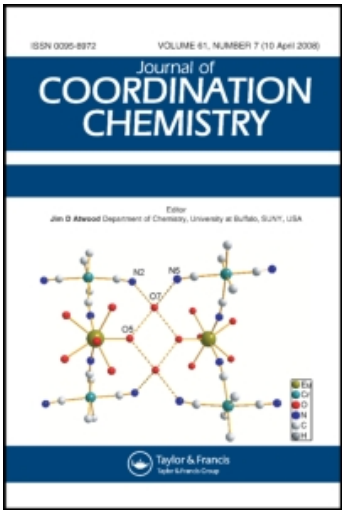

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Influence of decreasing solvent polarity (1,4-dioxane/water mixtures) on the stability and structure of complexes formed by copper(II), 2,2′-bipyridine or 1,10-phenanthroline and guanosine 5′-diphosphate: evaluation of isomeric equilibria†‡

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The stability constants of the 1:1 complexes formed between $Cu(Arm)^{2+}$, where Arm = 2,2′-bipyridine or 1,10-phenanthroline, and guanosine 5′-diphosphate $(GDP)^{3-}$ or its monoprotonated form $H(GDP)^{2-}$ were determined by potentiometric pH titrations in water and in water containing 30 or 50% (v/v) 1,4-dioxane (25°C; $I = 0.1$ M, $NaNO_3$). The stability of the binary $Cu(GDP)^{-}$ complex is enhanced due to macrochelate formation of the diphosphate-coordinated Cu^{2+} with N7 of the guanine residue as previously shown. In $Cu(Arm)(GDP)^{-}$ the N7 is released from Cu^{2+} and the stability enhancement of more than one log unit in aqueous solution is clearly attributable to intramolecular stack formation between the aromatic rings of Arm and the guanine moiety. Indeed, stacked isomers occur to more than 90% in equilibrium with open unstacked forms. Surprisingly, the same formation degrees of the stacks are observed for Cu(Arm)(dGMP) complexes, where $dGMP^{2-}$ = 2′-deoxyguanosine 5′-monophosphate, despite the fact that the overall stability of the latter species is by about 2.7 log units lower. In 1,4-dioxane–water mixtures stack formation is drastically reduced, probably due to hydrophobic solvation of the aromatic rings by the ethylene bridges of 1,4-dioxane. The relevance of these results regarding biological systems is indicated.

Keywords: Aromatic-ring stacking; Isomeric equilibria; Mixed solvents; Nucleotide complexes; Stability constants

1. Introduction

Nucleotides commonly participate in enzymatic reactions in the form of metal ion complexes [1, 2]. Such substrates need to be properly oriented in the active site cavities of the enzymes [3] where non-covalent interactions like hydrogen bonding [4–6] and π–π stacking between aromatic residues [4, 7–9] occur. Among nucleotides

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†This study is dedicated to Professor Dr Alfredo Mederos on the occasion of his retirement from the University of La Laguna (Spain) with the very best wishes for all of his future endeavors.

‡This is part 70 of the series *Ternary Complexes in Solution*; for parts 69 and 68 see [14] and [15], respectively.

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those with a purine residue, due to their two-ring aromatic moiety, are favored in forming relatively stable stacks, especially if compared with pyrimidine nucleotides.

2,2′-Bipyridine (Bpy) and 1,10-phenanthroline (Phen) (figure 1) as well as derivatives thereof have proven very helpful in evaluating the stacking capabilities of aromatic residues in metal ion complexes [7, 10] of amino acids [8, 9, 11, 12] and nucleotides [8, 11, 13]. The metal ion most often involved in these studies is Cu^{2+} [7, 14–17], next to Pt^{2+} [7, 8]. Therefore, in the present study that deals with mixed ligand complexes, we also used the ''indicator ligands'' Bpy or Phen. Copper(II) was selected because (i) many comparisons are possible (see above), (ii) its complexes with Bpy and Phen are of high stability [18, 19] and (iii) because it is itself a biologically important metal ion [20–22].

The nucleotide guanosine 5′-diphosphate (GDP^{3-}) (figure 1 [10, 23]), was selected for two reasons: (i) GDP^{3-} participates in many metabolic reactions; e.g., it plays a role in the so-called G-protein systems [24] which utilize guanosine 5′-triphosphate (GTP^{4-}) [25, 26] and where metal ions are involved in connected hydrolysis reactions [27–29]; (ii) being interested in extended comparisons [30], after the ternary complexes of the adenine-nucleotides [31] AMP^{2-} [32], ADP^{3-} [31] and ATP^{4-} [7, 33], it was logical to study those of the guanine-nucleotides. Only the mixed ligand complexes containing 2′-deoxyguanosine 5′-monophosphate ($dGMP^{2-}$) have received attention [34, 35].

Thus, we determined the stability constants of the ternary Cu(Arm)(H; GDP) and $Cu(Arm)(GDP)^{-}$ complexes, where Arm represents a heteroaromatic nitrogen base, i.e., Bpy or Phen, and evaluated our data with regard to the position of the intramolecular equilibrium (1), which quantifies the extent of π–π stacking interaction between Arm and the purine residue. The formation degree of the stacked (st) species $Cu(Arm)(GDP)^{-}_{st}$ is

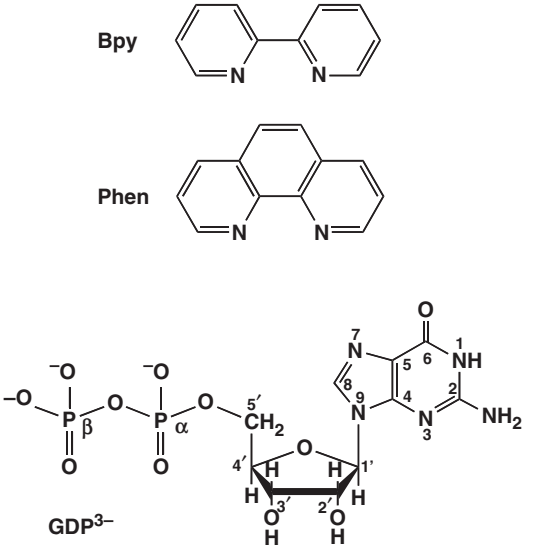
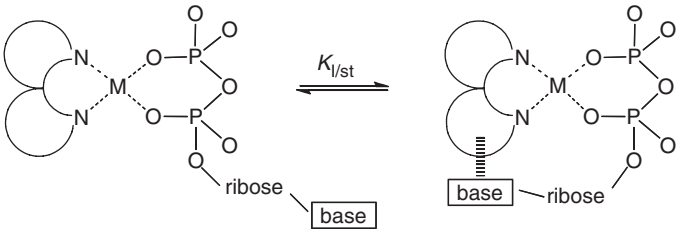


Figure 1. Chemical structures of the heteroaromatic nitrogen bases (Arm) used in this study, i.e. 2,2′-bipyridine (Bpy) and 1,10-phenanthroline (Phen), as well as of the nucleotide guanosine 5′-diphosphate (GDP^{3-}) which is shown in its dominating *anti*-conformation [10, 23].

calculated and compared with previous results for Cu(Arm)(dGMP)$_{st}$ [34]; the stacking intensities are comparable in both instances.



(1)

However, there is one more important aspect: it is now well-established that the so-called "effective" or "equivalent solution" dielectric constants or permittivities in proteins [36–40] or in the active site cavities of metalloenzymes [41] are reduced compared to the situation in bulk water. It is also generally agreed that different types of water exist in cells [42, 43] and that at the protein–water interface the activity of water is decreased [6, 44] due to the presence of aliphatic and aromatic amino acid side chains [45]. Estimates for the dielectric constants (ε) in such biological locations range from *ca.* 30 to 70 [36, 38, 41], compared to about 80 in bulk water; hence, by employing aqueous solutions that contain *ca.* 30 to 50% 1,4-dioxane, one may expect to simulate to some degree the situation in active site cavities [11]. The dielectric constants of the two indicated solvent mixtures are *ca.* 60 and 35, respectively [41, 46–48], and quite a number of studies involving binary complexes exist [11, 33, 49–52], yet information about mixed ligand complexes is hardly available [33]. Therefore, we have also studied the stabilities of Cu(Arm)(H; GDP) and Cu(Arm)(GDP)$^-$ in the two mentioned solvent mixtures and shown that the extent of intramolecular stacking decreases in the presence of 1,4-dioxane.

2. Results and discussion

Great care was taken in this study to measure the various equilibrium constants under conditions where no self-association occurs [7, 53, 54]. The measurements were made with solutions 0.6 mM in GDP; this guarantees [53, 54] that indeed the properties of the monomeric species are studied. The concentration of the heteroaromatic N bases, Bpy or Phen, was also 0.6 mM, such that self-association is negligibly small for these ligands as well [33]; in fact, this is even more true for Cu(Bpy)$^{2+}$ and Cu(Phen)$^{2+}$ due to charge repulsion [55]. Furthermore, it is known [7, 33, 56] that the presence of 1,4-dioxane, due to hydrophobic solvation of the aromatic rings, inhibits stacking. Hence, the results presented definitely refer to the monomeric species.

2.1. *Some comments on the acidity constants of H_2(GDP)$^-$*

At pH above *ca.* 2.5, which is relevant for this study, GDP^{3-} (figure 1) accepts two protons to give H_2(GDP)$^-$ [52]. Considering further that the (N1)H unit may lose a proton as well, the following three deprotonation reactions are of relevance:

$$H_2(GDP)^- \rightleftharpoons H(GDP)^{2-} + H^+ \tag{2a}$$

$$K^{H}_{H_2(GDP)} = [H(GDP)^{2-}][H^+]/[H^2(GDP)^-] \quad (2b)$$

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

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

$$GDP^{3-} \rightleftharpoons (GDP - H)^{4-} + H^+ \quad (4a)$$

$$K^{H}_{GDP} = [(GDP - H)^{4-}][H^+]/[GDP^{3-}]. \quad (4b)$$

The expression $(GDP - H)^{4-}$ in equation (4) is to be read as GDP^{3-} *minus* H^+; i.e. it refers to the deprotonation of the (N1)H unit. The acidity constants of equations (2)–(4) have recently been determined [52] in water as well as in water containing 30 or 50% (v/v) 1,4-dioxane; these results have also been confirmed now and are assembled in table 1 [57].

It was concluded previously [52] that in aqueous solution one proton in $H_2(GDP)^-$ is located at the β-phosphate group and the second one to more than 90% at N7 of the guanine with a minor percentage at one of the primary sites of the diphosphate residue. This second proton is released first equation (2) giving $H(GDP)^{2-}$ which is then deprotonated from its β-phosphate group equation (3), and finally from its (N1)H site equation (4). As far as equilibria (3a) and (4a) are concerned, there is no difference in the location of the protons in $H(GDP)^{2-}$ in the various solvents but a decreased dielectric constant inhibits charge separation and consequently the pK_a values increase (table 1; columns 5 and 6).

However, in $H_2(GDP)^-$ the location of the protons changes. Of course, one of the two protons is still at the terminal β-phosphate group, but in 50% 1,4-dioxane/water the other proton is no longer preferably at the N7 site [52]. Thus, about 75% of the $H_2(GDP)^-$ isomers have two protons phosphate-bound and only *ca.* 25% of the isomers have one of the two protons at N7 (the other one remaining at the phosphate residue). In other words, a decreasing solvent polarity disfavors protonation at N7.

Table 1. Negative logarithms of the acidity constants of $H_2(GDP)^-$ [equations (2)–(4)] in dependence on the amount of 1,4-dioxane added to water, together with some properties of the solvents (25°C; $I = 0.1$ M, $NaNO_3$).[a,b]

% (v/v) 1,4-Dioxane	Mol fraction	ε[c]	$pK^{H}_{H_2(GDP)}$	$pK^{H}_{H(GDP)}$	pK^{H}_{GDP}
0	0	78.5	2.67 ± 0.02	6.38 ± 0.01	9.56 ± 0.03
30	0.083	52.7	2.75 ± 0.02	6.89 ± 0.02	9.97 ± 0.02
50	0.175	35.2	2.89 ± 0.04	7.09 ± 0.02	10.25 ± 0.04

[a] The error limits are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger; the error limits of derived data (in the text and other tables) were calculated according to the error propagation after Gauss.
[b] The acidity constants listed above are collected from our earlier work [52]; so-called practical, mixed or Brønsted constants are listed [57].
[c] The permittivities or dielectric constants (ε) are interpolated from the data given in refs [46–48].

2.2. *Stability constants of the various Cu^{2+} complexes*

The experimental data of the potentiometric pH titrations of the Cu^{2+}/Arm/GDP systems are completely described by equilibria (2), (3) and (5), (6), provided the evaluation is not carried into the pH range where formation of hydroxo complexes occurs. Due to the high stability of $Cu(Arm)^{2+}$ complexes [18, 19], the evaluation of the data becomes simple for these ternary complexes [31, 34] and corresponds to that of binary complexes. Therefore, for the equilibria and equations below, $M^{2+} = Cu^{2+}$, $Cu(Bpy)^{2+}$ or $Cu(Phen)^{2+}$:

$$M^{2+} + H(GDP)^{2-} \rightleftharpoons M(H;GDP) \tag{5a}$$

$$K^{M}_{M(H;GDP)} = [M(H;GDP)]/([M^{2+}][H(GDP)^{2-}]) \tag{5b}$$

$$M^{2+} + GDP^{3-} \rightleftharpoons M(GDP)^{-} \tag{6a}$$

$$K^{M}_{M(GDP)} = [M(GDP)^{-}]/([M^{2+}][GDP^{3-}]) \tag{6b}$$

In formulas such as Cu(H;GDP), the H^+ and GDP^{3-} are separated by a semicolon to facilitate reading; yet, they appear within the same parenthesis to indicate that the proton is at the ligand without defining its location. Of course, equilibria (5a) and (6a) are connected with each other via the deprotonation equilibrium (7a) and the corresponding acidity constant may be calculated with equation (8):

$$M(H;GDP) \rightleftharpoons M(GDP)^{-} + H^{+} \tag{7a}$$

$$K^{H}_{M(H;GDP)} = [M(GDP)^{-}][H^{+}]/[M(H;GDP)] \tag{7b}$$

$$pK^{H}_{M(H;GDP)} = pK^{H}_{H(GDP)} + \log K^{M}_{M(H;GDP)} - \log K^{M}_{M(GDP)} \tag{8}$$

The constants for equilibria (5a), (6a) and (7a) are listed in columns 4, 5 and 6 of table 2, respectively.

Table 2. Logarithms of the stability constants of the binary Cu(H;GDP) and $Cu(GDP)^{-}$ complexes as well as of their corresponding ternary Cu(Arm)(H;GDP) [equation (5)] and $Cu(Arm)(GDP)^{-}$ [equation (6)] counterparts in dependence on the amount of 1,4-dioxane added to water and as determined by potentiometric pH titrations (25°C; $I = 0.1$ M, $NaNO_3$), together with the negative logarithms of the acidity constants of the Cu(H;GDP) and Cu(Arm)(H;GDP) species [equations (7) and (8)] as well as the resulting values for $\Delta \log K_{Cu/Arm/GDP}$ [equations (10) and (11)].[a]

No.	M^{2+}	% (v/v)1,4-Dioxane[b]	$\log K^{M}_{M(H;GDP)}$	$\log K^{M}_{M(GDP)}$	$pK^{H}_{M(H;GDP)}$	$\Delta \log K_{Cu/Arm/GDP}$
1a	Cu^{2+}	0	3.39 ± 0.19	5.85 ± 0.04	3.92 ± 0.19	
1b	$Cu(Bpy)^{2+}$	0	3.94 ± 0.18	6.79 ± 0.05	3.53 ± 0.19	0.94 ± 0.06
1c	$Cu(Phen)^{2+}$	0	4.13 ± 0.25	7.00 ± 0.05	3.51 ± 0.26	1.15 ± 0.06
2a	Cu^{2+}	30	3.50 ± 0.08	6.21 ± 0.15	4.18 ± 0.17	
2b	$Cu(Bpy)^{2+}$	30	3.38 ± 0.06	6.43 ± 0.03	3.84 ± 0.07	0.22 ± 0.15
2c	$Cu(Phen)^{2+}$	30	3.21 ± 0.17	6.29 ± 0.03	3.81 ± 0.17	0.08 ± 0.15
3a	Cu^{2+}	50	3.80 ± 0.13	6.36 ± 0.05	4.53 ± 0.14	
3b	$Cu(Bpy)^{2+}$	50	3.82 ± 0.15	6.63 ± 0.03	4.28 ± 0.15	0.27 ± 0.06
3c	$Cu(Phen)^{2+}$	50	3.66 ± 0.08	6.53 ± 0.05	4.22 ± 0.10	0.17 ± 0.07

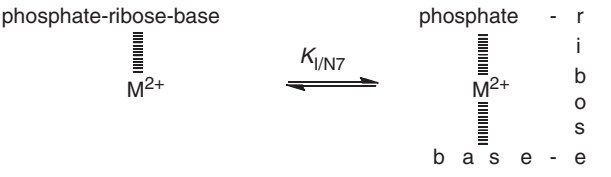
[a] For the error limits' see footnote "a" in table 1. Entries 1a, 2a and 3a are from our earlier work [52].
[b] For the solvent properties' see columns 2 and 3 in table 1.

Constants of the binary Cu^{2+}/GDP system in water and in water containing increasing amounts of 1,4-dioxane are taken from our recent work [52]; those for the ternary $Cu(Arm)^{2+}$/GDP systems have not been determined before [58, 59].

2.3. *Structural considerations on the monoprotonated ternary Cu(Arm)(H;GDP) complexes*

Since analysis of potentiometric pH titrations only yields the amount and distribution of the species of a net charged type, further information is required to locate the binding sites of the proton and the metal ions in the Cu(Arm)(H; GDP) species. A comparison of the acidity constants of $H_2(GDP)^-$ with those of Cu(Arm)(H; GDP) reveals, for all three solvents employed, the order $pK^H_{H_2(GDP)} < pK^H_{Cu(Arm)(H;GDP)} < pK^H_{H(GDP)}$ (cf. the values listed in tables 1 and 2). This means the $pK^H_{Cu(Arm)(H;GDP)}$ values are between about 0.8 and 1.4 pK units larger than those of $pK^H_{H_2(GDP)}$, yet they are also about 2.8 to 3.1 pK units lower than $pK^H_{H(GDP)}$. The corresponding order is observed if the acidity constant of the binary Cu(H;GDP) complex is compared with those of $H_2(GDP)^-$. Hence, the proton must be located in all instances at the terminal phosphate group in the Cu(H;GDP) and Cu(Arm)(H;GDP) complexes because metal ion coordination must give rise to an acidification [60, 61]. However, where is Cu^{2+} or $Cu(Arm)^{2+}$? Also at the phosphate group or at the nucleobase moiety? Are macrochelates formed or is intramolecular stacking involved in the ternary complexes?

For the binary Cu(H;GDP) complex it was previously concluded [52] that not only H^+ but also Cu^{2+} is at the diphosphate group and that in all three solvents the phosphate-coordinated metal ion also interacts with N7 forming a macrochelate as indicated in equilibrium (9)



(9)

though its formation degree could not be calculated. Considering that $Cu(Arm)^{2+}$ binding at the N7 site of a purine is strongly inhibited [31] (see also section 2.6), it is clear that the primary binding site in the ternary Cu(Arm)(H; GDP) complexes is also the diphosphate residue, which carries a proton. The question is: Is there a further interaction? Indeed, there must be one, as the stability data for aqueous solution show: The stability of the Cu(Arm)(H;GDP) complexes is larger than the one of the binary Cu(H;GDP) species (table 2; entries 1, column 4). Because this enhanced stability cannot be due to macrochelate formation, as indicated above, the only remaining possibility is intramolecular stacking between the purine residue and the aromatic rings of Arm in the ternary complexes. In accord with this interpretation is the fact that in the mixed solvents the stabilities of the ternary complexes decrease and approach that of the binary complex, because, due to the hydrophobic solvation of the aromatic rings by the ethylene bridges of 1,4-dioxane, the intramolecular stacking interaction will be inhibited though most likely not reduced to zero in the 1,4-dioxane/water mixture.

2.4. *Proof of an increased stability of the ternary Cu(Arm)(GDP)$^-$ complexes*

One way to quantify the stability of mixed ligand complexes [62–64] is to consider equilibrium (10a); the corresponding equilibrium constant [equation (10b)] is calculated with equation (11) as follows.

$$Cu(Arm)^{2+} + Cu(GDP)^- \rightleftharpoons Cu(Arm)(GDP)^- + Cu^{2+} \tag{10a}$$

$$10^{\Delta \log K} = \frac{[Cu(Arm)(GDP)^-][Cu^{2+}]}{[Cu(Arm)^{2+}][Cu(GDP)^-]} \tag{10b}$$

$$\Delta \log K = \log K^{Cu(Arm)}_{Cu(Arm)(GDP)} - \log K^{Cu}_{Cu(GDP)} \tag{11}$$

If further identification of $\Delta \log K$ for a certain equilibrium is needed, this will be given by additional subscripts like $\Delta \log K_{Cu/Arm/GDP}$.

According to the general rule for complex stabilities, $K_1 > K_2$, one expects that equilibrium (10a) lies to the left with negative values for $\Delta \log K$. This agrees with statistical considerations obtained for a Jahn–Teller distorted octahedral coordination sphere of Cu^{2+} to which two bidentate ligands A and B are coordinated; i.e. $\Delta \log K_{Cu/statist} \approx -0.9$ was estimated [63]. The values for the corresponding Cu(Bpy)(GDP)$^-$ and Cu(Phen)(GDP)$^-$ complexes according to equation (11) are listed for all three solvents in column 7 of table 2. From these results it follows that equilibrium (10a) is displaced significantly to the right! Consequently, these ternary complexes show increased stability, yet it is difficult to draw conclusions from these results with regard to their structure in solution, since the binary Cu(GDP)$^-$ complex exists in the form of the isomeric species indicated in equilibrium (9) [52]; thus, Cu(GDP)$^-$ is more stable than expected on the basis of the basicity of the diphosphate group of GDP^{3-}.

2.5. *Quantification of the stability enhancements for the ternary Cu(Arm)(GDP)$^-$ complexes*

Another way to evaluate a possibly increased stability of the ternary Cu(Arm)(GDP)$^-$ species, independently of the binary Cu(GDP)$^-$ complex, could rest on the well-known [65] linear relationship for families of closely related ligands. These neither exist for ternary Cu(Arm)(R-DP)$^-$ complexes, where R-DP^{3-} represents a simple diphosphate monoester, that is, R may be any residue which does not affect complex formation, nor for the 1,4-dioxane/water mixtures used as solvents in the present study. However, the parameters for the corresponding straight line for Cu(R-DP)$^-$ complexes have been determined [66]; they are given in equation (12):

$$\log K^{M}_{M(L)} = m \cdot pK^{H}_{H(L)} + b \tag{12a}$$

$$\log K^{Cu}_{Cu(R-DP)} = 1.283 \cdot pK^{H}_{H(R-DP)} - 2.939 \tag{12b}$$

The five R-DP ligands used in this determination [66] are indicated in figure 2. With a known $pK^{H}_{H(R-DP)}$ value, an expected stability constant for the corresponding Cu(R-DP)$^-$ complex can be calculated. Such a value for a binary Cu(R-DP)$^-$ complex can be corrected to obtain an expected stability constant for a ternary

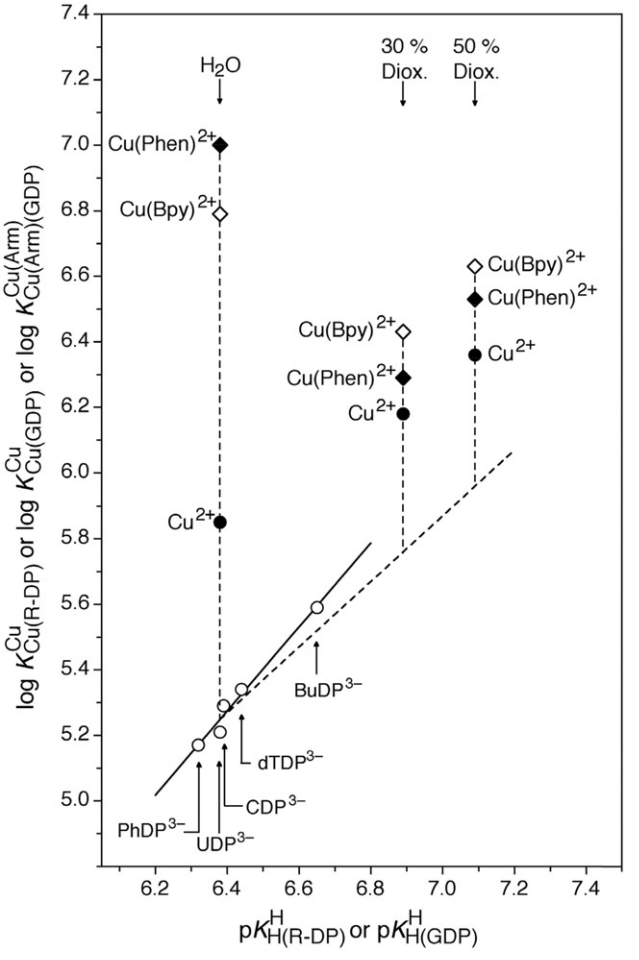


Figure 2. Evidence for an enhanced stability of the $Cu(GDP)^{-}$ (●) and $Cu(Arm)(GDP)^{-}$ (◇,♦) complexes in aqueous solution and in water containing 30 or 50% (v/v) 1,4-dioxane, based on the relationship between $\log K^{Cu}_{Cu(R-DP)}$ and $pK^{H}_{H(R-DP)}$ for the simple Cu(R-DP) complexes in aqueous solution (○), where $R\text{-}DP^{3-}$ = phenyl diphosphate ($PhDP^{3-}$), uridine 5′-diphosphate (UDP^{3-}), cytidine 5′-diphosphate (CDP^{3-}), thymidine [= 1-(2′-deoxy-β-D-ribofuranosyl)thymine] 5′-diphosphate ($dTDP^{3-}$), and *n*-butyl diphosphate ($BuDP^{3-}$) (from left to right) and the deduced straight reference line with $m = 1$ for the mixed solvents. The parameters of the least-squares line (solid line) through the indicated five data sets are given in equation (12b), those for the mixed solvents (broken line) in equation (17) (see text in section 2.5). The equilibrium constants for the $Cu^{2+}/H^{+}/R\text{-}DP^{3-}$ systems (○) are from [66] and the data points due to the $Cu^{2+}/H^{+}/GDP^{3-}$ (●) and $Cu(Arm)^{2+}/H^{+}/GDP^{3-}$ (◇,♦) systems are based on the values listed in tables 1 and 2. The vertical broken lines emphasize the stability differences from the reference lines; for details the text in sections 2.5 and 2.6 needs to be consulted. All the plotted equilibrium constants refer to solutions at 25°C and $I = 0.1$ M ($NaNO_3$).

$Cu(Arm)(R\text{-}DP)^{-}$ complex [31]. For the situation in mixed solvents the situation is even more complicated but sophisticated estimations are still possible, as shown subsequently.

Equation (12b) is valid in the pK_a range 6.2–6.8 and the error limit (three times the standard deviation) for a calculated $\log K^{Cu}_{Cu(R-DP)}$ value is ±0.04 [66]. Figure 2 shows that the binary $Cu(GDP)^{-}$ complex in water is about 0.6 log unit more stable than

expected on the basis of the basicity of the diphosphate residue. The corresponding ternary Cu(Bpy)(GDP)$^-$ and Cu(Phen)(GDP)$^-$ complexes, based on the same reference line, show an enhanced stability of about 1.5 and 1.7 log units, respectively (figure 2).

From previous experience one knows [62–64, 67] that an increased complex stability is expected for mixed ligand complexes formed by a divalent 3d metal ion, a heteroaromatic N base, and an O donor ligand. Hence, the open (op) Cu(Arm)(GDP)$^-_{op}$ isomer, depicted in equilibrium (1) at the left, already has an "intrinsic" increased stability due to the Arm/O,O-ligand combination [63, 68, 69]. This expected stability increase resulting from the Arm/O,O-ligand combination is certainly well represented by the Cu^{2+}/Arm/$MePP^{3-}$ system, where $MePP^{3-}$ represents methylphosphonylphosphate ($CH_3-P(O)_2^- - O - PO_3^{2-}$) [69], because the equatorial coordination sphere of Cu^{2+}, i.e. Cu(Arm)(O,O)$^-$, is identical in all these mixed ligand complexes. Hence, the previously obtained results for the $MePP^{3-}$ systems [69], as summarized in equations (13) and (14),

$$Cu(Bpy)^{2+} + Cu(MePP)^- \rightleftharpoons Cu(Bpy)(MePP)^- + Cu^{2+} \tag{13a}$$

$$\Delta \log K_{Cu/Bpy/MePP} = 0.42 \pm 0.05 \tag{13b}$$

$$Cu(Phen)^{2+} + Cu(MePP)^- \rightleftharpoons Cu(Phen)(MePP)^- + Cu^{2+} \tag{14a}$$

$$\Delta \log K_{Cu/Phen/MePP} = 0.45 \pm 0.05 \tag{14b}$$

can now be applied. The calculated stability of the open complex Cu(Arm)(GDP)$^-_{op}$ is given by equation (15):

$$\log K^{Cu(Arm)}_{Cu(Arm)(GDP)op} = \log K^{Cu}_{Cu(GDP)op} + \Delta \log K_{Cu/Arm/MePP} \tag{15}$$

The stability constant of the Cu(GDP)$^-_{op}$ complex follows from equation (12b) and $pK^H_{H(GDP)}$ (table 1), and values for $\Delta \log K_{Cu/Arm/MePP}$ are given in equations (13b) and (14b). Consequently, the "actual" (actl) stability increase due to any intramolecular interaction with the guanine residue in the ternary Cu(Arm)(GDP)$^-$ complexes is given by equation (16):

$$\log \Delta_{Cu/Arm/GDP/actl} = \log K^{Cu(Arm)}_{Cu(Arm)(GDP)exp} - \log K^{Cu(Arm)}_{Cu(Arm)(GDP)op} \tag{16}$$

The various terms which appear in equations (15) and (16) are listed for aqueous solution in entries 1a and 1b of table 3.

No reference line exists for binary Cu(R-DP)$^-$ complexes in the 1,4-dioxane/water solvents. However, the influence of a decreasing solvent polarity, i.e. an increasing amount of 1,4-dioxane added to aqueous solutions, on the stability of several Cu^{2+} and Zn^{2+} complexes in dependence on the acid–base properties of three monophosphate monoester ligands as well as of uridine 5′-triphosphate (UTP^{4-}), formate ($HCOO^-$), and acetate (CH_3COO^-) has been studied. [70]. These eight rather different systems show the astonishing result that, in all instances, straight lines with slopes close to one are observed. In fact, the average slope (m) for the indicated eight systems is $m = 1.00 \pm 0.15$ (3σ), thus one may expect that the slope for the Cu^{2+}/H^+/R-DP^{3-} system is also close to one.

Application of $pK^H_{H(GDP)} = 6.38$ to equation (12) gives for the Cu(GDP)$^-_{op}$ complex in water $\log K^{Cu}_{Cu(GDP)op} = 5.25 \pm 0.04$ (see also table 3). Use of this value together with the

Table 3. Quantification of the stability increase of the ternary Cu(Arm)(GDP)$^{-}$ complexes which is "actually" (actl) due to an intramolecular stacking interaction involving the guanine moiety of GDP^{3-} [equilibrium (1)] as defined by equation (16) for aqueous as well as 1,4-dioxane/water solutions at 25°C and $I = 0.1$ M ($NaNO_3$)[a].

No.	$Cu(Arm)^{2+}$	% (v/v) 1,4-Dioxane[a]	$\log K^{Cu(Arm)}_{Cu(Arm)(GDP)exp}$ [b]	$\log K^{Cu}_{Cu(GDP)op}$ [c]	$\Delta\log K_{Cu/Arm/MePP}$ [d]	$\log K^{Cu(Arm)}_{Cu(Arm)(GDP)op}$ [e]	$\log \Delta_{Cu/Arm/GDP/actl}$ [f]
1a	$Cu(Bpy)^{2+}$	0	6.79 ± 0.05	5.25 ± 0.04	0.42 ± 0.05	5.67 ± 0.06	1.12 ± 0.08
1b	$Cu(Phen)^{2+}$	0	7.00 ± 0.05	5.25 ± 0.04	0.45 ± 0.05	5.70 ± 0.06	1.30 ± 0.08
2a	$Cu(Bpy)^{2+}$	30	6.43 ± 0.03	5.76 ± 0.08	0.42 ± 0.05	6.18 ± 0.09	0.25 ± 0.09
2b	$Cu(Phen)^{2+}$	30	6.29 ± 0.03	5.76 ± 0.08	0.45 ± 0.05	6.21 ± 0.09	0.08 ± 0.09
3a	$Cu(Bpy)^{2+}$	50	6.63 ± 0.03	5.96 ± 0.11	0.42 ± 0.05	6.38 ± 0.12	0.25 ± 0.12
3b	$Cu(Phen)^{2+}$	50	6.53 ± 0.05	5.96 ± 0.11	0.45 ± 0.05	6.41 ± 0.13	0.12 ± 0.12

[a] See footnote "a" in table 1 regarding the error limits. For the solvent properties see columns 2 and 3 in table 1.
[b] These experimentally (exp) measured values are from table 2, column 5 [equation (6)].
[c] See equilibrium (9) at the left and equation (15); these values can be calculated with equations (12b), (17), and the $pK^{H}_{H(GDP)}$ values of table 1; they are also listed in table 3 (column 4) of ref. [52].
[d] See equations (13) and (14) as well as the text in section 2.5.
[e] These values were calculated according to equation (15).
[f] These values follow from the definition given in equation (16).

one for $pK^{H}_{H(GDP)}$ and $m = 1$ allows calculation for the intercept $b_{dioxane/water}$ of the straight reference line for dioxane/water mixtures and $Cu^{2+}/H^{+}/R\text{-}DP^{3-}$ systems [equation (17)]:

$$\log K^{Cu}_{Cu(GDP)op/Diox} = 1.00 \cdot pK^{H}_{H(GDP)/Diox} - 1.13 \quad (17)$$

Evidently, for the acidity constant in water, i.e. $pK^{H}_{H(GDP)} = 6.38$, one obtains from equation (17) the corresponding stability constant of the open isomer, $Cu(GDP)^{-}_{op}$, i.e. $\log K^{Cu}_{Cu(GDP)op} = \log K^{Cu}_{Cu(R-DP)} = 5.25$, also in water, in agreement with the calculations of equation (12). Therefore, the broken reference line in figure 2, valid for the 1,4-dioxane/water mixtures, has its starting point at $\log K^{Cu}_{Cu(R-DP)} = 5.25$ and $pK^{H}_{H(GDP)} = 6.38$. The vertical broken lines for 30 or 50% 1,4-dioxane/water represent the overall stability enhancements for the ternary $Cu(Arm)(GDP)^{-}$ complexes from which $\Delta \log K_{Cu/Arm/MePP}$ values [equations (13) and (14)] need to be deducted to obtain the stability enhancements solely due to the guanine residue. Previously it has been shown for several O-donor ligands that the values for $\Delta \log K_{Cu/Arm/O\text{-}Donor}$ are (within their error limits) rather independent of the solvent by going from water to 50% 1,4-dioxane/water mixtures [71]. Hence, in a first approximation, equations (13)–(16) can also be applied here; the corresponding values are summarized in entries 2 and 3 of table 3, indicating that equilibrium (1) is also of relevance, at least to some extent, in the 1,4-dioxane/water mixtures.

The data points for the binary $Cu^{2+}/H^{+}/GDP^{3-}$ system are inserted into figure 2 for comparison; the corresponding stability enhancements due to macrochelate formation [equilibrium (9)] involving N7 have been discussed recently [52].

2.6. *Evaluation of the stability enhancements of the ternary $Cu(Arm)(GDP)^{-}$ complexes and extent of intramolecular stack formation*

From figure 2, it is evident that the $Cu(Arm)(GDP)^{-}$ complexes in aqueous solution are considerably more stable than the binary $Cu(GDP)^{-}$. It follows further that the stability of all these complexes is overwhelmingly determined by the metal ion affinity of the diphosphate residue and that the observed stability enhancement must result from an additional interaction in the guanine residue. There are two possibilities for further interactions of the open isomer $Cu(Arm)(GDP)^{-}_{op}$: (i) A macrochelate could be formed with N7, i.e. $Cu(Arm)(GDP)^{-}_{cl/N7}$, as known for the binary $Cu(GDP)^{-}$ complex [equation (9)] [52]. (ii) The formation of an intramolecular stack, $Cu(Arm)(GDP)^{-}_{st}$, as occurs in the ternary Cu(Arm)(H; GDP) systems (section 2.3) and proven to occur in the Cu(Arm)(dGMP) complexes, where $dGMP^{2-}$ = 2′-deoxyguanosine 5′-monophosphate [equation (1)] [34]. A schematic and simplified structure of the $Cu(Phen)(GDP)^{-}_{st}$ species is shown in figure 3.

Based on the above considerations the equilibrium scheme (18) may be written as:

$$Cu(Arm)^{2+} + GDP^{3-} \underset{}{\overset{K^{Cu(Arm)}_{Cu(Arm)(GDP)_{op}}}{\rightleftharpoons}} Cu(Arm)(GDP)^{-}_{op} \quad \begin{matrix} \overset{K_{l/Arm/N7}}{\rightleftharpoons} Cu(Arm)(GDP)^{-}_{cl/N7} \\ \underset{K_{l/st}}{\rightleftharpoons} Cu(Arm)(GDP)^{-}_{st} \end{matrix} \quad (18)$$

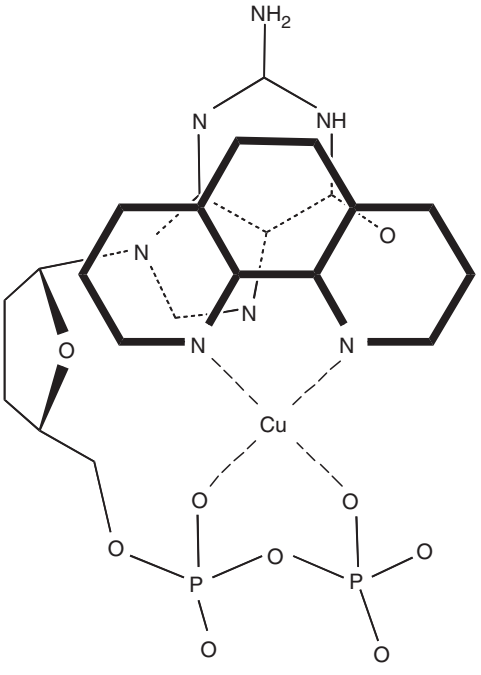


Figure 3. Schematic and simplified structure of the species with an intramolecular stack according to equilibrium (1) for Cu(Phen)(GDP)$^-$. It may be noted in this connection that in solution certainly a whole series of stacked complexes occurs in which the orientation of the aromatic rings toward each other differs somewhat; of course, the expression Cu(Arm)(GDP)$^-_{st}$ and the quantifications given for it (tables 3 and 4) encompass all these species.

The question which needs to be answered now is: are both branches in the equilibrium scheme (18) of comparable importance? Already in section 2.3 we have indicated that a N7 interaction of diphosphate-coordinated Cu(Arm)$^{2+}$ is inhibited. In fact, this inhibition amounts to $-(0.80 \pm 0.25)$ log units compared with the same interaction of Cu^{2+} as concluded recently [31, 34]. In fact, this value is an upper limit since there is evidence that the steric inhibition is actually more pronounced [72]. In any case, the enhanced stability of the binary Cu(GDP)$^-$ complex is solely due to macrochelate formation [52] and therefore, the expected stability of the Cu(Arm)(GDP)$^-$ complexes can be calculated as given in equation (19) under the assumption that only macrochelate formation and no stacking occurs:

$$\log K^{Cu(Arm)}_{Cu(Arm)(GDP)cl/N7} = \log K^{Cu}_{Cu(GDP)} + \Delta \log K_{Cu/Arm/MePP} - (0.80 \pm 0.25) \quad (19a)$$

$$= (5.85 \pm 0.04) + (0.44 \pm 0.07) - (0.80 \pm 0.25) \quad (19b)$$

$$= 5.49 \pm 0.26 \quad (19c)$$

The stability constant for the first term is from our previous work [52] (see also table 2) and the second term on the right hand side in equations (19a) and (19b) appears here since the Arm/O,O-ligand combination [the average of equations (13) and (14) is used] leads to an enhanced complex stability (see above and in section 2.5) which must be

taken into account in the calculation for $K^{Cu(Arm)}_{Cu(Arm)(GDP)cl/N7}$. The result in equation (19c) shows that the calculated stability for $Cu(Arm)(GDP)^{-}_{cl/N7}$ is at least [72] 1.3 and 1.5 log units less stable than the stability constants measured for the ternary $Cu(Bpy)(GDP)^{-}$ and $Cu(Phen)(GDP)^{-}$ complexes, respectively (see table 2; entries 1, column 5); hence, one may conclude that the upper branch of the equilibrium scheme (18) does not significantly contribute to the overall stability of these complexes in aqueous solution. In other words, the concentration of $Cu(Arm)(GDP)^{-}_{cl/N7}$ is negligible. Therefore, we are left with the lower branch of the equilibrium scheme (18) and this then means that the total actual stability increase as defined by equation (16) (table 3, column 8) has to be attributed to the intramolecular stack formation according to equilibrium (1). This conclusion is in accord with the results obtained for Cu(Arm)(dGMP) in aqueous solution where the extent of macrochelate formation was estimated as being $8 \pm 9\%$ and $6 \pm 7\%$ for $Cu(Bpy)(dGMP)_{cl/N7}$ and $Cu(Phen)(dGMP)_{cl/N7}$, respectively [34]. In other words, at best traces of macrochelates occur in the ternary Cu(Arm)(dGMP) complexes.

Of course, at this point one may recall that intramolecular stacking interactions may in addition be proven via spectrophotometric (charge transfer) [7, 11, 34, 73] and ^{1}H NMR shift measurements (upfield shifts) [7, 11, 74], as previously carried out for related mixed ligand–nucleotide systems [7, 11, 34, 73, 74]. However, we considered such attempts as superfluous in the present case because (i) intramolecular stack formation in Cu(Arm)(dGMP) was proven to occur by spectrophotometric measurements [34] and (ii) a crystal structure study showed for the ternary Cu^{2+}/Bpy/ADP system intramolecular stacks [75]. We considered it more important to gather quantitative information about the actual position of equilibrium (1) and the effect of the solvent composition on this equilibrium.

The above conclusion that macrochelates involving N7 are not of relevance in aqueous solution for the ternary $Cu(Arm)(GDP)^{-}$ complexes, can be confirmed by related reasonings but this time also including the results obtained for the 1,4-dioxane/water mixtures (table 2). At first one needs to calculate $K^{Cu(Arm)}_{Cu(Arm)(GDP)cl/N7}$ in analogy to equation (19), and this is done in equations (20) and (21) for water containing 30 or 50% 1,4-dioxane, respectively:

$$\log K^{Cu(Arm)}_{Cu(Arm)(GDP)cl/N7} = \log K^{Cu}_{Cu(GDP)} + \Delta \log K_{Cu/Arm/MePP} - (0.80 \pm 0.25) \quad (20a)$$

$$= (6.21 \pm 0.15) + (0.44 \pm 0.07) - (0.80 \pm 0.25) \quad (20b)$$

$$= 5.85 \pm 0.30 \quad (20c)$$

$$\log K^{Cu(Arm)}_{Cu(Arm)(GDP)cl/N7} = (6.36 \pm 0.05) + (0.44 \pm 0.07) - (0.80 \pm 0.25) \quad (21a)$$

$$= 6.00 \pm 0.26 \quad (21b)$$

Comparison of the results given in equations (19c), (20c) and (21b), valid for water and water containing 30 or 50% 1,4-dioxane, respectively, with the stability constants calculated for the open $Cu(Arm)(GDP)^{-}_{op}$ isomers, listed in column 7 of table 3, reveals that the values obtained for the open $Cu(Arm)(GDP)^{-}_{op}$ isomers are somewhat larger than those obtained for the macrochelated $Cu(Arm)(GDP)^{-}_{cl/N7}$ species. Hence, the

Table 4. Comparison of the extent of intramolecular stack formation in ternary Cu(Arm)(GDP)$^-$ complexes in dependence on the solvent employed and as calculated from stability constants determined by potentiometric pH titrations in water and in water containing 30 or 50% (v/v) 1,4-dioxane. Listed are the stability enhancements $\log \Delta_{Cu/Arm/GP/actl}$ [equation (16)], the intramolecular and dimensionless equilibrium constant $K_{I/st}$ [equations (1) and (22)] and the percentages of the stacked Cu(Arm)(GP) species [equation (23)]. The corresponding results [34] for the Cu(Arm)(dGMP) complexes are given for comparison (25°C; $I = 0.1$ M, $NaNO_3$).[a]

Cu(Arm)(GP)	% (v/v)1,4-Dioxane[a]	$\log \Delta_{Cu/Arm/GP/actl}$[b]	$K_{I/st}$	% Cu(Arm)$(GP)_{st}$
Cu(Bpy)(dGMP)	0	1.20 ± 0.09	14.85 ± 3.28	93.7 ± 1.3
Cu(Phen)(dGMP)	0	1.33 ± 0.10	20.38 ± 4.92	95.3 ± 1.1
Cu(Bpy)(GDP)$^-$	0	1.12 ± 0.08	12.18 ± 2.43	92.4 ± 1.4
Cu(Phen)(GDP)$^-$	0	1.30 ± 0.08	18.95 ± 3.68	95.0 ± 0.9
Cu(Bpy)(GDP)$^-$	30	0.25 ± 0.09	0.78 ± 0.36	44 ± 12
Cu(Phen)(GDP)$^-$	30	0.08 ± 0.09	0.20 (0/0.48)[c]	17 (0/32)[c]
Cu(Bpy)(GDP)$^-$	50	0.25 ± 0.12	0.78 ± 0.49	44 ± 16
Cu(Phen)(GDP)$^-$	50	0.12 ± 0.13	0.32 (0/0.78)[c]	24 (0/44)[c]

[a] See footnote "a" of table 1 regarding the error limits. For the solvent properties see columns 2 and 3 in table 1.
[b] The first two entries are from the second column in table 4 of ref. [34]; all other values are from table 3 (column 8) of this study.
[c] The values in parenthesis are the lower and upper limits, respectively.

latter isomers do not contribute remarkably to the overall stability of the ternary Cu(Arm)(GDP)$^-$ complexes in all three solvents; this would only be the case if $K^{Cu(Arm)}_{Cu(Arm)(GDP)cl/N7} > K^{Cu(Arm)}_{Cu(Arm)(GDP)op}$. This result confirms again that only the lower pathway, involving stacking, in the equilibrium scheme (18) needs to be considered. The stability enhancements $\log \Delta_{Cu/Arm/GDP/actl}$ (equation (16)) listed in column 8 of table 3 are attributed completely to intramolecular stacking interactions (figure 3) in the ternary Cu(Arm)(GDP)$^-$ complexes.

These values for $\Delta \log K_{Cu/Arm/GDP/actl}$ allow quantification of the position of equilibrium (1) by following previous routes and by applying equation (22) (for details see, e.g., refs. [11, 14, 32, 65, 76]), where log Δ represents $\log \Delta_{Cu/Arm/GDP/actl}$:

$$K_{I/st} = [Cu(Arm)(GDP)^-_{st}]/[Cu(Arm)(GDP)^-_{op}] \quad (22a)$$

$$= 10^{\log \Delta} - 1 \quad (22b)$$

Of course, once $K_{I/st}$ is known, the percentage of the stacked isomer in equilibrium (1) can be calculated with equation (23):

$$\%\ Cu(Arm)(GDP)^-_{st} = 100 \cdot K_{I/st}/(1 + K_{I/st}) \quad (23)$$

The corresponding results are assembled in table 4 together with the corresponding data obtained previously [34] for the Cu(Arm)(dGMP) systems.

3. Conclusions

Several of the results summarized in table 4 warrant emphasis: of utmost interest is the fact that in the corresponding Cu(Arm)(dGMP) and Cu(Arm)(GDP)$^-$ systems (top four entries of table 4) the formation degrees of the intramolecular stacks are very large

but identical within their error limits and this despite the fact that the actual global stability constants differ significantly, e.g. $\log K^{Cu(Phen)}_{Cu(Phen)(dGMP)} = 4.27 \pm 0.08$ [34] and $\log K^{Cu(Phen)}_{Cu(Phen)(GDP)} = 7.00 \pm 0.05$ (table 2). Of course, these differences are a reflection of the much higher metal ion affinity of a diphosphate residue compared to that of a monophosphate residue.

Another point of interest is that the larger Phen is expected to stack somewhat better with the guanine residue than Bpy and indeed, for aqueous solutions this is observed. However, for mixed 1,4-dioxane/water mixtures, two points need to be noted: (i) most importantly, the extent of stacking is drastically inhibited by increasing amounts of 1,4-dioxane though most likely it does not completely disappear. (ii) In contrast to the situation in water, the results for the mixed solvents indicate that intramolecular stacking in the Cu(Phen)(GDP) systems is somewhat more inhibited than in those which contain Bpy. The reason most probably is that Phen is more strongly solvated by the ethylene bridges of 1,4-dioxane than Bpy.

To conclude, the most interesting result of this study, from our point of view, is the fact that the stacking tendency of a nucleobase moiety in a nucleotide complex does not depend on the denticity or length of the phosphate residue, in agreement with earlier indications [31]. However, the relevance of this kind of adduct formation for recognition reactions in nature is evident; e.g., in protein–nucleotide/nucleic acid interactions the role of Bpy or Phen may be taken over by the phenyl or indole moieties of phenylalanyl or tryptophanyl residues, respectively. In the same context one should note that a decrease of solvent polarity favors phosphate-metal ion binding whereas stacking interactions of the nucleobase residue appear as being perturbed (figure 2), yet in an active site cavity of an enzyme exactly this process could lead to a reorientation of the nucleotide substrate by replacing stacking (partly) by hydrophobic interactions, simulated in the present study by the ethylene bridges of 1,4-dioxane.

4. Experimental

The heteroaromatic amines, i.e. 2,2′-bipyridine and 1,10-phenanthroline monohydrate (both *pro analysi*), were obtained from Merck AG, Darmstadt, Germany. All other materials used in the experiments, including CO_2-free water, were from the same sources as previously [52, 66, 77]. The exact concentrations of the stock solutions were determined as described [52, 66].

The potentiometric pH titrations were carried out with the same equipment and in the same way as described [66]; this also holds for the evaluation of the experimental data [66, 77]. The titration apparatus was calibrated with aqueous buffer solutions [52]. The given acidity constants are so-called practical, mixed or Brønsted constants [57]; no corrections were applied for the change in solvent from water to 1,4-dioxane/water mixtures [52]. The stability constants presented are, as usual, concentration constants. It needs to be noted further that always the differences in NaOH consumption between solutions with and without ligand [57] were evaluated.

In the determination of the stability constants of the Cu(Arm)(H; GDP) and $Cu(Arm)(GDP)^-$ complexes [equations (5) and (6)], the same concentrations (0.6 mM) (1 : 1 : 1 ratios) were used as previously [52], but Cu^{2+} was replaced by Cu^{2+}/Arm (1 : 1 ratio). Under the experimental conditions the formation of the $Cu(Arm)^{2+}$ complexes is

practically complete due to their high stability [18, 19]; in fact, titrations containing only HNO_3 or HNO_3 plus Cu^{2+}/Arm were identical in the lower pH range, and therefore the evaluation of the titration data of the ternary systems could be done in the same way as for the binary ones. The data collection was initiated at a pH of about 2.7 and stopped at the onset of the formation of $Cu(Arm)^{2+}$-hydroxo complexes which was evident from the titrations without GDP.

The listed stability constants of the Cu(Arm)(H; GDP) and $Cu(Arm)(GDP)^{-}$ complexes are the results of at least four independent pairs of titrations in each solvent.

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References

[1] J.J.R. Fraústo da Silva, R.J.P. Williams. *The Biological Chemistry of the Elements*, pp. 1–561, Clarendon Press, Oxford (1991).
[2] A. Sigel, H. Sigel (Eds). *Interactions of Metal Ions with Nucleotides, Nucleic Acids and Their Constituents*, Vol. 32 *of Metal Ions in Biological Systems*, pp. 1–814, Marcel Dekker, New York (1996).
[3] H. Sigel. *Chem. Soc. Rev.*, **33**, 191 (2004).
[4] L.W. Tari, A. Matte, H. Goldie, L.T.J. Delbaere. *Nat. Struct. Biol.*, **4**, 990 (1997).
[5] R.K.O. Sigel, E. Freisinger, B. Lippert. *J. Biol. Inorg. Chem.*, **5**, 287 (2000).
[6] R.K.O. Sigel, A.M. Pyle. *Chem. Rev.*, **107**, 97 (2007).
[7] O. Yamauchi, A. Odani, H. Masuda, H. Sigel. *Met. Ions Biol. Syst.*, **32**, 207 (1996).
[8] T. Yajima, G. Maccarrone, M. Takani, A. Contino, G. Arena, R. Takamido, M. Hanaki, Y. Funahashi, A. Odani, O. Yamauchi. *Chem. Eur. J.*, **9**, 3341 (2003).
[9] T. Yajima, R. Takamido, Y. Shimazaki, A. Odani, Y. Nakabayashi, O. Yamauchi. *Dalton Trans.*, 299 (2007).
[10] K. Aoki. *Met. Ions Biol. Syst.*, **32**, 91 (1996).
[11] H. Sigel. *Pure Appl. Chem.*, **61**, 923 (1989).
[12] H. Sigel. *Pure Appl. Chem.*, **76**, 375 (2004).
[13] H. Sigel, R. Griesser. *Chem. Soc. Rev.*, **34**, 875 (2005).
[14] A. Fernández-Botello, A. Holý, V. Moreno, B.P. Operschall, H. Sigel. *Inorg. Chim. Acta*, **361**, (2008 in press).
[15] H. Sigel, B.P. Operschall, S.S. Massoud, B. Song, R. Griesser. *Dalton Trans.*, 5521 (2006).
[16] E. Bugella-Altamirano, D. Choquesillo-Lazarte, J.M. González-Pérez, M.J. Sánchez-Moreno, R. Marín-Sánchez, J.D. Martín-Ramos, B. Covelo, R. Carballo, A. Castiñeiras, J. Niclós-Gutíerrez. *Inorg. Chim. Acta*, **339**, 160 (2002).
[17] A. Fernández-Botello, A. Holý, V. Moreno, H. Sigel. *J. Inorg. Biochem.*, **98**, 2114 (2004).
[18] H. Irving, D.H. Mellor. *J. Chem. Soc.*, 5222 (1962).
[19] G. Anderegg. *Helv. Chim. Acta*, **46**, 2397 (1963).
[20] I. Bertini, A. Sigel, H. Sigel (Eds). *Handbook on Metalloproteins*, pp. 709–959, Marcel Dekker, New York (2001).
[21] N. Fatemi, B. Sarkar. *Inorg. Chim. Acta*, **339**, 179 (2002).
[22] A. Sigel, H. Sigel, R.K.O. Sigel (Eds). *Neurodegenerative Diseases and Metal Ions*, Vol. 1 of *Metal Ions in Life Sciences*, pp. 1–463, J. Wiley & Sons, Chichester (2006).
[23] A. Mucha, B. Knobloch, M. Jeżowska-Bojczuk, H. Kozlowski, R.K.O. Sigel. *Chem. Eur. J.*, **14**, 6663 (2008).
[24] G. Schatz. *FEBS Lett.*, **572**, 1 (2004).
[25] H.A. Lester, A. Karschin. *Ann. Rev. Neurosci.*, **23**, 89 (2000).
[26] J. Garzon, I. De Antonio, P. Sanchez-Blazquez. *Methods Enzymol. B*, **314**, 3 (2000).
[27] H.R. Kalbitzer. *Met. Ions Biol. Syst.*, **22**, 81 (1987).

[28] G.H. Reed, R.R. Poyner. *Met. Ions Biol. Syst.*, **37**, 183 (2000).
[29] J.D. Crowley, D.A. Traynor, D.C. Weatherburn. *Met. Ions Biol. Syst.*, **37**, 209 (2000).
[30] R.K.O. Sigel, H. Sigel. *Met. Ions Life Sci.*, **2**, 109 (2007).
[31] E.M. Bianchi, S.A.A. Sajadi, B. Song, H. Sigel. *Inorg. Chim. Acta*, **300–302**, 487 (2000).
[32] S.S. Massoud, R. Tribolet, H. Sigel. *Eur. J. Biochem.*, **187**, 387 (1990).
[33] R. Tribolet, R. Malini-Balakrishnan, H. Sigel. *J. Chem. Soc., Dalton Trans.*, 2291 (1985).
[34] M.S. Lüth, L.E. Kapinos, B. Song, B. Lippert, H. Sigel. *J. Chem. Soc., Dalton Trans.*, 357 (1999).
[35] M.S. Lüth, B. Song, B. Lippert, H. Sigel. *Inorg. Chem.*, **39**, 1305 (2000).
[36] D.C. Rees. *J. Mol. Biol.*, **141**, 323 (1980).
[37] G.R. Moore. *FEBS Lett.*, **161**, 171 (1983).
[38] N.K. Rogers, G.R. Moore, M.J.E. Sternberg. *J. Mol. Biol.*, **182**, 613 (1985).
[39] E. Demchuk, R.C. Wade. *J. Phys. Chem.*, **100**, 17373 (1996).
[40] G. Iversen, Y.I. Kharkats, J. Ulstrup. *Mol. Phys.*, **94**, 297 (1998).
[41] H. Sigel, R.B. Martin, R. Tribolet, U.K. Häring, R. Malini-Balakrishnan. *Eur. J. Biochem.*, **152**, 187 (1985); see also Footnote on p. 258 in M. Bastian, H. Sigel. *Inorg. Chim. Acta*, **178**, 249 (1990).
[42] T. Head-Gordon. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 8308 (1995).
[43] J. Israelachvili, H. Wennerström. *Nature*, **379**, 219 (1996).
[44] L. De Meis. *Biochim. Biophys. Acta*, **973**, 333 (1989).
[45] B.L. Vallee, R.J.P. Williams. *Proc. Natl. Acad. Sci. U.S.A.*, **59**, 498 (1968).
[46] G. Åkerlöf, O.A. Short. *J. Am. Chem. Soc.*, **58**, 1241 (1936).
[47] G. Åkerlöf, O.A. Short. *J. Am. Chem. Soc.*, **75**, 6357 (1953).
[48] F.E. Critchfield, J.A. Gibson Jr, J.L. Hall. *J. Am. Chem. Soc.*, **75**, 1991 (1953).
[49] G. Liang, H. Sigel. *Inorg. Chem.*, **29**, 3631 (1990).
[50] D. Chen, F. Gregáň, A. Holý, H. Sigel. *Inorg. Chem.*, **32**, 5377 (1993).
[51] H. Sigel, B. Song. *Met. Ions Biol. Syst.*, **32**, 135 (1996).
[52] E.M. Bianchi, R. Griesser, H. Sigel. *Helv. Chim. Acta*, **88**, 406 (2005).
[53] H. Sigel, S.S. Massoud, N.A. Corfù. *J. Am. Chem. Soc.*, **116**, 2958 (1994).
[54] K.H. Scheller, H. Sigel. *J. Am. Chem. Soc.*, **105**, 5891 (1983).
[55] P.R. Mitchell. *J. Chem. Soc., Dalton Trans.*, 1079 (1980).
[56] R. Tribolet, H. Sigel. *Biophys. Chem.*, **27**, 119 (1987).
[57] H. Sigel, A.D. Zuberbühler, O. Yamauchi. *Anal. Chim. Acta*, **255**, 63 (1991).
[58] *IUPAC Stability Constants Database*, Release 5, Version 5.71 (compiled by L.D. Pettit and H.K.J. Powell <pettit@acadsoft.co.uk>), Academic Software, Timble, Otley, West Yorkshire, UK (2007).
[59] *NIST Critically Selected Stability Constants of Metal Complexes*, Reference Database 46, Version 7.0 (data collected and selected by R.M. Smith and A.E. Martell), US Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD, USA (2003).
[60] H. Sigel, B. Lippert. *Pure Appl. Chem.*, **70**, 845 (1998).
[61] R. Griesser, G. Kampf, L.E. Kapinos, S. Komeda, B. Lippert, J. Reedijk, H. Sigel. *Inorg. Chem.*, **42**, 32 (2003).
[62] H. Sigel. *Chimia*, **21**, 489 (1967).
[63] H. Sigel. *Angew. Chem., Int. Ed. Engl.*, **14**, 394 (1975).
[64] H. Sigel. *Inorg. Chem.*, **19**, 1411 (1980).
[65] R.B. Martin, H. Sigel. *Comments Inorg. Chem.*, **6**, 285 (1988).
[66] S.A.A. Sajadi, B. Song, F. Gregáň, H. Sigel. *Inorg. Chem.*, **38**, 439 (1999).
[67] R. Malini-Balakrishnan, K.H. Scheller, U.K. Häring, R. Tribolet, H. Sigel. *Inorg. Chem.*, **24**, 2067 (1985).
[68] H. Sigel. In *Coordination Chemistry–20*, D. Banerjea (Ed.), pp. 27–45, IUPAC by Pergamon Press, Oxford (1980).
[69] B. Song, S.A.A. Sajadi, F. Gregáň, N. Prónayová, H. Sigel. *Inorg. Chim. Acta*, **273**, 101 (1998).
[70] G. Liang, N.A. Corfù, H. Sigel. *Z. Naturforsch. B*, **44**, 538 (1989).
[71] H. Sigel, R. Malini-Balakrishnan, U.K. Häring. *J. Am. Chem. Soc.*, **107**, 5137 (1985).
[72] L.E. Kapinos, B. Song, H. Sigel. *Chem. Eur. J.*, **5**, 1794 (1999).
[73] P.R. Mitchell, H. Sigel. *J. Am. Chem. Soc.*, **100**, 1564 (1978).
[74] P.R. Mitchell, B. Prijs, H. Sigel. *Helv. Chim. Acta*, **62**, 1723 (1979).
[75] W.S. Sheldrick. *Z. Naturforsch.*, **B37**, 863 (1982).
[76] H. Sigel. *Coord. Chem. Rev.*, **144**, 287 (1995).
[77] E.M. Bianchi, S.A.A. Sajadi, B. Song, H. Sigel. *Chem. Eur. J.*, **9**, 881 (2003).