

EXPERIMENTAL SECTION

The reaction mixtures initially consisted of 2 μL of carrier DNA (1 μg), 3 μL of nucleosomal DNA (146 base pairs in length) labeled at the 5' ends with radioactive phosphorous, and 65 μL of a suspension of calcium phosphate precipitate. The calcium phosphate precipitate was prepared by mixing solutions of calcium chloride and potassium phosphate, in a Tris-chloride buffer of pH 8.0, to final concentrations of 26 mM phosphate and 16 mM calcium. The DNA was allowed to adsorb to the calcium phosphate precipitate for 1 hour; control experiments show that this is sufficient time for all DNA to be bound. To the mixture was then added the cutting reagent: either 0.2 units of DNase I, or 20 μL of 25 mM ferrous ammonium sulfate, 50 mM EDTA. To the iron-containing mixtures was then added 10 μL of 0.3% H_2O_2 to initiate the reaction. The reaction mixtures were incubated at 37°C for the appropriate times, quenched by addition of excess EDTA and thiourea (a hydroxyl radical scavenger), ethanol precipitated, dissolved in formamide-dye mixture, denatured, and electrophoresed.

ACKNOWLEDGEMENTS

We are grateful to the Chicago Community Trust/Searle Scholars Program, the Research Corporation, and the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health (Grant SO7 RR07041) for support of this work.

REFERENCES

- [1] C.R. CANTOR, A. EFSTRATIADIS, *Nucleic Acids Res.*, **12**, 8059 (1984).
- [2] D. RHODES, A. KLUG, *Nature*, **286**, 573 (1980).
- [3] R.P. HERTZBERG, P.B. DERVAN, *Biochemistry*, **23**, 3934 (1984).
- [4] J.K. BARTON, A.L. RAPHAEL, *J. Am. Chem. Soc.*, **106**, 2466 (1984).
- [5] B.E. BOWLER, L.S. HOLLIS, S.J. LIPPARD, *J. Am. Chem. Soc.*, **106**, 6102 (1984).



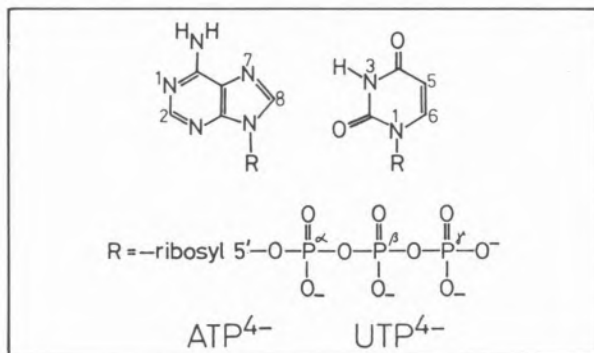
PS5.59 — MO

ROGER TRIBOLET
HELMUT SIGEL
Institute of Inorganic Chemistry
University of Basel
Spitalstrasse 51, CH-4056 Basel
Switzerland

SOLVENT-INFLUENCE ON THE INTRAMOLECULAR EQUILIBRIA OCCURRING IN COMPLEXES OF ADENOSINE 5'-TRIPHOSPHATE

Metal ion complexes of nucleotides are substrates for many enzymic reactions. In fact, nucleotides, especially adenosine 5'-triphosphate (ATP^{4-}), are quite versatile ligands [1-3]. Part of this versatility is connected with the occurrence of intramolecular equilibria in their binary and ternary complexes. To learn how the position of these equilibria is influenced by the polarity of the solvent, we have studied the effect of dioxane on the stability and structure of the complexes formed in the Cu^{2+} /1,10-phenanthroline (Phen)/ATP system. Such knowledge is important, because enzymic reactions usually take place in active-site cavities of proteins, and there is evidence [4] that the polarity in these cavities is decreased compared with the polarity of aqueous solutions.

The following results and conclusions are based on potentiometric pH titrations carried out in water and in 30% or 50% (v/v) dioxane-water mixtures ($I = 0.1$, NaClO_4 ; 25°C) [5]. For the eva-



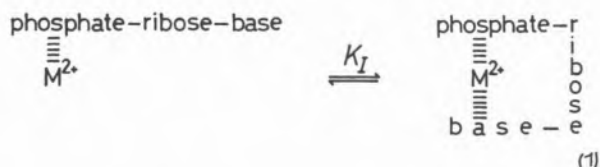
luation of many of the data it was necessary to study not only $\text{Cu}^{2+}/\text{Phen}/\text{ATP}$, but to include also the $\text{Cu}^{2+}/\text{Phen}/\text{uridine } 5'\text{-triphosphate (UTP}^{4-})$ system [5]. We are considering in the following the position of three intramolecular equilibria occurring with ATP complexes; all are affected by dioxane:

1) Location of the Proton in $\text{Cu}(\text{H}\cdot\text{ATP})^-$

The proton in $\text{Cu}(\text{H}\cdot\text{ATP})^-$ may be at N-1 of the adenine moiety or at the terminal γ -phosphate group; *i.e.* there is an equilibrium between isomeric complexes. In aqueous solution the isomer with the proton at N-1 is dominating, while in water-dioxane mixtures the complexes with the proton at the γ -phosphate are favored. This indicates, as one might expect, that with decreasing solvent polarity the formation of non-charged sites is promoted.

2) Extent of Macrocholate-Formation in $\text{Cu}(\text{ATP})^{2-}$

Equilibrium (1) involves for $\text{Cu}(\text{ATP})^{2-}$ an isomer with phosphate-coordination only, and a macrocholate, $\text{Cu}(\text{ATP})_{\text{cl}}^{2-}$, in which N-7 is also coordina-



ted to Cu^{2+} . The formation degree of this macrochelated isomer is influenced by dioxane as indicated:

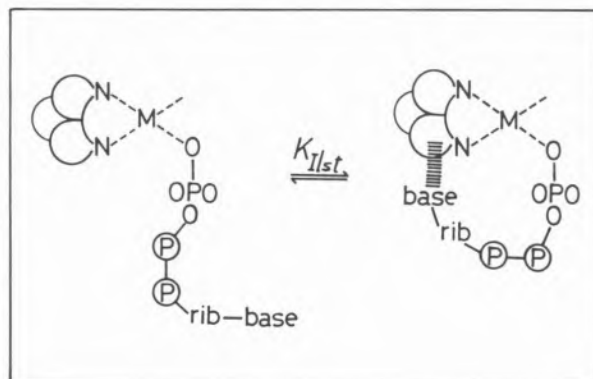
% (v/v) Dioxane:	0	30	50
% $\text{Cu}(\text{ATP})_{\text{cl}}^{2-}$:	68 ± 4	45 ± 6	24 ± 9

(the error limits correspond to an uncertainty of ± 0.05 in the log K values; see [5])

The concentration of the macrochelated isomer decreases evidently with increasing amounts of dioxane in the solvent mixture. This is probably the result of an increasing hydrophobic solvation of the adenine moiety of ATP^{4-} by the ethylene groups of dioxane, rendering the metal ion coordination to N-7 more difficult.

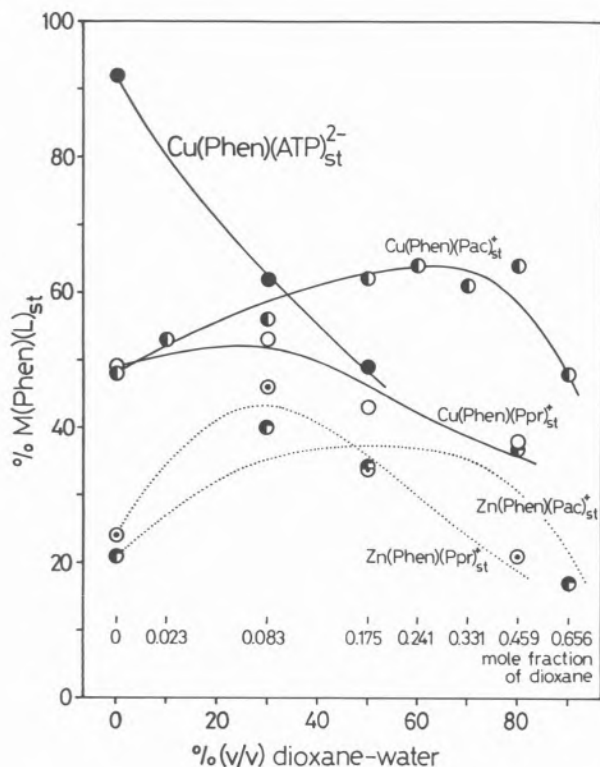
3) Intramolecular Stack-Formation in $\text{Cu}(\text{Phen})(\text{ATP})^{2-}$

The formation of an intramolecular, *i.e.* metal ion-bridged, stack between the aromatic rings of 1,10-phenanthroline and the adenine residue of ATP^{4-} is indicated in equilibrium (2):



The formation of the stacked isomer, $\text{Cu}(\text{Phen})(\text{ATP})_{\text{st}}^{2-}$, is affected by dioxane, but by far not as much as the binary adduct formed between Phen and ATP^{4-} . A change from water to 50% (v/v) dioxane-water decreases the stability of the binary $(\text{Phen})(\text{ATP})^{4-}$ adduct by a factor of $< 1/10$, while the metal ion-bridged ternary adduct is disfavored only by a factor of about $1/2$ (see figure).

The observation that dioxane influences the formation of intramolecular stacks differently than simple unbridged stacks is interesting and should be viewed in a wider frame: In the figure the formation degree of the intramolecular stack according to equilibrium (2) is plotted in dependence on the percentage of dioxane (added to an aqueous solution) for $\text{Cu}(\text{Phen})(\text{ATP})^{2-}$, as well as for $\text{M}(\text{Phen})(2\text{-phenylacetate})^+$ and $\text{M}(\text{Phen})(3\text{-phenylpropionate})^+$, where $\text{M} = \text{Cu}^{2+}$ or Zn^{2+} . It is evident that dioxane (or ethanol) [6] is even able to *promote* intramolecular stack formation in ternary Cu^{2+} or Zn^{2+} complexes; this contrasts with any experience regarding binary stacking adducts [5-7]. The reason for this observation must be connected with the presence of the metal ion; it appears that under certain structural conditions (the distance in the ligand between the coordinating atom and the stacking moiety is obviously important; see [5,6,8]) the stacked isomer is stabilized by the organic solvent molecules.



Figure

Formation degree of the intramolecular aromatic-ring stack (eq. (2)) in $\text{Cu}(\text{Phen})(\text{ATP})_{\text{st}}^{2-}$ and several other ternary complexes containing Cu^{2+} (full lines) or Zn^{2+} (dotted lines) in dependence on the percentage of dioxane added to the aqueous reagent mixture. The data are taken from refs. [5] and [6] ($I=0.1$; 25°C). Abbreviations: L, ligand; M, metal ion; Pac, 2-phenylacetate; Phen, 1,10-phenanthroline; Ppr, 3-phenylpropionate

ACKNOWLEDGEMENTS

The support of this work by the Swiss National Science Foundation is gratefully acknowledged.

REFERENCES

- [1] K.H. SCHELLER, F. HOFSTETTER, P.R. MITCHELL, B. PRIJS, H. SIGEL, *J. Am. Chem. Soc.*, **103**, 247-260 (1981).
- [2] K.H. SCHELLER, H. SIGEL, *J. Am. Chem. Soc.*, **105**, 5891-5900 (1983).
- [3] H. SIGEL, K.H. SCHELLER, R.M. MILBURN, *Inorg. Chem.*, **23**, 1933-1938 (1984).
- [4] H. SIGEL, R.B. MARTIN, R. TRIBOLET, U.K. HÄRING, R. MALINI-BALAKRISHNAN, submitted for publication.
- [5] R. TRIBOLET, R. MALINI-BALAKRISHNAN, H. SIGEL, *J. Chem. Soc., Dalton Trans.* (1985), in press.
- [6] H. SIGEL, R. MALINI-BALAKRISHNAN, U.K. HÄRING, submitted for publication.
- [7] a) K.A. CONNORS, S.-R. SUN, *J. Am. Chem. Soc.*, **93**, 7239-7244 (1971);
b) B. FARZAMI, Y.H. MARIAM, F. JORDAN, *Biochemistry*, **16**, 1105-1110 (1977).
- [8] H. SIGEL, R. TRIBOLET, K.H. SCHELLER, *Inorg. Chim. Acta*, **100**, 151-164 (1985).



PS5.60 — TU

W. KLEIBÖHMER

B. WENCLAWIAK

B. KREBS

Institute of Inorganic Chemistry

University of Münster, D-4400 Münster

F.R.G.

REACTION OF $[\text{cis-Pt}(\text{NH}_3)_2\text{Cl}_2]$ WITH RIBOSE DINUCLEOSIDE MONOPHOSPHATES. HPLC INVESTIGATION ON THE TIME DEPENDENT FORMATION OF THE REACTION PRODUCTS

The coordination of the aquated antitumor drug $[\text{cis-Pt}(\text{NH}_3)_2\text{Cl}_2]$ to the chromosomal DNA in the cell is generally accepted to be its primary way of action. Early investigations have shown that the preferred binding site is the N7 atom or occasionally the N1 atom of the purine bases and the N3 atom of the pyrimidine bases.

Even the reaction of $[\text{cis-Pt}(\text{NH}_3)_2\text{Cl}_2]$ (*cis*-DDP) with short DNA fragments like ApA, ApG, GpA and GpG results in a variety of products. The time dependent formation of these products has been investigated by reversed phase liquid chromatography. 1:1 mmolar ratios of the dinucleotides and *cis*-DDP have been reacted for several hours at 37°C in dark vials. Every hour a certain injection onto the column was chromatographed. The HPLC measurements of the reaction system ApA/*cis*-DDP show that two major products are formed during a reaction time of 48 hours. In the reaction system of GpA and *cis*-DDP we observe the formation of an intermediate with highest intensity after 13 hours. It was eluted after 30 minutes, whereas the major product peak is eluted after 16 minutes. The formation of an intermediate is also observed in the ApG/*cis*-DDP system. It has its maximum absorbance after 8 hours and is eluted after the main product peak. GpG forms