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Biologically Important Compounds as Ligands: Binary and Ternary Complexes of 5-Amino-7-hydroxy-triazolo[4,5—*d*]pyrimidine („8-Azaguanine") in Solution

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Metal complexation equilibria in the binary complexing systems of the type *M*—*L* [*M* = Cu(II), Ni(II), Co(II), Zn(II), Cd(II), and UO_2(VI); *L* = *AZN* = 8-azaguanine] have been examined potentiometrically. The work has further been extended to investigating the ternary complexing systems of the type *M*—*A*—*L* [*A* = 2,2′-bipyridine, 1,10-phenanthroline or nitrilotriacetic acid]. Measurements were done at 25 °C and at an ionic strength of 0.1*M* ($NaClO_4$) in 50% (*v*/*v*) aqueous ethanol medium. Stabilities of the ternary complexes as compared to those of the corresponding binary complexes of *AZN* are also discussed.

Biologisch relevante Verbindungen als Liganden: Binäre und ternäre Komplexe von 5-Amino-7-hydroxy-triazolo[4,5—d]pyrimidin („8-Azaguanin") in Lösung

Es wurden Komplexierungsgleichgewichte vom binären Typ *M*—*L* [*M* = = Cu(II), Ni(II), Co(II), Zn(II), Cd(II) und UO_2(VI); *L* = *AZN* = 8-Azaguanin] potentiometrisch untersucht. Die Untersuchungen wurden auf ternäre Systeme vom Typ *M*—*A*—*L* ausgedehnt [*A* = 2,2′-Bipyridin, 1,10-Phenanthrolin oder Nitrilotriessigsäure]. Die Messungen wurden bei 25 °C bei Ionenstärken von 0,1*M* ($NaClO_4$) in 50% wäßr. Ethanol durchgeführt. Die Stabilität der ternären Komplexe im Vergleich zu den entsprechenden binären wird diskutiert.

Introduction

8-Azaguanine (*AZN*; 5-amino-7-hydroxy-triazolo[4,5—*d*]pyrimidine) is one of the unnatural purine bases, not available normally in biological systems.

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The action of such unnatural bases seems to be twofold: (i) bases generally block some stage or the other in the process of biosynthesis of the normal purine nucleotides, e.g. inhibition of biosynthesis of guanosine monophosphate (*GMP*) by *AZN*[1], and (ii) the bases themselves after conversion to nucleotides, are incorporated in varying degrees into *RNA* and/or *DNA*, ultimately resulting in an abnormal form of nucleic acid, e.g. incorporation of *AZN* at the expense of guanine into the *RNA* of tobacco mosaic virus (*TMV*)[2] and to still a larger extent into the *RNA* of bacillus cereus[3].

It was considered to be of interest to investigate the avidity of *AZN* with various metal ions especially in the context of the hypothesis that metal ions are involved in the stabilization of the *Watson* and *Crick* double helix of *DNA* by way of some bonding to phosphate groups and others to the purine and pyrimidine bases present[4].

The chelating behaviour of various purine bases have been widely studied, but work on metal-*AZN* complexes is scanty[5]. Here, the results of studies on the interaction of Cu(II), Ni(II), Co(II), Zn(II), Cd(II), and UO_2(VI) ions in binary complexation with *AZN*, and also their ternary complex formation involving *AZN* as a secondary and 2,2′-bipyridine (*BIPY*). 1,10-phenanthroline (*PHEN*) or nitrilotriacetic acid (*NTA*) as a primary ligand are reported. *BIPY*, *PHEN*, and *NTA* are all biologically important[6–8]. pH-metric titration technique of *Irving* and *Rossotti*[9] and its modification by *Chidambaram* and *Bhattacharya*[10] have been employed. All the experiments were carried out at 25 °C and at an ionic strength of 0.1*M* ($NaClO_4$) and 50% (*v*/*v*) aqueous-ethanol medium.

Experimental

Materials

Solutions were prepared using reagent grade chemicals and doubly distilled water, either by direct weighing or by standardization employing usual methods: (a) metal ions: 0.01*M* perchlorates of Cu(II), Ni(II), Co(II), Zn(II), Cd(II) and UO_2(VI) in 0.02*M* perchloric acid; (b) 1.0*M* sodium perchlorate; (c) 0.02*M* perchloric acid; (d) 0.1*M* sodium hydroxide; (e) primary ligand (*A*): 0.01*M* each of *BIPY*, *PHEN* and *NTA*; and (f) secondary ligand (*L*): 0.01*M* *AZN* in 0.02*M* NaOH.

Procedure

All the measurements were carried out at 25 °C using a Leeds and Northrup pH-meter with a glass calomel electrode assembly.

The following mixtures were prepared for binary and ternary systems:

(*A*) 5.0 ml perchloric acid (0.02*M*) + 5.0 ml sodium perchlorate (1.0*M*) + 15.0 ml water + 25.0 ml ethanol,

(*B*) 10.0 ml perchloric acid (0.02*M*) + 5.0 ml sodium perchlorate (1.0*M*) + 5.0 ml *L* (0.01*M* in 0.02*M* NaOH) + 5.0 ml water + 25.0 ml ethanol,

(C) 9.0 ml perchloric acid (0.02M) + 5.0 ml sodium perchlorate (1.0M) + 1.0 ml metal perchlorate (0.01M in 0.02M perchloric acid) + 5.0 ml L (0.01M in 0.02M NaOH) + 5.0 ml water + 25.0 ml ethanol,

(D) 5.0 ml perchloric acid (0.02M) + 5.0 ml sodium perchlorate (1.0M) + 5.0 ml A (0.01M) + 10.0 ml water + 25.0 ml ethanol,

(E) 5.0 ml metal perchlorate (0.01M in 0.02M perchloric acid) + 5.0 ml sodium perchlorate (1.0M) + 5.0 ml A (0.01M) + 10.0 ml water + 25.0 ml ethanol, and

(F) 5.0 ml perchloric acid (0.02M) + 5.0 ml metal perchlorate (0.01M in 0.02M perchloric acid) + 5.0 ml sodium perchlorate (1.0M) + 5.0 ml A (0.01M) + 5.0 ml L (0.01M in 0.02M NaOH) + 25.0 ml ethanol.

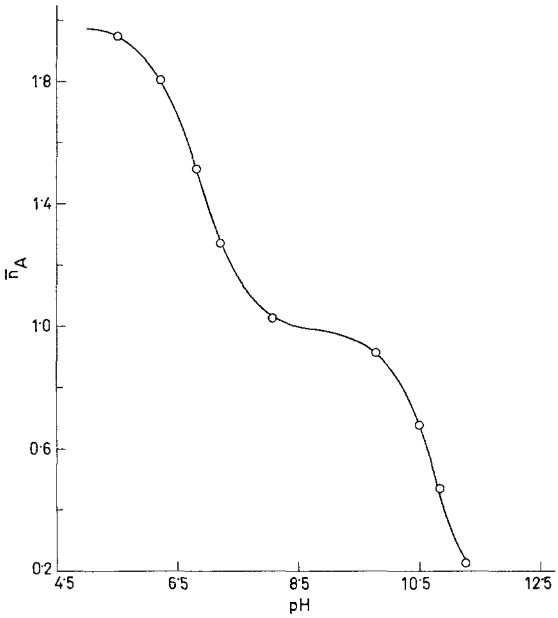


Fig. 1. Formation curve: AZN protonation system

The mixtures A to F were individually titrated against 0.1M NaOH (Figs. of the titration curves A–F are omitted to economize space).

In the calculations for the mixed ligand formation constants with NTA, an allowance for the three protons liberated by way of complexation with various metal ions in the formation of $(MNTA)^-$ was made. $BIPY$ and $PHEN$ are neutral ligands.

Calculations

M-L Systems

The titration curves A, B and C were employed for evaluating[9] the average number of protons bound per free ligand ion ($\bar{n}_A$), average number of ligands attached per metal ion ($\bar{n}$) and free ligand exponent

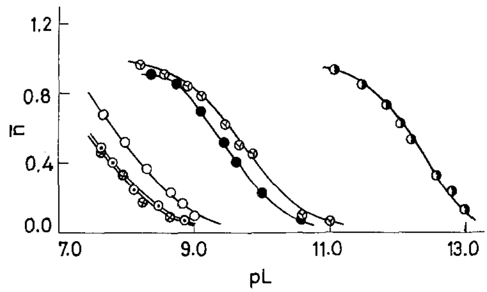


Fig. 2. Formation curves: [*M-AZN*] systems -◑-◑- Cu(II), -⊗-⊗- UO_2(VI), -●-●- Ni(II), -○-○- Co(II), -⊙-⊙- Zn(II), -⊕-⊕- Cd(II)

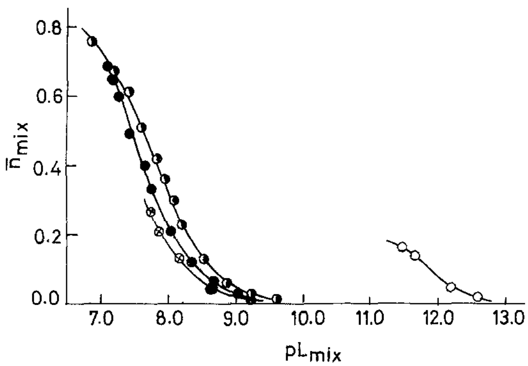


Fig. 3. Formation curves: [*M-BIPY-AZN*] systems -○-○- Cu(II), -◑-◑- Ni(II), -●-●- Co(II), -⊗-⊗- Zn(II)

(*pL*). The formation curves corresponding to proton-ligand (Fig. 1) and metal-ligand (Fig. 2) were then plotted. Approximate values of the formation constants obtained by interpolation at half $\bar{n}_A(\bar{n})$ value method and more precise values determined by the average value method are recorded in Table 1.

The absence of protonated and polynuclear species was confirmed by using several concentrations of the reactants, where the results obtained were identical. In all the systems precipitation occurred soon after the 1:1 (*M*:*L*) stage and studies beyond this range were impossible. Hence, ML_2 and the hydroxo species likely to be formed after this stage could not be considered.

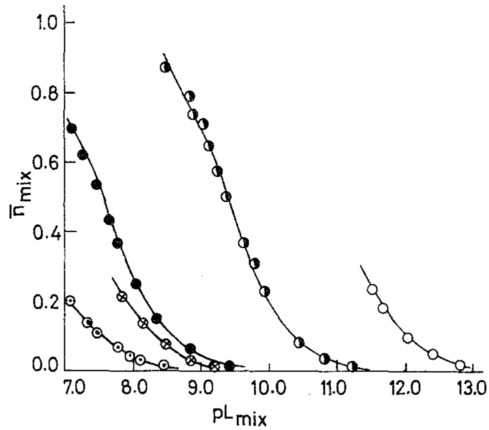


Fig. 4. Formation curves: [*M-PHEN-AZN*] systems -○-○- Cu(II), -◑-◑- Ni(II), -●-●- Co(II), -⊗-⊗- Zn(II), -⊙-⊙- Cd(II)

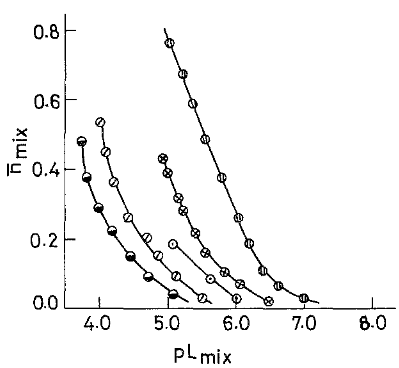


Fig. 5. Formation curves: [*M-NTA-AZN*] systems -⦶-⦶- Cu(II), -⊗-⊗- Ni(II), -⊙-⊙- Co(II), -⊘-⊘- Zn(II), -⊖-⊖- Cd(II)

M-A-L Systems

Curve *F* departs from curve *B* only after the complete formation of 1:1 $(M \cdot BIPY)^{2+}$ or $(M \cdot PHEN)^{2+}$ complex species and before the formation of their hydroxo species (*vide* curves *A*, *D* and *E*). $(M \cdot NTA)^{-}$ does not undergo hydroxo complex formation even at higher pH values. Thus, the average number of secondary ligand molecules attached per *MA* ions, $\bar{n}_{mix}$ were calculated using:

$$\bar{n}_{mix} = \frac{(v^{vi} - v^{ii})(N^0 + E^0)}{(V^0 + v')\,\bar{n}_A\, TC_{MA}^{0}}$$

Table 1. *Stability constants of binary and ternary metal complexes* [50% (*v*/*v*) aqueous-ethanol medium, $\mu = 0.1$]

Reactions	log(Equil. Const.) Method[a]	Method[b]
$L^{2-} + H^+ \rightleftharpoons LH^-$	10.90	10.77
$LH^- + H^+ \rightleftharpoons LH_2$	6.67	6.81
$Cu^{2+} + L^{2-} \rightleftharpoons CuL$	12.30	12.24
$UO_2^{2+} + L^{2-} \rightleftharpoons UO_2L$	9.70	9.69
$Ni^{2+} + L^{2-} \rightleftharpoons NiL$	9.47	9.47
$Co^{2+} + L^{2-} \rightleftharpoons CoL$	8.00	8.07
$Zn^{2+} + L^{2-} \rightleftharpoons ZnL$	7.60	7.68
$Cd^{2+} + L^{2-} \rightleftharpoons CdL$	—	7.64
$(Cu \cdot BIPY)^{2+} + L^{2-} \rightleftharpoons (Cu \cdot BIPY \cdot L)$	—	10.84
$(Ni \cdot BIPY)^{2+} + L^{2-} \rightleftharpoons (Ni \cdot BIPY \cdot L)$	7.60	7.56
$(Zn \cdot BIPY)^{2+} + L^{2-} \rightleftharpoons (Zn \cdot BIPY \cdot L)$	—	7.35
$(Co \cdot BIPY)^{2+} + L^{2-} \rightleftharpoons (Co \cdot BIPY \cdot L)$	7.45	7.45
$(Cu \cdot PHEN)^{2+} + L^{2-} \rightleftharpoons (Cu \cdot PHEN \cdot L)$	—	11.07
$(Zn \cdot PHEN)^{2+} + L^{2-} \rightleftharpoons (Zn \cdot PHEN \cdot L)$	—	7.36
$(Ni \cdot PHEN)^{2+} + L^{2-} \rightleftharpoons (Ni \cdot PHEN \cdot L)$	9.35	9.36
$(Co \cdot PHEN)^{2+} + L^{2-} \rightleftharpoons (Co \cdot PHEN \cdot L)$	7.55	7.57
$(Cd \cdot PHEN)^{2+} + L^{2-} \rightleftharpoons (Cd \cdot PHEN \cdot L)$	—	6.59
$(Cu \cdot NTA)^- + L^{2-} \rightleftharpoons (Cu \cdot NTA \cdot L)^{3-}$	5.55	5.53
$(Zn \cdot NTA)^- + L^{2-} \rightleftharpoons (Zn \cdot NTA \cdot L)^{3-}$	4.03	4.03
$(Ni \cdot NTA)^- + L^{2-} \rightleftharpoons (Ni \cdot NTA \cdot L)^{3-}$	—	4.83
$(Co \cdot NTA)^- + L^{2-} \rightleftharpoons (Co \cdot NTA \cdot L)^{3-}$	—	4.55
$(Cd \cdot NTA)^- + L^{2-} \rightleftharpoons (Cd \cdot NTA \cdot L)^{3-}$	—	3.67

[a] Interpolation at half $\bar{n}_A(\bar{n})$ value method.
[b] Average value method.

where v^{vi}, v^{ii} and v' are the volumes of alkali consumed to reach the same pH value in the curves F, B and A respectively, TC_{MA^0} = total initial concentration of MA (which is equivalent to initial metal ion concentration taken in mixture E or F); V^0 is the initial vol. of the titration mixture; E^0 is the initial concentration of perchloric acid and N^0 is the concentration of alkali used.

Values of $\bar{n}_A$ at different pH were available from the binary complexing system. From the values of $\bar{n}_{mix}$, pL_{mix} was calculated by:

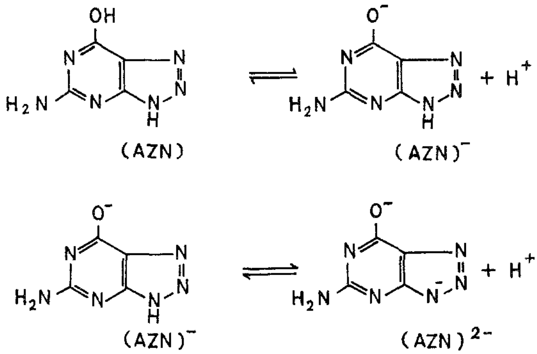
$$pL_{mix} = \log_{10}\left[\frac{\sum_{n=0}^{n=j} \beta_n^H \left(\frac{1}{\text{antilog B}}\right)^n}{TC_{L^\circ} - \bar{n}_{mix}\, TC_{MA^\circ}} \cdot \frac{V^0 + v^{vi}}{V^0}\right]$$

$\bar{n}_{mix}$ was plotted against pL_{mix} to get formation curves (Figs. 3–5), and values of formation constants are recorded in Table 1.

Results and Discussion

Proton-Ligand System

The departure between curves B and A is equivalent to the dissociation of two protons in AZN (phenolic and imino H). Dissociation of the imino proton in purines at higher pH values is not uncommon[11].



Binary M-AZN Complexes

In all the binary systems only 1:1 (M:L) complexes could be detected. Opacity, turbidity or precipitation occurred at higher pH and the constants for 1:2 (M:L) complexes could not be obtained. The order of stability of 1:1 complexes (Table 1) is:

$$Cu(II) > UO_2(VI) > Ni(II) > Co(II) > Zn(II) > Cd(II)$$

which is as usual. Generally with O^-, O^- donors UO_2(VI) forms stronger complexes than Cu(II), but this may be reversed in ligands with O^-, N^- donors[12], as in this case.

Ternary M-A-AZN Complexes

The systems Cd(II)-*BIPY-AZN*, UO_2(VI)-*BIPY-AZN*, UO_2(VI)-*PHEN-AZN* and UO_2(VI)-*NTA-AZN* could not be studied either due to occurrence of precipitation, opacity or turbidity, or the necessary conditions[10] were not applicable.

From statistical considerations, the stabilities of ternary complexes in *M-BIPY* or *PHEN-L* systems should be appreciably lower than the first step formation constant of ML as the concentration of electrons around the metal ion in $(M\text{-}BIPY)^{2+}$ would be more than in $[M(H_2O)_n]^{2+}$ owing to *BIPY* being more strongly coordinating than H_2O. But it is noted here that values are not much lower (Table 1); the reason being in $(M \cdot BIPY)^{2+}$ species the M-N bond is influenced not only by $L \rightarrow M$ σ-interaction, but there also occurs to some extent $M \rightarrow L$ $(d\pi$—$p\pi)$-interaction, which does not permit the concentration of electrons around the metal ion to increase significantly[10]. Results with 1,10-phenanthroline as a primary ligand (Table 1) are similar to those with 2,2′-bipyridine, perhaps due to the structural similarity between *PHEN* and *BIPY*.

On the other hand, the formation constant corresponding to the association of *AZN* with $(M \cdot NTA)^-$ is much less than the first step formation constant of *ML* (Table 1). This appears to be due to *Coulomb* repulsion between the L^{2-} and NTA^{3-} anions, which lowers the stability of the mixed ligand chelates. No such repulsion, however, is encountered during the process of *ML* formation.

The order of stability of mixed ligand complexes follows the same pattern as in the binary *AZN* complexes.

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