

Influence of charge on the stability of 5'-cytidine monophosphate in solution

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1. Introduction

The nucleotides constitute the back bone structure of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The importance of metal ions in biological processes like transcription, replication and translation reactions are very well established. Therefore, the attention the metal-nucleotide interactions have received, especially during the last decade is not surprising (Marzilli *et al* 1980; Hodgson 1977; Gillert and Ban 1977; Martin and Mariam 1979; Wilson *et al* 1982; Orenburg *et al* 1980; Scheller *et al* 1981). Among the nucleotides, mostly the nucleotide triphosphates have been studied (Taqi Khan and Rabindra Reddy 1973a, b, 1975, 1976; Taqui Khan and Martell 1967; Wallas 1958). However, very little is known on metal-mononucleotide interactions and that too with bivalent metal ions. The data with metals of higher oxidation states are not available in the literature. Therefore, it was considered important to investigate the interaction of 5'-cytidine monophosphate with various metal ions. Earlier we have studied the interaction of 5'-cytidine monophosphate with transition and alkaline earth metal ions along with other related systems (Rabindra Reddy *et al* 1985). In the present manuscript, we have extended our investigations to the trivalent lanthanides to study the influence of charge on the stability of the mononucleotides.

2. Experimental

5'-Cytidine monophosphate was obtained from the Sigma Chemical Company, U.S.A. All rare earth oxides were of Johnson Mathey's spectral grade and a stock solution was prepared by dissolving a known weight of the oxide in pure nitric acid. The lighter lanthanides were standardized with the disodium salt of EDTA (Schwarzenbach 1957). The heavier lanthanides were estimated gravimetrically as oxides (Kolthoff and Elving 1963). Carbonate-free sodium hydroxide was prepared and was standardized by titration with potassium acid phthalate.

The experimental method employed consisted of the potentiometric titration of the

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ligand with sodium hydroxide solution in the absence and in the presence of the metal ion being investigated. All titrations with metal ions were carried out in a 1 : 1 ratio of metal ions and the ligand at $35 \pm 0.1^\circ\text{C}$. However, the experimental details can be found in one of our recent papers (Rabindra Reddy *et al* 1984).

3. Calculations

3.1 Acid dissociation constants

The acid dissociation constants K_a and K_{2a} of the ligand were calculated with the aid of the graphical method (Martell and Calvin 1952). In the graphical method a relationship between K_a and K_{2a} can be expressed in terms of known quantities by (1):

$$B = (K_{2a} \cdot B/A) - (1/K_a) \quad (1)$$

where

$$A = \frac{\{(a-1)T_L - [\text{H}^+]\}}{\{(2-a)T_L/[\text{H}^+]\} - 1} \quad \text{and} \quad B = \frac{\{(a-1)T_L + [\text{H}^+]\}}{\{[\text{H}^+](aT_L + [\text{H}^+])\}}$$

A plot of B versus B/A gives a straight line with slope equal to K_{2a} and intercept equal to $1/K_a$.

3.2 Stability constants

In order to calculate the stability constants of the binary complexes of La(III), Pr(III), Nd(III), Sm(III), Gd(III), Dy(III) and Er(III) with 5'-cytidine monophosphate in a 1 : 1 ratio, the following equations were used (excluding the charges).



together with related equilibria,



$$K_{\text{ML}}^{\text{M}} = \frac{[\text{M}][\text{L}]}{[\text{ML}]} \quad (4)$$

$$[\text{L}] = \frac{\{(2-m)T_L - [\text{H}^+] + [\text{OH}^-]\}}{(2[\text{H}^+]^2/K_{1a}K_{2a}) + ([\text{H}^+]/K_{2a})}$$

$$[\text{M}] = [\text{L}] \{([\text{H}^+]^2/K_{1a}K_{2a}) + ([\text{H}^+]/K_{2a}) + 1\}$$

$$[\text{ML}] = T_L - [\text{M}]$$

where,

$[\text{L}]$ = concentration of free ligand;

$[\text{M}]$ = concentration of free metal ion;

$[\text{ML}]$ = concentration of complex formed;

m = moles of base added per mole of the metal ion;

a = moles of base added per mole of ligand;

T_L = total concentration of the ligand species present in solution.

4. Results

The ligand titration curves of 5'-cytidine monophosphate given in figure 1 (X) shows an inflection at $a = 2$ indicating the simultaneous dissociation of two protons. The acid dissociation constants were calculated graphically, as described in the calculation section and were presented in table 1. The titration curve of Dy(III)-5'-cytidine monophosphate in a 1:1 ratio is given in figure 1 (Y). Invariably a precipitate appeared before the inflection point could be reached (at $a \approx 1.8$) for all the systems studied. However, it was assumed that a normal 1:1 complex, K_1 , is formed between the buffer regions $a = 0$ and $a = 2$ and the stability constants were calculated with the help of (4) by taking experimental points far below the precipitation regions. The assumption of a simultaneous formation of a protonated and normal complex gave negative results. The constants thus calculated are presented in table 1.

5. Discussion

The closeness in the pK_a value of 5'-cytidine monophosphate with those of cytosine (4.20) (Taqi Khan and Krishnamurthy 1974) and uridine (4.19) (Rabindra Reddy and Malleswara Rao 1985) suggests that the dissociation of the first proton is from the same site *i.e.*, N_3-H . The second ionization of 5'-cytidine monophosphate is from the phosphate moiety, whose value of 6.56 is consistent with the values reported for similar systems. The dissociation steps of the 5'-cytidine monophosphate are given in figure 2.

The stability constants of 5'-cytidine monophosphate with rare earths decrease in the

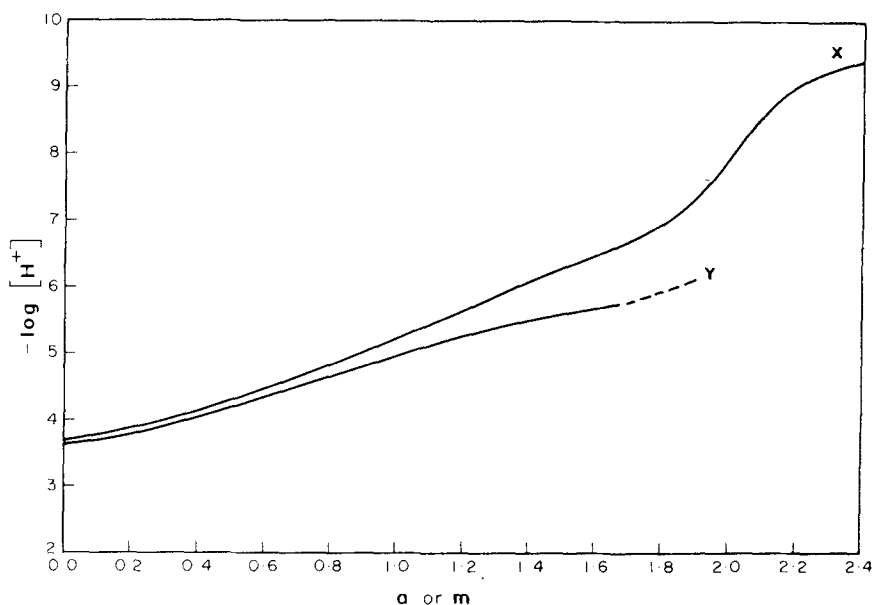
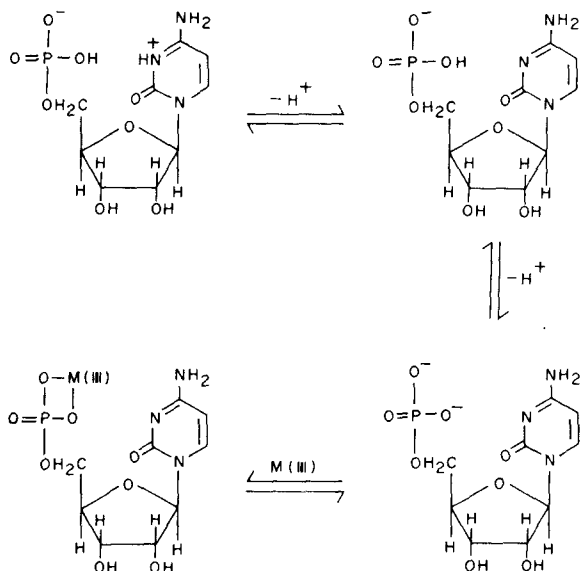


Figure 1. Interaction of Dy(III) with 5'-cytidine monophosphate in a 1:1 ratio and 0.10 M (KNO_3) ionic strength at 35°C; X = free ligand; Y = Dy(III)-5'-cytidine monophosphate a and m = moles of base added per mole of ligand and metal ion respectively.

Table 1. Stability constants associated with interaction of metal with 5'-cytidine monophosphate in a 1:1 ratio.

Metal	Metal 5'-CMP log K_1
La(III)	3.73 ± 0.02
Pr(III)	3.84 ± 0.02
Nd(III)	4.01 ± 0.03
Sm(III)	4.11 ± 0.03
Gd(III)	4.20 ± 0.03
Dy(III)	4.21 ± 0.04
Er(III)	4.35 ± 0.04

$\mu = 0.10 \text{ M (KNO}_3\text{)}$; temperature
 $= 35^\circ\text{C}$; $pK_a = 4.40$, $pK_{2a} = 6.56$.

**Figure 2.** Schematic representation of the dissociation and association steps of 5'-CMP, M(III) = trivalent metal ion.

order: Er(III) > Dy(III) > Gd(III) > Sm(III) > Nd(III) > Pr(III) > La(III). The stability constants are inversely proportional to their ionic radii, a trend that is observed for a variety of ligands with lanthanons. It is of interest here to compare the stability constants of trivalent lanthanons with those of bivalent transition metal ions. 5'-cytidine monophosphate forms more stable complexes with the former as compared to the latter (Rabindra Reddy *et al* 1985). This may be explained on the basis of differences in the charge of the metal ions. The more positively charged lanthanides permit closer

approach of the ligands and larger electrostatic attraction resulting in the formation of stable complexes with the mononucleotide as compared to the less charged transition and alkaline earth metal ions. However, the magnitude of the stability data suggests that in the tri- and bivalent metal ions the nature of bonding may be similar. Based on this we propose a tentative structure for the lanthanide-5'-cytidine monophosphate interactions (figure 2).

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