J Biol Inorg Chem (2004) 9: 365–373
DOI 10.1007/s00775-004-0533-4

ORIGINAL ARTICLE

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A quantitative appraisal of the ambivalent metal ion binding properties of cytidine in aqueous solution and an estimation of the *anti–syn* energy barrier of cytidine derivatives

Received: 19 December 2003 / Accepted: 19 February 2004 / Published online: 19 March 2004

Abstract The recently defined $\log K^{M}_{M(L)}$ versus $pK^{H}_{H(L)}$ straight-line plots for L = pyridine-type (PyN) and *ortho*-aminopyridine-type (*o*PyN) ligands now allow the evaluation in a quantitative manner of the stability of the 1:1 complexes formed between cytidine (Cyd) and Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} or Cd^{2+} (M^{2+}); the corresponding stability constants, $K^{M}_{M(Cyd)}$, including the acidity constant, $K^{H}_{H(Cyd)}$, for the deprotonation of the $(N3)H^{+}$ site had been determined previously under exactly the same conditions as the mentioned plots. Since the stabilities of the $M(PyN)^{2+}$ and $M(oPyN)^{2+}$ complexes of Ca^{2+} and Mg^{2+} are practically identical, it is concluded that complex formation occurs in an outer-sphere manner, and this is in accord with the fact that in the p K_a range 3–7 metal ion binding is independent of $K^{H}_{H(PyN)}$ or $K^{H}_{H(oPyN)}$. $Ca(Cyd)^{2+}$ and $Mg(Cyd)^{2+}$ are more stable than the corresponding (outer-sphere) $M(PyN)^{2+}$ complexes and this means that the C2 carbonyl group of Cyd must participate, next to N3 which is most likely outer-sphere, in metal ion binding, leading thus to chelates; these have formation degrees of about 50% and 35%, respectively. $Co(Cyd)^{2+}$ and $Ni(Cyd)^{2+}$ show no increased stability based on the $\log K^{M}_{M(oPyN)}$ versus $pK^{H}_{H(oPyN)}$ plots; hence, the (C2)O group does not participate in metal ion binding, but the inner-sphere coordination to N3 is strongly inhibited by the $(C4)NH_2$ group. In the $M(Cyd)^{2+}$ complexes of Mn^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}, this inhibiting effect on M^{2+} binding at N3 is partially compensated by participation of the (C2)O group in complex formation and the corresponding chelates have formation degrees between about 30% (Zn^{2+}) and 83% (Cu^{2+}). The different structures of the mentioned chelates are discussed in relation to available crystal structure analyses. (1) There is evidence (crystal structure studies: Cu^{2+}, Zn^{2+}, Cd^{2+}) that four-membered rings form, i.e. there is a strong M^{2+} bond to N3 and a weak one to (C2)O. (2) By hydrogen bond formation to (C2)O of a metal ion-bound water molecule, six-membered rings, so-called semichelates, may form. (3) For Ca^{2+} and Mg^{2+}, and possibly Mn^{2+}, and their Cyd complexes, six-membered chelates are also likely with (C2)O being inner-sphere (crystal structure) and N3 outer-sphere. (4) Finally, for these metal ions also complexes with a sole outer-sphere interaction may occur. All these types of chelates are expected to be in equilibrium with each other in solution, but, depending on the metal ion, either the one or the other form will dominate. Clearly, the cytidine residue is an ambivalent binding site which adjusts well to the requirements of the metal ion to be bound and this observation is of relevance for single-stranded nucleic acids and their interactions with metal ions. In addition, the *anti–syn* energy barrier has been estimated as being in the order of 6–7.5 kJ/mol for cytidine derivatives in aqueous solution at 25 °C.

Keywords *Anti–syn* barrier · Cytidine complexes Isomeric equilibria · Nucleic acids · Stability constants

Abbreviations ADP^{3-} adenosine 5′-diphosphate · AMP^{2-} adenosine 5′-monophosphate · ATP^{4-} adenosine 5′-triphosphate · CDP^{3-} cytidine 5′-diphosphate · *cl* closed · CMP^{2-} cytidine 5′-monophosphate · $3'\text{-}CMP^{2-}$ cytidine 3′-monophosphate · CTP^{4-} cytidine 5′-triphosphate · *Cyd* cytidine · *DNA* deoxyribonucleic acid · *I* ionic strength · K_a acidity constant · K_I intramolecular equilibrium constant · *L* general ligand · M^{2+} general divalent metal ion · NTP^{4-} nucleoside 5'-triphosphate · *op* open · *oPyN ortho*-aminopyridine-type ligand · *PyN* pyridine-type ligand · *t*-RNA transfer ribonucleic acid · *Tu* tubercidin (7-deazaadenosine)

In honor of Professor Liang-Nian Ji on the occasion of his 70th birthday in friendship and with best wishes.

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Introduction

The cytosine residue is, along with the adenine, guanine and uracil/thymine moieties, one of the four main nucleobases which occur in RNA or DNA [1, 2], where it participates in stacking interactions and hydrogen bonding. Consequently, it is not surprising to find that the structure of DNA double and triple helices is affected, for example, by protonation of N3 of the cytosine moiety [3]. This N3, often considered as a pyridine-type nitrogen [4, 5], can also bind metal ions, as is evident, for example, from the crystal structure analysis of yeast phenylalanine transfer ribonucleic acid (*t*-RNA) [6, 7, 8, 9], which revealed a strong binding of Pb^{2+} to N3 and a weaker one to (C2)O. This result is in accord with solution studies which showed that in nucleic acids next to the N7/(C6)O site of guanine the N3/(C2)O unit of cytosine has the strongest affinity for Pb^{2+} [10, 11] and according to the *Stability Ruler* of Martin [12, 13, 14] the same order may be surmised for Zn^{2+}.

The ambivalent properties of the cytosine residue are evident from crystal structure studies: (1) in the *cis*-$(NH_3)_2Pt(3'\text{-}CMP)_2^2$ complex (3′-CMP^{2-} = cytidine 3′-monophosphate),*cis*-$(N3)_2Pt$ binding is observed [15, 16], whereas (2) in $Ba(CMP)\cdot 8.5H_2O$ (CMP^{2-} = cytidine 5′-monophosphate) the alkaline earth metal ion is bonded to (C2)O (and the sugar moiety) [15, 17]. Between these two "extremes" are cases (3) where both N3 *and* (C2)O participate, e.g. in the tetrakis(1-methylcytosine)copper(II) perchlorate dihydrate [18] the four N3 atoms of the nucleobases form a CuN_4 plane with the more weakly bound C2 carbonyls above and below this plane.

Considering that biological reactions take place in solution [1, 19, 20], it is of relevance to know if this ambivalent behavior persists in aqueous solution, especially as in metabolic reactions not only nucleic acids but next to other cytosine derivatives also the nucleotides CMP^{2-}, cytidine 5′-diphosphate (CDP^{3-}) and cytidine 5′-triphosphate (CTP^{4-}) participate [21]. These nucleotides interact with the biologically relevant metal ions (mainly) via their phosphate residues [22, 23, 24, 25] and this interaction determines the stability of these complexes.

A detailed appraisal of the ambivalent properties of the cytosine residue is now possible because straight-line plots of log $K^{M}_{M(L)}$ versus $pK^{H}_{H(L)}$ [27] have recently been determined [26] for simple pyridine-type and also *ortho*-aminopyridine-type ligands (L) like 2-aminopyridine, etc. (see Fig. 1) [28]. This achievement allows a reevaluation of the stability constants of the 1:1 complexes formed between cytidine (Cyd) and the divalent metal ions (M^{2+}) Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}. These constants are defined according to equilibrium 1 and equation 2:

$$M^{2+} + Cyd \rightleftharpoons M(Cyd)^{2+} \quad (1)$$

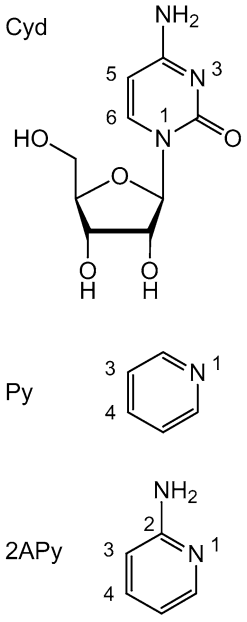


Fig. 1 Chemical structures of cytidine (Cyd), pyridine (Py) and 2-aminopyridine (2APy). Cyd is shown in its predominating *anti* conformation [28]; in the *syn* conformation the (C2)O group projects onto the ribose ring

$$K^{M}_{M(Cyd)} = [M(Cyd)^{2+}]/([M^{2+}][Cyd]) \quad (2)$$

They were determined via potentiometric pH titrations somewhat over 10 years ago in this laboratory [29] under exactly the same experimental conditions as the mentioned straight-line plots (aqueous solutions; 25 °C; I = 0.5 M, $NaNO_3$) [26, 29]. The related acidity constant for the release of the proton from the $(N3)H^+$ site of $H(Cyd)^+$, $pK^{H}_{H(Cyd)} = 4.24 \pm 0.02$ [29], is defined according to equilibrium 3 and equation 4:

$$HCyd^+ \rightleftharpoons H^+ + Cyd \quad (3)$$

$$K^{H}_{H(Cyd)} = [H][Cyd]/[H(Cyd)^+] \quad (4)$$

Formerly, approximated straight-line plots for pyridine-type ligands (PyN) had already been used [29], but no corresponding plots for *ortho*-aminopyridine-type ligands (*o*PyN) had been available; only the equilibrium constants for $H(tubercidin)^+$ and some $M(Tu)^{2+}$ complexes [Tu = tubercidin (7-deazaadenosine)] were known [29, 30]. If one considers the structures of the ligands seen in Fig. 1, it is evident that the *o*-amino group is expected [31] to sterically inhibit M^{2+} binding to the pyridine-N; on the other hand, participation of the carbonyl oxygen [32, 33, 34, 35] at C2 in complex formation should give rise to an increased complex stability compared to one of the sterically inhibited $M(oPyN)^{2+}$ complexes.

Results and discussion

Qualitative appraisal of the stability of $M(Cyd)^{2+}$ complexes

For families of structurally related ligands, plots of $\log K^{M}_{M(L)}$ versus $pK^{H}_{H(L)}$ result in straight lines [27, 36, 37, 38] and these are defined by Eq. 5:

$$\log K^{M}_{M(L)} = m \times pK^{H}_{H(L)} + b \quad (5)$$

where m represents the slope and b the intercept with the y axis. The parameters for m and b, together with the error limits of the values calculated for $\log K^{M}_{M(L)}$, where L = PyN or *o*PyN, in the pH range 3–7 are listed in [26]. Combination of these results [26] with the points due to $\log K^{M}_{M(Cyd)}$ and $pK^{H}_{H(Cyd)}$, taken from [29], lead to the plots of Fig. 2.

Several conclusions can immediately be reached. (1) The $M(oPyN)^{2+}$ complexes of all eight metal ions are less stable than the $M(PyN)^{2+}$ species. (2) The stabilities of the $M(oPyN)^{2+}$ and $M(PyN)^{2+}$ complexes for Ca^{2+} and Mg^{2+} differ only little, and in the pH range 3–7 they are independent of the p K_a value of the pyridine derivative considered; this indicates [26] that complex formation takes place in an outer-sphere manner. (3) Surprisingly, the $Ca(Cyd)^{2+}$ and $Mg(Cyd)^{2+}$ complexes are even more stable than the sterically unhindered $M(PyN)^{2+}$ species; this proves for these alkaline earth metal ions the importance of the C2 carbonyl interac-

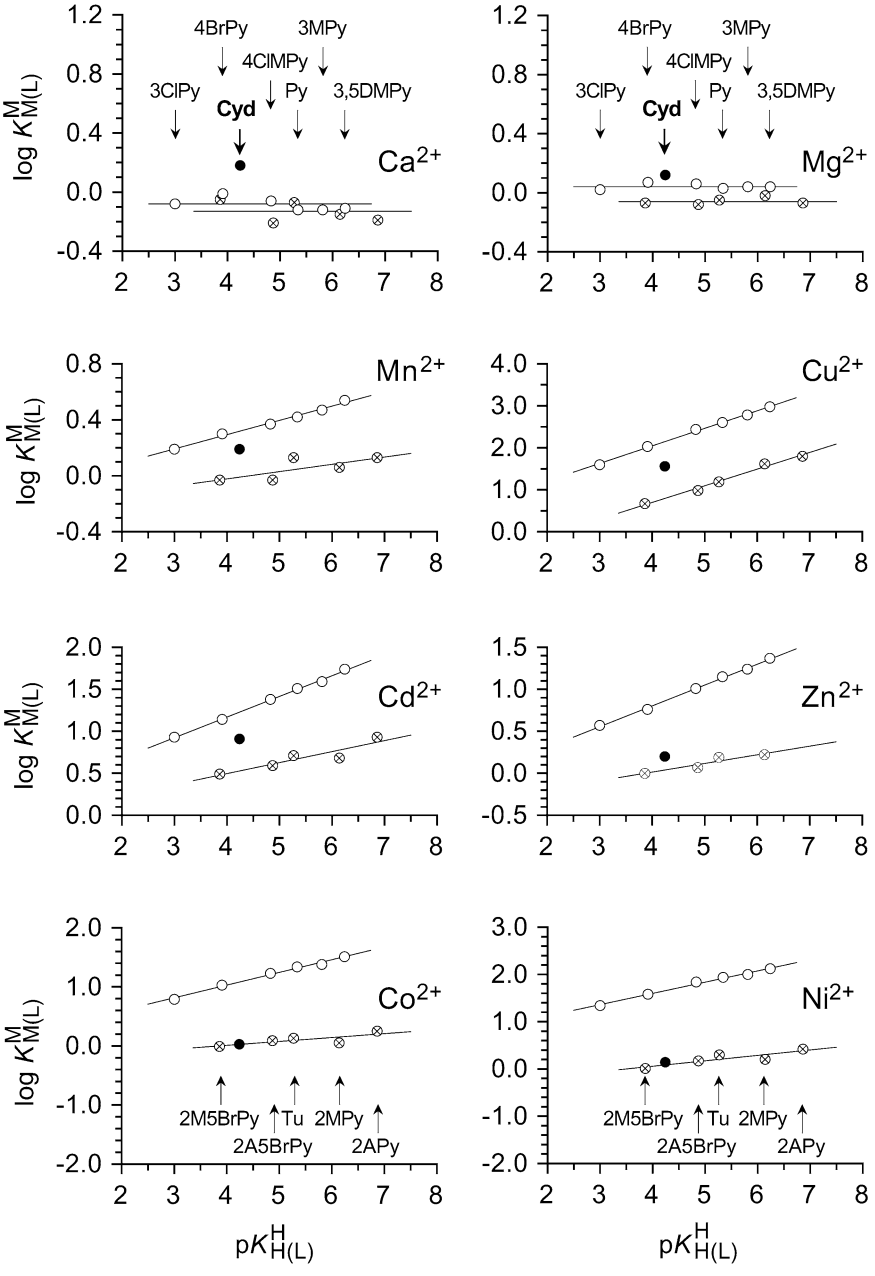


Fig. 2 Evidence for the varying coordinating properties of cytidine (*solid circle*) depending on the metal ion involved. This observation is based on the $\log K^{M}_{M(L)}$ versus $pK^{H}_{H(L)}$ relationship for simple pyridine-type (*open circles*) as well as *ortho*-aminopyridine-type (*circled crosses*) ligands; the reduced stability of the complexes formed with the latter ligands reflects the steric inhibition due to an *ortho*-amino (or -methyl) group. The least-squares straight-reference lines for the simple pyridine-type ligands are defined by the equilibrium constants for the systems containing (*open circles*; *at the top from left to right*): 3-chloropyridine (3ClPy), 4-bromopyridine (4BrPy), 4-(chloromethyl)pyridine (4ClMPy), pyridine (Py), *β*-picoline (= 3-methylpyridine, 3MPy) and 3,5-lutidine (= 3,5-dimethylpyridine, 3,5DMPy); and those for the *ortho*-aminopyridine-type ligands by the constants for the systems containing (*circled crosses*; *at the bottom from left to right*): 2-methyl-5-bromopyridine (2M5BrPy), 2-amino-5-bromopyridine (2A5BrPy), tubercidin (= 7-deaza-adenosine, Tu), α-picoline (= 2-methylpyridine, 2MPy) and 2-aminopyridine (2APy). All plotted equilibrium constants refer to aqueous solution at 25 °C and I = 0.5 M ($NaNO_3$); the data for Cyd are from [29] and those for the pyridine derivatives from [26]

tion. (4) The remaining six $M(Cyd)^{2+}$ complexes fall in two categories: one where the stability of the complexes corresponds to that of the $M(oPyN)^{2+}$ species (Co^{2+}, Ni^{2+}) and another one with stabilities between those of the $M(oPyN)^{2+}$ and $M(PyN)^{2+}$ species (Mn^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}), where the steric inhibition of the *o*-amino group is evidently in part compensated by a (C2)O/M^{2+} interaction.

Quantitative evaluation of the stabilities of the $M(Cyd)^{2+}$ complexes

Table 1 contains the stability constants determined previously [29] for the $M(Cyd)^{2+}$ complexes (column 2) together with the calculated stability constants for simple $M(PyN)^{2+}$ species (column 3) based on $pK^{H}_{H(Cyd)} = pK^{H}_{H(PyN)} = 4.24(\pm 0.02)$ [29] and the straight-line parameters given in [26], as well as the stability constants for the $M(oPyN)^{2+}$ complexes (column 4) resulting from the corresponding calculations for a sterically hindered *o*PyN derivative, also with $pK^{H}_{H(oPyN)} = 4.24$.

The evaluation of the extent of the steric effect of an *o*-amino group on M^{2+} binding to a pyridine-type nitrogen with p K_a = 4.24 is best done by calculating the differences between the stabilities of the $M(oPyN)^{2+}$ and $M(PyN)^{2+}$ complexes (column 2 of Table 2), i.e. $\log K^{M}_{M(oPyN)} - \log K^{M}_{M(PyN)}$ (Table 1, columns 3 and 4). This difference is zero within the error limits for the two Ca^{2+} complexes and varies between about −0.1 to −1.6 log units for the other complexes. This demonstrates that the inhibiting effects of the *o*-amino group for the various metal ions differ significantly and it indicates further, as stated before, that outer-sphere N-binding is important for Ca^{2+} while inner-sphere N-binding becomes increasingly relevant for the other metal ions and is most likely strongly dominating for Co^{2+}, Ni^{2+} and Cu^{2+}. However, for Mn^{2+} species, both binding modes appear to be in equilibrium with each other; this is also indicated by the small slopes ($m_{Mn/PyN} = 0.102 \pm 0.005$ and $m_{Mn/oPyN} = 0.052 \pm 0.026$ [26]) observed for the Mn^{2+} complexes (see also Fig. 2).

Comparison of the complex stabilities for the $M(Cyd)^{2+}$ complexes with those of the simple $M(PyN)^{2+}$ species, i.e. $\log K^{M}_{M(Cyd)} - \log K^{M}_{M(PyN)}$ (Table 2, column 3), confirms the increased stabilities of the $Ca(Cyd)^{2+}$ and $Mg(Cyd)^{2+}$ complexes. In none of the other cases is the C2 carbonyl group able to offset via a M^{2+} interaction the inhibiting steric effect of the *o*-amino group. The sterically inhibiting effect of an *ortho*-carbonyl group is small, as has been repeatedly concluded [39, 40].

The stability-enhancing effect of the (C2)O group is best seen by comparing the complex stabilities of the $M(Cyd)^{2+}$ complexes with those of *o*-amino-inhibited $M(oPyN)^{2+}$ species (Table 1, columns 2 and 4), i.e. by calculating the difference $\log K^{M}_{M(Cyd)} - \log K^{M}_{M(oPyN)}$ (see column 4 in Table 2). These results demonstrate, in accord with Fig. 2, that there is no stability enhancement within the error limits for the $Co(Cyd)^{2+}$ and $Ni(Cyd)^{2+}$ complexes, whereas varying stability enhancements result for the other metal ions, depending on their affinity toward carbonyl oxygens.

Quantification of the extent of participation of the carbonyl group in metal ion binding

The differing involvement of the (C2)O group in metal ion binding may be quantified in the following way. The stability constants for the $M(oPyN)^{2+}$ complexes

Table 1 Comparison of the experimentally determined stability constants, $\log K^{M}_{M(Cyd)}$ (column 2), of some $M(Cyd)^{2+}$ complexes (Eq. 2) with the stability constants $\log K^{M}_{M(PyN)}$ (column 3), calculated for the complexes of simple pyridine-type ligands (PyN), and with the constants $\log K^{M}_{M(oPyN)}$ (column 4) calculated for the complexes of the sterically inhibited *ortho*-aminopyridine-type ligands (*o*PyN) having the same basicity as N3 of Cyd (aqueous solution; 25 °C; I = 0.5 M, $NaNO_3$)[a,b]

M^{2+}	$\log K^{M}_{M(Cyd)}$	$\log K^{M}_{M(PyN)}$	$\log K^{M}_{M(oPyN)}$
Ca^{2+}	0.18 ± 0.06	−0.08 ± 0.07	−0.13 ± 0.13
Mg^{2+}	0.12 ± 0.04	0.04 ± 0.04	−0.06 ± 0.06
Mn^{2+}	0.19 ± 0.08	0.32 ± 0.02	−0.01 ± 0.07
Co^{2+}	0.03 ± 0.08	1.08 ± 0.03	0.03 ± 0.08
Ni^{2+}	0.14 ± 0.12	1.66 ± 0.03	0.08 ± 0.10
Cu^{2+}	1.56 ± 0.06	2.15 ± 0.04	0.79 ± 0.07
Zn^{2+}	0.20 ± 0.11	0.86 ± 0.02	0.04 ± 0.08
Cd^{2+}	0.91 ± 0.07	1.23 ± 0.02	0.53 ± 0.09

[a]The values in column 2 are from [29]; those of columns 3 and 4 were calculated with $pK^{H}_{H(Cyd)} = 4.24 \pm 0.02$ [29] and the straight-line equations defined in [26]

[b]The error limits given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger

Table 2 Logarithmic differences between the calculated stability constants for M^{2+} complexes of a pyridine-type ligand (PyN) and an *ortho*-aminopyridine-type ligand (*o*PyN) with the same basicity as N3 in cytidine (column 2), thus quantifying the steric inhibition for an N3 coordination due to the (C4)NH_2 group in cytidine. Columns 3 and 4 provide the differences between $\log K^{M}_{M(Cyd)}$ and the calculated stability constants $\log K^{M}_{M(PyN)}$ and $\log K^{M}_{M(oPyN)}$, respectively[a]

M^{2+}	$\log K^{M}_{M(oPyN)} - \log K^{M}_{M(PyN)}$	$\log K^{M}_{M(Cyd)} - \log K^{M}_{M(PyN)}$	$\log K^{M}_{M(Cyd)} - \log K^{M}_{M(oPyN)}$
Ca^{2+}	−0.05 ± 0.15	0.26 ± 0.09	0.31 ± 0.14
Mg^{2+}	−0.10 ± 0.07	0.08 ± 0.06	0.18 ± 0.07
Mn^{2+}	−0.33 ± 0.07	−0.13 ± 0.08	0.20 ± 0.11
Co^{2+}	−1.05 ± 0.09	−1.05 ± 0.09	0.00 ± 0.11
Ni^{2+}	−1.58 ± 0.10	−1.52 ± 0.12	0.06 ± 0.16
Cu^{2+}	−1.36 ± 0.08	−0.59 ± 0.07	0.77 ± 0.09
Zn^{2+}	−0.82 ± 0.08	−0.66 ± 0.11	0.16 ± 0.14
Cd^{2+}	−0.70 ± 0.09	−0.32 ± 0.07	0.38 ± 0.11

[a]All differences are based on the data listed in Table 1; the error limits were calculated according to the error propagation after Gauss

(Table 1, column 4) represent the monodentate binding of the pyridine nitrogen (N3) of cytidine toward M^{2+}; we define this species as the "open" isomer, $M(Cyd)^{2+}_{op}$. The experimentally measured stability constants $K^{M}_{M(Cyd)}$ (Eq. 2) are of course overall constants which encompass all $M(Cyd)^{2+}$ species present in solution, independent of their structure, and including those with a carbonyl interaction. The latter mentioned species are chelates and we define them as "closed", i.e. $M(Cyd)^{2+}_{cl}$. One may add that a simple monodentate (C2)O/M^{2+} binding is highly unlikely because, for example, for $Ca(Cyd)^{2+}$ and $Mg(Cyd)^{2+}$ a carbonyl–M^{2+} interaction must occur but from the properties of the corresponding $M(PyN)^{2+}$ complexes it is evident that a water molecule is between N and M^{2+} (see Fig. 2 and the preceding section: the slope of the straight-reference lines equals zero!); hence, so-called semichelates result. Of course, the interaction could also occur with both sites, N3 and (C2)O, in an outer-sphere manner. In any case, the intramolecular equilibrium 6:

$$M(Cyd)^{2+}_{op} \rightleftharpoons M(Cyd)^{2+}_{cl} \quad (6)$$

exists and its position is quantified by the dimensionless intramolecular equilibrium constant K_I (Eq. 7):

$$K_I = \left[M(Cyd)^{2+}_{cl}\right] / \left[M(Cyd)^{2+}_{op}\right] \quad (7)$$

The stability enhancement due to the additional (C2)O/M^{2+} interaction is defined by the stability difference expressed in Eq. 8:

$$\begin{aligned} \log \Delta &= \log K^{M}_{M(Cyd)} - \log K^{M}_{M(Cyd)_{op}} \\ &= \log K^{M}_{M(Cyd)} - \log K^{M}_{M(o PyN)} \end{aligned} \quad (8)$$

These log Δ values correspond to the vertical distances in Fig. 2 between the points due to a given $M(Cyd)^{2+}$ (solid circle) and its reference line (circled crosses) and are listed in column 4 of Table 2.

Previously it has been shown [27, 35, 41, 42, 43] that the stability enhancement log Δ is interlinked with K_I by Eq. 9:

$$K_I = 10^{\log \Delta} - 1 \quad (9)$$

Of course, knowledge of K_I allows the calculation of the formation degree of the closed species in equilibrium 6 via Eq. 10:

$$\%M(Cyd)^{2+}_{cl} = 100 \times K_I/(1 + K_I) \quad (10)$$

The results for log Δ, K_I and $\%M(Cyd)^{2+}_{cl}$ are listed in Table 3 (columns 2, 3 and 4).

It needs to be emphasized that the $M(Cyd)^{2+}_{cl}$ species are a *mixture* of several chelated isomers and, among these, four-membered rings may occur if N3 and (C2)O are both inner-sphere bound (evidence from solid state studies exists for Cu^{2+}, Zn^{2+} and Cd^{2+} species; see Conclusions). By inclusion of a coordinated water, so-called semichelates may form and these now contain a six-membered ring with a hydrogen bond. This outer-sphere interaction may occur with (C2)O if N3 is inner-sphere, but the reversed situation is likely as well, especially with Ca^{2+}, but also with Mg^{2+} and Mn^{2+}. In fact, Ca^{2+}–carbonyl interactions are legion and often relatively strong (2.2–2.3 Å) [44]. Finally, to complete the picture, outer-sphere binding of M^{2+} to both sites, N3 and (C2)O, is possible to some extent and, indeed, hydrogen bonding is well known for both (C2)O and N3 of the cytosine residue [45].

Table 3 Increased complex stability, log Δ (Eq. 6), and extent of chelate formation according to equilibrium 6 for the $M(Cyd)^{2+}$ systems, as quantified by the dimensionless equilibrium constant K_I (Eqs. 7 and 9) and the percentage of $M(Cyd)^{2+}_{cl}$ (Eq. 10) (aqueous solution; 25 °C; $I = 0.5$ M, $NaNO_3$)[a]

M^{2+}	log Δ[b]	K_I	$\%M(Cyd)^{2+}_{cl}$
Ca^{2+}	0.31 ± 0.14	1.04 ± 0.66	51 ± 16
Mg^{2+}	0.18 ± 0.07	0.51 ± 0.24	34 ± 11
Mn^{2+}	0.20 ± 0.11	0.58 ± 0.40	37 ± 16
Co^{2+}	0.00 ± 0.11	0.00 ± 0.25	~0
Ni^{2+}	0.06 ± 0.16	0.15 ± 0.42	~0
Cu^{2+}	0.77 ± 0.09	4.89 ± 1.22	83 ± 4
Zn^{2+}	0.16 ± 0.14	0.45 ± 0.47	31 ± 22
Cd^{2+}	0.38 ± 0.11	1.40 ± 0.61	58 ± 11

[a] For the error limits, see footnotes "b" and "a" of Tables 1 and 2, respectively
[b] From column 4 of Table 2

Clearly, the percentages listed in column 4 of Table 3 encompass all the mentioned "closed" species. For $Co(Cyd)^{2+}_{cl}$ and $Ni(Cyd)^{2+}_{cl}$ the formation degrees are zero within the error limits, in accord with the conclusions reached in the first section of Results and discussion. However, for all the other $M(Cyd)^{2+}$ complexes, including those with Ca^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+}, the formation degrees of the closed species are significant, varying between about 30% and 50%, and reaching a maximum of about 83% for $Cu(Cyd)^{2+}_{cl}$.

Estimation of the size of the *anti–syn* energy barrier in cytidine derivatives

The data presented in this work, together with some related earlier results, allow an evaluation of the energy barrier between the *anti* and *syn*conformations of the cytidine residue (see Fig. 1) by following the route developed earlier [46]. This previous calculation can be repeated with an increased precision because now more reliable stability constants are available for $Cu(Cyd)^{2+}$ and $Cu(adenosine)^{2+}$.

The evaluation is based [46] on a comparison of the stabilities of the $Cu(ATP)^{2-}$ ($\log K^{Cu}_{Cu(ATP)} = 6.34 \pm 0.03$ [46]) and $Cu(CTP)^{2-}$ ($\log K^{Cu}_{Cu(CTP)} = 6.03 \pm 0.05$ [46]) complexes (error limits always 3σ). Since ATP^{4-} (adenosine 5′-triphosphate) exists in solution predominantly in the *anti* conformation (see Fig. 3) [15, 28, 47], N7 of the adenine residue can be reached by a

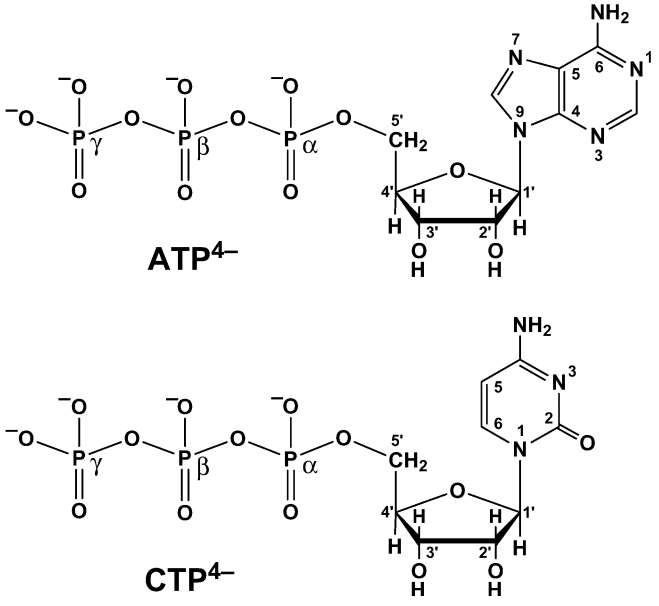
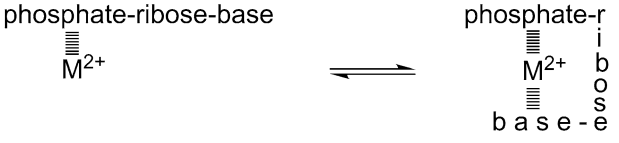


Fig. 3 Chemical structures of adenosine 5′-triphosphate (ATP^{4-}) and cytidine 5′-triphosphate (CTP^{4-}) in their predominating *anti* conformations [15, 28, 47]

phosphate-coordinated metal ion and macrochelates according to equilibrium 11 may form [46, 48]:



(11)

Indeed, macrochelate formation for $Cu(ATP)^{2-}$ (67 ± 3% [48]) is reflected in an increased complex stability which amounts, based on a pure triphosphate–Cu^{2+} coordination, to log $\Delta_{Cu/ATP} = 0.48 \pm 0.04$ [46, 48]. This contrasts with the situation in $M(CTP)^{2-}$ complexes, where N3, the potential binding site, is directed away from the phosphate groups, as is seen in Fig. 3, since in the dominating *anti* conformation the N1–C6 bond of pyrimidines projects onto or near the ribose ring [47]. This means that for macrochelate formation in M(CTP) complexes it is necessary to use part (or all) of the N3–M^{2+} interaction energy to turn the *anti* into the *syn*conformation. Indeed, Cu^{2+} is the only metal ion, among those studied [46], with a large enough affinity for N3 to force part of CTP^{4-} in the $Cu(CTP)^{2-}$ species into a *syn* conformation. The observed stability increase, log $\Delta_{Cu/CTP} = 0.17 \pm 0.06$ [46], corresponds to a formation degree of 32 ± 9% for the macrochelate. In fact, the affinity of Cu^{2+} for N3 of the cytosine residue is quite pronounced: the stability of $Cu(Cyd)^{2+}$ (log $K^{Cu}_{Cu(Cyd)} = 1.56 \pm 0.06$;Table 1) is considerably larger than that of $Cu(adenosine)^{2+}$ with an N7 coordination (log $k^{Cu}_{Cu(Ado/N7)} = 0.69 \pm 0.10$; note that here the micro stability constant [5] needs to be used; the error limit is an estimate). Hence, by using the difference between these latter stability constants, (1.56 ± 0.06) − (0.69 ± 0.10) = 0.87 ± 0.12, and the stability difference between $Cu(ATP)^{2-}$ and $Cu(CTP)^{2-}$, which is due to the different extents of macrochelate formation, (6.34 ± 0.03) − (6.03 ± 0.05) = 0.31 ± 0.06, it is possible to estimate the energy barrier between the *anti* and *syn* conformations of CTP^{4-}. As ATP^{4-}exists predominantly in the *anti* conformation (Fig. 3), the overall value of 1.18 ± 0.13 log units [= (0.87 ± 0.12) + (0.31 ± 0.06)] represents an estimate for this barrier corresponding to $\Delta G° = 6.7 \pm 0.7$ kJ/mol (for the relation between $\Delta G°$ and log K, see [35]).

A corresponding evaluation is possible based on the stability constants of Cu(AMP) (log $K^{Cu}_{Cu(AMP)} = 3.17 \pm 0.02$ [43]) and Cu(CMP) (log $K^{Cu}_{Cu(CMP)} = 2.88 \pm 0.09$ [49, 50]) (AMP^{2-} = adenosine 5′-monophosphate). The stability enhancement for the Cu(AMP) complex, log $\Delta_{Cu/AMP} = 0.30 \pm 0.06$, reflects a formation degree of the macrochelate of 50 ± 7% [43, 50]. In contrast, the stability of the Cu(CMP) complex is solely determined by the basicity of the phosphate group of CMP^{2-} [22, 49] and, if at all, only traces of macrochelates (Eq. 11) with N3 are formed. Therefore, our estimate can provide only a *lower* limit for the energy barrier between the *anti* and *syn*conformations [22, 49, 50]. By using the above difference between the stabilities of the nucleoside complexes, i.e. 0.87 ± 0.12 log units, as well as the difference between the stabilities of the Cu(AMP) and Cu(CMP) complexes, i.e. (3.17 ± 0.02) − (2.88 ± 0.09) = 0.29 ± 0.09, one obtains the overall value of 1.16 ± 0.15 log units [= (0.87 ± 0.12) + (0.29 ± 0.09)], which represents the lower limit of the *anti–syn* energy barrier for CMP^{2-}. By taking into account the error limit, one obtains 1.01 log units and thus $\Delta G° \geq 5.8$ kJ/mol at 25 °C. This limiting value is close to the earlier estimate [46] for this barrier, which was 6 kJ/mol, and also in excellent agreement with the estimate calculated above.

One may also use the stability constants of $Cu(ADP)^{-}$ (log $K^{Cu}_{Cu(ADP)} = 5.61 \pm 0.03$ [43]) and $Cu(CDP)^{-}$([23]) (ADP^{3-} = adenosine 5′-diphosphate) for a further estimation. However, although $Cu(ADP)^{-}$ forms macrochelates according to equilibrium 11 [43], one obtains also in this case only a lower limit since the stability of $Cu(CDP)^{-}$ is again solely determined by the basicity of the diphosphate residue [23]. The differences (5.61 ± 0.03) − (5.29 ± 0.08) = 0.32 ± 0.08 and 0.87 ± 0.12 (see above) result in an overall value of 1.19 ± 0.14 log units, which provides the lower limit of 1.05 log units and thus $\Delta G° \geq 6.0$ kJ/mol.

To conclude (and giving special weight to the evaluation based on the nucleoside 5′-triphosphates), the energy difference between the *anti* and *syn*conformations of cytidine derivatives in aqueous solution at 25 °C amounts to about 6–7.5 kJ/mol.

Conclusions

The evaluations presented prove that the cytosine residue is truly an ambivalent liganding site; the type of its interactions in complexes in aqueous solution depends on the metal ion and it is amazing to see how well the presented solution studies are complemented by crystal structure analyses. Therefore, three representative examples referring to M^{2+} complexes formed with CMP^{2-} or $dCMP^{2-}$ are shown in Fig. 4 [17, 51, 52]. In all instances, in agreement with the discussions in the preceding section, the (d)CMP^{2-}ligands are present in the solid state in their more stable *anti* conformation. Consequently, no macrochelates form; instead, one metal ion coordinates to the phosphate residue and another one to the N3/(C2)O site(s) and thus polymeric structures result in the solids. However, under these circumstances, metal ions may coordinate to the cytosine residue in an unrestricted manner, just as they do in solution to cytidine and thus the following comparisons are possible.

Since the stability constant of the $Co(Cyd)^{2+}$complex fits on the reference line defined for *o*-aminopyridine-type binding (Fig. 2), no increased stability is observed (Table 3) and consequently no chelates are formed. Thus, one has to conclude that Cyd coordinates via N3 in a monodentate fashion to Co^{2+}. Exactly this binding mode is observed in the solid state (Fig. 4A) where Co^{2+}, with a distorted tetrahedral coordination sphere, forms a strong bond to N3 (1.987 Å) and no further interaction with the nucleobase occurs [51, 53]. For $Ni(Cyd)^{2+}$also a sole N3–Ni^{2+} interaction must be surmised, based on the stability data (Fig. 2 and Table 3).

The larger Cd^{2+} is able to form a four-membered ring in Cd(dCMP) [15, 45] in the solid state (Fig. 4B), although the N3–Cd^{2+} distance is somewhat shorter (2.295 Å) than the (C2)O–Cd^{2+} one (2.641 Å) [52]. However, a considerably more distorted four-membered ring exists in the solid-state structure of Zn(CMP), in which Zn^{2+} is five-fold coordinated (distorted trigonal-bipyramidal) [45] with a strong bond to N3 (2.04 Å) and a rather weak one to (C2)O (2.69 Å) [54]. Very similar bond lengths are observed in the Zn^{2+} complexes of the monomethyl phosphate esters of CMP and dCMP [55]. Of course, in solution, equilibria may form with $Cd(Cyd)^{2+}$ and $Zn(Cyd)^{2+}$ which also involve semichelates with N3 inner-sphere bound and a water molecule between $M^{2+}$and (C2)O. In any case, in accord with the bond lengths, chelate formation with nearly 60% is substantial for $Cd(Cyd)^{2+}$, whereas it is much less pronounced for $Zn(Cyd)^{2+}$ with about 30% only (Table 3).

Similarly, in tetrakis(1-methylcytosine)copper(II) perchlorate dihydrate [18] the N3–Cu^{2+} distances are on average 2.026 Å, whereas the C2 oxygens are above and below the $Cu(N3)_4$ plane with an average distance of 2.734 Å; this means, here again, distorted four-membered rings exist, though the distortion may be less severe because apical interactions of Cu^{2+} occur a priori in a larger distance and are weaker [56]. Corresponding observations have been made with tetrakis(cytosine)copper(II) perchlorate dihydrate [57], and other Cu^{2+}complexes of cytosine derivatives as well [58, 59, 60, 61, 62]. Of course, in aqueous solution, $Cu(Cyd)^{2+}$ may also form a semichelate, N3 being inner-sphere bound, next to the open isomer which occurs in

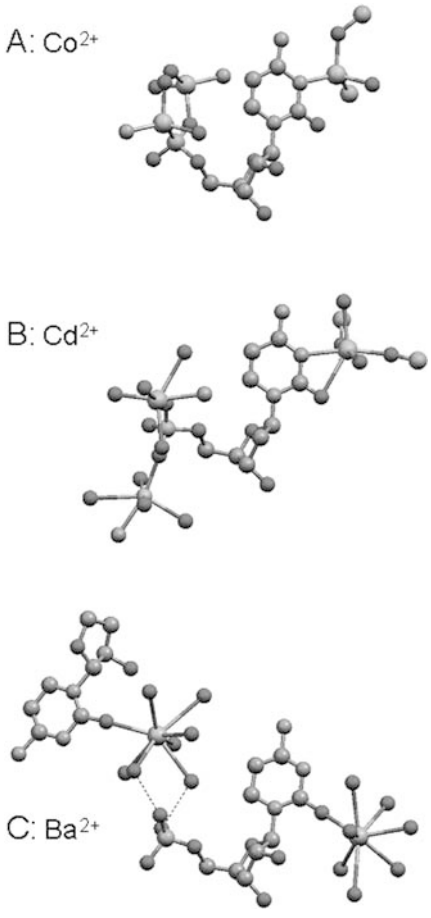


Fig. 4A–C Crystal structures of three metal ion complexes formed with cytidine 5′-monophosphate (CMP^{2-}) or 2′-deoxycytidine 5′-monophosphate ($dCMP^{2-}$) exhibiting different metal ion-binding modes. All metal ions in the above structures are represented by somewhat *larger balls* than the other atoms. In all three structures the cytosine ring shows approximately the same orientation and the crucial metal ion (with other atoms in its coordination sphere) is always located at the *right hand side*. Oxygen atoms are represented by *black balls* and hydrogen atoms are omitted for clarity, though in **C** two hydrogen bonds are indicated. **A** In the polymeric Co(CMP) [51] the tetrahedral metal ion is bound to N3 of the cytosine residue (bond length for N3–Co^{2+}: 1.99 Å) as well as to two oxygens of phosphate groups and to one water molecule. **B** In the polymeric Cd(dCMP) [52], binding of the octahedral Cd^{2+} occurs to both N3 (2.30 Å) and (C2)O (2.64 Å); the other four sites are occupied by two oxygens of phosphate groups and two water molecules. **C** In $Ba(CMP)\cdot 8.5H_2O$ [17] the octa-coordinated metal ion is inner-sphere bound to (C2)O (2.59 Å) and also to seven water molecules which are involved in hydrogen bonding. The coordinates for the structures were downloaded from the Cambridge Structural Database and the structures were drawn with the Mercury 1.1.2 program from the Cambridge Crystallographic Data Centre

equilibrium with a formation degree of $17 \pm 4\%$ only (Table 3, column 4: 100–83%).

Another interesting case is Mn(CMP), for which in the solid state only a rather strong (C2)O–Mn^{2+} interaction (2.08 Å [63]) was found [15, 45]; this is clearly responsible for the increased stability (see Fig. 2) of $Mn(Cyd)^{2+}$, most likely together with an outer-sphere interaction to N3; in accord herewith, chelate formation for $Mn(Cyd)^{2+}$ amounts to about 35% (Table 3). The suggested outer-sphere interaction with N3 is further supported by the small slope ($m = 0.052 \pm 0.026$ [26]) observed for the Mn^{2+} complexes of the *o*-aminopyridine-type ligands (see Fig. 2); such a small slope means that complex stability is nearly independent of the basicity of the N site and this is indicative for outer-sphere interactions.

In $Ca(Cyd)^{2+}$ and $Mg(Cyd)^{2+}$, like in $Mn(Cyd)^{2+}$, metal ion binding to the (C2)O group leads to an increased stability and a rather pronounced chelate formation of about 35% and 50%, respectively (Table 3). Indeed, the fact that in Ba(CMP)·$8.5H_2O$ one of the three Ba^{2+} ions interacts with the nucleobase via (C2)O [15, 17, 45] in an inner-sphere manner (2.59 Å) (see Fig. 4C) supports the conclusions regarding $Ca(Cyd)^{2+}$ and $Mg(Cyd)^{2+}$, for which in solution an additional outer-sphere interaction with N3 is likely, as this would result in a six-membered semichelate (see the third section of Results and discussion). In accord herewith, outer-sphere binding of Mg^{2+} to N3 of a cytosine residue has been observed in a recent crystal structure analysis of yeast phenylalanine *t*-RNA [64]. However, since outer-sphere binding of Mg^{2+} to carbonyl oxygens, e.g. of uracil residues [64, 65], is also quite common, one may conclude that with Mg^{2+} and Ca^{2+} (and possibly with Mn^{2+} as well) in equilibrium also sole outer-sphere (chelate) complexation occurs.

To conclude, cytosine is clearly a very versatile binding moiety: For metal ions like Ca^{2+}, Mg^{2+} and Mn^{2+} the interaction is expected to occur preferably via (C2)O, whereas for Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} an N3 inner-sphere binding is of relevance; chelates form in all instances except with Co^{2+} and Ni^{2+}. In other words, the cytosine residue is well prepared by evolution to interact with different metals by adjusting to the preferences of a given ion. This is especially important for single-stranded nucleic acids, where the cytosine unit is known to bind to such diverse metal ions as Pb^{2+} [6, 7, 8, 9] and Mg^{2+} [64].

Acknowledgements The competent technical assistance of Mrs Rita Baumbusch and Mrs AstridSigel in the preparation of this manuscript is gratefully acknowledged. This study was supported by the Swiss National Foundation and within the COST D20 programme by the Swiss Federal Office for Education and Science.

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