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Beryllium(II) binding to ATP and ADP: Potentiometric determination of the thermodynamic constants and implications for *in vivo* toxicity

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Abstract

Highly toxic beryllium(II) is divalent metal ion with a high charge density, making it a potential target for binding to bio-molecules rich in O donor groups. In aqueous solution Be^{2+} binds to ATP and ADP to form 1:1 Be^{2+}:ATP and Be^{2+}:ADP complexes in relatively acidic media. At neutral pH the complex formed undergoes hydrolysis. Be^{2+} binding to ATP and ADP is much stronger than Ca^{2+} and Mg^{2+} binding. The high affinity of Be^{2+} toward ATP and ADP binding suggests a mechanism relevant to understanding the *in vivo* chemical toxicity of this metal.

Introduction

There has been an increased interest in recent years in developing an understanding of the bio-coordination chemistry of Be^{2+} in relation to its *in vivo* toxicity. The hydrated cation, $Be^{2+}{}_{aq}$, or one of its ligated forms such as $BeF_4{}^{2-}$ or $Be(OH)_2$, has been suggested to bind at the specific Ca^{2+} binding sites of ATPases, and that this is an important chemical mechanism responsible for the toxicity exhibited by $Be^{2+}{}_{aq}$. Previous studies demonstrated that exposure of cultured macrophages to nM concentrations of $BeCl_2$ induced large oscillating increases in intracellular Ca^{2+} concentrations (Lewis *et al.* 1999). The source of Ca^{2+} was largely from the extracellular environment suggesting that inhibition of membrane Ca^{2+} dependent ATPases was a possible mechanism by which $BeCl_2$ caused the increases in intracellular Ca^{2+} concentrations. It is possible that the observed oscillations in the Ca^{2+} concentrations were related first to Be^{2+} assisted dissociation of Ca^{2+} from ATPase by directly competing with Ca^{2+} for ATPase binding, and then the marked increases in intracellular Ca^{2+} displaced the Be^{2+}, restoring the activity of the ATPases. The competition between Be^{2+} and Ca^{2+} for ATPase binding can strongly affect Ca^{2+} active transport mediated by the Ca^{2+}-ATPase (membrane protein) present in sacroplasmic reticulum, which catalyzes the transport of Ca^{2+} (Hasselbach & Makinose 1961; MacLennan *et al.* 1985; Campbell *et al.* 1991). The Ca^{2+} ion is transported in an energy dependent process involving the hydrolysis of one ATP per two Ca^{2+} ions transported (Inesi *et al.* 1988; Bigelow & Ines: 1992). The first step in the global process consists of two Ca^{2+} ions binding to the ATPase enzyme. This reaction is facilitated by the presence of high Ca^{2+} affinity sites in the protein that can likely bind Be^{2+} in a competitive way.

Be^{2+} interference with ATPase catalyzed reactions can result from the formation of M-ADP-BeX complexes (M = divalent metal cation). Such species have been reported to be responsible for the ATPase inhibition in myosine ATPase (Phan & Reisler 1992) and P-glycoprotein ATPase (Sankaran *et al.* 1997). Herein, we report the thermodynamic parameters for Be^{2+} binding to ATP and ADP, and their possible significance with respect to *in vivo* Be^{2+} toxicity.

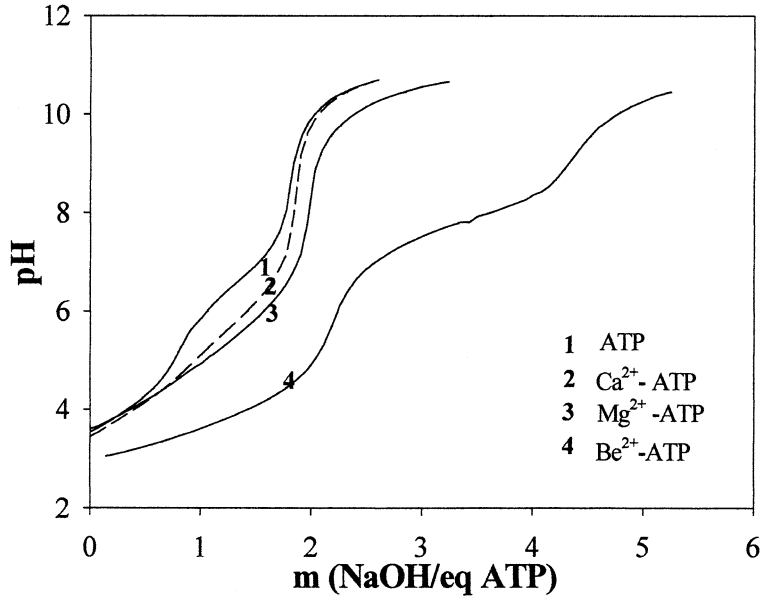


Fig. 1. Potentiometric pH titration curves for 1:1 molar ratios of metal ions to ATP. m is the number of equivalents of base (NaOH) added per mole of ATP. Conditions: [ATP] = 1.57×10^{-3} M, [Ca^{2+}] = 1.60×10^{-3} M, [Mg^{2+}] = 1.65×10^{-3} M, [Be^{2+}] = 1.62×10^{-3} M, and T = 25 °C. All solutions contain 0.10 M $NaClO_4$.

Experimental

Materials

All solutions were prepared in deionized water. NaOH stock solution was prepared by dilution from a 0.1 M NaOH stock solution and standardized by titration with HCl standard solution. Stock solutions of 1.0 M $NaClO_4$ were prepared from solid sodium perchlorate hydrate (Aldrich 99+%) and standardized by passing through a Dowex 50 W-X8 strong acid cation-exchange column in H^+ form. This stock solution was diluted as required to adjust the ionic strength to $I = 0.1$ M. All ADP and ATP solutions were prepared prior to their utilization from their sodium salts ($NaH_2ADP \cdot 3H_2O$ and $Na_2H_2ATP \cdot H_2O$; Aldrich 99+%). Metal ion solutions (0.02 M) for calcium and magnesium were prepared from their perchlorate salts ($Ca(ClO_4)_2$ and $Mg(ClO_4)_2$) and standardized by titration with EDTA.

Methods

All pH measurements were made using a Corning 250 pH/ion meter equipped with an Orion ROSS pH electrode (model 8103 ROSS Combination Electrode) filled with 3.0 M NaCl. Titrations were conducted under N_2 in a double-jacketed titration vessel maintained at 25 °C and continually stirred. All titrations were performed by adding 0.0096 M NaOH in 0.02 ml increments using a SCOTT Titronic 96 burette. A 10 ml aliquot of a ca. 1.5 mM solution of each ligand was titrated with a 0.0096 M NaOH solution. The deprotonation constants were obtained by refining the experimental data with the software SUPERQUAD. The metal-ligand binding constants were obtained from titrations of the metal complex solution prepared in 1:1 metal to ligand ratio.

Results and discussion

Both ATP and ADP form stable Be-ATP and Be-ADP complexes in acidic media. Potentiometric titrations carried out at 1:1 M^{2+}:ATP ratio (M = Ca, Mg, Be; Figure 1, curves 2–4) show pH *vs* added base behavior distinctly different from the titration of free ATP (Figure 1, curve 1). Titration curve 4 for Be^{2+} shows two inflection points at m = 2 and m = 4 with two sharp pH jumps at about pH = 5 and pH = 9, demonstrating the formation of multiple species. The Be^{2+} complex formed in the first buffer region (pH = 3 to 4.5) is stable up to pH = 5; at higher pH, the competition of OH^- leads to the formation of hydroxo species. The complex formed in the second buffer region between about pH = 6.5 and pH = 9 involves two protons. Titration data for Ca^{2+} and Mg^{2+} ATP binding (Fig-

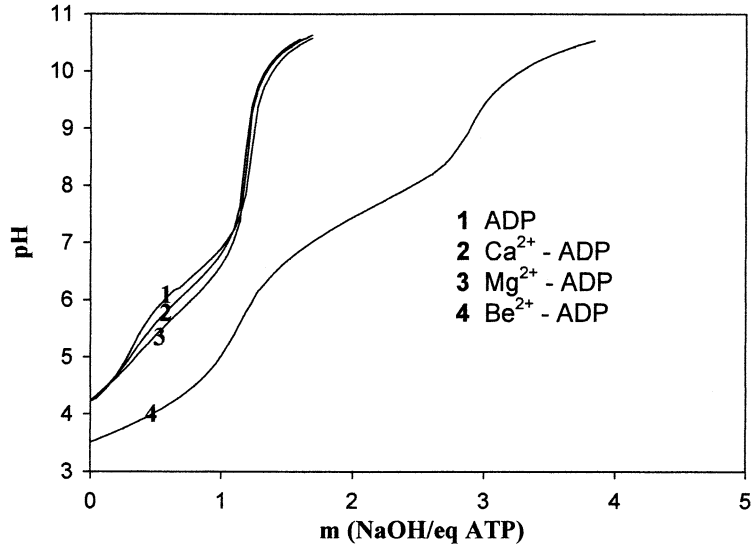


Fig. 2. Potentiometric pH titration curves for 1:1 molar ratios of metal ions to ADP. m is the number of equivalents base (NaOH) added per mole of ADP. Conditions: Curve 1, [ADP] = 2.1×10^{-3} M. Curve 2 [ADP] = 1.71×10^{-3} M, [Ca^{2+}] = 1.84×10^{-3} M. Curve 3 [ADP] = 1.81×10^{-3} M, [Mg^{2+}] = 2.0×10^{-3} M. Curve 4 [ADP] = 2.0×10^{-3} M, [Be^{2+}] = 1.85×10^{-3} M, and T = 25 °C. All solutions contain 0.10 M $NaClO_4$.

ure 1, curves 2 and 3) show a progressive pH increase up to about pH = 7, followed by a sharp pH increase at about pH = 8, suggesting that the complexes are formed around neutral pH. Titration data for the free ligand ATP and all M^{2+}:ATP or ADP (see Figure 2) experiments were modeled numerically by considering two protonation equilibria for the ligand (ATP or ADP) and the formation of Ca^{2+}ATP, Mg^{2+}ATP, Be^{2+}ATP, Be^{2+}ATP(OH) and $Be^{2+}ATP(OH)_2$ (and the corresponding ADP) complexes, as illustrated in equations (1)–(4).

$$ATP^{4-} + H^{+} \rightleftharpoons ATPH^{3-} \qquad \beta_{011} \qquad (1)$$

$$ATP^{4-} + 2H^{+} \rightleftharpoons ATPH_2^{2-} \qquad \beta_{012} \qquad (2)$$

$$ATP^{4-} + M^{2+} \rightleftharpoons ATPM^{2-} \qquad \beta_{110} \qquad (3)$$

$$ATPM^{2-} + OH^{-} \rightleftharpoons ATPM(OH)^{3-} \qquad \beta_{11\text{-}1} \qquad (4)$$

Protonation constants for ADP and ATP are listed in Table 1 and are consistent with previous literature reports (Shanbhag & Choppin 1987). The overall formation constants for the complexes formed with the deprotonated ligand were determined through numerical fitting of the data by the software SUPERQUAD. The calculated stability constants for the complexes formed are presented in Table 2. Our results for Ca^{2+} and Mg^{2+} binding are consistent with literature reports, (Shanbhag & Choppin 1987) which verifies our direct comparison of Be^{2+} complexation with these *in vivo* cations.

Table 1. Protonation constants for ATP and ADP, $I = 0.1$ ($NaClO_4$), $T = 25$ °C.

	log β_{011} [a]	log β_{012} [a]
ATP	6.62 ± 0.02	10.49 ± 0.06
	6.51[b]	10.54[b]
ADP	6.41[b]	10.36[b]

[a]Defined in equations [1] and [2].
[b]Data from Shanbhag & Choppin (1987); $I = 0.1$ $NaClO_4$, $T = 25$ °C.

Figure 3 demonstrates that the affinity of divalent metal ions for ATP binding is linearly correlated with the affinity of the metal ion for the OH^{-} ion. These data demonstrate very strong Be^{2+} binding to ATP relative to all of the other metals plotted. The structures of M-ATP and M-ADP complexes are not well identified as a result of the multiple binding mode capability of the ATP (see ATP structure) (Huang & Tsai 1962; Bishop *et al.* 1981; Shanbhag & Choppin 1987). Taking OH^{-} as the archetypical hard oxygen donor ligand, this linear correlation in Figure 3 suggests binding of these metals to ATP (and ADP) at the O donor sites.

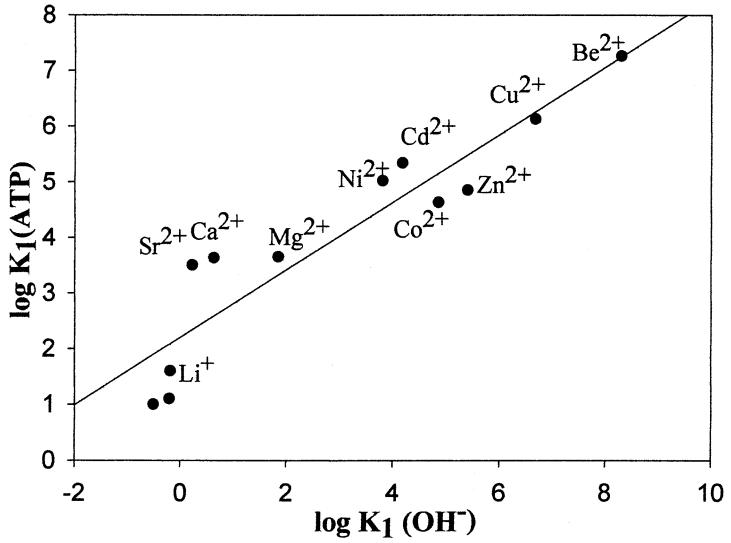
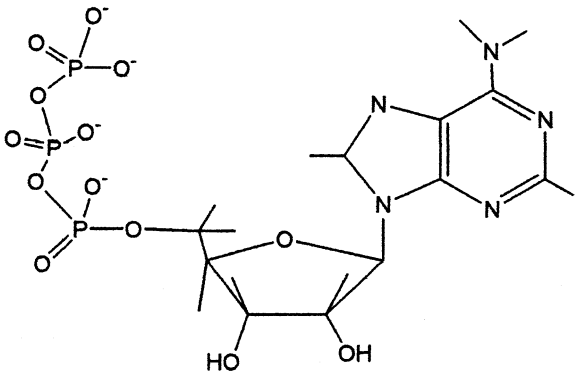


Fig. 3. Plot of log K_1 values for ATP vs. log $K_1(OH^-)$ values for various metal ions. Data for Be^{2+}, Mg^{2+}, and Ca^{2+} are from this work; all other data are from references Huang & Tsai (1962), Martell & Smith (1974–1982), Bishop *et al.* (1981), Shanbhag & Choppin (1987).



Structure of adenosine triphosphate (ATP)

The thermodynamic stability constants for ATP and ADP binding to Be^{2+} are several orders of magnitude higher than Ca^{2+} and Mg^{2+} binding (Figure 3). This demonstrates that Be^{2+}, even in relatively low concentrations compared to Ca^{2+} and Mg^{2+}, will effectively block these metals from ATP binding. Our results demonstrate that in the presence of Be^{2+} the active transport of Ca^{2+} will be altered by the formation of Be-ATP complexes. For example, the data in Table 2 illustrate that the ratio of free intracellular $Be_{aq}{}^{2+}$ to $Ca_{aq}{}^{2+}$ concentrations need only be 0.0001 to 1 for 50% of the Ca^{2+} bound to ATP to be displaced. Comparable ratios apply to Mg^{2+} displacement and to competitive binding to ADP.

Adenosine phosphates are present in all forms of life in mM concentrations and play an important role in enzyme activity. There is substantial evidence that the nucleotide participates in enzyme activity as a metal-nucleotide complex. Our results show that Be^{2+} is a potential target for ATP binding and that Be^{2+} out competes Ca^{2+} and Mg^{2+} for ATP and ADP binding, suggesting a possible mechanism for *in vivo* beryl-

Table 2. Binding constant of Mg^{2+}, Ca^{2+} and Be^{2+} to ATP and ADP, $I = 0.1$ ($NaClO_4$), $T = 25$ °C

ATP	log β_{110}[a]	log β_{11-1}[b]
Ca^{2+}	3.63 ± 0.40	
	3.70[c]	
Mg^{2+}	3.54 ± 0.11	
	4.03[c]	
Be^{2+}	7.62 ± 0.05	0.00 ± 0.15
ADP		
Ca^{2+}	2.94 ± 0.49	
	2.90[c]	
Mg^{2+}	3.48 ± 0.10	
	3.28[c]	
Be^{2+}	7.23 ± 0.07	0.21 ± 0.10

[a]Defined in equation [3].
[b]Defined in equation [4].
[c]Data from reference Shanbhag & Choppin (1987); $I = 0.1$ $NaClO_4$, $T = 25$ °C. Values of log β_{110} from this reference use log $\beta_{012} = 10.54$ for ATP and log $\beta_{012} = 10.36$ for ADP.

lium toxic activity through interference with enzyme activity involving ATP.

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