase, using Morrison and White's initial conditions [3], where ATP_t = 1 mM and $ADP_t = CP_t$ = 5 mM. Let $ATP_t + ADP_t = D$, and $CP_t + Cr_t = D'$ then $ADP_t = CP_t = D - ADP_t$ and $Cr_t = D' - D + ATP_t$ whence, at equilibrium, $K(app) = (D-ATP_t)^2/ATP_t(D' - D + ATP_t)$. This equation, which is a quadratic in ATP_t, can be solved. With ATP_t known, ADP_t and CP_t are also known; then

$$Mg_t = [Mg^{2+}](1 + Ks_{atp}ATP_t/R' + Ks_{adp}ADP_t/P' + Ks_{cp}CP_t/Z') .$$

The curve relating K(app) to total magnesium concentration for creatine kinase using the equation with Morrison and White's conditions is shown in fig. 2, with Morrison and White's experimental points superimposed. Fig. 2 also shows the theoretical curve for aconitase with $C_t + I_t$ = 1 mM, with the authors experimental points superimposed. Only qualitative agreement is available for the adenylate kinase equilibrium (see Bowen and Kerwin [4]). All these derived relationships are sensitive to the values of the stability constants used; Bowen and Kerwin used tris buffer which is known to affect the value of the stability constant of $MgATP^{2-}$ [5].

* Logarithms of stability constants used in the calculations and their sources, are as follows for K_C, 5.84 [6]; K_i, 5.75 [7]; K_{atp}, 6.53 [8]; K_{adp}, 6.44 [8]; K_{amp}, 6.21 [8]; Ks_c, 3.377 [9]; Ks_i, 2.522 [9]; Ks_{atp}, 4.845 [3]; Ks_{adp}, 3.60 [3]; Ks_{amp}, 1.95 [8] Ks_{cp}, 1.6 [3]. $K(app)_o$ for aconitase was taken as 12.79 [9], and for adenylate kinase as 0.375 [4]. K_T for creatine kinase was taken as 0.030 [3]. A pH of 7.5 was standard in all the calculations.

Other modifications of the equations allow $K(app)_o$ and the substrate-magnesium stability constants to be calculated from a set of experimental results. Details of this treatment as applied to the aconitase equilibrium will be published elsewhere.

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