Extent of metal ion-sulfur binding in complexes of thiouracil nucleosides and nucleotides in aqueous solution

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This study is dedicated to Professor Dr. Osamu Yamauchi on the occasion of his 70th birthday in friendship and with best wishes for all his future endeavors

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Abstract

Previously published stability constants of several metal ion (M^{2+}) complexes formed with thiouridines and their 5'-monophosphates, together with recently obtained log $K^{M}_{M(U)}$ *versus* pK^{H}_{U} plots for M^{2+} complexes of uridinate derivatives (U^{-}) allowed now a quantitative evaluation of the effect that the exchange of a (C)O by a (C)S group has on the stability of the corresponding complexes. For example, the stability of the Ni^{2+}, Cu^{2+} and Cd^{2+} complexes of 2-thiouridinate is increased by about 1.6, 2.3, and 1.3 log units, respectively, by the indicated exchange of groups. Similar results were obtained for other thiouridinates, including 4-thiouridinate. The structure of these complexes and the types of chelates formed (involving $(N3)^{-}$ and (C)S) are discussed. A recently advanced method for the quantification of the chelate effect allows now also an evaluation of several complexes of thiouridinate 5'-monophosphates. In most instances the thiouracilate coordination dominates the systems, allowing only the formation of small amounts of phosphate-bound isomers. Among the complexes studied only the one formed by Cu^{2+} with 2-thiouridinate 5'-monophosphate leads to significant amounts of the macrochelated isomer, which means that in this case Cu^{2+} is able to force the nucleotide from the *anti* to the *syn* conformation, allowing thus metal ion binding to both potential sites and this results in the formation of about 58% of the macrochelated isomer. The remaining 42% are species in which Cu^{2+} is overwhelmingly coordinated to the thiouracilate residue; Cu^{2+} binding to the phosphate group occurs in this case only in trace amounts.

Keywords

Chelate formation • Isomeric equilibria • Metal ion-sulfur coordination • Thiouridine 5'-monophosphates • Thiouridines

1. Introduction

Thioderivatives of purine and pyrimidine nucleobases are of potential therapeutic value [1]. If incorporated into nucleic acids, such bases change the properties; e.g., thiolation of uracil residues, especially in RNA wobble positions, affects the conformation of the nucleic acid in solution [2] and this has implications for recognition processes. Of course, the substitution of oxygen by sulfur also changes the metal ion binding properties of uracil derivatives [3]; e.g., the affinity toward Zn^{2+} is expected to increase [4–6]. In line herewith are attempts to recruit divalent metal ions for DNA by incorporation of 4-thio-2'-deoxythymidine or 4-thio-2'-deoxyuridine into DNA [7].

Considering further that 4-thiouridine (U4S) is found in bacterial and archaeal tRNA [8,9], a quantification of metal ion binding to such sulfur sites is certainly desirable. This aim can now be achieved due to recent correlations for uridinate derivatives (U^-), i.e., for log $K^{M}_{M(U)}$ *versus* pK^{H}_{U} straight-line plots [10]. These parameters allow to evaluate previous very exact equilibrium constant measurements of 2-thiouridine (U2S), 4-thiouridine (U4S) and 2,4-dithiouridine (U2S4S) (see Fig. 1 [11,12]) for their complexes formed with Ni^{2+}, Cu^{2+} and Cd^{2+} [3,13], providing indirectly also information for the corresponding Zn^{2+} species [4–6], and to quantify in detail the stability enhancement of these complexes which results from the exchange of an O by a S atom.

insert Figure 1 close to here

The indicated achievements allowed further to evaluate available equilibrium data of complexes of thiouridinate 5'-monophosphates [14] by the application of a recently developed new method for the quantification of macrochelate formation [15] which also provides information about the amounts of metal ions which are either solely phosphate- or nucleobase-coordinated [16]. For example, the complexes of 2-thiouridinate 5'-monophosphate and Cu^{2+} exist, as shown now, to a significant amount as macrochelates; among the 'open', i.e., not chelated, species, the one with nucleobase binding dominates whereas the one with phosphate coordination alone occurs only as a minority species.

2. Results and discussion

2.1. Extent of the stability enhancement of several thiouridine complexes

For all uridine derivatives (U), including the thio ones, the following two equilibria are of relevance for the present study:

$$U \rightleftharpoons (U - H)^- + H^+ \quad (1a)$$

$$K^{H}_{U} = [(U - H)^-][H^+]/[U] \quad (1b)$$

$$M^{2+} + (U - H)^- \rightleftharpoons M(U - H)^+ \quad (2a)$$

$$K^{M}_{M(U-H)} = [M(U - H)^+]/([M^{2+}][(U - H)^-]) \quad (2b)$$

It should be noted that deprotonation of a hydroxyl group at the ribose ring occurs only with pK_a = 12.5 [17] and is thus not of relevance in the present context.

For families of structurally related ligands (L), plots of log $K^{M}_{M(L)}$ *versus* p$K^{H}_{H(L)}$ result in straight lines [18]; this is also true for L = $(U - H)^-$ ligands if only those with O donors in the uracil residue are considered [10]. Hence Eq. (3) holds:

$$\log K^{M}_{M(U-H)} = m \cdot pK^{H}_{U} + b \quad (3)$$

The parameters *m* and *b* for the straight lines of 11 different divalent metal ions and their complexes are listed in Ref. [10]. In Fig. 2 log $K^{M}_{M(U-H)}$ *versus* pK^{H}_{U} is plotted for the Ni^{2+} and Cd^{2+} complexes of several uridinate derivatives, together with some related data [19,20] which will be discussed in Section 2.2.

insert Figure 2 close to here

For the present it is important to note that the stabilities of the $M(U2S - H)^+$ and $M(U4S - H)^+$ complexes are significantly above the reference lines which hold for the $M(U - H)^+$ complexes of simple uracil derivatives. This observation demonstrates that the thiouracil residues compared to the parent uracil residue have a much higher affinity toward Ni^{2+} and Cd^{2+}; the same observation is made for Cu^{2+} (not shown in Fig. 2). In fact, the vertical dotted lines of the $M(US - H)^+$ data points to the various reference lines reflect this stability enhancement.

The mentioned stability enhancement can be quantified based on Eq. (4):

$$\log \Delta = \log K^{M}_{M(US-H)} - \log K^{M}_{M(U-H)calc} \quad (4)$$

The first stability constant given on the right hand side of Eq. (4) is the one experimentally measured for the various $M(US - H)^+$ complexes. The second stability constant is calculated based on Eq. (3) and the known parameters [10] of *m* and *b* by application of the acidity constant pK^{H}_{US} (Eq. (1)) valid for the deprotonation of the N(3)H site of the thiouridines.

In Table 1 the acidity constants (column 3) of uridine and four of its thioderivatives (see Fig. 1) are listed [3,10,13,21,22]. The corresponding measured stability constants are given in column 5 whereas the calculated stability constants based on the basicity of $N(3)^-$ are compiled in column 6. Application of these data to Eq. (4) provides the stability enhancements of the complexes formed with the various thiouridines (Table 1, column 7). Of course, for the $M(Urd - H)^+$ complexes no stability enhancement is observed. These stability data fit within the error limits on the reference lines.

insert Table 1 close to here

The observed stability enhancement is most pronounced for the $Cu(U2S - H)^+$ complex. The slightly higher stability enhancement of the Cu(U2S5Ac – H) species is a simple charge effect of the acetate substituent in position 5. Interestingly, for the $Ni(U2S - H)^+$ complex the stability enhancement is more pronounced by about 0.3 log units compared to the one formed with $(U4S - H)^-$. For the complexes of Cd^{2+} the situation is just reversed. This may have to do with the size of these two cations because Cd^{2+} is larger and the (C4)S site is more exposed than (C2)S and therefore somewhat more easily accessible.

Of interest is also the stability of the $Ni(U2S4S - H)^+$ complex. Ni^{2+} cannot bind simultaneously to both S sites for sterical reasons. Therefore, it is revealing to consider the stability enhancements of the individual binding sites[§] in $(U2S - H)^-$ and $(U4S - H)^-$ and to use these as micro stability enhancements to calculate an expected stability enhancement for

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[§] Clearly, if these were identical then the extra stability enhancement would simply amount to 0.3 log unit (equal to a factor of two) for the complex of the $(U2S4S - H)^-$ species.

$Ni(U2S4S - H)^+$, i.e., $10^{(1.64\pm0.08)} + 10^{(1.35\pm0.08)} = 10^{(1.82\pm0.08)}$; indeed, this value is within the error limits practically identical with the determined one, i.e., $10^{(1.95\pm0.09)}$, for $Ni(U2S4S - H)^+$ (Table 1; entry 4, column 7). This result indicates that the affinity of Ni^{2+} to $(N3)^-$ is not very pronounced because a "symmetrical" species based on a Ni^{2+}-$(N3)^-$ coordination and a twofold S interaction should give rise to an additional stability enhancement.

It is interesting to note that while $(U2S - H)^-$ forms very stable complexes with Cu^{2+} (see Table 1, entries 2b and 5), both analogues with a (C4)S unit (entries 3 and 4) immediately reduce Cu^{2+} to Cu^+ [13]. This observation indicates that some negative charge from $(N3)^-$ is delocalized toward (C4)S because thionate groups are known to reduce Cu^{2+} effectively. This does not mean that such a charge delocalization does not also occur toward (C2)S but it appears to be less pronounced.

2.2. Some considerations on the structure of the complexes formed with thiouridinates

It seems wise to begin this discussion with some reconsiderations about the complexes of cytidine (Cyd) [20]. The data point for $Ni(Cyd)^{2+}$ falls on the reference line defined by complexes of *ortho*-aminopyridine derivatives [20] as is evident from Fig. 2. This means that the (C2)O carbonyl group of Cyd does not affect the stability of $Ni(Cyd)^{2+}$, hence, in this species Ni^{2+} is solely coordinated to N3. The same has been surmised for the Ni^{2+} complexes of the uridinate derivatives [10]. This is different for the Cd^{2+} (see Fig. 2) and Cu^{2+} complexes. Both $M(Cyd)^{2+}$ species are more stable than is expected based on the reference lines of the *ortho*-aminopyridine derivatives [20]. Furthermore, the reference line for the $Cd(U - H)^+$ complexes is located even above the reference line due to the complexes formed with simple pyridine-type derivatives. This is evidence that the carbonyl groups are also involved in this case in metal ion binding [10]. For the Cyd complexes of Cu^{2+} and Cd^{2+} formation degrees for the chelates of 83 ± 4 and 58 ± 11% have been calculated, respectively [20]. For the complexes formed with $(Urd - H)^-$-type ligands this formation degree is most likely even higher [10].

From X-ray crystal structure studies (for details see [20]) it is known that Cd^{2+} and Cu^{2+} are able to form 4-membered chelates with the cytosine residue involving the N3 and (C2)O sites.

Co^{2+} binds in a monodentate fashion to N3 and the same was surmised for Ni^{2+}, including the $(U - H)^-$ systems [10]. For the present study this means that Cu^{2+} and Cd^{2+} most likely form 4-membered rings involving $(N3)^-$ and (C2)S (Fig. 3, Structure A). In fact, it is known that such low-membered chelates are more easily formed if a (C)O is replaced by a (C)S site [1]. For Ni^{2+} most probably the S sites in the $(US - H)^-$ ligands are the crucial ones though spectroscopic data indicate that also in this case some interaction with the $(N3)^-$ site occurs [13]. Of course, there is the possibility that also semichelates with a water molecule between the S-coordinated Ni^{2+} and $(N3)^-$ form (see Fig. 3, Structure B). It is evident that the total amount of chelates formed with Cd^{2+} and Cu^{2+} must be larger than the numbers given above for the corresponding Cyd complexes due to the very significant stability enhancements of about 1.3 and 2.3 log units, respectively (Table 1, entries 2b and 2c, column 7). Considering that the stability enhancements for the Ni^{2+} and Cd^{2+} complexes are in a comparable order (see Table 1), chelate formation also for Ni^{2+} must be assumed.

The most likely structures of these chelates for the $(U2S - H)^-$ ligand, as an example, are summarized in Fig. 3: Structure A is believed to be important in accordance with the mentioned

insert Figure 3 close to here

X-ray structures, even in solution. For Ni^{2+}, and possibly also to some extent Cd^{2+} and Cu^{2+}, Structure B may be of relevance. Structures as indicated in C involving a sulfur-hydrogen bond are in aqueous solution probably not very important because S, in contrast to O, is not an excellent site for H bond formation.

2.3. Definitions regarding the stabilities of thiouridine 5'-monophosphates

The structures of the two thionucleotides to be discussed in this section, i.e., $U2SMP^{2-}$ and $U4SMP^{2-}$, are shown in Fig. 4 below. The equilibrium constants to be considered are defined in Eqs. (5)–(8):

$$H(USMP)^- \rightleftharpoons H^+ + USMP^{2-} \quad (5a)$$

$$K^{H}_{H(USMP)} = [H^+][USMP^{2-}]/[H(USMP)^-] \quad (5b)$$

$$USMP^{2-} \rightleftharpoons H^+ + ((US - H)MP)^{3-} \quad (6a)$$

$$K^{H}_{USMP} = [H^+][((US - H)MP)^{3-}]/[USMP^{2-}] \quad (6b)$$

$$M^{2+} + USMP^{2-} \rightleftharpoons M(USMP) \quad (7a)$$

$$K^{M}_{M(USMP)} = [M(USMP)]/([M^{2+}][USMP^{2-}]) \quad (7b)$$

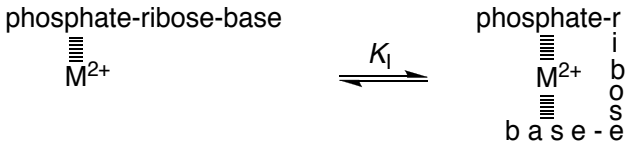
$$M^{2+} + ((US - H)MP)^{3-} \rightleftharpoons M[(US - H)MP]^- \quad (8a)$$

$$K^{M}_{M[(US - H)MP]} = [M[(US - H)MP]^-]/([M^{2+}][((US - H)MP)^{3-}]) \quad (8b)$$

However, of relevance for the present evaluations are only the acidity constants defined in Eqs. (5) and (6) as well as the stability constant of the $M[(US - H)MP]^-$ complex (Eq. (8)) in which (N3)H is deprotonated (see Table 3, vide infra). M(USMP) complexes (Eq. (7)) also form, at least in part: For example, Cu(U2SMP) exists, but it is better written as Cu·(U2S – H)MP·H to indicate that Cu^{2+} is at the thiouracil residue and the proton at the phosphate group; deprotonation of this H^+ occurs with $pK_a = 5.67$ [14]. This means, Cu^{2+} at the uracil moiety acidifies the proton at the $P(O)_2(OH)^-$ group of $H(U2SMP)^-$ by $\Delta pK_a = pK^{H}_{H(U2SMP)} - pK^{H}_{Cu\cdot(U2S - H)MP\cdot H} = (6.10 \pm 0.02) - (5.67 \pm 0.05) = 0.43 \pm 0.05$; a value which is in excellent agreement with the reasonings discussed below in Section 2.4.

It may be further added here that the deprotonation of the monoprotonated phosphate groups in $H(U2SMP)^-$ and $H(U4SMP)^-$ occurs with $pK^{H}_{H(U2SMP)} = 6.10 \pm 0.02$ and $pK^{H}_{H(U4SMP)} = 6.07 \pm 0.03$, respectively [14]; these values are very similar to that of the parent $H(UMP)^-$ species which has $pK^{H}_{H(UMP)} = 6.15 \pm 0.01$ [23]. This is very different for the deprotonation of the (N3)H sites in these compounds: For $U2SMP^{2-}$ and $U4SMP^{2-}$ $pK^{H}_{U2SMP} = 8.30 \pm 0.01$ and $pK^{H}_{U4SMP} = 8.23 \pm 0.02$, respectively [14] hold, showing that, if compared with UMP^{2-}, i.e., $pK^{H}_{UMP} = 9.45 \pm 0.02$ [23], here the exchange of (C)O by a (C)S group has a very pronounced effect.

The question that arises in the present case is: Does any macrochelate formation as indicated in equilibrium (9) occur?



(9)

Evidently a simultaneous coordination of a metal ion to one of the (C)S sites and the phosphate group could occur only if the thionucleotides are transformed from their dominating *anti* into the *syn* conformation (see Fig. 4). This energy barrier is in the order of about 7 kJ/mol as is known from related nucleotides [20]. Consequently, the parent UMP^{2-} does not form any macrochelates with the common divalent metal ions [24,25]. Does the exchange of a (C)O site by a (C)S unit change the situation?

insert Figure 4 close to here

This question can best be answered by a new evaluation method which was recently introduced to quantify the chelate [15] or macrochelate effect [16]. This effect refers to an enhanced stability of a complex formed by a ligand offering two or more donor atoms if these two (or more) donor atoms participate simultaneously in complex formation. The method is based on an 'expected' stability constant which is calculated from the micro stability constants of the individual binding sites possibly involved, in the present case this is a (C)S unit and the phosphate group. This expected constant is defined in Eq. (10):

$$K^{M}_{M[(US-H)MP]expected} = \frac{[((US-H)MP\cdot M)^{-}] + [(M\cdot(US-H)MP)^{-}]}{[M^{2+}][((US-H)MP)^{3-}]} \quad (10a)$$

$$= k^{M}_{(US-H)MP\cdot M} + k^{M}_{M\cdot(US-H)MP} \quad (10b)$$

Phosphate coordination is represented in these isomeric species by $((US-H)MP\cdot M)^{-}$ and thiouracil binding by $(M\cdot(US-H)MP)^{-}$.

2.4. Evaluations of the M[(US – H)MP]⁻ complexes with regard to the chelate effect

The first of the micro stability constants which appear in Eq. (10b) can be calculated based on previous research obtained with phosph(on)ate ligands ($R\text{-}PO_3^{2-}$) and their complexes [26,27]. Plots of $\log K^{M}_{M(R\text{-}PO_3)}$ *versus* $pK^{H}_{H(R\text{-}PO_3)}$ resulted in straight lines defined in analogy to Eq. (3). The corresponding parameters for the three metal ions considered here are given in Eqs. (11) to (13):

$$\log K^{Ni}_{Ni(R\text{-}PO_3)} = 0.245\cdot pK^{H}_{H(R\text{-}PO_3)} + 0.422 \quad (11)$$

$$\log K^{Cu}_{Cu(R\text{-}PO_3)} = 0.465 \cdot pK^{H}_{H(R\text{-}PO_3)} - 0.015 \quad (12)$$

$$\log K^{Cd}_{Cd(R\text{-}PO_3)} = 0.329 \cdot pK^{H}_{H(R\text{-}PO_3)} + 0.399 \quad (13)$$

The error limits (3σ) of log stability constants calculated with given $pK^{H}_{H(R\text{-}PO_3)}$ values and Eqs. (11), (12) and (13) are ±0.05, ±0.06 and ±0.05, respectively, in the pH range 5–8 [26–28].

For the calculation of a stability constant based on Eqs. (11)–(13) the acidity constant $pK^{H}_{H(R\text{-}PO_3)}$ is needed. As discussed above, for monoprotonated 2-thiouridine 5'-monophosphate, $H(U2SMP)^-$, the acidity constants $pK^{H}_{H(U2SMP)} = 6.10 \pm 0.02$ (Eq. (6)) and $pK^{H}_{U2SMP} = 8.30 \pm 0.01$ (Eq. (7)) hold [14]. Evidently it is the first value that is of relevance here but it is also clear that this value needs to be corrected for the charge effect that the $(N3)^-$ site exercises on the deprotonation of the $P(O)_2(OH)^-$ group because the complexes are formed with both ligand sites being deprotonated and have such the composition $M[(U2S - H)MP]^-$.

This charge effect follows from a comparison of the pK^{H}_{U2SMP} (Eq. (6)) and pK^{H}_{U2S} values (Eq. (1)) because the first acidity constant involves the effect of the twofold negatively charged phosphate group on the release of the proton from the (N3)H site, i.e., Eq. (14) holds:

$$\Delta pK_a = pK^{H}_{U2SMP} - pK^{H}_{U2S} = (8.30 \pm 0.01) - (8.05 \pm 0.01) = 0.25 \pm 0.01 \quad (14)$$

Because such effects must be reciprocal, it is not surprising that the same effect was observed for a distance-wise very similar deprotonation of the monoprotonated phosphate group of orotidinate 5'-monophosphate ($pK^{H}_{H(OMP)} = 6.40 \pm 0.02$ [29]) and uridine 5'-monophosphate ($pK^{H}_{H(UMP)} = 6.15 \pm 0.01$ [23]), i.e., $\Delta pK_a = 0.25 \pm 0.02$. Hence, for the micro acidity constant of U2SMP valid for the deprotonation of the monoprotonated phosphate group under conditions where the thiouracil residue is deprotonated $pk^{(U2S - H)MP}_{(U2S - H)MP \cdot H} = (6.10 \pm 0.02) + (0.25 \pm 0.01) = 6.35 \pm 0.02$ follows. This microconstant represents the basicity of the phosphate group of $((U2S - H)MP)^{3-}$ and may be used together with the straight-line plots of Eqs. (11)–(13). These results are given in column 4 of Table 2. The analogous calculations for $H(U4SMP)^-$ with the acidity constants $pK^{H}_{H(U4SMP)} = 6.07 \pm 0.03$ and $pK^{H}_{U4SMP} = 8.23 \pm 0.02$ lead with Eq. (15)

$$\Delta pK_a = pK^{H}_{U4SMP} - pK^{H}_{U4S} = (8.23 \pm 0.02) - (8.01 \pm 0.01) = 0.22 \pm 0.02 \quad (15)$$

to the micro acidity constant $pk^{(U4S-H)MP}_{(U4S-H)MP\cdot H}$ = (6.07 ± 0.03) + (0.22 ± 0.02) = 6.29 ± 0.04 which is needed for the straight-line equations.

insert Table 2 close to here

However, the stability constants given in column 4 of Table 2 need to be corrected for the charge effect that the uncoordinated but negatively charged thiouracilate residue exercises on a metal ion bound to the PO_3^{2-} group. This effect amounts to 0.40 ± 0.15 log units [16,26,29] (in excellent agreement with the value given in Section 2.3 in the paragraph which follows Eqs. (5)–(8)). Hence, this value needs to be added to the constants listed in column 4 to give the micro stability constants for the $((US-H)MP\cdot M)^-$ species formed at the phosphate site of the $((US-H)MP)^{3-}$ thionucleotides (Table 2, column 5).

Values for the second micro stability constant in Eq. (10b) are obtained by employing the known (see Table 1) stability constants of the thiouridinate complexes, $M(US-H)^+$. These values are provided in column 6 of Table 2 and they need to be corrected for the difference in basicity of the uracilate residue in the thiouridinates and in the $((US-H)MP)^{3-}$ species. These differences have already been expressed in Eqs. (14) and (15) for U2SMP and U4SMP, respectively. Application of the slopes of the log $K^M_{M(U)}$ *versus* pK^H_U straight-line plots ([10]; see also Fig. 2) leads to the basicity-corrected log $K^M_{M(US-H)B,cor}$ values listed in column 7. It is evident from Table 2 that these corrections are very minor. More important is the effect that the uncomplexed, twofold negatively charged PO_3^{2-} group has on M^{2+} binding at the thiouracil residue; this charge effect amounts to about 0.60 ± 0.15 log units [16] and may be compared with the mentioned 0.40 ± 0.15 log units due to the singly charged $P(O)_2(OH)^-$ group (see above). Addition of 0.60 log units to the values in column 7 gives the logarithms of the micro stability constants log $k^M_{M\cdot(US-H)MP}$ listed in column 8; they quantify the metal ion affinity of the thiouracilate residue in the $((US-H)MP)^{3-}$ ligands.

Now the expected stability of the $M[(US-H)MP]^-$ complexes can be calculated according to Eq. (10b). These values are listed in column 5 of Table 3. In other words, now the expected

insert Table 3 close to here

stability constants log $K^M_{M[(US-H)MP]expected}$ (Eq. 10) for $M[(US-H)MP]^-$ complexes without

any macrochelate formation, but based solely on the M^{2+} affinity of the two individual binding sites of $((US - H)MP)^{3-}$ are known, and therefore we can define the chelate effect [15,16] according to equation (16) by comparing the expected stability constant with the one actually measured:

$$\log Chelate = \log K^{M}_{M[(US - H)MP]} - \log K^{M}_{M[(US - H)MP]expected} \quad (16)$$

The values for the three terms which appear in Eq. (16) are listed in columns 6, 4 and 5, respectively, in Table 3.

A view on the log *Chelate* values in column 6 of Table 3 reveals that a chelate effect is only observed for the $Cu[(U2S - H)MP]^{-}$ complex; possibly there is also a small effect for the $Cd[(U2S - H)MP]^{-}$ species. This means that only Cu^{2+} (and possibly Cd^{2+}) is (are) able to bind to the $(C2)S/(N3)^{-}$ unit and simultaneously to the phosphate group, thus forcing U2SMP into the less stable *syn* conformation. It is not surprising that no indication for any macrochelate formation in the complexes of the $((U4S - H)MP)^{3-}$ ligand is observed because the structure of this ligand (see Fig. 4) is such that a metal ion bound at the $(C4)S/(N3)^{-}$ unit can hardly reach the phosphate group and *vice versa*. It is not clear why in the latter case the expected values are relatively high compared to the measured ones (Table 3, entries 2), but still it is certain that no hint for macrochelate formation occurs.

2.5. Formation degrees of the various $M[(US - H)MP]^{-}$ isomers

Because the definition of the macrochelate effect as given in Eq. (16) is based on the micro stability constants of individual metal ion binding sites (Eq. (10b)), knowledge of log *Chelate* allows not only the calculation of the formation degree of the macrochelate but also of the species in which M^{2+} is solely bound either to the thiouracilate moiety or to the phosphate group of $((US - H)MP)^{3-}$. Evidently this kind of knowledge is meaningful for biological systems [30–32] because it demonstrates how metal ions can switch from one site to another through macrochelate formation [16].

At this point it is helpful to note that from Eq. (16) Eq. (17) follows:

$$10^{\log Chelate} = K^{M}_{M[(US - H)MP]} / K^{M}_{M[(US - H)MP]expected} \quad (17a)$$

$$= K^{M}_{M[(US-H)MP]} / (k^{M}_{(US-H)MP\cdot M} + k^{M}_{M\cdot(US-H)MP}) \quad (17b)$$

$$= \frac{[M[(US-H)MP]^{-}]}{[((US-H)MP\cdot M)^{-}] + [(M\cdot(US-H)MP)^{-}]} \quad (17c)$$

According to this definition (Eq. 17) $10^{\log Chelate}$ is the dimensionless equilibrium constant that quantifies the position of equilibrium (18):

$$\{((US-H)MP\cdot M)^{-} + (M\cdot(US-H)MP)^{-}\} \rightleftharpoons M[(US-H)MP]^{-} \quad (18)$$

In this equilibrium the left side contains the sum of the 'open' species resulting from metal ion binding to the individual sites of $((US-H)MP)^{3-}$, whereas $M[(US-H)MP]^{-}$ at the right hand side represents the *total* amount of complexes formed, including the chelates.

For log *Chelate* = 0 in Eq. (16), the ratio given in Eq. (17c) equals one; this means, as it should be, that no chelates exist and that all $M[(US-H)MP]^{-}$ species are present as the open $((US-H)MP\cdot M)^{-}$ and $(M\cdot(US-H)MP)^{-}$ isomers. For all situations in which log *Chelate* > 0, the ratio will be larger than 1 and this then means that macrochelates exist [16]. For example, for log *Chelate* = 0.3, a value of 2:1 follows for the ratio given in Eq. (17c). This means that 50% of all $M[(US-H)MP]^{-}$ complexes exist in the form of macrochelates.

Of course, the two open species are also in equilibrium with each other. Clearly, the position of this equilibrium is defined by the ratio of the two micro stability constants given in Eq. (10b), the values of which are listed in columns 5 and 8 of Table 2. Application of this information allows one to calculate the formation degrees of all the species present in a $M[(US-H)MP]^{-}$ system, that is, the amount of 'closed' or macrochelated species present, and consequently, also of the *total* amount of open species, $M[(US-H)MP]^{-}_{op/tot}$. Application of the micro stability constants to these percentages gives then those of the two open species at their individual binding sites. The corresponding results are listed in Table 4.

insert Table 4 close to here

Several conclusions are possible from these results: (i) In the case of the $Cu[(U2S-H)MP]^{-}$ species the macrochelates dominate with approximately 58%; however, from the in total 42% of the open species practically all have Cu^{2+} coordinated to the thiouracilate residue and purely phosphate-bound species occur only in trace amounts. (ii) The upper and lower limits given of

the various isomers for the Cd[(U2S – H)MP]$^-$ system (Table 4, entry 1c) demonstrate how relatively small changes in log *Chelate* (as long as the value is below 1) heavily affect the formation degrees of the isomers. (iii) As one might have expected, in all those instances where macrochelates form only in trace amounts or not at all, thiouracilate coordination dominates heavily, but even though, small amounts of solely phosphate-coordinated complexes are still formed.

3. Conclusions

Despite all the shortcomings which arise from the estimates of the charge effects (given with generous error limits) on the micro stability constants employed for the individual binding sites (see Table 2), it is evident that meaningful results are still obtained: Replacement of one of the carbonyl oxygens in an uracil residue by a sulfur atom significantly affects the metal ion binding properties of the corresponding nucleosides or nucleotides. Clearly, the presence of a sulfur atom strongly enhances the stabilities of the complexes formed with Ni^{2+}, Cu^{2+} or Cd^{2+}.

In contrast, for alkaline earth ions, like Mg^{2+} or Ca^{2+}, which have a very low affinity toward sulfur sites, it is expected that, for example, Mg[(U2S – H)MP]$^-$ behaves very similar as Mg(UMP – H)$^-$, except that the thio species loses its proton from the (N3)H site at a much lower pH and may thus exist in the physiological pH range.

For the biologically important Zn^{2+} no information is available, but based on the *Stability Ruler* of Martin [4–6] it is expected that the properties of its complexes are approximately between those described herein for the Ni^{2+} and Cd^{2+} complexes. In other words, Zn^{2+} is expected to bind heavily to the thiouracilate residue. Again, due to the higher acidity of the sulfur-containing ligands, these (N3)H-deprotonated species are expected to occur to some extent also in the physiological pH range as it is the case with the Ni^{2+} and Cd^{2+} complexes [14] of the thiouridinate 5'-monophosphates.

4. Abbreviations and definitions

See Figures 1 and 4 for the uracil derivatives; *I*, ionic strength; K_a, acidity constant; L, general ligand; M^{2+}, general divalent metal ion; R-PO_3^{2-}, simple phosphate monoester or phosphonate ligand with a residue R that does not affect metal ion binding; U, uracil derivative (see Fig. 1) which often also includes the thio derivatives, if so, this is clear from the context; US, thiouracil derivative. Formulas like U2SMP (see Fig. 4) written without a charge represent the species in general.

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Table 1

Negative logarithms of the acidity constants (Eq. (1)) of uridine and its thio derivatives (U) shown in Fig. 1, together with the stability constant comparisons for several $M(U - H)^-$ complexes between the measured stability constants (Eq. (2)) and the calculated ones based on Eq. (3) with the resulting stability differences log Δ as defined by Eq. (4)[a]

No.	U	pK_U^H	M^{2+}	log $K^M_{M(U-H)}$	log $K^M_{M(U-H)calc}$	log Δ
1a	Urd	9.18 ± 0.02	Ni^{2+}	1.76 ± 0.06	1.70 ± 0.07	0.06 ± 0.09
b			Cu^{2+}	4.13 ± 0.20	4.13 ± 0.21	0
c			Cd^{2+}	3.16 ± 0.04	3.21 ± 0.05	–0.05 ± 0.06
2a	U2S	8.05 ± 0.01	Ni^{2+}	2.99 ± 0.04	1.35 ± 0.07	1.64 ± 0.08
b		8.05 ± 0.04	Cu^{2+}	5.91 ± 0.06	3.62 ± 0.21	2.29 ± 0.22
c		8.05 ± 0.01	Cd^{2+}	4.11 ± 0.03	2.77 ± 0.05	1.34 ± 0.06
3a	U4S	8.01 ± 0.01	Ni^{2+}	2.68 ± 0.04	1.33 ± 0.07	1.35 ± 0.08
b			Cd^{2+}	4.34 ± 0.01	2.75 ± 0.05	1.59 ± 0.05
4	U2S4S	7.17 ± 0.01	Ni^{2+}	3.02 ± 0.05	1.07 ± 0.07	1.95 ± 0.09
5	U2S5Ac$^-$	8.55 ± 0.04	Cu^{2+}	6.45 ± 0.06	3.85 ± 0.21	2.60 ± 0.22

[a] The values in entries 1 are from Ref. [10] (25ºC; I = 0.1 M, $NaNO_3$) (3σ). The stability constant for $Cu(Urd - H)^+$ (entry 1b) is calculated from the log $K^{Cu}_{Cu(U-H)}$ *versus* pK_U^H plot (see [10]); this result agrees well with log $K^{Cu}_{Cu(Urd-H)}$ = 4.2 ± 0.2 (25ºC; I = 1 M, $NaNO_3$) [21]) and 4.32 ± 0.06 (20ºC; I = 0.1 M, KNO_3 [22]). – Entries 2b and 5 are from Ref. [3]; all the other values in entries 2, 3 and 4 are from Ref. [13] (25ºC; I = 0.2 M, KCl) (1σ). – The error limits listed above (σ = standard deviation) are those given in the various studies. The error limits of the derived data, in the present case for log Δ, were calculated according to the error propagation after Gauss. This also holds for the errors given in the other tables of this study.

Table 2

Estimation of the various micro stability constants needed for the application of Eq. (10b) regarding the independent metal ion-binding sites, i.e., the thiouracilate residue and the phosphate group of the $((US - H)MP)^{3-}$ ligands (see Fig. 4)[a]

No.	$((US - H)MP)^{3-}$	M^{2+}	log $K^{M}_{M(R\text{-}PO_3)}$	log $k^{M}_{(US-H)MP\cdot M}$	log $K^{M}_{M(US-H)}$	log $K^{M}_{M(US-H)B,cor}$	log $k^{M}_{M\cdot(US-H)MP}$
1a	$((U2S - H)MP)^{3-}$	Ni^{2+}	1.98 ± 0.05	2.38 ± 0.16	2.99 ± 0.04	3.07 ± 0.04	3.67 ± 0.16
b		Cu^{2+}	2.94 ± 0.06	3.34 ± 0.16	5.91 ± 0.06	6.02 ± 0.06	6.62 ± 0.16
c		Cd^{2+}	2.49 ± 0.05	2.89 ± 0.16	4.11 ± 0.03	4.21 ± 0.03	4.81 ± 0.15
2a	$((U4S - H)MP)^{3-}$	Ni^{2+}	1.96 ± 0.05	2.36 ± 0.16	2.68 ± 0.04	2.75 ± 0.04	3.35 ± 0.16
b		Cd^{2+}	2.47 ± 0.05	2.87 ± 0.16	4.34 ± 0.01	4.43 ± 0.01	5.03 ± 0.15

[a] For details see text in Section 2.4. – Note, the values given in column 4 were calculated with Eqs. (11)–(13) and those listed in column 6 are from Table 1.

Table 3

Comparison of the measured (Eq. (8)) and calculated (expected) stability constants of several $M[(US - H)MP]^-$ complexes and their evaluation towards the chelate effect as defined by Eq. (16)[a]

No.	$((US - H)MP)^{3-}$	M^{2+}	$\log K^{M}_{M[(US-H)MP]}$		log *Chelate*
			measured	expected	
1a	$((U2S - H)MP)^{3-}$	Ni^{2+}	3.51 ± 0.04	3.69 ± 0.15	0
b		Cu^{2+}	7.00 ± 0.04	6.62 ± 0.16	0.38 ± 0.16
c		Cd^{2+}	4.93 ± 0.03	4.82 ± 0.15	0.11 ± 0.15
2a	$((U4S - H)MP)^{3-}$	Ni^{2+}	2.85 ± 0.08	3.39 ± 0.15	0
b		Cd^{2+}	4.76 ± 0.04	5.03 ± 0.15	0

[a] For further details see text in Section 2.4. The measured constants in column 4 are from Ref. [14] (25ºC; I = 0.1 M, KNO_3) and the expected values in column 5 were calculated according to Eq. (10b) by using the micro stability constants listed in columns 5 and 8 of Table 2.

Table 4

Summary of the formation degrees of the macrochelated (closed) species, $M[(US - H)MP]^-_{cl}$, of the total open species, $M[(US - H)MP]^-_{op/tot}$, as well as of the open phosphate- and thiouracilate-coordinated complexes, $((US - H)MP{\cdot}M)^-$ and $(M{\cdot}(US - H)MP)^-$, as calculated (Eq. (17)) based on the extent of the chelate effect (Eq. (16)) and the micro stability constants for individual binding of the metal ions (Table 2, columns 5 and 8) either to the phosphate group or the thiouracilate residue (Eq. (10)) in aqueous solution[a]

No.	$((US - H)MP)^{3-}$	M^{2+}	log *Chelate*	% $M[(US - H)MP]^-_{cl}$	% $M[(US - H)MP]^-_{op/tot}$	% $((US - H)MP{\cdot}M)^-$	% $(M{\cdot}(US - H)MP)^-$
1a	$((U2S - H)MP)^{3-}$	Ni^{2+}	0	0	100	5	95
b		Cu^{2+}	0.38 ± 0.16	58	42	0.02	42
c		Cd^{2+}	0.11 ± 0.15	22 (45/0)	78 (55/100)	1 (0.7/1.2)	77 (54/99)
2a	$((U4S - H)MP)^{3-}$	Ni^{2+}	0	0	100	9	91
b		Cd^{2+}	0	0	100	1	99

[a] The values in column 4 are from Table 3 (column 6). The percentage of $M[(US - H)MP]^-_{op/tot}$ follows from Eq. (17c) because the total amount of $M[(US - H)MP]^-$ present equals 100%; hence, it follows further that % $M[(US - H)MP]^-_{cl}$ = 100 – % $M[(US - H)MP]^-_{op/tot}$. Application of the micro stability constants (Table 2, columns 5 and 8) of the $((US - H)MP{\cdot}M)^-$ and $(M{\cdot}(US - H)MP)^-$ species to the total amount of $M[(US - H)MP]^-_{op/tot}$ present, allows calculation of the percentages of the individually bound open isomers (see also text in Section 2.5). The listed values are only estimations due to the large error limits of the micro stability constants, but the above results still prove that both isomers exist in equilibrium. As an example for entry 1c the upper and lower limits are listed as they follow from log *Chelate* = 0.26 (= 0.11 + 0.15) and 0.00 (= 0.11 – 0.15), respectively.

Figure Legends

Fig. 1. Chemical structures of uridine (Urd), 2-thiouridine (U2S), 4-thiouridine (U4S), 5-carboxymethyl-2-thiouridine (U2S5Ac$^-$), 2,4-dithiouridine (U2S4S) and cytidine (Cyd). All pyrimidine nucleosides are shown in their dominating *anti* conformation [11,12]. The uridine derivatives are abbreviated as U and in the (N3)$^-$-deprotonated, anionic form as (U – H)$^-$, which is to be read as U *minus* H$^+$; of course, the resulting negative charge at N3 can be delocalized in part to the neighboring (C)O or (C)S units. Further abbreviations and definitions are given in the legend of Fig. 4 and in Section 4.

Fig. 2. Evidence of an increased stability of several M(US – H)$^-$ complexes of Ni^{2+} and Cd^{2+} (⊗) based on the comparison of their data points (values from Table 1; 25ºC; I = 0.2 M, KCl) with the log $K^{M}_{M(U-H)}$ *versus* pK^{H}_{U} straight-line relationships (+) [10] for uridinate-type ligands (U – H)$^-$ and their complexes formed with Ni^{2+} and Cd^{2+}, as well as with the corresponding relationships between log $K^{M}_{M(L)}$ and p$K^{H}_{H(L)}$ for simple pyridine-type ligands PyN (●) and the sterically inhibited *o*-amino(methyl)pyridine-type ligands *o*PyN (■) [19]; the data points for the M^{2+}/Cyd systems (○) [20] are given for further comparisons (25ºC; I = 0.5 M, $NaNO_3$). The least-squares straight reference lines are drawn according to Eq. (3). The plotted equilibrium constants refer for the M^{2+}/(U – H)$^-$ systems (+) to (from left to right) 5-fluorouridine, 5-chloro-2'-deoxyuridine, uridine and thymidine (= 2'-deoxy-5-methyluridine) (25ºC; I = 0.1 M, $NaNO_3$) [10], for the PyN systems (L) (●) to 3-chloropyridine, 4-bromopyridine, 4-(chloromethyl)pyridine, pyridine, 3-methylpyridine and 3,5-dimethylpyridine [19], and for the *o*-substituted *o*PyN systems (L) (■) to 2-methyl-5-bromopyridine, 2-amino-5-bromopyridine, tubercidine (= 7-deazaadenosine), 2-methylpyridine and 2-aminopyridine [19] (25ºC; I = 0.5 M, $NaNO_3$). The reduced stability of the M(*o*PyN)$^{2+}$ complexes, compared to the M(PyN)$^{2+}$ ones, reflects the steric inhibition of an *o*-amino (or *o*-methyl) group. The change in I from 0.1 to 0.5 M is of no significance because the small connected shifts in log K and pK_a go "parallel" to each other.

Fig. 3. Possible metal ion-binding modes in the chelates formed in equilibrium by the $M(U2S - H)^{+}$ complexes (as an example) in aqueous solution (see Section 2.2). The negative charge in the 2-thiouridinate structures is shown on N3, but it can be delocalized in part on the neighboring (C)O and (C)S groups. Of course, in Structure C a further semichelate may be formed involving a coordinated water molecule and the (C4)O group.

Fig. 4. Chemical structures of 2-thiouridine 5'-monophosphate ($U2SMP^{2-}$) and 4-thiouridine 5'-monophosphate ($U4SMP^{2-}$) as well as of their parent nucleotide uridine 5'-monophosphate (UMP^{2-}) shown in their dominating *anti* conformation [11,12]. Deprotonation of the thiouridine 5'-monophosphates ($USMP^{2-}$) at the (N3)H site leads to 3-fold negatively charged species which are written as $((US - H)MP)^{3-}$ to indicate that the proton is released from the thiouracil residue.

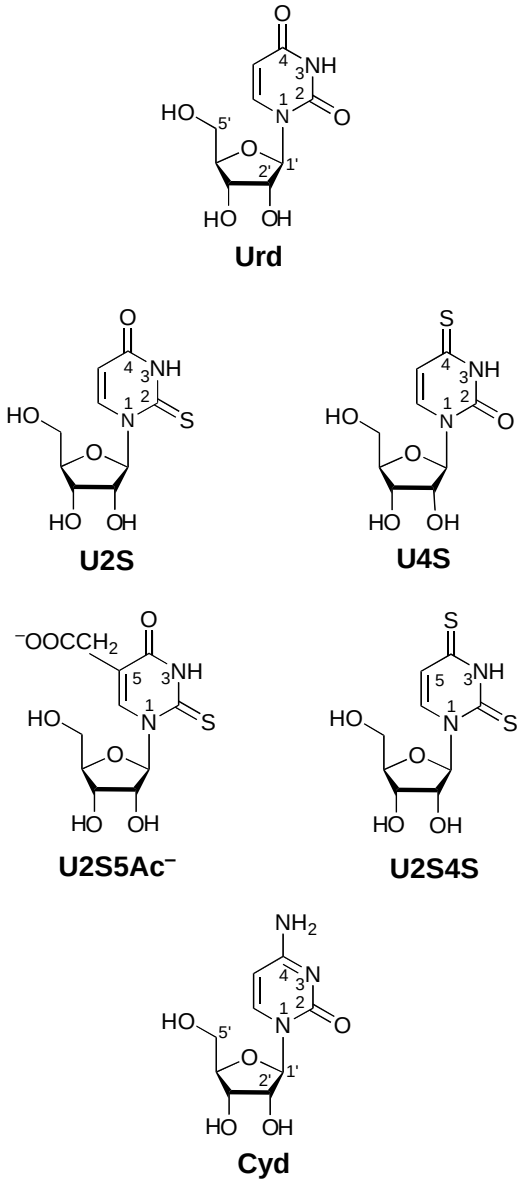


Figure 1

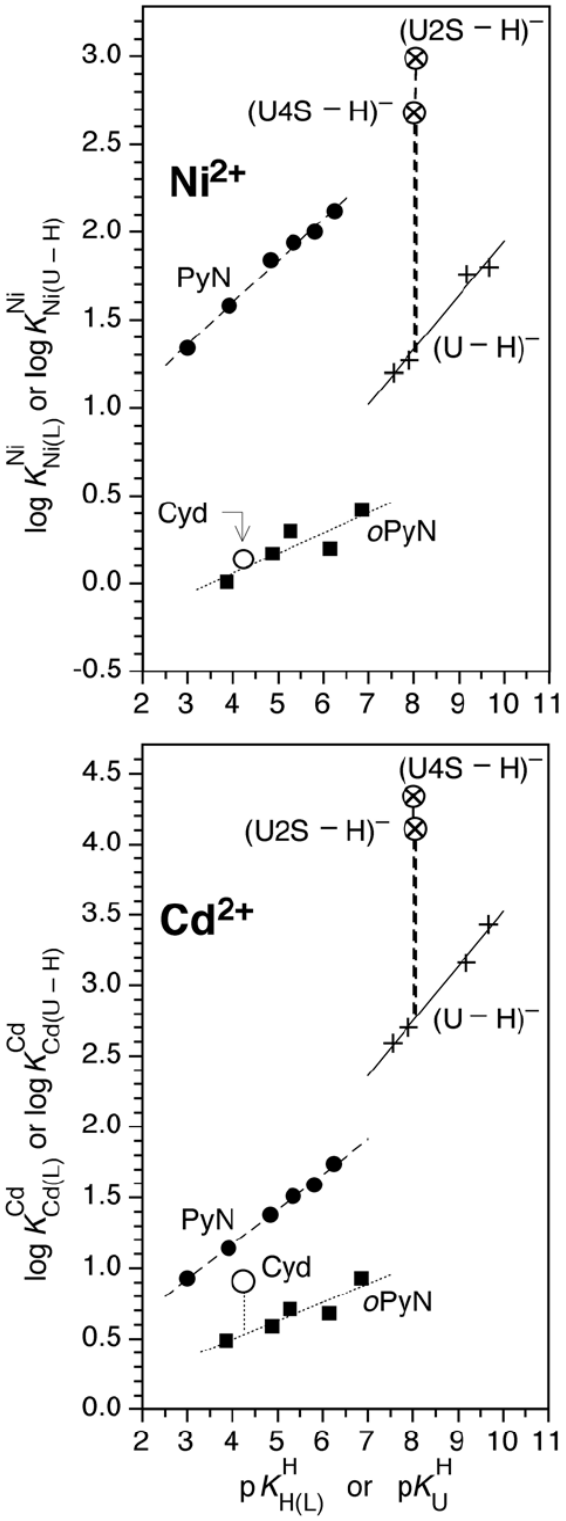

(U2S − H)⁻
(U4S − H)⁻
Ni2+
PyN
(U − H)⁻
Cyd
oPyN
log K^Ni_Ni(L) or log K^Ni_Ni(U − H)
(U4S − H)⁻
(U2S − H)⁻
Cd2+
(U − H)⁻
PyN
Cyd
oPyN
log K^Cd_Cd(L) or log K^Cd_Cd(U − H)
p K^H_H(L) or p K^H_U

Figure 2

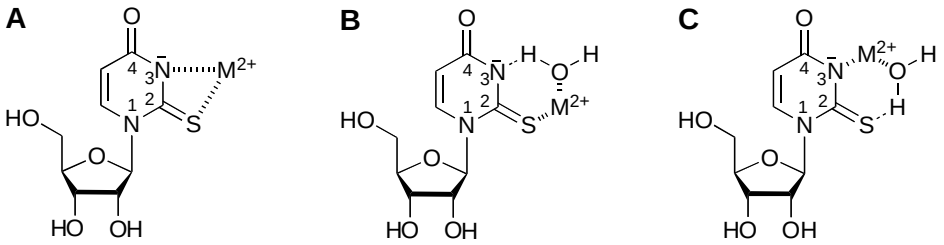
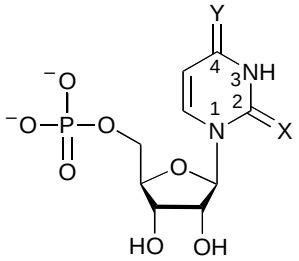


Figure 3



U2SMP^{2-}: X = S, Y = O
U4SMP^{2-}: X = O, Y = S
UMP^{2-}: X = Y = O

Figure 4