Extent of Intramolecular Aromatic-Ring Stacking in Ternary Cu^{2+} Complexes Formed by 2,2′-Bipyridyl or 1,10-Phenanthroline and Flavin Mononucleotide (FMN^{2-})[1,2]

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The stability constants of the 1:1 complexes formed between $Cu(Arm)^{2+}$, where Arm = 2,2′-bipyridyl or 1,10-phenanthroline, and flavin mononucleotide (= FMN^{2-} = riboflavin 5′-phosphate) were determined by potentiometric pH titrations in aqueous solution at 25 °C and I = 0.1 M ($NaNO_3$). The experimental conditions were carefully selected such that only the monomeric complex species formed. On the basis of previously established log K versus pK_a straight-line plots (Chen, D.; et al. *J. Chem. Soc., Dalton Trans.* **1993**, 1537−1546) for the corresponding ternary complexes of simple phosphate monoesters and phosphonate derivatives, $R-PO_3^{2-}$, where R is a noncoordinating residue, it is shown that the stability of the ternary Cu(Bpy)(FMN) and Cu(Phen)(FMN) complexes is considerably higher than is expected on the basis of the basicity of the phosphate group of FMN^{2-}. By comparison with the stability of the ternary Cu(Arm)(G1P) complexes, where G1P = glycerol 1-phosphate, which had previously been studied (Liang, G.; et al. *J. Am. Chem. Soc.* **1992**, *114*, 7780−7785) and in which the coordination sphere of Cu^{2+} is identical with the one in Cu(Arm)(FMN), it can unequivocally be shown that the mentioned enhanced stability of the Cu(Arm)(FMN) complexes is solely due to the formation of intramolecular stacks; their formation degree reaches for Cu(Bpy)(FMN) and Cu(Phen)(FMN) about 80 and 90%, respectively. These, as well as recent results regarding the self-stacking of FMN^{2-} (Bastian, M.; Sigel, H. *Biophys. Chem.* in press) show that the flavin moiety is ideally suited for stacking and charge-transfer interactions, which are so important for the flavin coenzymes in nature.

Introduction

In many flavoenzymes charge-transfer or stacking interactions between the 7,8-dimethylisoalloxazine (i.e., the flavin) moiety and other aromatic residues of (the) protein(s) or the substrate-(s) occur.[5,6] Indeed, the flat structure[7] of the isoalloxazine unit is ideal for this kind of interaction. Furthermore, flavoenzymes, often being metal ion-dependent, catalyze redox reactions[5,8] via the 7,8-dimethylisoalloxazine (dmia) residue; in fact, they are often at the crossroads of such events[9] and therefore much studied.[5,10] With these facts in mind, we selected flavin mononucleotide (FMN^{2-}; Figure 1),[11] one of the flavo-coenzymes, which occurs in a large number of proteins,[8] for our investigations. After completing our studies on binary M(FMN) complexes,[12] we decided to focus on mixed ligand−

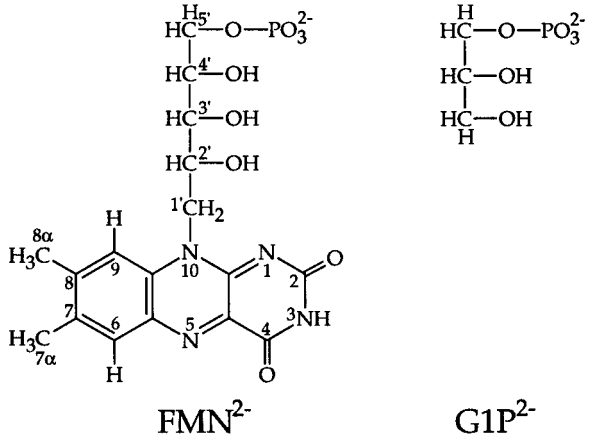

Figure 1. Chemical structures of flavin mononucleotide (=FMN^{2-} = riboflavin 5'-phosphate) and of glycerol 1-phosphate ($G1P^{2-}$; previously D,L-G1P was used[11] but this is without consequences for the present comparisons).

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(1) Abbreviatons: 5′-AMP^{2-}, adenosine 5′-monophosphate; Arm, heteroaromatic nitrogen base, e.g. Bpy or Phen; Bpy, 2,2′-bipyridyl; dmia, 7,8-dimethylisoalloxazine residue; FMN^{2-}, flavin mononucleotide (=riboflavin 5′-phosphate); $G1P^{2-}$, glycerol 1-phosphate; I, ionic strength of a solution; M^{2+}, general divalent metal ion also including in part $Cu(Arm)^{2+}$; Phen, 1,10-phenanthroline. Species which are given 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 versions applies is always clear from the context.

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Table 1. Logarithms of the Stability Constants of the Binary Cu(R−PO_3) (Eq 2)[11,12] and Ternary Cu(Arm)(R−PO_3) Complexes (Eq 3), where R−PO_3^{2-} = FMN^{2-} or $G1P^{2-}$ (see Figure 1), As Determined by Potentiometric pH Titrations in Aqueous Solution, Together with the Resulting Values for Δ log $K_{Cu/Arm/R-PO_3}$ (Eq 4) at 25 °C and I = 0.1 M ($NaNO_3$)[a,b]

M^{2+}	log $K^M_{M(FMN)}$	Δ log $K_{M/Arm/FMN}$	log $K^M_{M(G1P)}$	Δ log $K_{M/Arm/G1P}$
Cu^{2+}	3.07 ± 0.06		2.83 ± 0.05	
$Cu(Bpy)^{2+}$	3.56 ± 0.02	0.49 ± 0.06	2.90 ± 0.05	0.07 ± 0.07
$Cu(Phen)^{2+}$	3.89 ± 0.04	0.81 ± 0.07	2.92 ± 0.05	0.09 ± 0.07

[a] The acidity constants for $H(FMN)^-$ are p$K^H_{H(FMN)}$ = 6.18 ± 0.01 (eq 1) and pK^H_{FMN} = 10.08 ± 0.05 (this latter value is due to the release of the proton from the H(N3) site; see Figure 1),[12] and for $H(G1P)^-$ p$K^H_{H(G1P)}$ = 6.23 ± 0.01; the latter value, as well as the stability constants of the $G1P^{2-}$ complexes, is from ref 11. [b] 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 the derived data, in the present case for Δ log $K_{Cu/Arm/R-PO_3}$, were calculated according to the error propagation after Gauss.

metal ion complexes consisting of FMN^{2-} and a heteroaromatic nitrogen base, i.e., 2,2′-bipyridyl (Bpy) or 1,10-phenanthroline (Phen), to see if intramolecular stacking could be observed.

As metal ion we selected Cu^{2+} because it is of biological relevance,[13] and, also important in the present context, it coordinates well with Bpy or Phen[14] as well as with phosphate groups.[3,15] Regarding the formation of a stable bridge between the two mentioned kinds of ligands, this is important because only then intramolecular stacking can be expected to occur.[16] A further reason why the use of Cu^{2+} as bridging metal ion in the present study was attractive is the recent observation[17] that the degradation of DNA by riboflavin is enhanced by the presence of Cu^{2+}.

An intramolecular stacking interaction of the indicated kind in a mixed ligand complex must lead to an enhanced complex stability.[16,18] To be certain about such an event, we used for comparison the corresponding mixed ligand complexes of glycerol 1-phosphate ($G1P^{2-}$), which we had previously studied.[11] $G1P^{2-}$ contains the same basic structure in the vicinity of the phosphate group like FMN^{2-}, but no aromatic residue (see Figure 1), and thus it is ideal for the indicated purpose. Indeed, the results show that aromatic-ring stacking contributes significantly to the stability of the Cu(Arm)(FMN) complexes, whereas that of the Cu(Arm)(G1P) species is solely governed by the affinity of the phosphate group to the binary $Cu(Arm)^{2+}$ complexes.

Experimental Section

Materials. The monosodium salt of riboflavin 5′-phosphate (FMN; pure) was obtained from Serva Feinbiochemica GmbH, Heidelberg, Germany. The disodium salt of 1,2-diaminoethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (Na_2H_2Edta) (for its use see ref 12), 2,2′-bipyridyl, 1,10-phenanthroline, potassium hydrogen phthalate, HNO_3, NaOH (Titrisol), and the nitrate salts of Na^+ and Cu^{2+} (all pro analysi) were from Merck AG, Darmstadt, Germany. The buffers used for pH calibration (pH 4.64, 7.00, and 9.00; based on the NBS scale, now NIST) were from Metrohm AG, Herisau, Switzerland.

The titer of the NaOH used for the titrations was determined with potassium hydrogen phthalate. All solutions were prepared using distilled, CO_2-free water, and the exact concentrations of all stock solutions including the one with FMN, which was freshly prepared daily, were determined as described.[12]

Potentiometric pH Titrations. The pH titrations were carried out exactly as described, and also the same apparatus was used (25 °C; I = 0.1 M, $NaNO_3$).[12]

The conditions were identical with those employed previously[12] for the determination of the acidity constants, $K^H_{H(FMN)}$ and K^H_{FMN}, for $H(FMN)^-$: i.e., 50 mL of aqueous 0.54 mM HNO_3 and $NaNO_3$ (I = 0.1 M) in the presence and absence of 0.3 mM FMN were titrated with 1 mL of 0.03 M NaOH.[12] Part of the $NaNO_3$ was now replaced in the solutions by $Cu(NO_3)_2$ and the heteroaromatic nitrogen base, keeping I at 0.1 M. The [FMN]:[Cu^{2+}/Arm] ratios were 1:11 and 1:5.6.

In the pH range (about 3.5−5.5) used for the calculations of the stability constants of the mixed ligand complexes, complex formation between Cu^{2+} and Bpy or Phen is already complete due to the high stability of the corresponding binary complexes;[14] this was evident from the identity of the titration curves obtained from a pair of solutions, one which only contained HNO_3 and the other with Cu^{2+}/Arm in addition. Of course, in the upper pH range such a pair of titrations begins to differ due to the formation of hydroxo complexes of $Cu(Arm)^{2+}$; at the corresponding pH, the collection of data for the calculations was stopped. Hence, in the calculations only complex formation between $Cu(Arm)^{2+}$ and FMN^{2-} had to be considered; i.e., each of the systems could be treated as a binary one. Consequently, the stability constants, $K^{Cu(Arm)}_{Cu(Arm)(FMN)}$, were computed[12] for each pair of titrations by taking into account the species H^+, $H(FMN)^-$, FMN^{2-}, $Cu(Arm)^{2+}$, and Cu(Arm)(FMN). Throughout the data were collected every 0.1 pH unit from about 5% complex formation to the beginning of hydrolysis of $Cu(Arm)(aq)^{2+}$. The values calculated individually for log $K^{Cu(Arm)}_{Cu(Arm)(FMN)}$ showed no dependence on pH or on the excess amount of $Cu(Arm)^{2+}$.

For the Cu^{2+}/Bpy and Cu^{2+}/Phen systems 9 and 13 independent pairs of titrations were made, respectively, which were independently carried out with different equipment (but of the same kind) by at least two persons. The individual results were averaged for the final results given in section 2 (Table 1, vide infra).

Results and Discussion

1. Definition of the Conditions and Equilibria To Be Considered. From our recent studies[19] regarding the self-stacking properties of FMN^{2-} it is evident that the experimental conditions for the quantification of the stabilities of ternary Cu(Arm)(FMN) complexes need to be selected carefully, to guarantee that the properties of the monomeric species really are studied. In the experiments described below [FMN] = 0.3 mM was used; this means, on the basis[19] of K = 27 ± 15 M^{-1}, one calculates that more than 98% of the total FMN^{2-} species are present in the monomeric form. If one assumes the "extreme" association constant, K = 100 M^{-1} (for details see ref 19), the calculation shows that even then still about 95% of FMN^{2-} is present as monomers. This then guarantees that the results presented below indeed refer to monomeric FMN species.

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Finally, it may be added that the self-association tendency of positively charged $M(Arm)^{2+}$ species is very small.[20]

The potentiometric pH titrations carried out in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$) with FMN in the presence of Cu^{2+} can be completely evaluated by taking into account[12] equilibria 1a and 2a. The analogous situation applies for the

$$H(FMN)^- \rightleftharpoons FMN^{2-} + H^+ \quad (1a)$$

$$K^{H}_{H(FMN)} = [FMN^{2-}][H^+]/[H(FMN)^-] \quad (1b)$$

$$Cu^{2+} + FMN^{2-} \rightleftharpoons Cu(FMN) \quad (2a)$$

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

ternary systems consisting of Cu^{2+}, Arm, and FMN, because the formation of the binary $Cu(Arm)^{2+}$ complexes is already complete at the onset of the formation of the ternary complexes (see Experimental Section). This means that as long as the evaluation of the experimental data is not carried into the pH range where formation of hydroxo complexes occurs, aside from eq 1, only equilibrium 3a needs to be considered.

$$Cu(Arm)^{2+} + FMN^{2-} \rightleftharpoons Cu(Arm)(FMN) \quad (3a)$$

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

2. Evidence for an Enhanced Stability of the Ternary Cu(Arm)(FMN) Complexes. The results regarding FMN^{2-} and eqs 2 and 3 are listed in column 2 of Table 1. Column 4 contains the corresponding results[11] for glycerol 1-phosphate ($G1P^{2-}$; Figure 1). As the acidity constants $pK^{H}_{H(FMN)} = 6.18$ for $H(FMN)^-$ (cf. ref 12) and $pK^{H}_{H(G1P)} = 6.23$ for $H(G1P)^-$ (cf. ref 11) are quite similar, the stability constants of the corresponding complexes may in a first approximation directly be compared: From the results in Table 1 it is thus immediately obvious that the stability of the Cu(Bpy)(FMN) and Cu(Phen)(FMN) complexes is special.

The relative stability of mixed ligand or ternary complexes toward their binary parent complexes is best quantified[3,4,21] by considering the stability difference defined in eq 4. In case a

$$\Delta \log K_{Cu} = \log K^{Cu(Arm)}_{Cu(Arm)(R-PO_3)} - \log K^{Cu}_{Cu(R-PO_3)} \quad (4)$$

further identification of $\Delta \log K_{Cu}$ for a certain equilibrium is needed, this will be given by additional subscripts like $\Delta \log K_{Cu/Arm/R-PO_3}$. Of course, the difference of the logarithms of two stability constants as in eq 4 is again a constant; it quantifies the position of equilibrium 5. The corresponding dimensionless

$$Cu(Arm)^{2+} + Cu(R-PO_3) \rightleftharpoons Cu(Arm)(R-PO_3) + Cu^{2+} \quad (5)$$

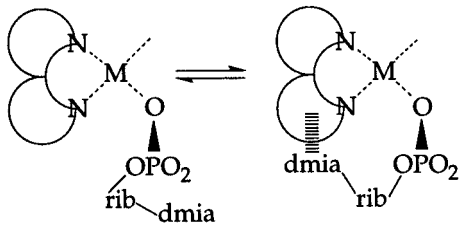
equilibrium constant is then defined by eq 6. According to the

$$10^{\Delta \log K_{Cu}} = \frac{([Cu(Arm)(R-PO_3)][Cu^{2+}]}{[Cu(Arm)^{2+}][Cu(R-PO_3)]} \quad (6)$$

general rule for complex stabilities, $K_1 > K_2$, one expects[21] that equilibrium 5 is on its left side with negative values for $\Delta \log K_{Cu}$ (eq 4), which is in agreement with statistical considerations: For the coordination of a monodentate ligand to the Jahn−Teller distorted Cu^{2+}, at which two binding sites are already occupied, one estimates[22] $\Delta \log K_{Cu/statist} \simeq -0.5$ (cf. also ref 21a).

It is evident that the results listed in column 5 of Table 1 for $\Delta \log K_{Cu/Arm/G1P}$ are close to zero or slightly positive; i.e., the above equilibrium 5 is about midway or slightly shifted to its right side in contrast to the expected behavior according to the statistics. However, this increased stability observed for the Cu(Arm)(G1P) complexes corresponds to previous experiences; i.e., it is expected for mixed ligand complexes formed by a divalent transition metal ion and a heteroaromatic N base and an O donor ligand.[21−23] The synergism operating here is clearly important for biological systems.[23]

However, the results of column 3 in Table 1 are truly surprising: The values for $\Delta \log K_{Cu/Arm/FMN}$ are on the order of 0.5−0.8; i.e., the corresponding dimensionless equilibrium constants (eq 6) are about 3−6 and consequently, equilibrium 5 is considerably displaced toward its right side. From Figure 1 it is evident that the decisive difference between the ligands $G1P^{2-}$ and FMN^{2-} is only the flavin moiety; as metal ions like Cu^{2+} do not directly interact with the 7,8-dimethylisoalloxazine residue of FMN^{2-},[12] the described observation is a first clear hint for a stacking interaction between the coordinated Arm and the flavin ring system in the ternary Cu(Arm)(FMN) complexes. Consequently, the position of the intramolecular equilibrium 7, in which dmia represents the 7,8-dimethylisoalloxazine residue of FMN^{2-}, needs to be considered.



(7)

3. Comparison of the Measured with the Expected Stabilities for the Ternary Cu(Arm)(FMN) Complexes. The stability of the ternary Cu(Arm)(FMN) and Cu(Arm)(G1P) complexes may be evaluated further by making use of the previously established[24] (cf. also ref 3) straight-line correlations for $\log K^{Cu(Arm)}_{Cu(Arm)(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ plots. For comparison, the straight-line equation for the binary $Cu(R-PO_3)$ complexes[25,26] is also given:

$$\log K^{Cu}_{Cu(R-PO_3)} = 0.465pK^{H}_{H(R-PO_3)} - 0.015 \quad (8)$$

$$\log K^{Cu(Bpy)}_{Cu(Bpy)(R-PO_3)} = 0.465pK^{H}_{H(R-PO_3)} + 0.009 \quad (9)$$

$$\log K^{Cu(Phen)}_{Cu(Phen)(R-PO_3)} = 0.465pK^{H}_{H(R-PO_3)} + 0.018 \quad (10)$$

The error limits of log stability constants calculated with given $pK^{H}_{H(R-PO_3)}$ values and eq 8, 9, or 10 are ±0.06, ±0.07, and

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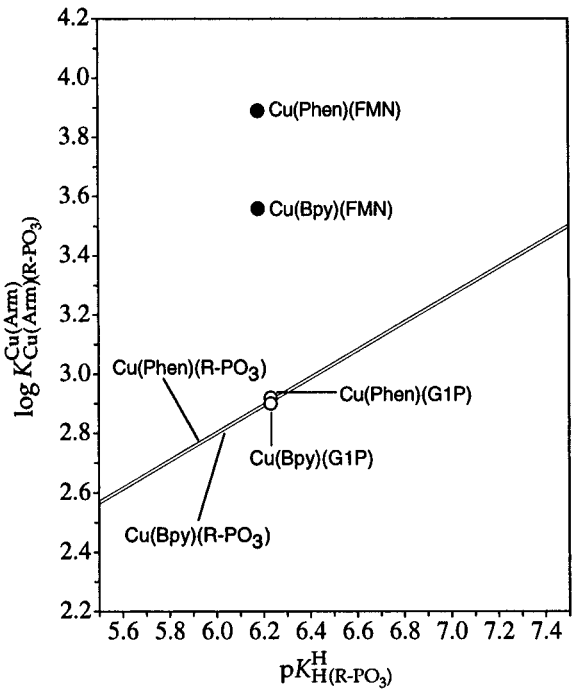

Figure 2. Evidence that the stability of the Cu(Arm)(G1P) complexes (○) is solely determined by the phosphate−metal ion coordination, whereas the Cu(Bpy)(FMN) and Cu(Phen)(FMN) complexes (●) own an additional stability, which is attributed to intramolecular aromatic-ring stacking, based on the relationship between log $K^{Cu(Arm)}_{Cu(Arm)(R-PO_3)}$ and $pK^{H}_{H(R-PO_3)}$ for the ternary Cu(Bpy)(G1P) and Cu(Phen)(G1P) (○), as well as for the Cu(Bpy)(FMN) and Cu(Phen)(FMN) (●) complexes in aqueous solution at $I = 0.1$ M ($NaNO_3$) and 25 °C. The plotted data are from Table 1. The two reference lines represent the log K versus pK_a relationship for Cu(Arm)(R−PO_3) complexes (eqs 9, 10); it should be emphasized that R−PO_3^{2-} symbolizes here phosphate monoesters (or phosphonates) with an R group unable to undergo any kind of hydrophobic, stacking, or other type of interaction.

±0.06 (3σ) log units, respectively, in the pK_a range 5−8 (see Tables 5, 6 in ref 25 and Table 5 in ref 24).

The reference lines as defined by eqs 9 and 10 are seen in Figure 2, where also the stability constants log $K^{Cu(Arm)}_{Cu(Arm)(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ are plotted for the two ligands considered here, i.e., R−PO_3^{2-} = FMN^{2-} and $G1P^{2-}$ (Figure 1). It is evident that the data points for Cu(Bpy)(G1P) and Cu(Phen)-(G1P) fit exactly on the corresponding reference lines. This is very different for the two Cu(Arm)(FMN) complexes; their data points are far above the reference lines, proving an increased complex stability, and this must mean[16,18] that aside from the phosphate−Cu^{2+} coordination a further interaction within the ternary complexes occurs.

The vertical differences just discussed, i.e., between the mentioned data points and their reference lines (cf. Figure 2), can be defined according to eq 11, in which the first term on

$$\log \Delta_{Cu/Arm/R-PO_3} = \log K^{Cu(Arm)}_{Cu(Arm)(R-PO_3)} - \log K^{Cu(Arm)}_{Cu(Arm)(R-PO_3)calc} \quad (11a)$$

$$= \log K^{Cu(Arm)}_{Cu(Arm)(R-PO_3)} - \log K^{Cu(Arm)}_{Cu(Arm)(R-PO_3)op} \quad (11b)$$

the right hand side is the experimentally determined stability constant (analogous to eq 3), whereas a value for log $K^{Cu(Arm)}_{Cu(Arm)(R-PO_3)calc}$ (eq 11a) can be calculated for any R−PO_3^{2-} ligand with the acidity constant, $pK^{H}_{H(R-PO_3)}$, and the straight line eqs 9 and 10. Of course, this latter value quantifies the stability of the ternary complex in which $Cu(Arm)^{2+}$ is only interacting with the phosphate residue of FMN^{2-}; this species is also schematically shown on the left side of equilibrium 7; often complexes of this kind are designated as "open" species, i.e., Cu(Arm)$(FMN)_{op}$, and consequently, eq 11a may be rewritten as given in eq 11b. The values for the terms appearing in eq 11 are listed in Table 2, including those for the binary Cu(FMN) (cf. ref 12) as well as the binary and ternary $G1P^{2-}$ complexes.[11]

Table 2. Stability Constant Comparisons for the Cu(R−PO_3) (Analogous to Eq 2) and Cu(Arm)(R−PO_3) Complexes (Analogous to Eq 3), where R−PO_3^{2-} = FMN^{2-} or $G1P^{2-}$, between the Measured Stability Constants (exptl) from Table 1 and the Calculated Stability Constants for a Pure and Unaltered Phosphate−Metal Ion Coordination (calcd) Based on the Basicity of the −PO_3^{2-} Groups[a] in FMN^{2-} or $G1P^{2-}$ and the Straight-Line Equations Given in Eqs 8−10 (for Aqueous Solutions at 25 °C and $I = 0.1$ M)[b]

		log $K^{M}_{M(R-PO_3)}$		
R−PO_3^{2-}	M^{2+}	exptl	calcd	log $\Delta_{M/R-PO_3}$[c]
FMN^{2-}	Cu^{2+}	3.07 ± 0.06	2.86 ± 0.06	0.21 ± 0.08
	$Cu(Bpy)^{2+}$	3.56 ± 0.02	2.88 ± 0.07	0.68 ± 0.07
	$Cu(Phen)^{2+}$	3.89 ± 0.04	2.89 ± 0.06	1.00 ± 0.07
$G1P^{2-}$	Cu^{2+}	2.83 ± 0.05	2.88 ± 0.06	−0.05 ± 0.08
	$Cu(Bpy)^{2+}$	2.90 ± 0.05	2.91 ± 0.07	−0.01 ± 0.09
	$Cu(Phen)^{2+}$	2.92 ± 0.05	2.91 ± 0.06	0.01 ± 0.08

[a] The corresponding acidity constants are given in footnote *a* of Table 1. [b] Regarding the error limits see footnote *b* of Table 1. [c] See eq 11.

The results given in the lower part of column 5 in Table 2 confirm the results of Figure 2; i.e., the values for log $\Delta_{Cu/G1P}$ and log $\Delta_{Cu/Arm/G1P}$ are zero within their error limits. This proves that $G1P^{2-}$ coordinates in all three complexes only via its phosphate group; in other words, there is no indication under the given experimental conditions for the formation of seven-membered chelates involving the neighboring hydroxy group (cf. Figure 1).[11] This conclusion is very important because it excludes the formation of such chelates also for the complexes of FMN^{2-} as this ligand contains the identical structural unit (cf. Figure 1).

Therefore, the slight stability increase of about 0.2 log unit observed for the binary Cu(FMN) complex (Table 2, column 5) also has to be attributed to the presence of the flavin ring; previously it was concluded that here a "dielectric constant" alteration is operating. As this effect regarding binary M(FMN) complexes was discussed in detail in ref 12, it shall not be considered further in the present context. However, as far as the values for log $\Delta_{Cu/Arm/FMN}$ are concerned, which are on the order of about 0.7−1 log unit (Table 2), these clearly reflect the intramolecular ligand−ligand interaction as follows from the comparison with the corresponding values for the Cu(Arm)-(G1P) complexes.

At this point it may be helpful to recall that in Cu(Arm)-$(FMN)_{op}$ FMN^{2-} actually represents a R−PO_3^{2-} ligand, where R is a noninteracting group (like the glycerol residue in $G1P^{2-}$), as is also seen in the open form in equilibrium 7. In other words, Cu(Arm)$(FMN)_{op}$ may also be written as Cu(Arm)(R−PO_3). Furthermore, $10^{\log \Delta_{Cu}}$, as defined by eq 11, is actually the ratio of two equilibrium constants; consequently, $10^{\log \Delta_{Cu}}$ itself must be a constant which defines the position of an equilibrium;[16b] indeed, it is the following one:

$$FMN^{2-} + Cu(Arm)(R-PO_3) \rightleftharpoons Cu(Arm)(FMN) + R-PO_3^{2-} \quad (12)$$

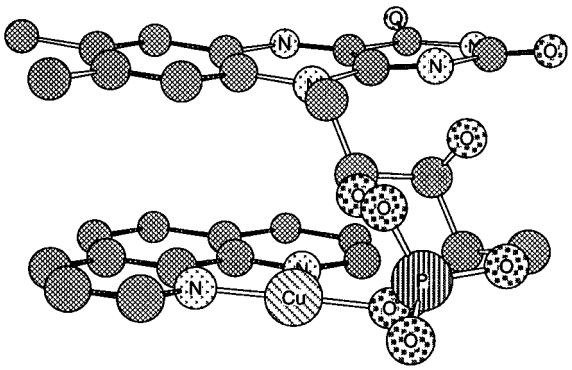

Figure 3. Simplified structure of a stacked species of the Cu(Phen)(FMN) complex in which the intramolecular stack is formed between the aromatic rings of 1,10-phenanthroline and the flat flavin residue. The structure was drawn with the program Chem3D Plus (Version 3.1.1) from Cambridge Scientific Computing Inc. It may be noted in this connection that in solution certainly a whole series of stacked complexes occur in which the orientation of the aromatic-ring planes toward each other differs somewhat; of course, the expression $Cu(Arm)(FMN)_{st}$ and the quantifications given for it encompass all of these species.

It is evident that the coordination spheres of the Cu^{2+} ions on both sides of equilibrium 12 are identical; consequently, the values for log $\Delta_{Cu/Arm/FMN}$ (eq 11) are a true reflection of the extent of the intramolecular stack formation in the Cu(Arm)(FMN) species. Of course, log $\Delta_{Cu/Arm/FMN} > 0$ (i.e., $10^{\log\Delta_{Cu}} > 1$) means that equilibrium 12 is shifted toward its right side; clearly, from this it follows further that in these cases mixed ligand complexes with an intramolecular stack exist.

A second view of Table 2 confirms, in connection with the above discussion on equilibrium 12, again the stack formation between the heteroaromatic amine and the flavin rings: for Cu(Phen)(FMN) a larger stability increase is expected and actually observed, if compared with that due to Cu(Bpy)(FMN) (see the upper part of column 5 in Table 2); this result clearly has its origin in the bigger size of the Phen ligand which allows a more pronounced overlap with the flavin residue than does Bpy. A tentative structure of the stacked Cu(Phen)(FMN) complex is depicted in Figure 3.

4. Extent of Intramolecular Stack Formation in Cu(Arm)(FMN) Species. If the two isomers shown in equilibrium 7 are designated as $Cu(Arm)(FMN)_{op}$ and $Cu(Arm)(FMN)_{st}$ (st = stacked), the dimensionless constant of this equilibrium is defined by equation 13. Values for K_I may be calculated (for

$$K_I = [Cu(Arm)(FMN)_{st}]/[Cu(Arm)(FMN)_{op}] \quad (13)$$

details see refs 16 and 27) with eqs 14 or 15.

$$K_I = \frac{K^{Cu(Arm)}_{Cu(Arm)(FMN)}}{K^{Cu(Arm)}_{Cu(Arm)(FMN)_{op}}} - 1 \quad (14)$$

$$K_I = 10^{\log\Delta_{Cu/Arm/R-PO_3}} - 1 \quad (15)$$

By taking into account eq 11, which defines the enhanced complex stability, eq 14 may be rewritten as eq 15. Both eqs 14 and 15 are applicable provided the stability of the open form in equilibrium 7 can be quantified. This is the case as we have seen in section 3, and the corresponding values are listed in Table 2. Hence, K_I (eqs 14, 15) can be calculated, and knowledge of this value then also allows one to obtain the percentage of the folded or stacked form of equilibrium 7 according to eq 16.

$$\%\ Cu(Arm)(FMN)_{st} = 100K_I/(1 + K_I) \quad (16)$$

Table 3. Extent of Intramolecular Stack Formation (Eq 7) in the Ternary Cu(Arm)(FMN) Complexes As Quantified by the Dimensionless Equilibrium Constant K_I (Eqs 13−15) and the Percentage of the Stacked Species, $Cu(Arm)(FMN)_{st}$ (Eq 16) (25 °C; $I = 0.1$ M, $NaNO_3$)[a]

$Cu(Arm)^{2+}$	log $\Delta_{Cu/Arm/FMN}$[b]	K_I	% $Cu(Arm)(FMN)_{st}$
$Cu(Bpy)^{2+}$	0.68 ± 0.07	3.79 ± 0.77	79 ± 3
$Cu(Phen)^{2+}$	1.00 ± 0.07	9.00 ± 1.61	90 ± 2

[a] Regarding the error limits see footnote *b* of Table 1. [b] From column 5 of Table 2; see eq 11.

The results of the calculations based on eqs 14−16 are summarized in Table 3. Of course, no entries for the Cu(Arm)(G1P) complexes appear in Table 3, as their log $\Delta_{Cu/Arm/G1P}$ values are zero within the error limits (see Table 2). However, the entries for the Cu(Arm)(FMN) complexes prove that the intramolecular equilibrium 7 is far on its right side. This means, the $Cu(Bpy)(FMN)_{st}$ and $Cu(Phen)(FMN)_{st}$ species occur with a formation degree of about 80 and 90%, respectively. It is interesting to note that these values are within the error limits identical with those determined for $Cu(Bpy)(5'\text{-AMP})_{st}$ (81 ± 4%) and $Cu(Phen)(5'\text{-AMP})_{st}$ (90 ± 2%).[16b] Moreover, for the antiviral adenosine monophosphate analogue, 9-(2-phosphonomethoxyethyl)adenine (=$PMEA^{2-}$), and its corresponding mixed ligand complexes also very similar observations have been made.[24,26]

Conclusions

The previous results of the self-association study[19] of FMN^{2-} as well as the present ones regarding the extent of intramolecular stack formation in the Cu(Bpy)(FMN) and Cu(Phen)(FMN) complexes prove that the 7,8-dimethylisoalloxazine residue is well-suited for stacking and charge-transfer interactions. In this respect as well as in its metal ion binding properties in general,[12] FMN^{2-} has much in common with nucleotides.[28]

The contribution of the stacked species to the free energy change for complex formation is given by $\Delta G° = -RT\ln(1 + K_I)$,[18] where K_I quantifies the intramolecular equilibrium 7 between the open and the stacked complexes (eq 13). This then means for 25 °C $\Delta G° = -5.71 \log \Delta_{Cu/Arm/FMN}$; in this latter expression log $\Delta_{Cu/Arm/FMN}$ quantifies the stability increase due to the intramolecular stack formation (eq 11). Hence, the stacking interaction contributes in Cu(Bpy)(FMN) and Cu(Phen)(FMN) −3.9 and −5.7 kJ/mol, respectively, to the stability of these ternary complexes. Furthermore, there is now growing evidence that stacking interactions are enthalpically driven,[29−31] though the nature of the attracting forces[32] between the heterocycles within a stack is still discussed. In any case, interactions of the described kind are strong enough to guarantee selectivity in a biological system,[29] yet at the same time they are weak enough to prevent that by such an interaction the system is falling into an energetically unfavorable sink; in other words, such interactions are ideal for enzymic turnover reactions.

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