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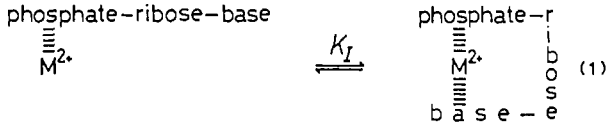
Solvent-Dependent Metal Ion–Nucleic Base Recognition. Extent of Macrochelate Formation in the Binary Copper(II) Complexes of Adenosine 5′-Monophosphate (AMP) and Adenosine 5′-Triphosphate (ATP) in Water–Dioxane Mixtures

It is now well established that the "effective" or "equivalent solution" dielectric constants in proteins[1,2] or active-site cavities of enzymes[3] are reduced compared to that in bulk water; i.e., the activity of water is decreased[4] due to the presence of aliphatic and aromatic amino acid side chains at the protein–water interface. Estimates for the dielectric constants in such locations range from ~30 to 70;[1-3] hence, by employing aqueous solutions that contain ~10–50% 1,4-dioxane,[5] one may expect to simulate to some degree the situation in active-site cavities.[6]

After study of the stability of complexes of simple phosphate monoester ligands in aqueous solution[7] and in water–dioxane mixtures,[8] the position of the intramolecular, concentration-independent equilibrium 1 for Cu(5′-AMP) can now be quantified[9]



(1)

in dependence on increasing amounts of dioxane in the aqueous reaction mixture, and be compared with the also varying extent of adenine N-7 back-binding[10-16] in Cu(5′-ATP)$^{2-}$,[17] the latter

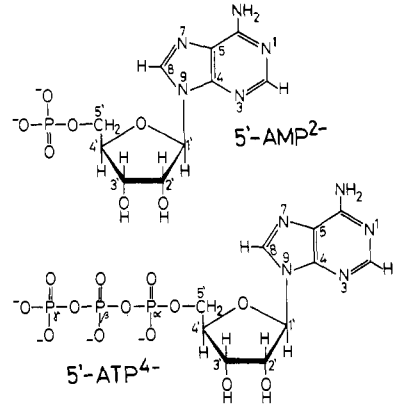


Figure 1. Chemical structures of the adenine-nucleotides (AN), 5′-AMP^{2-} and 5′-ATP^{4-}, in their dominating anti conformation.[13a,19]

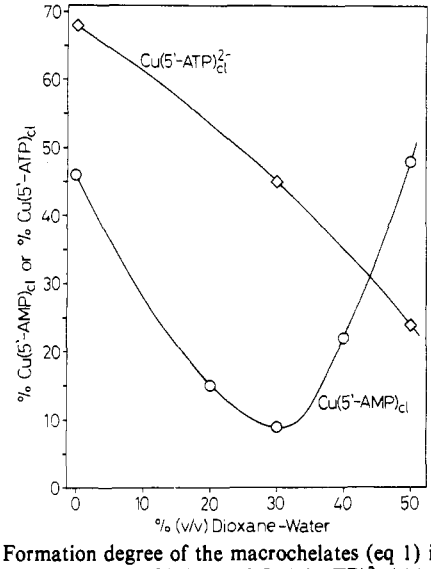


Figure 2. Formation degree of the macrochelates (eq 1) in the binary complex systems Cu(5′-AMP) (○) and Cu(5′-ATP)$^{2-}$ (◇) as a function of the percentage of 1,4-dioxane added to the aqueous reagent mixtures (I = 0.1 M ($NaNO_3$); 25 °C).

complex being possibly a natural active form of Cu^{2+}.[18] The results for these Cu^{2+}/adenine–nucleotide (AN) systems (Figure

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(10) With purine–nucleoside 5′-monophosphates[11] and 5′-triphosphates,[12] macrochelates may be formed involving oxygen(s) of the phosphate residue and N-7 of the purine moiety;[13,14] for several complexes of 5′-AMP^{2-} (cf. ref 15) and 5′-ATP^{4-} (cf. ref 16) with divalent metal ions, the isomer distribution of eq 1 is established.
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Table I. Negative Logarithms of the Acidity Constants for the 2-Fold Protonated Adenine Nucleotides (AN) H_2(5′-AMP)* and H_2(5′-ATP)$^{2-}$, Comparison of the Measured Log Stability Constants, log $K^{Cu}_{Cu(AN)}$, of the Cu(AN) Complexes for 5′-AMP^{2-} and 5′-ATP^{4-} with the Calculated Stability, log $K^{Cu}_{Cu(AN)_{op}}$ (See Text), for the Cu(AN) Isomers with Only Cu^{2+}–Phosphate Coordination, and Extent of the Intramolecular Macrochelate Formation (Eq 1) in the Cu(AN) Species As Quantified by K_I (Eq 2) and % $Cu(AN)_{cl}$ (See Text) in Dependence on the Amount of 1,4-Dioxane Added to Water (I = 0.1 M ($NaNO_3$); 25 °C)[a,b]

AN	% (v/v) dioxane	mol fract	ϵ[c]	$pK^H_{H_2(AN)}$[a]	$pK^H_{H(AN)}$[a]	log $K^{Cu}_{Cu(AN)}$[a]	log $K^{Cu}_{Cu(AN)_{op}}$[a]	log Δ	K_I	% $Cu(AN)_{cl}$
AMP^{2-}	0	0	78.5	3.84 ± 0.02	6.21 ± 0.01	3.14 ± 0.01	2.87 ± 0.08	0.27 ± 0.08	0.86 ± 0.35	46 ± 10
	20	0.050	61.3	3.58 ± 0.01	6.74 ± 0.01	3.56 ± 0.01	3.49 ± 0.04	0.07 ± 0.04	0.17 ± 0.11	15 ± 8
	30	0.083	52.7	3.47 ± 0.02	7.00 ± 0.01	3.86 ± 0.02	3.82 ± 0.03	0.04 ± 0.04	0.10 ± 0.09	9 ± 8
	40	0.124	44.1	3.44 ± 0.01	7.31 ± 0.01	4.30 ± 0.02	4.19 ± 0.06	0.11 ± 0.06	0.29 ± 0.19	22 ± 11
	50	0.175	35.2	3.42 ± 0.02	7.48 ± 0.01	4.73 ± 0.04	4.45 ± 0.02	0.28 ± 0.04	0.91 ± 0.20	48 ± 5
ATP^{4-}	0	0	78.5	4.01 ± 0.01	6.49 ± 0.01	6.32 ± 0.04	5.83	0.49 ± 0.05	2.09 ± 0.36	68 ± 4
	30	0.083	52.7	3.68 ± 0.02	6.82 ± 0.01	6.40 ± 0.05	6.14	0.26 ± 0.05	0.82 ± 0.21	45 ± 6
	50	0.175	35.2	3.59 ± 0.02	6.90 ± 0.02	6.34 ± 0.05	6.22	0.12 ± 0.05	0.32 ± 0.15	24 ± 9

[a] Definition of equilibrium constants (charges are omitted): $K^H_{H_2(AN)} = [H][H(AN)]/[H_2(AN)]$, $K^H_{H(AN)} = [H][AN]/[H(AN)]$, $K^{Cu}_{Cu(AN)} = ([Cu(AN)_{cl}] + [Cu(AN)_{op}])/[Cu][AN]$, and $K^{Cu}_{Cu(AN)_{op}} = [Cu(AN)_{op}]/[Cu][AN]$. [b] The error limits for the acidity and stability constants are 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The error limits given for log Δ, K_I, and % $Cu(AN)_{cl}$ were calculated according to the error propagation after Gauss by using the errors listed in the seventh and eighth column. The data for ATP are from ref 17; here the errors for log Δ, K_I, and % $Cu(ATP)_{cl}$ are estimates; for the situation in water, see also ref 16. The data for AMP in water are from ref 15. [c] The dielectric constants for the dioxane–water mixtures are interpolated from the data given in ref 5.

1)[13a,19] are intriguing: macrochelate formation (eq 1) for Cu(AMP) passes through a *minimum* with a formation degree of ~10% in 30% (v/v) dioxane–water, and ~45 and ~50% in water and 50% dioxane–water, respectively, while for $Cu(ATP)^{2-}$ it decreases gradually from ~70% in water to ~25% in 50% dioxane–water.

The experimental data of the potentiometric pH titrations of the Cu^{2+}/AMP systems[20] could be fully explained at pH >3 by taking into account the species H^+, $H_2(AMP)^{\pm}$, $H(AMP)^-$, AMP^{2-}, Cu^{2+}, and Cu(AMP) [$H_3(AMP)^+$ (cf. ref 19) and Cu(H·AMP)$^+$ (cf. refs 15,21) are negligible]. The dimensionless equilibrium constant, K_I, of equilibrium 1 is calculated[9,15] with eq 2, which involves the log stability-constant difference log Δ

$$K_I = \frac{[Cu(AMP)_{cl}]}{[Cu(AMP)_{op}]} = \frac{K^{Cu}_{Cu(AMP)}}{K^{Cu}_{Cu(AMP)_{op}}} - 1 = 10^{\log\Delta} - 1 \quad (2)$$

= log $K^{Cu}_{Cu(AMP)}$ − log $K^{Cu}_{Cu(AMP)_{op}}$. The overall stability constant $K^{Cu}_{Cu(AMP)}$ is experimentally accessible, while the stability constant of the open isomer, $K^{Cu}_{Cu(AMP)_{op}}$, is more difficult to obtain. The latter problem is resolved by constructing log $K^{Cu}_{Cu(R\text{-}MP)}$ versus $pK^H_{H(R\text{-}MP)}$ plots for the Cu(R-MP) complexes with 4-nitrophenyl phosphate, phenyl phosphate and D-ribose 5′-monophosphate (=R-MP) from the data given in ref 8 for the four mixed solvents employed; the corresponding least-squares regression lines allow calculation of the stability constant of the open isomer for any known acidity constant (pK_a value)[7,9,15] of a monoprotonated phosphate residue, $H(R\text{-}MP)^-$. Finally, with K_I the percentage of the closed or macrochelated form, $Cu(AMP)_{cl}$, of eq 1 may be calculated: % $Cu(AMP)_{cl} = 100K_I/(1 + K_I)$. All results, including those for $Cu(ATP)^{2-}$,[17] are summarized in Table I. To facilitate the overview, the percentages of the macrochelated Cu(AMP) and $Cu(ATP)^{2-}$ species are also plotted in Figure 2 versus the percentage of dioxane present in the dioxane–water mixtures.

The stability of the Cu(AMP) and $Cu(ATP)^{2-}$ complexes is obviously mainly determined in all solvents by the phosphate-group basicity (Table I). However, it is also clear that addition of dioxane to the aqueous solutions of the complexes alters the extent of macrochelate formation considerably and not in a predictable way. A minimum is reached for $Cu(AMP)_{cl}$ in 30% (v/v) dioxane–water, while the extent of $Cu(ATP)_{cl}^{2-}$ decreases with increasing dioxane concentrations (Figure 2). These different influences of dioxane on the formation of the macrochelates must be due to a combination of effects. Among these effects are certainly the alterations of the metal ion affinity of the binding sites due to the decreasing solvent polarity; another aspect is the hydrophobic (lipophilic) solvation of the purine moiety of the nucleotides by the ethylene groups of 1,4-dioxane, which may shield a binding site, e.g. N-7, in a certain concentration range of dioxane.

Considering the substrate and product structures in enzymic reactions, the above observations are meaningful, and they are also to be expected for other metal ion–nucleotide complexes: it is evident that at a protein–water interface subtle polarity changes are enough to favor the one or other structure (eq 1).[6] Moreover, one wonders how far is also metal ion binding to nucleic acids[22] affected by changes in the polarity of the surrounding solvent? In this respect, it should be noted that not only the nucleic base residues alter their coordinating properties but also the metal ion affinity of phosphate residues increases drastically with a decreasing solvent polarity as is evident from the data given for phosphate binding in column 8 of Table I. Finally, the influence of solvent changes on the reactivity was already proven[23] for the metal ion facilitated ATP dephosphorylation.

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(24) Work done at the University of Basel during a leave from the Academy of Beijing Traditional Medicine, People's Republic of China.

University of Basel
Institute of Inorganic Chemistry
Spitalstrasse 51
CH-4056 Basel, Switzerland

Guogang Liang[24]
Helmut Sigel*

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