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COMPARISON OF THE PROPERTIES OF THE Ca^{2+} AND Cd^{2+} COMPLEXES OF GUANOSINE 5'-MONOPHOSPHATE (GMP) IN AQUEOUS SOLUTION

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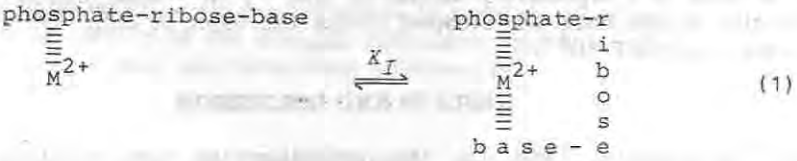
ABSTRACT. The stability constants of 1:1 complexes formed between Ca^{2+} or Cd^{2+} and GMP^{2-} were determined by potentiometric pH titration in aqueous solution (I = 0.1 M, $NaNO_3$; 25°C). For comparison, the corresponding data for the AMP^{2-} complexes are included and taken from our earlier work. The extent of macrochelate formation in these complexes is calculated; the macrochelates form by an interaction of the phosphate-coordinated metal ion with N-7 of the purine base-residue. In Cd(GMP) and Cd(AMP) this base-backbinding is considerable, while only traces of macrochelates are formed with Ca(GMP) and none for Ca(AMP). The different properties of the Ca^{2+} and Cd^{2+} complexes show that great care must be exercised if Cd^{2+} is used as a probe for Ca^{2+} sites in systems containing nucleotides.

INTRODUCTION

The physiological role of nucleotides is closely linked to the presence of metal ions (1,2). This has initiated much interest in the corresponding metal ion complexes. For example, the solution structures of several metal ion (M^{2+}) complexes of adenosine 5'-triphosphate (ATP^{4-}) (3) and of 1,N^6-ethenoadenine nucleotides (4) are relatively well established.

Presently we are investigating the stability and structure of nucleoside monophosphate (NMP^{2-}) complexes in solution. We have shown previously that for the complexes of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} with cytidine 5'-monophosphate (CMP^{2-}) or uridine 5'-monophosphate (UMP^{2-}) the stability is solely determined by the basicity of the phosphate residue (5). However, in the case of adenosine 5'-monophosphate (AMP^{2-}) the 3d ions, as well as Zn^{2+} and Cd^{2+} form macrochelates (6) according to equation 1:



(1)

Our next aim is to study the complexes of guanosine 5'-monophosphate (GMP^{2-}) and to compare their stabilities and structures with those of the corresponding and previously studied (6) AMP^{2-} complexes (Fig. 1). We report here the stability and extent of macrochelate formation for the Ca(GMP) and Cd(GMP) complexes.

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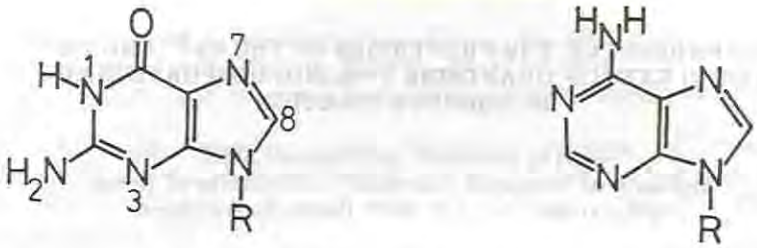

R = -ribosyl 5'-monophosphate

GMP^{2-} AMP^{2-}

Fig. 1. Chemical structure of the nucleoside 5'-monophosphates (NMP^{2-}) considered in this study, i.e. of guanosine 5'-monophosphate (GMP^{2-}) and adenosine 5'-monophosphate (AMP^{2-}).

A comparison of the properties of Ca(GMP) and Cd(GMP) is of interest for the following reason: Ca^{2+} has important roles in a number of physiological functions, as for instance, in the excitation of nerves and muscles (7). Since this ion is not very suitable for spectroscopic studies, Ca^{2+} sites in biological systems are often probed by other metal ions such as Cd^{2+}. The effective ionic radii of Ca^{2+} only slightly exceed those of Cd^{2+} for a given coordination number (8) and this has led to the use of $^{113}Cd^{2+}$ as a NMR probe for Ca^{2+}. The results presented now confirm previous conclusions (8,9) that great care should be exercised if Cd^{2+} is used as a probe for Ca^{2+} sites.

EXPERIMENTAL

The disodium salt of guanosine 5'-monophosphate was purchased from Sigma Chemical Co., St. Louis, Mo, USA. All the other reagents were the same as used previously (5).

The potentiometric pH titrations were performed and evaluated exactly as described (5) for binary M(NMP) complexes of pyrimidine-nucleoside 5'-monophosphates and of some simple phosphate monoesters. The stability constants, $K^{M}_{M(GMP)}$, of the complexes were computed by taking into account the species H^+, $H_2(GMP)^{\pm}$, $H(GMP)^-$, GMP^{2-}, M^{2+} and $M(GMP)$ (5,6). The collection of the data was stopped before the onset of M^{2+}_{aq} hydrolysis and the formation of $(GMP-H)^{3-}$.

RESULTS AND DISCUSSION

The experimental data for the potentiometric pH titrations could be satisfactorily explained by taking into account the following three equilibria:

$$H_2(NMP)^{\pm} \rightleftharpoons H(NMP)^- + H^+ \qquad (2a)$$

$$K^{H}_{H_2(NMP)} = \frac{[H(NMP)^-][H^+]}{[H_2(NMP)]} \qquad (2b)$$

$$H(NMP)^{-} \rightleftharpoons NMP^{2-} + H^{+} \quad (3a)$$

$$K^{H}_{H(NMP)} = \frac{[NMP^{2-}][H^{+}]}{[H(NMP)^{-}]} \quad (3b)$$

$$M^{2+} + NMP^{2-} \rightleftharpoons M(NMP) \quad (4a)$$

$$K^{M}_{M(NMP)} = \frac{[M(NMP)]}{[M^{2+}][NMP^{2-}]} \quad (4b)$$

The corresponding equilibrium constants are listed in Table 1 together with the previous results (6) obtained for the Ca(AMP) and Cd(AMP) complexes.

Table 1. Comparison of the measured stability, $K^{M}_{M(NMP)}$ (equation 4b), of the Ca^{2+} and Cd^{2+} 1:1 complexes of GMP^{2-} and AMP^{2-} (cf.[a]) with the calculated stability, $K^{M}_{M(NMP)_{op}}$, for an isomer with only a M^{2+}/phosphate coordination[b], and extent of a possible stability increase as defined by log Δ (equation 5). All constants refer to aqueous solutions at 25°C and I = 0.1 M ($NaNO_3$)[c].

M(NMP)	log $K^{M}_{M(NMP)}$	log $K^{M}_{M(NMP)_{op}}$[b]	log Δ
Ca(GMP)	1.53 ± 0.01	1.46 ± 0.05	0.07 ± 0.05
Ca(AMP)	1.46 ± 0.01	1.46 ± 0.05	0.00 ± 0.05
Cd(GMP)	2.98 ± 0.02	2.45 ± 0.06	0.53 ± 0.06
Cd(AMP)	2.68 ± 0.02	2.44 ± 0.06	0.24 ± 0.06

[a]Acidity constants (equations 2 and 3): $pK^{H}_{H_2(GMP)} = 2.43 \pm 0.05$, $pK^{H}_{H(GMP)} = 6.25 \pm 0.02$; $pK^{H}_{H_2(AMP)} = 3.84 \pm 0.02$, $pK^{H}_{H(AMP)} = 6.21 \pm 0.01$. The proton in $H(NMP)^{-}$ is at the phosphate group; addition of a further proton gives $H_2(NMP)^{\pm}$ with the proton at N-7 in $H_2(GMP)$ and at N-1 in $H_2(AMP)^{\pm}$.

[b]Calculated with $pK^{H}_{H(NMP)}$ and the baseline equations of Figure 2 (see legend).

[c]All equilibrium constants were determined by potentiometric pH titrations. The range of error given with the constants is 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. All constants involving AMP are taken from reference (6).

Macrochelate formation as shown in equation 1 will result in increased complex stability (9), i.e. by a greater stability than determined by the basicity of the phosphate group alone (6). This enhanced complex stability (log Δ) is defined in equation 5:

$$\log \Delta = \log K^{M}_{M(NMP)} - \log K^{M}_{M(NMP)_{op}} \quad (5)$$

where log $K^{M}_{M(NMP)}$ includes all M(NMP) species present and log $K^{M}_{M(NMP)_{op}}$ quantifies the stability of the open form as shown in equation 1. The value of the former can be determined experimentally (equation 4a) while the latter

can be computed from log $K^{M}_{M(R-MP)}$ versus $pK^{H}_{H(R-MP)}$ plots based on simple phosphate monoester ligands ($R\text{-}MP^{2-}$).
It is evident from Fig. 2 that the Cd(GMP) and Cd(AMP) complexes are considerably more stable than expected, strongly suggesting the existence of the machrochelated species shown in equation 1. This is in contrast to the points (Fig.2) due to Ca(GMP) and Ca(AMP) which are much closer to the baseline.

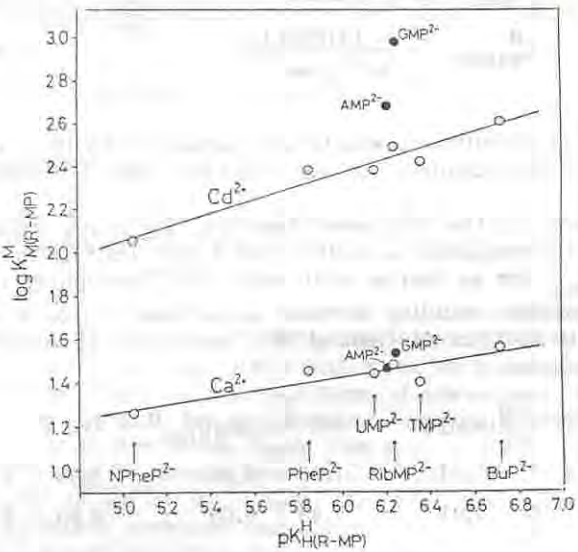

Fig. 2. Relationship between log $K^{M}_{M(R-MP)}$ and $pK^{H}_{H(R-MP)}$ for the 1:1 complexes of Ca^{2+} and Cd^{2+} with some simple phosphate monoester ligands ($R\text{-}MP^{2-}$): 4-nitrophenyl phosphate ($NPheP^{2-}$), phenyl phosphate ($PheP^{2-}$), uridine 5'-monophosphate (UMP^{2-}), D-ribose 5'-monophosphate ($RibMP^{2-}$), thymidine 5'-monophosphate (TMP^{2-}) and n-butyl phosphate (BuP^{2-}) (from left to right) (o). The least squares lines are drawn through the corresponding data sets, which are taken from reference (5); the equations for these baselines are for Ca^{2+}, $y = (0.156 \pm 0.039).\ pK_a + (0.487 \pm 0.239)$, and for Cd^{2+}, $y = (0.317 \pm 0.042).\ pK_a + (0.467 \pm 0.253)$. The points due to the complexes formed with GMP^{2-} and AMP^{2-} (•) are inserted for comparison; the corresponding equilibrium constants are given in Table 1. All plotted equilibrium constant values refer to aqueous solutions at 25°C and I = 0.1 M ($NaNO_3$).

A precise mathematical evaluation is possible by using the straight-line equations of Fig. 2 (see legend) and the $pK^{H}_{H(NMP)}$ values of $H(GMP)^{-}$ and $H(AMP)^{-}$. With this information the stability of the Ca^{2+} and Cd^{2+} complexes for a sole phosphate coordination, i.e. of $M(NMP)_{op}$, may be calculated. The results are listed in the third column of Table 1. The column at the right in Table 1 contains the values for log Δ as defined in equation 5. It is obvious that the vertical distances between the points due to Ca(NMP) or Cd(NMP) and the baselines in Fig. 2 are identical with log Δ.

The increased complex stability as defined by log Δ can be used to calculate the equilibrium constant, K_I, (6,9) as shown in equation 6, where $M(NMP)_{op}$ and $M(NMP)_{cl}$ represent the 'open' and 'closed' forms shown in equation 1.

$$K_I = \frac{[M(NMP)_{cl}]}{[M(NMP)_{op}]} = \frac{K^M_{M(NMP)}}{K^M_{M(NMP)_{op}}} - 1 = 10^{\log \Delta} - 1 \qquad (6)$$

The results of these calculations are summarized in Table 2. The percentages for $M(NMP)_{cl}$ are also evaluated by using the relationship $100 \cdot K_I/(1+K_I)$.

Table 2. Extent of the intramolecular macrochelate formation according to equation 1 (see also equation 6) for the M(NMP) complexes of Table 1.

M(NMP)	log Δ	K_I	% $M(NMP)_{cl}$
Ca(GMP)	0.07 ± 0.05	0.17 ± 0.14	15 ± 10
Ca(AMP)	0.00 ± 0.05	0.00 ± 0.12	0 (<11)
Cd(GMP)	0.53 ± 0.06	2.39 ± 0.49	70 ± 4
Cd(AMP)	0.24 ± 0.06	0.74 ± 0.25	43 ± 8

The results of Table 2 show that the extent of macrochelate formation is larger for Cd(GMP) than for Cd(AMP). This enhanced backbinding to N-7 (see Fig. 1) in GMP^{2-} corresponds to the higher basicity of the GMP N-7 compared to the AMP N-7 (10). In the case of the Ca^{2+} complexes there is no evidence for any base-backbinding in Ca(AMP) whereas there appears to be some chelate formation in Ca(GMP).

Based on our experience in the coordination chemistry of nucleotides (3-6) we assume that in the macrochelates most of the Cd/N-7 interaction occurs inner-sphere, while in the case of Ca(GMP) probably an outer-sphere species is formed, i.e. a water molecule coordinated to Ca^{2+} is also hydrogen bonded to N-7. The hydrogen bonding qualities of N-7 are well known in the solid state (11). There is also the possibility, as suggested from X-ray structural studies (12-14), that the carbonyl O at C-6 forms a hydrogen bond with a coordinated water molecule in the M(GMP) complexes and that this further favors the formation of macrochelates. An overview on the possible structures of the macrochelates formed with purine-nucleoside 5'-monophosphates via N-7 and the phosphate group has been given recently (6).

In conclusion, there is the possibility that the macrochelate, $M(NMP)_{cl}$, consists of different forms and that these forms occur in equilibrium with each other (6). It is clear that the percentage given for $M(NMP)_{cl}$ in Table 2 is the total amount of macrochelate that occurs in a given case. However, independent of these finer details regarding the possible structures of the macrochelate, it is evident that in Cd(GMP) and Cd(AMP) the extent of base-backbinding is considerably larger than in the corresponding Ca(NMP) complexes. Therefore, great care should be exercised if in nucleotide systems Cd^{2+} is employed as a probe for Ca^{2+} sites.

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REFERENCES

1. H. Sigel (Ed.), "Nucleotides and Derivatives: Their Ligating Ambivalency", Vol. 8 of "Metal Ions in Biological Systems", Marcel Dekker, Inc., New York & Basel (1979).
2. H. R. Kalbitzer, Met. Ions Biol. Syst., **22,** 81 (1987).
3. H. Sigel, Eur. J. Biochem., **165,** 65 (1987).
4. H. Sigel, Chimia, **41,** 11 (1987).
5. S. S. Massoud and H. Sigel, Inorg. Chem., **27,** 1447 (1988).
6. H. Sigel, S. S. Massoud, and R. Tribolet, J. Am. Chem. Soc., in press.
7. H. Sigel (Ed.), "Calcium and Its Role in Biology", Vol. 17 of "Metal Ions in Biological Systems", Marcel Dekker, Inc., New York & Basel (1984).
8. R.B. Martin, Met. Ions Biol. Syst., **17,** 1 (1984).
9. R. B. Martin and H. Sigel, Comments Inorg. Chem., **6,** 285 (1988).
10. R. B. Martin, Acc. Chem. Res., **18,** 32 (1985).
11. M. D. Poojary, N. S. Begum, H. Manohar, and R. Bau, J. Chem. Soc., Chem. Commun., 821 (1985).
12. R. W. Gellert and R. Bau, Met. Ions Biol. Syst., **8,** 1 (1979).
13. P. De Meester, D. M. L. Goodgame, A. C. Skapski, and B. T. Smith, Biochim. Biophys. Acta, **340,** 113 (1974).
14. K. Aoki, Acta Cryst., **B32,** 1454 (1976).