Influence of Decreasing Solvent Polarity (1,4-Dioxane/Water Mixtures) on the Acid–Base and Copper(II)-Binding Properties of Guanosine 5′-Diphosphate[1])

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Dedicated to Professor Dr. *André E. Merbach,* on the occasion of his 65th birthday, with admiration for his seminal contributions to chemistry and all best wishes for his future endeavors

The acidity constants of twofold protonated guanosine 5′-diphosphate, $H_2(GDP)^-$, and the stability constants of the [Cu(H;GDP)] and $[Cu(GDP)]^-$ complexes were determined in H_2O as well as in 30 or 50% (*v*/*v*) 1,4-dioxane/H_2O by potentiometric pH titrations (25°; $I = 0.1$M, $NaNO_3$). The results showed that in H_2O one of the two protons of $H_2(GDP)^-$ is located mainly at the N(7) site and the other one at the terminal β-phosphate group. In contrast, for 50% 1,4-dioxane/H_2O solutions, a micro acidity-constant evaluation evidenced that *ca.* 75% of the $H_2(GDP)^-$ species have both protons phosphate-bound, because the basicity of pyridine-type N sites decreases with decreasing solvent polarity whereas the one of phosphate groups increases. In the [Cu(H;GDP)] complex, the proton and the metal ion are in all three solvents overwhelmingly phosphate-bound, and the release of this proton is inhibited by decreasing polarity of the solvent. Based on previously determined straight-line plots of $\log K^{Cu}_{Cu(R-DP)}$ *vs.* $pK^{H}_{H(R-DP)}$ (where R represents a non-interacting residue in simple diphosphate monoesters $ROP(O^-)(=O)-O-P(=O)(O^-)_2$, $R-DP^{3-}$), which were now extended to mixed solvents (based on analogies), it is concluded that, in all three solvents, the $[Cu(GDP)]^-$ complex is more stable than expected based on the basicity of the diphosphate residue. This increased stability is attributed to macrochelate formation of the phosphate-coordinated Cu^{2+} with N(7) of the guanine residue. The formation degree of this macrochelate amounts in aqueous solution to *ca.* 75% (being thus higher than that of the Cu^{2+} complex of adenosine 5′-diphosphate) and in 50% (*v*/*v*) 1,4-dioxane/H_2O to *ca.* 60%, *i.e.*, the formation degree of the macrochelate is only relatively little affected by the change in solvent, though it needs to be emphasized that the overall stability of the $[Cu(GDP)]^-$ complex increases with decreasing solvent polarity. By including previously studied systems in the considerations, the biological implications are shortly discussed, and it is concluded that Nature has here a tool to alter the structure of complexes by shifting them on a protein surface from a polar to an apolar region and *vice versa*.

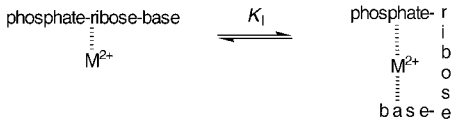
1. Introduction. – It is now well-established that the so-called ‘effective’ or ‘equivalent solution’ dielectric constants or permittivities in proteins [1–4] or in the

[1]) Abbreviations used: ADP^{3-}, adenosine 5′-diphosphate; AMP^{2-}, adenosine 5′-monophosphate; ATP^{4-}, adenosine 5′-triphosphate; GDP^{3-}, guanosine 5′-diphosphate; GMP^{2-}, guanosine 5′-monophosphate; GTP^{4-}, guanosine 5′-triphosphate; Guo, guanosine; *I*, ionic strength; K_a, general acidity constant; L, general ligand; M^{2+}, divalent metal ion; NDP^{3-}, nucleoside 5′-diphosphate; NTP^{4-}, nucleoside 5′-triphosphate; Nu, general nucleotide; $R-DP^{3-}$, diphosphate monoester, R being a non-interacting organic residue; UTP^{4-}, uridine 5′-triphosphate. Species written in the text without a charge either do not carry one or represent the species in general (*i.e.*, independent from their protonation degree); which of the two possibilities applies is always clear from the context. 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. A formula like $(GDP - H)^{4-}$ means that the compound has lost a further proton and it is to be read as GDP^{3-} *minus* H^+.

active-site cavities of metalloenzymes [5] are reduced compared to the situation in bulk water. It is also generally agreed that different types of water exist in cells [6]. At the protein-water interface, the activity of water is decreased [7] due to the presence of aliphatic and aromatic amino acid side chains [8], and, in such a low-dielectric medium, inner-sphere binding of metal ions to the ligating groups of proteins, especially in the case of negatively charged residues, is favored [9]. It has also been pointed out with regard to biological systems [10] that metal ions like to be coordinated to a hydrophilic shell that is then embedded within a larger hydrophobic shell.

Estimates for the dielectric constant (ε) in such biological locations range from *ca.* 30 to 70 [1][3][5] compared to *ca.* 80 in bulk water; hence, by employing aqueous solutions that contain *ca.* 20–50% 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 [5][12]. Other solvents that have been used in studies [5][13] devoted to a decreased solvent polarity are H_2O/EtOH mixtures. However, for the present, we prefer the use of 1,4-dioxane for two reasons: *i*) H_2O containing 20 or 50% (*v*/*v*) EtOH gives ε values only of *ca.* 70 or 50, respectively [5][13]; to achieve ε values of *ca.* 60 or 35, H_2O mixtures containing *ca.* 35 or 80% EtOH would be needed, respectively. *ii*) Because EtOH is a smaller and less-rigid molecule compared to 1,4-dioxane, one expects that its hydrophobic solvating properties by the Et residue are somewhat more pronounced than those of the CH_2CH_2 bridges of 1,4-dioxane, and this would not be in accord with the aim of this study where not the hydrophobic solvation but the decreased solvent polarity is in the center of interest.

Among the few available studies in which the effect of a decreasing solvent polarity has been investigated [13–17] is one that deals with the Cu^{2+} complex of adenosine 5′-monophosphate (AMP^{2-}) [16]. In this study, it has been shown that, by changing the solvent from H_2O to 50% (*v*/*v*) 1,4-dioxane/H_2O, the overall stability of the [Cu(AMP)] complex increases gradually by 1.6 log units. However, despite a rather regular stability increase due to the change in solvent, the formation degree of the macrochelate according to the equilibrium of *Eqn. 1* passes through a minimum in *ca.* 30% (*v*/*v*) 1,4-dioxane/H_2O; in other words, there is a significant change in the structure of this complex. It should be noted that macrochelate formation of a phosphate-coordinated metal ion by interaction with N(7) is commonly observed in complexes of transition-metal ions with purine nucleotides [18–20]. In contrast to the indicated properties of [Cu(AMP)], the overall stability of the Cu^{2+} complex formed with adenosine 5′-triphosphate (ATP^{4-}) is only relatively little affected by the change in solvent composition [21], whereas the formation degree of its macrochelate decreases rather significantly by going from H_2O to 50% (*v*/*v*) 1,4-dioxane/H_2O.



(1)

Since guanosine is known to have a significantly larger affinity toward divalent transition-metal ions than adenosine (*cf.* the data in [22] with those in [23]) [24], we

selected for the present study a guanosine derivative, *i.e.*, guanosine 5′-diphosphate (GDP^{3-}), the structure of which is shown together with the one of AMP^{2-} in *Fig. 1* [25][26]. GDP plays a role in the so-called G-protein systems [27] which utilize guanosine 5′-triphosphate (GTP^{4-}) [28] and where metal ions are involved in the hydrolysis reactions [29][30]. Cu^{2+} was selected because of its high affinity toward the N of guanosine in aqueous solution [18][23][31] and also because of its role in biology [32][33].

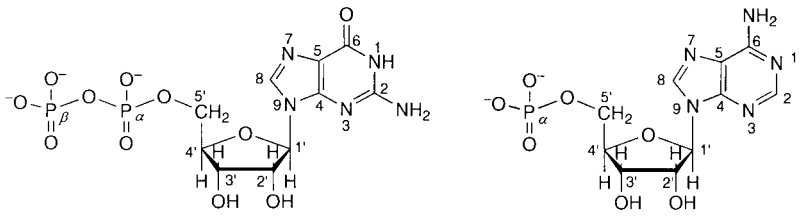


Fig. 1. *Chemical structure of guanosine 5′-diphosphate* (GDP^{3-}) *and adenosine 5′-monophosphate* (AMP^{2-}) *in their dominating* anti *conformations* [25][26]

By potentiometric pH titrations, we studied now the effect of increasing amounts of 1,4-dioxane added to aqueous solutions on the acid–base properties of GDP^{3-}. Indeed, this effect is rather pronounced, and the species $H_2(GDP)^-$ has different structures in H_2O and in 50% (*v*/*v*) 1,4-dioxane/H_2O. It is, thus, no surprise that also the properties of the $[Cu(H;GDP)]$[1]) and $[Cu(GDP)]^-$ complexes are affected by the change in solvent.

2. Results and Discussion. – 2.1. *Preamble.* Great care was taken in this study to measure the various equilibrium constants under conditions where no self-association occurs [24][34][35]. The measurements were made with solutions being 0.6 mM in GDP; this guarantees [24][34] that indeed the properties of the monomeric species are studied. It may be added that it is also known [21][36] that the presence of 1,4-dioxane, due to hydrophobic solvation of the aromatic rings, inhibits stacking.

2.2. *Acidity Constants of $H_2(GDP)^-$.* In the pH range of *ca.* 2.5 to 11, relevant for this study, GDP^{3-} (see *Fig. 1*) accepts two protons to give $H_2(GDP)^-$. Based on the experience with $H_2(GMP)^{\pm}$ [24] and $H_2(GTP)^{2-}$ [37] in aqueous solution, one may in a first approximation assume that the first of the two protons of $H_2(GDP)^-$ is released from the H–N(7)$^+$ site and the second one from the terminal β-phosphate group. Considering further that the H–N(1) unit may lose a proton as well, the following three deprotonation reactions need to be taken into account:

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

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

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

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

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

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

Indeed, with *Eqns. 2b*, *3b*, and *4b*, the experimental data could be perfectly fitted in the mentioned pH range and in the solvents H_2O and 30 or 50% (*v*/*v*) 1,4-dioxane/H_2O. The corresponding acidity constants are listed in *Table 1* together with some related data [38–40].

Table 1. *Negative Logarithms of the Acidity Constants of $H_2(GDP)^-$ (Eqns. 2–4), of Monoprotonated Guanosine* (Guo), *and of also Twofold Protonated and Related Nucleotides* (Nu) *in Dependence on the Amount of 1,4-Dioxane Added to Water, as well as Some Properties of the Solvents*[a])[1]). The constants were measured by potentiometric pH titrations at 25°, $I = 0.1$M ($NaNO_3$).

Entry	$H(Guo)^+$ or $H_2(Nu)$	1,4-Dioxane		ε[b])	$pK^{H}_{H(Guo)}$ or $pK^{H}_{H_2(Nu)}$	$pK^{H}_{H(Nu)}$	pK^{H}_{Nu}	Ref.
		% (*v*/*v*)	mol fraction		H–N(7)$^+$[c])[d])	$P(O)_2(OH)^-$[c])	H–N(1)[c])	
1	$H(Guo)^+$	0	0	78.5	2.11 ± 0.04		9.22 ± 0.01	[24]
2	$H_2(GMP)^{\pm}$	0	0	78.5	2.48 ± 0.04	6.25 ± 0.02	9.49 ± 0.02	[24]
3	$H_2(GDP)^-$	0	0	78.5	2.67 ± 0.02	6.38 ± 0.01	9.56 ± 0.03	–[f])
4	$H_2(GDP)^-$	30	0.083	52.7	2.75 ± 0.02	6.89 ± 0.02	9.97 ± 0.02	–[f])
5	$H_2(GDP)^-$	50	0.175	35.2	2.89 ± 0.04	7.09 ± 0.02	10.25 ± 0.04	–[f])
6	$H_2(GTP)^{2-}$	0	0	78.5	2.94 ± 0.02	6.50 ± 0.02	9.57 ± 0.02	[27]
7	$H_2(AMP)^{\pm}$	0	0	78.5	3.84 ± 0.02[d])	6.21 ± 0.01		[38]
8	$H_2(AMP)^{\pm}$	50	0.175	35.2	3.42 ± 0.02[d])	7.48 ± 0.01		[16]
9	$H_2(ATP)^{2-}$	0	0	78.5	4.01 ± 0.01[d])	6.49 ± 0.01		[21]
10	$H_2(ATP)^{2-}$	50	0.175	35.2	3.59 ± 0.02[d])	6.90 ± 0.02		[21]
11	$H(UTP)^{3-}$	0	0	78.5	–[e])	6.46 ± 0.01		[21]
12	$H(UTP)^{3-}$	50	0.175	35.2	–[e])	6.92 ± 0.01		[21]

[a]) So-called practical, mixed, or *Brønsted* constants [39] are listed, see *Exper. Part*. The errors given are *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b]) The dielectric constants (ε) for the 1,4-dioxane/H_2O mixtures are interpolated from the data given in [12]. [c]) Site at which the deprotonation reaction occurs. The $P(O)_2(OH)^-$ unit refers always to the terminal phosphate group in a nucleotide. [d]) In the case of the adenine nucleotides, the proton is released from the H–N(1)$^+$ site. [e]) The uracil residue cannot be protonated in aqueous solution [40]; further protonation of $H(UTP)^{3-}$ occurs at the phosphate chain. [f]) Measured in this work.

The results of *Table 1* allow the following conclusions: *1*) From the acidity constants in *Column 7* of *Entries 6*, *9*, and *11*, it follows that the kind of nucleobase has very little influence on the pK_a value due to the release of the last proton from the phosphate chain, *i.e.*, from the terminal γ-phosphate groups of the NTPs. This conclusion is confirmed by the pK_a values in *Entries 2* and *7* due to $H(GMP)^-$ and $H(AMP)^-$, respectively. Considering the large distance between the nucleobases and the phosphate residues, this result is expected, and it means that the pK_a values for $P(O)_2(OH)^-$ groups may directly be compared with each other independent of the nucleobases of the nucleotides.

2) The release of the proton from the H–N(7)$^+$ site of the guanine moiety is increasingly inhibited by increasing charge on the phosphate residue: Guo < GMP <

GDP < GTP (*Column 6*, *Entries 1–3* and *6*). The same effect, though clearly less pronounced, is observed for the deprotonation of the H–N(1) site (*Column 8*). This is also expected because removal of a positive charge should become increasingly difficult if the negative charge in the vicinity increases. Naturally, the effect on H–N(1) should be smaller, because this unit is further away from the phosphate residue than the $H-N(7)^+$ site. Note, purine nucleotides exist in solution in the *anti* conformation (see *Fig. 1*).

3) Evidently, for a decreasing solvent polarity, which corresponds to an increasing hydrophobic medium, one expects that charge separation becomes more difficult and indeed, this is observed: with increasing 1,4-dioxane concentration, the pK_a values for the $P(O)_2(OH)^-$ groups increase as well (*Column 7* of *Table 1*, *Entries 3–5* and *7–12*).

4) However, the smaller the phosphate residue, the larger is the effect of a decreasing solvent polarity: The ΔpK_a values for the differences between the acidity constants measured in H_2O and in 50% 1,4-dioxane/H_2O are $1.27 > 0.71 > 0.41 \approx 0.46$ for $H(AMP)^-$, $H(GDP)^{2-}$, $H(ATP)^{3-}$, and $H(UTP)^{3-}$, respectively (*Entries 7 vs. 8*, *3 vs. 5*, *9 vs. 10*, and *11 vs. 12* of *Column 7*). Considering that the charges and the number of O-atoms increase by going from a mono- *via* a di- to a triphosphate, it is expected that the extent of solvation by H_2O increases within this series as well. Clearly, the stronger the solvation by H_2O the less should be the effect of 1,4-dioxane, and this is exactly observed.

5) That a low-polarity or -permittivity medium inhibits charge separation is also evident from the properties of the H–N(1) site in GDP^{3-}: The pK_a value increases from 9.56 ± 0.03 in H_2O to 10.25 ± 0.04 in 50% 1,4-dioxane/H_2O (*Column 8* of *Table 1*, *Entries 3–5*). The fact that this ΔpK_a value of 0.69 ± 0.05 is identical within the error limits to the one observed for the $H(GDP)^{2-}$ species, *i.e.*, $\Delta pK_a = 0.71 \pm 0.02$ ($= (7.09 \pm 0.02) - (6.38 \pm 0.01)$), should not give rise to speculation; this result is fortuitous! For example, for $H(GMP)^-$, the change in ΔpK_a equals 1.23 ± 0.06 due to the same change in solvent, whereas the corresponding value for the H–N(1) site of GMP^{2-} amounts only to $\Delta pK_a = 0.81 \pm 0.04$[2]).

6) To say it more generally, in conclusions *3*) and *4*), we have seen that, in accord with previous observations [41], the basicity of negatively charged O sites increases with a decreasing solvent polarity, *i.e.*, it becomes more difficult to remove the proton. This contrasts with the also known [41] properties of pyridine-type N-sites, in which the basicity of N decreases with decreasing solvent polarity; *i.e.*, the pyridinium moiety becomes less stable. In accord herewith, the pK_a value for the $H-N(1)^+$ site decreases in $H_2(AMP)^\pm$, which is a pyridine-type site, by $\Delta pK_a = 0.42 \pm 0.03$ in going from H_2O to 50% 1,4-dioxane/H_2O as a solvent; similarly, for the same site in $H_2(ATP)^{2-}$ and the same change in solvent, $\Delta pK_a = 0.42 \pm 0.02$ (*Column 6* of *Table 1*; *Entries 7 vs. 8* and *9 vs. 10*). Hence, the property of the $H-N(1)^+$ unit of the adenine residue is as expected! Understandably, to solvate the positively charged $H-N(1)^+$ unit with H_2O molecules becomes increasingly difficult with increasing 1,4-dioxane concentrations.

7) In view of conclusion *6*), the apparent basicity increase of the $H-N(7)^+$ site of the guanine residue in $H_2(GDP)^-$ is not to be understood! There is no reason why an

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[2]) Results to be published.

imidazole N-atom should behave totally differently than a pyridine N-atom. Does the macro acidity constant measured for $H_2(GDP)^-$ in 50% 1,4-dioxane/H_2O involve a contribution of a proton released from the phosphate residue? To say it differently, is in $H_2(GDP)^-$ in H_2O one proton at N(7) and the other at the β-phosphate group (see *Sect. 2.3*) and does in 50% 1,4-dioxane/H_2O exist an isomer where both protons are at the diphosphate residue in $H_2(GDP)^-$? This then would explain why $pK^H_{H_2(GDP)}$ increases with an increasing 1,4-dioxane concentration (*Column 6* in *Table 1*, *Entries 3–5*). An answer is attempted in *Sect. 2.4*.

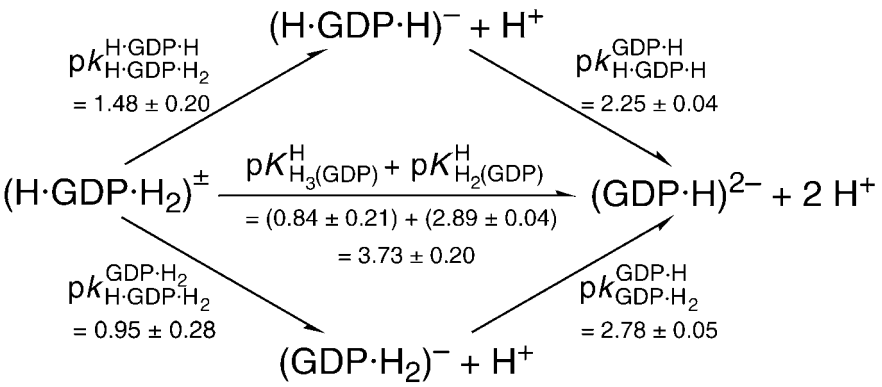
2.3. *Location of the Protons at $H_2(GDP)^-$ in Aqueous Solution*. First, it may be noted that the present results given for the acidity constants of $H_2(GDP)^-$ in aqueous solution (*Table 1*, *Entry 3*) are in fair agreement with a much earlier determination [42], and they also agree well with the previous results obtained for $H_2(GMP)^\pm$ [24] and $H_2(GTP)^{2-}$ [37].

Evidently, in monoprotonated guanosine, $H(Guo)^+$, the proton must be located at N(7) [43]. That this is also true in $H_2(GMP)^\pm$, in which a $P(O)_2(OH)^-$ residue is attached to the 5′-position, follows from the increase in basicity of N(7), which is due to the negative charge now present in its neighborhood. The ΔpK_a increase of 0.37 ± 0.06 ($= (2.48 \pm 0.04) - (2.11 \pm 0.04)$) is in accord with the expectations [44] (*Column 6* in *Table 1*, *Entries 1* and *2*).

For $H_2(GTP)^{2-}$, it has recently been shown *via* a micro acidity constant evaluation [37] that one proton is at the terminal γ-phosphate group and the other one is located to *ca.* 90% at N(7) with *ca.* 10% being also at the triphosphate chain. Since the overall basicity of the phosphate residues decreases in the order triphosphate > diphosphate > monophosphate, one may conclude, based on the results provided for $H_2(GMP)^\pm$ and $H_2(GTP)^{2-}$, that in $H_2(GDP)^-$ in aqueous solution, one proton is at the terminal β-phosphate group and the other overwhelmingly, *i.e.*, 90% being the lower limit, at the N(7) site.

2.4. *Evidence for an Isomer Equilibrium of the $H_2(GDP)^-$ Species in 50% 1,4-Dioxane/H_2O*. In conclusion *7*) of *Sect. 2.2*, we asked the question, is one of the protons in $H_2(GDP)^-$ in 1,4-dioxane/H_2O mixtures partially located at N(7) and partially at the already monoprotonated diphosphate chain, and is this the reason why $pK^H_{H_2(GDP)}$ increases (*Column 6*, *Table 1*, *Entries 3–6*) with increasing amounts of 1,4-dioxane added to H_2O? The only way to deal with such a problem is to try to quantify the intrinsic acid–base properties of the various sites in a molecule, which can accept and/or release protons, *via* micro acidity constants [45]. The corresponding micro acidity, constant scheme for the reaction of $H_3(GDP)^\pm$ to $H(GDP)^{2-}$ releasing two protons is given in the *Scheme*; in this reaction, the above-mentioned $H_2(GDP)^-$ species appear as intermediates. The *Scheme* summarizes the equilibrium for $H_3(GDP)^\pm$ defining the micro acidity constants k and giving their interrelationships with the macro acidity constants K according to the definitions given in the lower part [45][46]. The species $H_3(GDP)^\pm$, on its way to $H(GDP)^{2-}$, may release protons from the $H-N(7)^+$ site and/or from the diphosphate residue and therefore, we rewrite $H_3(GDP)^\pm$ as $(H \cdot GDP \cdot H_2)^\pm$; since deprotonation may occur at either site, we define the intermediate forms of $H_2(GDP)^-$ as $(H \cdot GDP \cdot H)^-$ and $(GDP \cdot H_2)^-$. There are four unknown microconstants but only the three independent *Eqns. a*, *b*, and *c* interrelating them with the macroconstants [45][46]. This means that one of these microconstants needs to be

Scheme. *Equilibrium Scheme for $H_3(GDP)^{\pm}$ Defining the Micro Acidity Constants* k *and Showing Their Interrelationships with the Macroconstants* K *and the Connection between $(H\cdot GDP\cdot H)^-$ and $(GDP\cdot H_2)^-$ and the Other Species Present*[a])



$$K^{H}_{H_3(GDP)} = k^{H\cdot GDP\cdot H}_{H\cdot GDP\cdot H_2} + k^{GDP\cdot H_2}_{H\cdot GDP\cdot H_2} \qquad \text{(a)}$$

$$\frac{1}{K^{H}_{H_2(GDP)}} = \frac{1}{k^{GDP\cdot H}_{H\cdot GDP\cdot H}} + \frac{1}{k^{GDP\cdot H}_{GDP\cdot H_2}} \qquad \text{(b)}$$

$$K^{H}_{H_3(GDP)}\cdot K^{H}_{H_2(GDP)} = k^{H\cdot GDP\cdot H}_{H\cdot GDP\cdot H_2}\cdot k^{GDP\cdot H}_{H\cdot GDP\cdot H} = k^{GDP\cdot H_2}_{H\cdot GDP\cdot H_2}\cdot k^{GDP\cdot H}_{GDP\cdot H_2} \qquad \text{(c)}$$

a) In $(H\cdot GDP\cdot H)^-$ and $(GDP\cdot H_2)^-$, one of the two protons is always bound to the terminal β-phosphate group and the other one either to the N(7) site or also to the diphosphate residue, respectively; $(H\cdot GDP\cdot H_2)^{\pm}$ and $(GDP\cdot H)^{2-}$ are also often written as $H_3(GDP)^{\pm}$ and $H(GDP)^{2-}$, respectively. The arrows indicate the direction for which the constants are defined, and the *Eqns. a–c* provide the connection between the macro- and microconstants. The macroconstant $pK^{H}_{H_2(GDP)} = 2.89 \pm 0.04$, valid for 50% 1,4-dioxane/H_2O (*Column 6* of *Table 1*, *Entry 5*), was measured by potentiometric pH titrations (25°; $I = 0.1$M, $NaNO_3$), and the microconstants $pk^{H\cdot GDP\cdot H}_{H\cdot GDP\cdot H_2} = 1.48 \pm 0.20$ and $pk^{GDP\cdot H}_{H\cdot GDP\cdot H} = 2.25 \pm 0.04$ were estimated as described in the text of *Sect. 2.4*. Knowledge of these three acidity constants allowed us to calculate with *Eqn. c* the missing values for the macroconstants given on the horizontal arrow in the *Scheme*; now, with the upper pathway complete, the micro acidity constants for the lower pathway could also be calculated by application of *Eqns. a* and *b*. The error limits of the various constants were calculated according to the error propagation after *Gauss* (see also *Footnote a* in *Table 1*).

either measured or estimated independently [37][46]. In fact, in the present case, where we shall concentrate on the solvent 50% 1,4-dioxane/H_2O, the situation appears at first sight to be rather discouraging because the only constant known is $pK^{H}_{H_2(GDP)} =$

2.89 ± 0.04, which is given on the horizontal arrow in the *Scheme*. Evidently, this means that two more constants need to be obtained or estimated before *Eqns. a*, *b*, and *c* can be applied.

It has been repeatedly noted that, for a set of related ligands, the difference between the pK_a values of the $P(O)_2(OH)^-$ and $P(O)(OH)_2$ residues is constant [47][48], even though the absolute pK_a values may differ depending on the systems considered. Similarly, for uridine 5′-triphosphate (UTP^{4-}), it has been observed [21] that the difference $\Delta pK_{a/UTP} = pK^H_{H(UTP)} - pK^H_{H_2(UTP)}$, both protons being released from the phosphate residue, is independent of the solvent, *i.e.*, it is within the error limits the same for H_2O and for 30 or 50% 1,4-dioxane/H_2O, namely $\Delta pK_{a/UTP} = 4.42 \pm 0.10$. This constancy implies, of course, that the solvent effect on the release of the first and the second protons from the phosphate residue is approximately the same. Hence, by taking the difference $\Delta pK_{a/GDP} = (7.09 \pm 0.02) - (6.38 \pm 0.01) = 0.71 \pm 0.02$, where the first value refers to 50% dioxane/H_2O and the second one to H_2O (see *Column 7* of *Table 1*, *Entries 3* and *5*), one may estimate a value for the release of the first proton from the twofold protonated phosphate residue of $H_3(GDP)^{\pm}$, provided the acidity constant for $H_3(GDP)^{\pm}$ in aqueous solution (where one proton is at N(7) and the other two are at the phosphate residue) is known. Indeed, this value has previously been estimated [49], *i.e.*, $pK^H_{H_3(GDP)} = 0.77 \pm 0.20$. Consequently, by adding the above given difference to this value for H_2O, one obtains for the mixed solvent the needed value, *i.e.*, $pk^{H\cdot GDP\cdot H}_{H\cdot GDP\cdot H_2} = (0.77 \pm 0.20) + (0.71 \pm 0.02) = 1.48 \pm 0.20$, which is shown in the upper pathway at the left of the *Scheme*.

A further estimate that is possible is for the deprotonation of the $H-N(7)^+$ unit in $(H\cdot GDP\cdot H)^-$. From the discussion in *Sect. 2.3* it follows that $pK^H_{H_2(GDP)} = 2.67 \pm 0.02$ (*Column 6* of *Table 1*, *Entry 3*) describes the situation in H_2O for the deprotonation of $H-N(7)^+$ in $(H\cdot GDP\cdot H)^-$ rather well since in $H_2(GDP)^-$, overwhelmingly N(7) and the terminal β-phosphate group are monoprotonated each. Hence, by using this value and by making the reasonable assumption that the deprotonations of the $H-N(1)^+$ and $H-N(7)^+$ sites are affected by a change in the solvent in about the same way, one can use the data available for $H_2(AMP)^{\pm}$ and $H_2(ATP)^{2-}$: for $H_2(AMP)^{\pm}$, the pK_a change due to the solvent change (*Column 6* of *Table 1*, *Entries 7–10*) amounts to $\Delta pK_{a/AMP} = (3.84 \pm 0.02) - (3.42 \pm 0.02) = 0.42 \pm 0.03$ and for $H_2(ATP)^{2-}$ to $\Delta pK_{a/ATP} = (4.01 \pm 0.01) - (3.59 \pm 0.02) = 0.42 \pm 0.02$ (see also conclusion *6*) in *Sect. 2.2*). Now a value for $pk^{GDP\cdot H}_{H\cdot GDP\cdot H}$ in 50% dioxane/H_2O may be estimated, *i.e.*, $pk^{GDP\cdot H}_{H\cdot GDP\cdot H} = pK^H_{H_2(GDP)} - \Delta pK_{a/AMP/ATP} = (2.67 \pm 0.02) - (0.42 \pm 0.03) = 2.25 \pm 0.04$. This value appears in the upper pathway at the right in the *Scheme*.

Now, the upper pathway in the *Scheme* can be completed, and also the missing macroconstants on the horizontal arrow can be obtained by applying *Eqn. c*), *i.e.*, $K^H_{H_3(GDP)} \cdot K^H_{H_2(GDP)} = 10^{-(1.48\pm0.20)} \cdot 10^{-(2.25\pm0.04)} = 10^{-(3.73\pm0.204)}$ and thus also $pK^H_{H_3(GDP)} = (3.73 \pm 0.204) - (2.89 \pm 0.04) = 0.84 \pm 0.21$; these estimated values given on the horizontal arrow in the *Scheme* refer to the corresponding deprotonation reactions in 50% 1,4-dioxane/H_2O. Application of *Eqns. a* and *b* allow now calculation of the micro acidity constants for the lower pathway of the *Scheme*. Comparison of the macroconstants with the micro acidity constants reveals that the lower pathway dominates because here the macro- and microconstants are more similar.

Since the values in the left part of the *Scheme* have rather large error limits, we use for the calculation of the ratio R, which quantifies the position of the intramolecular equilibrium of *Eqn. 5*, the right hand part of the *Scheme*, *i.e.*, *Eqn. 6*.

$$(\mathrm{GDP}\cdot\mathrm{H_2})^- \rightleftharpoons (\mathrm{H}\cdot\mathrm{GDP}\cdot\mathrm{H})^- \tag{5}$$

$$R = \frac{[(\mathrm{H}\cdot\mathrm{GDP}\cdot\mathrm{H})^-]}{(\mathrm{GDP}\cdot\mathrm{H_2})^-]} = \frac{[(\mathrm{GDP}\cdot\mathrm{H})^{2-}][\mathrm{H}^+]}{[(\mathrm{GDP}\cdot\mathrm{H_2})^-]} \cdot \frac{[(\mathrm{H}\cdot\mathrm{GDP}\cdot\mathrm{H})^-]}{[(\mathrm{GDP}\cdot\mathrm{H})^{2-}][\mathrm{H}^+]} \tag{6a}$$

$$= \frac{k^{\mathrm{GDP\cdot H}}_{\mathrm{GDP\cdot H_2}}}{k^{\mathrm{GDP\cdot H}}_{\mathrm{H\cdot GDP\cdot H}}} \tag{6b}$$

$$= \frac{10^{-(2.78\pm0.05)}}{10^{-(2.25\pm0.04)}} = \frac{1}{10^{(0.53\pm0.06)}} = \frac{1}{3.39\pm0.47} = \frac{23\pm3\%}{77\pm3\%} \tag{6c}$$

Though the above evaluation contains several assumptions, there is no doubt that it describes the correct trend for the formation of the isomers appearing in the equilibrium of *Eqn. 5*. This means, we may conclude that, in 50% 1,4-dioxane/H_2O *ca.* 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 the N(7) site (the other one remaining at the phosphate residue). This contrasts with the situation in H_2O where the isomer with one proton each at N(7) and at the terminal β-phosphate group strongly dominates (see *Sect. 2.3*).

To conclude, the suspicion indicated in conclusion *7* of *Sect. 2.2* that the change in solvent affects the location of the protons in $H_2(GDP)^-$, and thus the position of the isomer equilibrium of *Eqn. 5*, is correct. Moreover, considering that, in the $(H\cdot GDP\cdot H)^-$ isomer, three charged sites occur, *i.e.*, two negatively charged ones at the phosphate residue and a positively charged one at the $H-N(7)^+$ unit, and that, in the $(GDP\cdot H_2)^-$ isomer, only the singly charged site at the phosphate chain exists, it is understandable that, under conditions with a reduced H_2O activity, solvation of the charged sites becomes more difficult and, thus, the $(GDP\cdot H_2)^-$ isomer with the lower number of charged sites is favored.

2.5. *Stability Constants of the [Cu(H;GDP)] and [Cu(GDP)]$^-$ Complexes.* The experimental data of the potentiometric pH titrations of the Cu^{2+}/GDP systems are completely described by the equilibria of *Eqns. 2a*, *3a*, *7a*, and *8a*, if the evaluation is not carried into the pH range where formation of hydroxo complexes occurs (see *Exper. Part*). The acidity constant of the connected equilibrium of *Eqn. 9a* may be calculated with *Eqn. 10*. The results obtained for the equilibria of *Eqns. 7a*, *8a*, and *9a* are listed in *Columns 3*, *4*, and *5* of *Table 2*, respectively. For reasons of comparison, the corresponding results for the Cu^{2+}/ADP system in aqueous solution are also given [38]. The constants for the Cu^{2+}/GDP systems in H_2O and in H_2O containing increasing amounts of 1,4-dioxane have not been determined before [42].

$$\mathrm{Cu^{2+}} + \mathrm{H(GDP)^{2-}} \rightleftharpoons [\mathrm{Cu(H;GDP)}] \tag{7a}$$

$$K^{Cu}_{Cu(H;GDP)} = [[Cu(H;GDP)]]/([Cu^{2+}][H(GDP)^{2-}]) \quad (7b)^3)$$

$$Cu^{2+} + GDP^{3-} \rightleftharpoons [Cu(GDP)]^- \quad (8a)$$

$$K^{Cu}_{Cu(GDP)} = [[Cu(GDP)]^-]/([Cu^{2+}][GDP^{3-}]) \quad (8b)^3)$$

$$[Cu(H;GDP)] \rightleftharpoons [Cu(GDP)]^- + H^+ \quad (9a)$$

$$K^{H}_{Cu(H;GDP)} = [[Cu(GDP)]^-][H^+]/[[Cu(H;GDP)]] \quad (9b)^3)$$

$$pK^{H}_{Cu(H;GDP)} = pK^{H}_{H(GDP)} + \log K^{Cu}_{Cu(H;GDP)} - \log K^{Cu}_{Cu(GDP)} \quad (10)$$

Considering that the acidity constants of $H(GDP)^{2-}$ ($pK^{H}_{H(GDP)} = 6.38 \pm 0.01$; see *Table 1*) and $H(ADP)^{2-}$ ($pK^{H}_{H(ADP)} = 6.40 \pm 0.01$ [38]) are very similar, the stability constants of the corresponding $[Cu(GDP)]^-$ and $[Cu(ADP)]^-$ complexes can directly be compared. From the stability constants given in the fourth column of *Table 2*, it is evident that the $[Cu(GDP)]^-$ complex is somewhat more stable, confirming the expectation expressed in *Sect. 1*. Since $[Cu(ADP)]^-$ forms macrochelates according to *Eqn. 1*, the formation degree of the corresponding macrochelates for $[Cu(GDP)]^-$ must be higher; this is indeed the case, as will be shown in *Sect. 2.8*. It may be added here that, for the $[Zn(GDP)]^-$ and $[Cd(GDP)]^-$ complexes, the formation of macrochelates has been established by ^{1}H-NMR shift experiments in aqueous solution [34].

Table 2. *Logarithms of the Stability Constants of the [Cu(H;GDP)]* (*Eqn. 7*) *and [Cu(GDP)]*$^-$ (*Eqn. 8*) *Complexes in Dependence on the Amount of 1,4-Dioxane Added to Water and as Determined by Potentiometric pH Titrations* (25°; $I = 0.1$M, $NaNO_3$), *as well as the Negative Logarithms of the Acidity Constants of the [Cu(H;GDP)] Species* (*Eqns. 9* and *10*)[a])[b]). The corresponding constants for the Cu^{2+}/ADP system in H_2O are given for comparison [38].

NDP^{3-}	1,4-Dioxane[c]) [% (*v*/*v*)]	$\log K^{Cu}_{Cu(H;NDP)}$	$\log K^{Cu}_{Cu(NDP)}$	$pK^{H}_{Cu(H;NDP)}$
ADP^{3-}	0	2.77 ± 0.16	5.61 ± 0.03	3.56 ± 0.16
GDP^{3-}	0	3.39 ± 0.19	5.85 ± 0.04	3.92 ± 0.19
GDP^{3-}	30	3.50 ± 0.08	6.21 ± 0.15	4.18 ± 0.17
GDP^{3-}	50	3.80 ± 0.13	6.36 ± 0.05	4.53 ± 0.14

[a]) The error limits given are *three times* the standard error of the mean value; the limits of the derived data (*Column 5*) were calculated according to the error propagation after *Gauss*. [b]) For the acidity constants of $H_2(GDP)^-$, see *Table 1*. The acidity constants of $H_2(ADP)^-$ are $pK^{H}_{H_2(ADP)} = 3.92 \pm 0.02$ and $pK^{H}_{H(ADP)} = 6.40 \pm 0.01$ (25°; $I = 0.1$M, $NaNO_3$) [38]. [c]) For the mol fractions and dielectric constants of the 1,4-dioxane/H_2O mixtures, see *Table 1*.

2.6. *Structural Considerations on the [Cu(H;GDP)] Species.* Potentiometric pH titrations allow determination of the stability constants of the $[Cu(H;GDP)]$ complexes in the various solvents, but to locate the binding sites of the proton and the metal ion in these species, further information is needed. At first, one considers best

3) For convenience, the brackets of the complex formulae are omitted in mathematical equations.

the proton, because binding of a metal ion to a protonated ligand commonly leads to acidification of the ligand-bound proton [23][50][51]. Indeed, the acidity constants of the [Cu(H;GDP)] complexes given in *Column 5* of *Table 2* are on average *ca.* 2.6 pK units below p$K^{H}_{H(GDP)}$ (see *Table 1*) and *ca.* 1.4 pK units *above* p$K^{H}_{H_2(GDP)}$. Hence, in all the [Cu(H;GDP)] species, the proton must evidently be located at the terminal β-phosphate group of the diphosphate, because this is the most basic site.

However, where is the metal ion? Is it at N(7) of the guanine moiety or also at the diphosphate residue? Considering that the stability of the $[Cu(Guo)]^{2+}$ complex in H_2O amounts only to $\log K^{Cu}_{Cu(Guo)} = 2.15$ [23], it is evident that the charge effect of the diphosphate residue alone cannot account for the much higher stability observed for the [Cu(H;GDP)] species ($\log K^{Cu}_{Cu(H;GDP)} = 3.39$ in H_2O); hence, one has to conclude that Cu^{2+} is also located at the diphosphate residue and that this also holds for the situation in mixed solvents.

Furthermore, in *Sect. 2.8* we shall see that the formation of the macrochelates according to *Eqn. 1* varies for $[Cu(GDP)]^-$ between *ca.* 75% in H_2O and 60% in 50% 1,4-dioxane/H_2O. Since the main difference between $[Cu(GDP)]^-$ and [Cu(H;GDP)] is that, in the latter species, the β-phosphate group carries a proton, Cu^{2+} is of course in the [Cu(H;GDP)] species also in position to reach N(7) of the guanine residue and to form macrochelates. In fact, for [Cu(H;ADP)], it was shown [38] that Cu^{2+} is overwhelmingly coordinated to the diphosphate residue, which also carries the proton, and that *ca.* 50% of it form macrochelates. Though a quantitative evaluation for [Cu(H;GDP)] in the various solvents is not possible because not enough information exists, it is clear that macrochelate formation must also be substantial for this species in all three solvents considered, since $[Cu(Guo)]^{2+}$ [23] is more stable than $[Cu(Ado)]^{2+}$ [22].

2.7. *Evidence of Enhanced Stability of the [Cu(GDP)]$^-$ Complexes in Various Solvents.* The existence of the equilibrium of *Eqn. 1* is well-established for many complexes of purine nucleotides [18–21][23][31][38]. For example, the increased stability observed for various [M(AMP)] complexes, being due to macrochelate formation with N(7) of the already phosphate-coordinated metal ion, disappears as expected in all the corresponding complexes formed with tubercidin 5′-monophosphate (= 7-deaza-AMP^{2-}) since, in this ligand, N(7) is replaced by a CH unit [52]. Indeed, any kind of chelation [53] must be reflected in enhanced complex stability [14][18]. Of course, such macrochelates will hardly form to 100%. It is important to be aware that the formation degree of the macrochelated or 'closed' species, which we designate for the Cu^{2+} complexes of GDP^{3-} as $[Cu(GDP)]^-_{cl}$, is *independent* of the total complex concentration because the intramolecular equilibrium constant K_I, as defined by *Eqn. 11*, where $[Cu(GDP)]^-_{op}$ refers to the 'open' species in *Eqn. 1*, is dimension-less. Taking this into account, *Eqn. 8a* may be rewritten as given in *Eqn. 12*. The corresponding equilibrium constant is then defined by *Eqn. 13*. This expression contains as one term the stability constant of the open isomer, which is defined in *Eqn. 14*.

$$K_I = [[Cu(GDP)]^-_{cl}]/[[Cu(GDP)]^-_{op}] \quad (11)^3)$$

$$Cu^{2+} + GDP^{3-} \rightleftharpoons [Cu(GDP)]^-_{op} \rightleftharpoons [Cu(GDP)]^-_{cl} \quad (12)$$

$$K^{Cu}_{Cu(GDP)} = \frac{[[Cu(GDP)]^-]}{[Cu^{2+}][GDP^{3-}]} \quad (13a)^3)$$

$$= \frac{[[Cu(GDP)]^-_{op}] + [[Cu(GDP)]^-_{cl}]}{[Cu^{2+}][GDP^{3-}]} \quad (13b)^3)$$

$$K^{Cu}_{Cu(GDP)op} = [[Cu(GDP)]^-_{op}]/([Cu^{2+}][GDP^{3-}]) \quad (14)^3)$$

It is evident that any break-down of the values for $K^{Cu}_{Cu(GDP)}$, which has to reflect the contribution of the various terms necessary for further interpretation, requires that values for $K^{Cu}_{Cu(GDP)op}$, which cannot directly be measured, are obtainable. In contrast, $K^{Cu}_{Cu(GDP)}$ (*Eqns. 8* and *13*) is experimentally accessible. However, the existence of a linear relationship for families of structurally closely related ligands between $\log K^{M}_{M(L)}$ and $pK^{H}_{H(L)}$ is well-known [53] and exists also for $\log K^{M}_{M(R-DP)}$ *vs.* $pK^{H}_{H(R-DP)}$ plots [54], where $R-DP^{3-}$ represents a simple diphosphate monoester, that is, R may be any residue which does not affect complex formation. The parameters for the corresponding straight line for $[Cu(R-DP)]^-$ complexes have been determined [54]; they are given in *Eqn. 15:*

$$\log K^{M}_{M(L)} = m \cdot pK^{H}_{H(L)} + b \quad (15a)$$

$$\log K^{Cu}_{Cu(R-DP)} = 1.283 \cdot pK^{H}_{H(R-DP)} - 2.939 \quad (15b)$$

The five $R-DP^{3-}$ ligands used in this determination [54] are indicated in *Fig. 2*. Evidently, with a known $pK^{H}_{H(R-DP)}$ value, an expected stability constant for the corresponding $[Cu(R-DP)]^-$ complex can now be calculated. *Eqn 15b* 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 [54]. *Fig. 2* shows that the $[Cu(GDP)]^-$ complex in H_2O is by *ca.* 0.6 log units more stable than is expected on the basis of the basicity of the diphosphate residue. In fact, by application of *Eqn. 15b* and $pK^{H}_{H(GDP)}$, the stability enhancement, $\log \Delta_{Cu/GDP}$, for $[Cu(GDP)]^-$ can exactly be defined by *Eqn. 16*. The equality of the various terms in *Eqn. 16* is evident.

$$\log \Delta_{Cu/GDP} = \log K^{Cu}_{Cu(GDP)} - K^{Cu}_{Cu(R-DP)} \quad (16a)$$

$$= \log K^{Cu}_{Cu(GDP)} - \log K^{Cu}_{Cu(GDP)op} = \log \Delta \quad (16b)$$

Unfortunately, no reference lines for $Cu^{2+}/H^+/R-DP^{3-}$ systems in mixed solvents exist. 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 UTP^{4-}, formate ($HCOO^-$), and acetate ($MeCOO^-$) have been studied [55]. These in total eight rather different systems show the astonishing result that, in all instances, straight lines with slopes close to one are observed. This indicates that the solvent effect on proton binding and on metal-ion

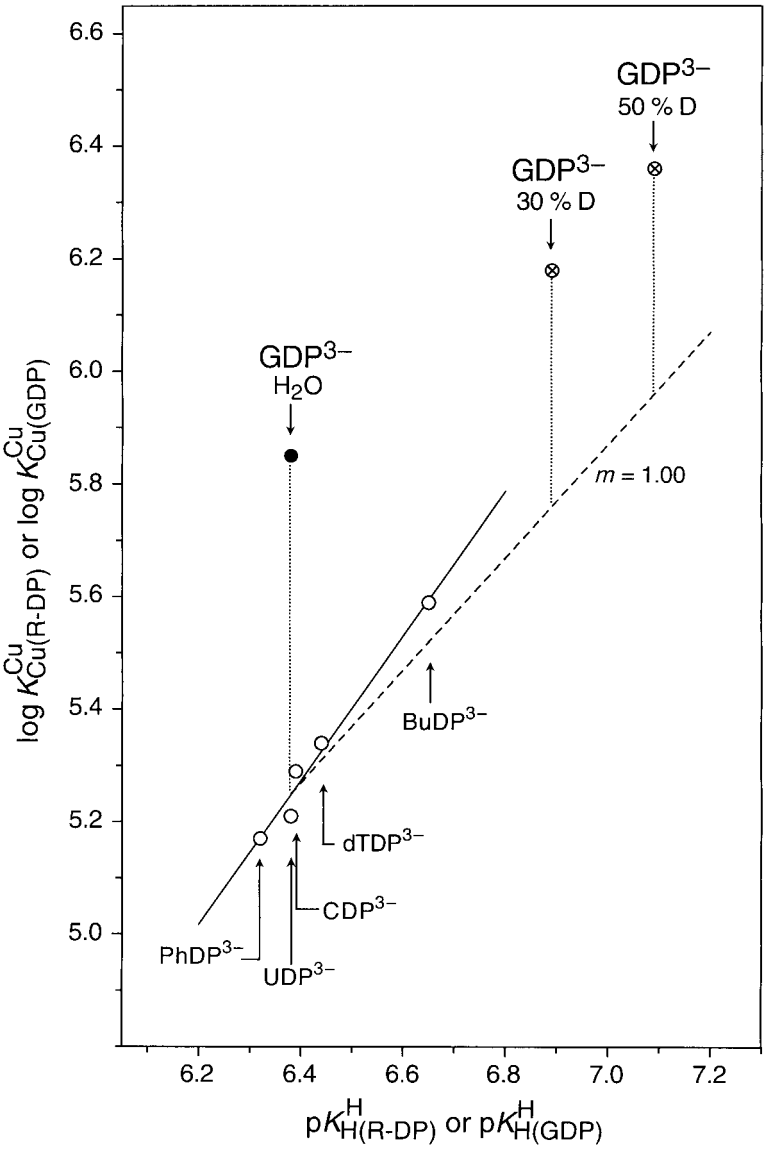


Fig. 2. *Evidence for an enhanced stability of the [Cu(GDP)]⁻ complex in H_2O* (●) *and in 30 or 50% (v/v) 1,4-dioxane(D)/H_2O* (⊗). Results based on the relationship between log $K^{Cu}_{Cu(R-DP)}$ and $pK^{H}_{H(R-DP)}$ for the simple $[Cu(R-DP)]^-$ complexes in H_2O (○), where $R-DP^{3-}$ = phenyl 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 butyl diphosphate ($BuDP^{3-}$) (from left to right) and the deduced straight reference line with $m = 1$ due to the mixed solvents. The parameters of the least-squares line (solid line) through the indicated five data sets are given in *Eqn. 15*, those for the mixed solvents (broken line) in *Eqn. 17* (see text in *Sect. 2.7*). The equilibrium constants for the $Cu^{2+}/H^+/R-DP^{3-}$ systems (○) are from [54] and the data points due to the $Cu^{2+}/H^+/GDP^{3-}$ systems (●, ⊗) are based on the values listed in *Tables 1* and *2*. The vertical dotted lines emphasize the stability difference from the reference lines; they equal log $\Delta_{Cu/GDP}$ as defined in *Eqn. 16* (see also *Table 3*, *Column 5*). All the plotted equilibrium constants refer to solutions at 25° and $I = 0.1$M ($NaNO_3$).

binding is approximately the same. In fact, the average slope (m) for the indicated eight systems is $m = 1.00 \pm 0.15$ (3σ); note, the error limit of the slope is deliberately given very generously, and one may thus expect that the slope for the $Cu^{2+}/H^{+}/R-DP^{3-}$ system is within these error limits.

Application of $pK^{H}_{H(GDP)} = 6.38$ (*Table 1*) to *Eqn. 15* gives for the $[Cu(GDP)]^{-}_{op}$ complex in H_2O $\log K^{Cu}_{Cu(GDP)op} = 5.25 \pm 0.04$. Use of this value together with the one for $pK^{H}_{H(GDP)}$ and $m = 1$ allows to calculate the intercept $b_{dioxane/water}$ of the straight reference line for dioxane/H_2O mixtures and $Cu^{2+}/H^{+}/R-DP^{3-}$ systems (see *Eqn. 17*).

$$\log K^{Cu}_{Cu(GDP)op/Diox} = (1.00 \pm 0.15) \cdot pK^{H}_{H(GDP)/Diox} - 1.13 \tag{17}$$

Evidently, for the acidity constant in H_2O, *i.e.*, $pK^{H}_{H(GDP)} = 6.38$, one obtains from *Eqn. 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 H_2O, in agreement with the calculations of *Eqn. 15*. Therefore, the broken reference line in *Fig. 2*, valid for the 1,4-dioxane/H_2O mixtures, has its starting point at $\log K^{Cu}_{Cu(R-DP)} = 5.25/pK^{H}_{H(GDP)} = 6.38$. The vertical dotted lines for 30 or 50% 1,4-dioxane/H_2O represent the stability enhancements according to *Eqn. 16* for the $[Cu(GDP)]^{-}$ complex in these two solvents. This establishes that the equilibrium of *Eqn. 1* is also of relevance under these conditions.

2.8. *Extent of Macrochelate Formation in Various Solvents for the [Cu(GDP)]$^{-}$ Complex*. With the results depicted in *Fig. 3* in mind, it is evident that values for the intramolecular equilibrium constant K_I (*Eqn. 11*) have to be the aim. In fact, combination of *Eqns. 11*, *13*, and *14* leads to *Eqn. 18*, which may be rearranged [52][53] to yield a further definition for K_I (*Eqn. 19*), in which the stability difference $\log \Delta$ is defined by *Eqn. 16*.

$$K^{Cu}_{Cu(GDP)} = K^{Cu}_{Cu(GDP)op} + K_I \cdot K^{Cu}_{Cu(GDP)op} \tag{18a}$$

$$= K^{Cu}_{Cu(GDP)op}\ (1 + K_I) \tag{18b}$$

$$K_I = \frac{K^{Cu}_{Cu(GDP)}}{K^{Cu}_{Cu(GDP)op}} - 1 = 10^{\log \Delta} - 1 \tag{19}$$

The equilibrium constant K_I can now be calculated through *Eqns. 16* and *19* as the values for $K^{Cu}_{Cu(GDP)}$ are known (*Table 2*, *Column 4*) and those for $K^{Cu}_{Cu(GDP)op}$ may be calculated with the acidity constants of $H(GDP)^{2-}$ in the various solvents (*Table 1*) and the corresponding straight reference lines defined in *Eqns. 15* (H_2O) and *17* (mixed solvents). It may be noted that the errors for the calculations carried out here with *Eqn. 17* (see *Table 3*) are based on the error limits given for the slope m.

As indicated before, the vertical distances emphasized by the dotted lines in *Fig. 2* are identical with the stability differences $\log \Delta_{Cu/GDP}$ as defined in *Eqn. 16*. Of course, the reliability of any calculation for K_I depends on the accuracy of the difference $\log \Delta_{Cu/GDP}$ which becomes the more important the more similar the two stability constants in *Eqn. 16* are. Therefore, only well-defined error limits allow quantitative evaluation of the extent of a possibly formed macrochelate. Of course, once K_I is

known, the percentage of the closed or macrochelated species occurring in the equilibrium of *Eqn. 1* follows from *Eqn. 20*.

$$\%[\mathrm{Cu(GDP)}]^{-}_{\mathrm{cl}} = 100 \cdot K_{\mathrm{I}} \,(1 + K_{\mathrm{I}}) \tag{20}$$

Application of this procedure [52][53] yields the results given in *Table 3*. The values in the final column show that macrochelate formation in aqueous solution is somewhat less-pronounced for $[\mathrm{Cu(ADP)}]^{-}_{\mathrm{cl}}$ than for $[\mathrm{Cu(GDP)}]^{-}_{\mathrm{cl}}$, in accordance with the expectation. Interestingly, the effect of the addition of 1,4-dioxane on the formation degree of the $[\mathrm{Cu(GDP)}]^{-}_{\mathrm{cl}}$ macrochelate is relatively small; the formation degree decreases only from *ca.* 75 to 60% going from H_2O to 50% 1,4-dioxane/H_2O (see *Table 3*, *Column 7*). However, it needs to be emphasized that, in contrast to the decrease in the formation degree of the macrochelate, the overall stability of the $[\mathrm{Cu(GDP)}]^{-}$ complex *increases* due to the mentioned change in solvent by *ca.* 0.5 log units (*Table 3*, *Column 3*).

Table 3. *Solvent Influence on the Extent of Macrochelate Formation According to the Equilibrium of* Eqn. 1 *for the [Cu(GDP)]⁻ Species as Quantified* via $\log \Delta_{Cu/GDP}$ (*Eqn. 16*) *by the Dimensionless and Intramolecular Equilibrium Constant* K_{I} (*Eqns. 11* and *19) and the Percentage of* $[\mathrm{Cu(GDP)}]^{-}_{\mathrm{cl}}$ (*Eqn. 20*) (25°; $I = 0.1$M, $NaNO_3$)[a]). The corresponding data for $[\mathrm{Cu(ADP)}]^{-}$ in H_2O [38] are given for comparison.

NDP^{3-}	1,4-Dioxane[b]) [% (*v*/*v*)]	$\log K^{Cu}_{Cu(NDP)}$[c])	$\log K^{Cu}_{Cu(NDP)op}$	$\log \Delta_{Cu/NDP}$	K_{I}	$\%[\mathrm{Cu(NPD)}]^{-}_{\mathrm{cl}}$
ADP^{3-}	0	5.61 ± 0.03	5.27 ± 0.04	0.34 ± 0.05	1.19 ± 0.25	54 ± 5
GDP^{3-}	0	5.85 ± 0.04	5.25 ± 0.04[d])	0.60 ± 0.06	2.98 ± 0.52	75 ± 3
GDP^{3-}	30	6.21 ± 0.15	5.76 ± 0.08[e])	0.45 ± 0.17	1.82 ± 1.10	65 ± 14
GDP^{3-}	50	6.36 ± 0.05	5.96 ± 0.11[e])	0.40 ± 0.12	1.51 ± 0.70	60 ± 11

[a]) For the error limits, see *Footnote a* in *Table 2*. [b]) For the mol fractions and dielectric constants of the 1,4-dioxane/H_2O mixtures, see *Table 1*. [c]) From *Column 4* of *Table 2*. [d]) Calculated with *Eqn. 15*. [e]) Calculated with *Eqn. 17*; the error limit is based on the error given for the slope *m* in *Eqn. 17*.

2.9. *Comparison of the Effect of a Decreasing Solvent Polarity on Macrochelate Formation of Several [Cu²⁺(Nucleotide)] Complexes.* In *Fig. 3* the percentages of the macrochelated species $[\mathrm{Cu(Nu)}]_{\mathrm{cl}}$, involving Cu^{2+} and the four 5′-nucleotides that have been studied [16][21] up to now, are plotted as a function of the percentage of 1,4-dioxane added to the aqueous solutions of the reagents. In all four instances, the overall stability of the complexes increases ([16][21] and *Table 2*) with increasing amounts of 1,4-dioxane, *i.e.*, a decreasing solvent polarity, as is expected for metal-ion complexes in which phosphate residues are the primary coordinating sites [18][55].

However, as far as macrochelation is concerned, the complexes behave very differently (see *Fig 3*): *i*) $[\mathrm{Cu(ATP)}]^{2-}_{\mathrm{cl}}$ decreases from nearly 70% in H_2O to *ca.* 25% in 50% 1,4-dioxane/H_2O. *ii*) $[\mathrm{Cu(AMP)}]_{\mathrm{cl}}$ passes through a minimum with a formation degree of *ca.* 10% in *ca.* 30% 1,4-dioxane/H_2O solutions and reaches in 50% 1,4-dioxane/H_2O again a formation degree of *ca.* 50%, which corresponds to that present in H_2O. This property of $[\mathrm{Cu(AMP)}]_{\mathrm{cl}}$ is not an isolated case, since the corresponding observation [56] regarding a minimum has also been made with the antiviral AMP^{2-} analogue, *i.e.*, the dianion of 9-[2-(phosphonomethoxy)ethyl]adenine ($PMEA^{2-}$) [57], which is used in the form of its bis(pivaloyloxymethyl) ester (*Adefovir dipivoxil*), as a

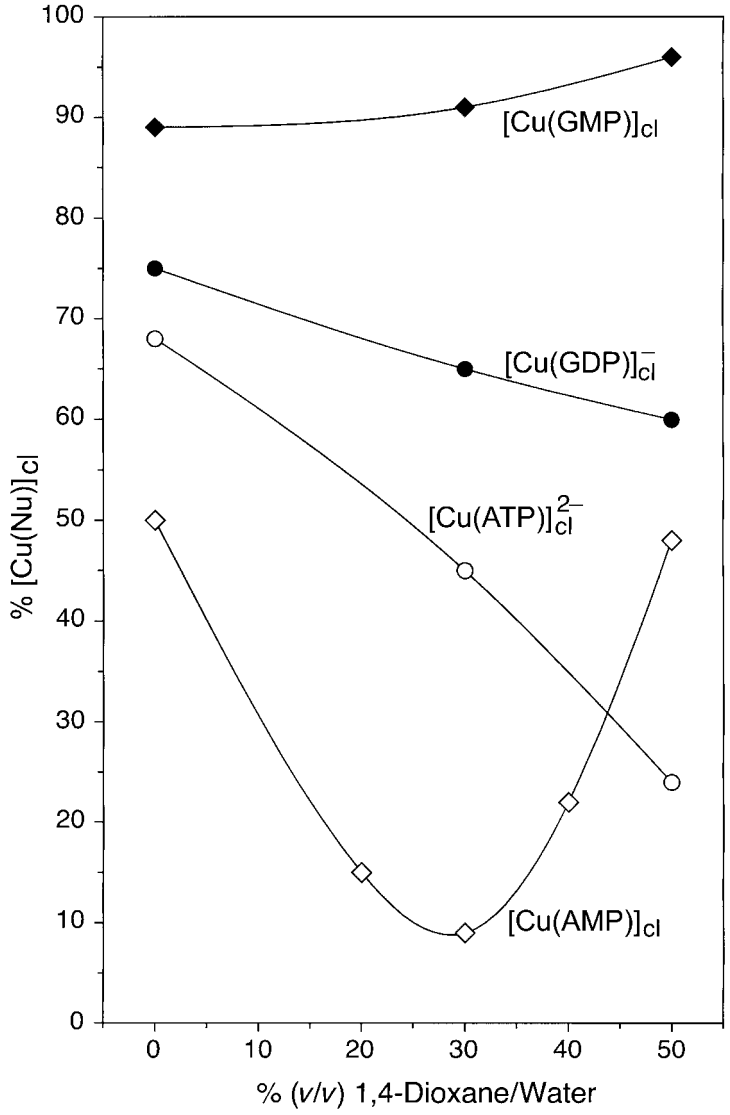


Fig. 3. *Formation degree of the macrochelates formed in the binary [Cu(AMP)]* [16][38], *[Cu(ATP)]*$^{2-}$ [21], *[Cu(GMP)]*^{2}), *and [Cu(GDP)]*$^{-}$ (see *Table 3*) *complex systems as a function of the percentage of 1,4-dioxane added to the aqueous reagent mixtures* (25°; $I = 0.1$M, $NaNO_3$)

drug for the treatment of chronic hepatitis B [58]. *iii*) Surprisingly, $[Cu(GDP)]^{-}_{cl}$ is only little affected by a decrease in solvent polarity, and, in the case of $Cu(GMP)_{cl}$, even a small enhancement of macrochelate formation is observed under these conditions.

These results are not easily explained: most likely, solvation of the adenine residue by the hydrophobic parts of 1,4-dioxane leads to an inhibition of the accessibility of

N(7) and, thus, to a decrease in macrochelate formation in $[Cu(ATP)]^{2-}$ and [Cu(AMP)] as well. However, the fact that, with $[Cu(AMP)]_{cl}$, the formation degree reaches a minimum and then increases again indicates that opposing solvent effects operate. It seems likely that, with decreasing H_2O activity, hydration of the not yet ligand-coordinated Cu^{2+} sites becomes increasingly difficult, and that this increases the affinity of Cu^{2+} for the N(7) site to such an extent that hindering 1,4-dioxane molecules are pushed away. If this picture is correct, then one should be able to observe also a minimum in the formation degree of $[Cu(ATP)]^{2-}_{cl}$ at larger 1,4-dioxane concentrations; unfortunately, the corresponding experiments cannot be made as the reactants become insoluble at higher dioxane concentrations.

Why is macrochelate formation of the Cu^{2+} complexes of the guanine nucleotides affected so little by increasing amounts of 1,4-dioxane? The guanine residue is clearly somewhat less hydrophobic than the adenine residue, and this probably means that its solvation by 1,4-dioxane is less intense and, therefore, screening of the N(7) site less significant. However, this can be only part of the explanation. The other reason is most likely the presence of the C(6)=O carbonyl group (see *Fig. 1*). There are indications that phosphate-coordinated metal ions like Cu^{2+} bind inner-sphere to N(7) and outer-sphere to C(6)=O [24]; if so, such a H-bond formed by a coordinated H_2O molecule to C(6)=O will be stabilized in an environment of reduced polarity!

The above considerations indicate further that the macrochelated $[Cu(Nu)]_{cl}$ species may actually not be a single one with a well-defined structure but that there are in fact several 'closed' isomers in equilibrium with each other [20][24][59]. In any case, the presented results establish that, by changing the polarity of the medium at a given site, *e.g.*, by a hydrophobic amino acid side chain, Nature has a tool to change the structure of complexes in solution. Such structural changes are certainly of relevance for enzyme/metal ion/substrate complexes.

3. Conclusions. – The effect that a change in the polarity of the solvent exerts on the acid–base properties of a compound can be rather dramatic: as seen for $H(GDP)^{2-}$ and some related acids, deprotonation of phosphate residues is inhibited by decreasing solvent polarity (see *Table 1*); for positively charged pyridinium-type N-sites, like the $H-N(7)^+$ unit of the guanine residue, just the opposite is true, *i.e.*, deprotonation is facilitated. Both effects are clearly linked to the decreasing solvation/hydration properties of aqueous solutions that contain increasing amounts of 1,4-dioxane. To say it differently, a low-polarity or -permittivity medium hinders charge separation, as is also evident from the properties of the H–N(1) site in GDP^{3-}; the pK_a values increase by going from H_2O to the 1,4-dioxane/H_2O solvent mixtures (*Table 1*).

The opposing solvent effects indicated above are clearly responsible for the observation that, in aqueous solution, one of the two protons of $H_2(GDP)^-$ is overwhelmingly located at the N(7) site, whereas the other one is bound to the terminal β-phosphate group of the diphosphate residue. Reduction of the solvent polarity by changing the dielectric constant from *ca.* 80 (H_2O) to 35 (50% 1,4-dioxane/H_2O) leads to a relocation of the protons in $H_2(GDP)^-$; now, the larger part of both protons is phosphate-bound. Clearly, Nature has here a tool to relocate protons, and thus, to affect reactivities, by moving a given site into a more-hydrophobic region or *vice versa* to a more hydrophilic one.

In the [Cu(H;GDP)] complex, the proton and the metal ion are both bound to the phosphate residue; in accord herewith as well as with the observations presented above, the release of the proton in [Cu(H;GDP)] is inhibited by decreasing solvent polarity, *i.e.*, with increasing amounts of 1,4-dioxane added to H_2O (see *Table 2*). At the same time, the stability of the $[Cu(GDP)]^-$ complex increases steadily with decreasing solvent polarity (*Table 2*). This observation is generally true for metal ions bound to phosphate or carboxylate groups.

As known from many previous studies [18][20][31][38][59], metal ions coordinated to the phosphate residue of purine nucleotides may form macrochelates with N(7) of the purine moiety as is reflected in increased complex stability. As we have seen, this is also true for $[Cu(GDP)]^-$ (see *Fig. 2* and *Table 3*). However, the astonishing observation is that the intramolecular equilibrium of *Eqn. 1* between the 'open' $[Cu(GDP)]^-_{op}$ isomer and the macrochelated $[Cu(GDP)]^-_{cl}$ species is only relatively little affected by the change in solvent (*Table 3*). This contrasts with the properties of the [Cu(AMP)] complex for which a minimum in the formation degree of the chelate in 30% 1,4-dioxane/H_2O is observed (see *Fig. 3*) [16]. Hence, depending on the complex considered, Nature may alter the structure of a substrate simply by moving it along a protein surface from a more-polar to a more-apolar region or *vice versa* [11]. It is most important to note in this context that the changes in free energy (ΔG^0) associated with such isomeric equilibria are very small; for example, the difference in ΔG^0 between a situation where all complex species are present in the 'open' form and the situation where 50% exist as (macro)chelates amounts at 25° only to -1.71 kJ/mol, which corresponds to a stability difference of $\log \Delta_{M/L} = 0.3$ [14].

The competent technical assistance of Mrs. *Astrid Sigel* in the preparation of this manuscript and helpful discussions with members of the *COST D20 Programme*, as well as research grants from the *Swiss National Science Foundation* and the *Swiss State Secretariat for Education and Research* (within *COST D20*) are gratefully acknowledged.

Experimental Part

General. The sodium salt of GDP was purchased from *Sigma Chemical Co.*, St. Louis, MO, USA, and 1,4-dioxane ('extra pure') was obtained from *Merck KGaA*, Darmstadt, Germany. All other materials used in the experiments, including CO_2-free H_2O, were from the same sources as previously [38][54]. The concentration of free, inorganic phosphate in GDP was determined [60] *via* molybdate reagent; it amounted to *ca.* 2% or less of GDP. The aq. stock solns. of the ligand were freshly prepared daily, and the pH was adjusted to *ca.* 8.0; the exact GDP concentration was newly determined each time by titrations with NaOH (see below).

Potentiometric pH Titrations. The pH titrations were carried out with the same equipment and in the same way as described in [54]; this also holds for the evaluation of the exper. data [38][54]. The titration apparatus was calibrated with aq. buffer solns. (pH 4.00, 7.00, and 9.00 [54]). The given acidity constants are the so-called practical, mixed, or *Brønsted* constants [39]; no corrections were applied for the change in solvent from H_2O to 1,4-dioxane/H_2O mixtures, though correction factors have been published for such [61] and related mixtures [62]. The stability constants presented are, as usual, concentration constants. It needs to be noted that always the differences in NaOH consumption between solns. with and without ligand [39] (see below) were evaluated.

The acidity constants $K^H_{H_2(GDP)}$ (*Eqn. 2*), $K^H_{H(GDP)}$ (*Eqn. 3*), and K^H_{GDP} (*Eqn. 4*) were determined by titrating 50 ml of aq. $3 \cdot 10^{-3}$ M HNO_3 (or 30 or 50% (*v*/*v*) 1,4-dioxane/H_2O) in the presence and absence of $6 \cdot 10^{-4}$ M GDP (25°; $I = 0.1$M, $NaNO_3$) under N_2 with 3 ml of 0.06M NaOH. For each pair of titrations, the data were evaluated for every 0.1 pH unit in the pH range 2.7–10.7. The final results for the acidity constants $K^H_{H_2(GDP)}$, $K^H_{H(GDP)}$, and K^H_{GDP} are the averages of at least eight independent pairs of titrations.

In the determination of the stability constants of the [Cu(H;GDP)] and $[Cu(GDP)]^-$ complexes (*Eqns. 7* and *8*), also the concentrations given above were applied. The Cu^{2+}/GDP ratio used in the experiments was 1:1.

The data were collected every 0.1 pH unit from the lowest pH reached in an experiment (usually pH 2.7) to the beginning of the hydrolysis of $[Cu(aq)]^{2+}$, which was evident from the titrations without ligand. The stability constants of the [Cu(H;GDP)] and $[Cu(GDP)]^{-}$ complexes are the results of four independent pairs of titrations.

REFERENCES

[1] D. C. Rees, *J. Mol. Biol*. **1980**, *141*, 323.
[2] G. R. Moore, *FEBS Lett.* **1983**, *161*, 171.
[3] N. K. Rogers, G. R. Moore, M. J. E. Sternberg, *J. Mol. Biol*. **1985**, *182*, 613.
[4] E. Demchuk, R. C. Wade, *J. Phys. Chem.* **1996**, *100*, 17373; G. Iversen, Y. I. Kharkats, J. Ulstrup, *Mol. Phys.* **1998**, *94*, 297.
[5] H. Sigel, R. B. Martin, R. Tribolet, U. K. Häring, R. Malini-Balakrishnan, *Eur. J. Biochem*. **1985**, *152*, 187; see also Footnote on p. 258 in M. Bastian, H. Sigel, *Inorg. Chim. Acta* **1990**, *178*, 249.
[6] J. G. Watterson, *Biochem. J.* **1987**, *248*, 615; L. I. Krishtalik, *J. Theor. Biol.* **1989**, *139*, 143; Y. Marcus, *J. Sol. Chem.* **1992**, *21*, 1217; T. Head-Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8308; J. Israelachvili, H. Wennerström, *Nature (London)* **1996**, *379*, 219.
[7] L. De Meis, *Biochim. Biophys. Acta* **1989**, *973*, 333.
[8] B. L. Vallee, R. J. P. Williams, *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *59*, 498; B. L. Vallee, R. J. P. Williams, *Chem. Brit.* **1968**, *4*, 397.
[9] T. Dudev, C. Lim, *J. Phys. Chem. B* **2000**, *104*, 3692.
[10] M. M. Yamashita, L. Wesson, G. Eisenman, D. Eisenberg, *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5648.
[11] H. Sigel, *Pure Appl. Chem.* **1989**, *61*, 923.
[12] G. Åkerlöf, O. A. Short, *J. Am. Chem. Soc.* **1936**, *58*, 1241; G. Åkerlöf, O. A. Short, *J. Am. Chem. Soc.* **1953**, *75*, 6357; F. E. Critchfield, J. A. Gibson Jr., J. L. Hall, *J. Am. Chem. Soc.* **1953**, *75*, 1991.
[13] H. Sigel, R. Malini-Balakrishnan, U. K. Häring, *J. Am. Chem. Soc.* **1985**, *107*, 5137.
[14] H. Sigel, L. E. Kapinos, *Coord. Chem. Rev.* **2000**, *200–202*, 563.
[15] C. P. Da Costa, B. Song, F. Gregáň, H. Sigel, *J. Chem. Soc., Dalton Trans.* **2000**, 899; G. Liang, D. Chen, M. Bastian, H. Sigel, *J. Am. Chem. Soc.* **1992**, *114*, 7780.
[16] G. Liang, H. Sigel, *Inorg. Chem*. **1990**, *29*, 3631.
[17] H. Sigel, R. Tribolet, *J. Inorg. Biochem.* **1990**, *40*, 163; G. Liang, H. Sigel, *Z. Naturforsch.*, *B* **1989**, *44*, 1555; G. Liang, R. Tribolet, H. Sigel, *Inorg. Chem.* **1988**, *27*, 2877.
[18] H. Sigel, B. Song, *Met. Ions Biol. Syst*. **1996**, *32*, 135.
[19] W. Wirth, J. Blotevogel-Baltronat, U. Kleinkes, W. S. Sheldrick, *Inorg. Chim. Acta* **2002**, *339*, 14.
[20] H. Sigel, *Pure Appl. Chem.* **2004**, *76*, 375.
[21] R. Tribolet, R. Malini-Balakrishnan, H. Sigel, *J. Chem. Soc., Dalton Trans.* **1985**, 2291.
[22] H. Sigel, N. A. Corfù, L.-n. Ji, R. B. Martin, *Comments Inorg. Chem.* **1992**, *13*, 35.
[23] B. Song, J. Zhao, R. Griesser, C. Meiser, H. Sigel, B. Lippert, *Chem.–Eur. J.* **1999**, *5*, 2374.
[24] H. Sigel, S. S. Massoud, N. A. Corfù, *J. Am. Chem. Soc*. **1994**, *116*, 2958.
[25] R. B. Martin, Y. H. Mariam, *Met. Ions Biol. Syst.* **1979**, *8*, 57; K. Aoki, *Met. Ions Biol. Syst.* **1996**, *32*, 91.
[26] R. Tribolet, H. Sigel, *Eur. J. Biochem.* **1987**, *163*, 353.
[27] G. Schatz, *FEBS Lett.* **2004**, *572*, 1.
[28] N. H. Keep, M. Barnes, I. Barsukov, R. Badii, L.-Y. Lian, A. W. Segal, P. C. E. Moody, G. C. K. Roberts, *Structure (London)* **1997**, *5*, 623; H. A. Lester, A. Karschin, *Annu. Rev. Neurosci.* **2000**, *23*, 89; J. Garzon, I. De Antonio, P. Sanchez-Blazquez, *Methods Enzymol. B* **2000**, *314*, 3.
[29] H. R. Kalbitzer, *Met. Ions Biol. Syst*. **1987**, *22*, 81.
[30] G. H. Reed, R. R. Poyner, *Met. Ions Biol. Syst.* **2000**, *37*, 183; J. D. Crowley, D. A. Traynor, D. C. Weatherburn, *Met. Ions Biol. Syst.* **2000**, *37*, 209.
[31] H. Sigel, E. M. Bianchi, N. A. Corfù, Y. Kinjo, R. Tribolet, R. B. Martin, *Chem.–Eur. J.* **2001**, *7*, 3729.
[32] N. Fatemi, B. Sarkar, *Inorg. Chim. Acta* **2002**, *339*, 179.
[33] 'Handbook on Metalloproteins', Eds. I. Bertini, A. Sigel, and H. Sigel, M. Dekker, Inc., New York, 2001, pp. 1.
[34] K. H. Scheller, H. Sigel, *J. Am. Chem. Soc.* **1983**, *105*, 5891.
[35] O. Yamauchi, A. Odani, H. Masuda, H. Sigel, *Met. Ions Biol. Syst*. **1996**, *32*, 207.
[36] R. Tribolet, H. Sigel, *Biophys. Chem.* **1987**, *27*, 119.

[37] H. Sigel, E. M. Bianchi, N. A. Corfù, Y. Kinjo, R. Tribolet, R. B. Martin, *J. Chem. Soc., Perkin Trans. 2* **2001**, 507.
[38] E. M. Bianchi, S. A. A. Sajadi, B. Song, H. Sigel, *Chem.–Eur. J.* **2003**, *9*, 881.
[39] H. Sigel, A. D. Zuberbühler, O. Yamauchi, *Anal. Chim. Acta* **1991**, *255*, 63.
[40] C. F. Moreno-Luque, E. Freisinger, B. Costisella, R. Griesser, J. Ochocki, B. Lippert, H. Sigel, *J. Chem. Soc., Perkin Trans. 2* **2001**, 2005.
[41] H. Sigel, *Chimia* **1967**, *21*, 489; H. Sigel, D. B. McCormick, *Acc. Chem. Res.* **1970**, *3*, 201.
[42] R. M. Bock, N. S. Ling, S. A. Morell, S. H. Lipton, *Arch. Biochem. Biophys.* **1956**, *62*, 253; R. M. Smith, A. E. Martell, Y. Chen, *Pure Appl. Chem.* **1991**, *63*, 1015; 'NIST Critically Selected Stability Constants of Metal Complexes', Reference Database 46, Version 6.0, compiled by R. M. Smith and A. E. Martell, US Department of Commerce, National Institute of Standards and Technology, Gaithersburg MD (USA), 2001.
[43] G. Kampf, L. E. Kapinos, R. Griesser, B. Lippert, H. Sigel, *J. Chem. Soc., Perkin Trans. 2* **2002**, 1320.
[44] M. Bastian, H. Sigel, *J. Coord. Chem.* **1991**, *23*, 137.
[45] R. B. Martin, *Met. Ions Biol. Syst.* **1979**, *9*, 1.
[46] B. Song, R. K. O. Sigel, H. Sigel, *Chem.–Eur. J.* **1997**, *3*, 29.
[47] M. J. Sánchez-Moreno, R. B. Gómez-Coca, A. Fernández-Botello, J. Ochocki, A. Kotynski, R. Griesser, H. Sigel, *Org. Biomol. Chem.* **2003**, *1*, 1819.
[48] A. Fernández-Botello, A. Holý, V. Moreno, H. Sigel, *Polyhedron* **2003**, *22*, 1067; A. Fernández-Botello, R. Griesser, A. Holý, V. Moreno, H. Sigel, in preparation.
[49] E. M. Bianchi, Ph.D. Thesis, University of Basel, 2003; E. M. Bianchi, H. Sigel, in preparation.
[50] H. Sigel, *Pure Appl. Chem.* **2004**, *76*, 1869; H. Sigel, B. Lippert, *Pure Appl. Chem.* **1998**, *70*, 845.
[51] R. Griesser, G. Kampf, L. E. Kapinos, S. Komeda, B. Lippert, J. Reedijk, H. Sigel, *Inorg. Chem.* **2003**, *42*, 32.
[52] H. Sigel, S. S. Massoud, R. Tribolet, *J. Am. Chem. Soc*. **1988**, *110*, 6857.
[53] R. B. Martin, H. Sigel, *Comments Inorg. Chem.* **1988**, *6*, 285.
[54] S. A. A. Sajadi, B. Song, F. Gregáň, H. Sigel, *Inorg. Chem.* **1999**, *38*, 439.
[55] G. Liang, N. A. Corfù, H. Sigel, *Z. Naturforsch., B* **1989**, *44*, 538.
[56] D. Chen, F. Gregáň, A. Holý, H. Sigel, *Inorg. Chem.* **1993**, *32*, 5377.
[57] H. Sigel, *Chem. Soc. Rev.* **2004**, *33*, 191; H. Sigel, *Pure Appl. Chem*. **1999**, *71*, 1727.
[58] R. B. Qaqish, K. A. Mattes, D. J. Ritchie, *Clin. Therapeutics* **2003**, *25*, 3084.
[59] H. Sigel, *Eur. J. Biochem.* **1987**, *165*, 65.
[60] H. Sigel, *Coord. Chem. Rev.* **1990**, *100*, 453; see p. 459.
[61] Y. K. Agrawal, *Talanta* **1973**, *20*, 1354; L. G. van Uitert, C. G. Haas, *J. Am. Chem. Soc.* **1953**, *75*, 451; L. G. van Uitert, W. C. Fernelius, *J. Am. Chem. Soc.* **1954**, *76*, 5887.
[62] R. G. Bates, 'Determination of pH: Theory and Practice', Wiley, Inc., New York, 1973, pp. 243.

Received October 22, 2004