T2

The Use of Chromium(III) and Cobalt(III) Complexes of Adenosine Diphosphate to Elucidate the Catalytic Mechanism of Creatine Kinase

V. L. PECORARO, J. RAWLINGS and W. W. CLELAND*

Department of Biochemistry, University of Wisconsin, Madison, Wis. 53706, U.S.A.

Complexes of the type α,β-bidentate $Cr(H_2O)_{4-n}(NH_3)_nADP$ (for n = 0, 3, 4) and $Co(H_2O)_{4-m}(NH_3)_m$ (for m = 3, 4) have been used over the pH range 5.5 to 7.8 to probe the chemical mechanism of rabbit muscle creatine kinase. In addition, V, V/K and Km values have been obtained for the normal substrate, MgADP, over the same pH range.

The cobalt and chromium nucleotides were synthesized following the procedures given in ref. 1–5. Separation of the diasteromers into Λ and Δ screw sense isomers was by cycloheptaamylose chromatography (pH 5.5, 4 °C, 10 m*M* MES, 2.5 cm × 2 m). The first band of the column (Δ isomer) was used in all inhibition studies. Kinetic assays with creatine kinase used the hexokinase-glucose-6-phosphate dehydrogenase coupled assay in which the appearance of NADPH at 340 nm was monitored. The pH was maintained with 50 m*M* MES buffer between pH 5.5–6.8 while 50 mM HEPES was used from pH 7.0–7.8. No buffer effects were observed. All of the cobalt and chromium nucleotides acted as competitive inhibitors *versus* MgADP and were fit to the equation $v = VS/\{K(1 + I/Kis) + S\}$.

The Kis and Km values for the metal nucleotides as a function of pH are shown in Fig. 1. The data indicated that metal–nucleotide binding to the enzyme is strongest below an approximate pK of 6.4. This pK is not associated with the metal nucleotide complexes nor a binding group of the enzyme. It appears that the pK of the acid–base catalyst (thought to be histidine [6]) is about 6.4 in the absence of nucleotide, and is raised to about 7.2 in the presence of a nucleotide, presumably as a result of a protein conformation change which allows a hydrogen bond to form between the histidine and the phosphorylated nitrogen of creatine phosphate. This change in pK upon nucleotide binding necessarily causes tighter nucleotide binding at low pH. Additionally, it is observed that chromium nucleotides are bound more strongly than the analogous cobalt nucleotides and that the binding affinity decreases as ammonia ligands are substituted for water in the metal coordination sphere.

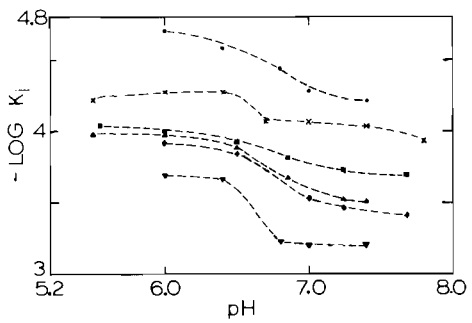


Fig. 1. Dependence of –log Ki as a function of pH for $Cr(H_2O)_4ADP$ (•); $Mg(H_2O)_4ADP$ (×); $Cr(NH_3)_3(H_2O)ADP$ (■); $Co(NH_3)_3H_2OADP$ (▲); $Cr(NH_3)_4ADP$ (♦); and $Co(NH_3)_4ADP$ (▼).

1 M. L. DePamphilis and W. W. Cleland, *Biochemistry, 12*, 3714 (1973).
2 R. D. Cornelius and W. W. Cleland, *Biochemistry, 17*, 3279 (1978).
3 R. D. Cornelius, P. A. Hart and W. W. Cleland, *Inorg. Chem., 16*, 2799 (1977).
4 D. Dunaway-Mariano and W. W. Cleland, *Biochemistry, 19*, 1496 (1980).
5 D. Dunaway-Mariano and W. W. Cleland, *Biochemistry, 19*, 1506 (1980).
6 P. F. Cook, G. L. Kenyon and W. W. Cleland, *Biochemistry, 20*, 1204 (1981).

T3

Gold(III) and Gold(I) Complexes of Purine and Pyrimidine Nucleosides Studied by IR, ^1H NMR and ^{197}Au Mössbauer Spectroscopies

G. H. M. CALIS

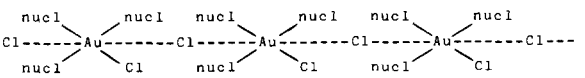
University of Nijmegen, Department of Physical Chemistry, Nijmegen, The Netherlands

and N. HADJILIADIS

University of Ioannina, Inorganic Chemistry Laboratory, Department of Chemistry, Ioannina, Greece

The interactions of metal ions and especially of platinum with nucleosides are of great importance. There are few analogous studies with gold ions [1, 2]. In this paper we report on the reactions of chloroauric acid ($HAuCl_4$) with inosine = ino, guanosine = guo, triacetylinosine = trino and triacetylguanosine = trguo, in aqueous or methanolic solutions [3]. They were characterized with elemental analyses, conductivity measurements, IR, ^1H NMR and ^{197}Au Mössbauer spectra. They correspond to the formulae $Au(nucl)_3Cl_2$, $Au(nucl)Cl_3$ and $Au(nucl\text{-}H^+)Cl_2$ for Au(III) and $Au(nucl)_2Cl$ for Au(I). Based on the results, possible structures for all the complexes were proposed.

The ^{197}Au Mössbauer spectra in the solid state seem to agree with the other data in most of the cases. Thus, a polymeric structure of the type



is proposed for the $Au(nucl)_3Cl_2$ complexes, implying six coordinate Au(III) ions, with the N(7) site of the nucleosides involved in bonding, based mainly on the ^{197}Au Mössbauer spectra. Polymeric structures were also proposed for the $Au(nucl\text{-}H^+)Cl_2$ complexes, from the combined IR and ^{197}Au Mössbauer spectra, involving in bonding the N(7) and O(6) sites of the ligands. The $Au(nucl)Cl_3$ complexes correspond to a square planar arrangement around Au(III) with the N(7) site again involved in bonding.

Finally, for the $Au(nucl)_2Cl$ the ^{197}Au Mössbauer spectra do not seem to agree with the other data, suggesting sp hydridization and two coordination of the type nucl–Au–Cl and not a three coordination of the type nucl–Au–Cl, as it was previously proposed
nucl
[3]. The results of the different techniques will be compared and discussed.

1 M. Bressan, R. Ettore and P. Rigo, *J. Magn. Reson.*, *26*, 43 (1977).
2 D. W. Gibson, M. Beer and R. J. Barnett, *Biochem.*, *10*, 3669 (1971).
3 N. Hadjiliadis, G. Pneumatikakis and R. Basosi, *J. Inorg. Biochem.*, *14*, 115 (1981).

T4

^{1}H-NMR Study on Self-Association and Macrochelate Formation in Metal Ion Systems of Nucleoside 5′-Diphosphates

KURT H. SCHELLER and HELMUT SIGEL*

Institute of Inorganic Chemistry, University of Basle, CH-4056 Basle, Switzerland

Nucleotides and their complexes are substrates for many enzymic reactions [1]. The self-association *via* aromatic ring stacking of nucleoside 5′-mono- [2] and 5′-triphosphates [3] is now well established and the structures for the complexes of these nucleotides (=N) in solution are relatively well characterized [3, 4]. Much less is known about nucleoside 5′-diphosphates (NDP^{3-}).

Therefore, the concentration dependence of the chemical shifts for the protons H-2, H-8, and H-1′ of ADP^{3-} and IDP^{3-}, H-8 and H-1′ of GDP^{3-}, and H-5, H-6, and H-1′ of CDP^{3-} and UDP^{3-} has been measured in D_2O at 27 °C. The results are consistent with the isodesmic model [3] of indefinite non-cooperative stacking (eqn. 1); the association constants for

$$(N)_n + N \rightleftharpoons (N)_{n+1} \quad K = [(N)_{n+1}]/[(N)_n][N] \quad (1)$$

NDP^{3-} are between 1.8 (ADP^{3-}) and about 0.6 M^{-1} (UDP^{3-}). In agreement with earlier results [3] obtained under the same conditions for nucleosides and nucleoside 5′-triphosphates, the self-stacking tendency of the base moieties of the nucleic acids decreases in the series adenine > guanine ≳ hypoxanthine > cytosine ~ uracil. Due to the repulsion of the negatively charged phosphate moieties, the self-association is always less pronounced for NDP^{3-} compared with the corresponding nucleoside.

Accordingly, the addition of Mg^{2+} to ADP^{3-} favors self-stacking, as is obvious from Fig. 1: the curvature of the lines is more pronounced for the $Mg(ADP)^-$ system ($K = 6.4\ M^{-1}$) than for the ADP^{3-} system. Generally, the self-stacking tendency of the NDP^{3-} systems is promoted by a factor of about 2–3 by the coordination of Mg^{2+} to the phosphate moiety. However, the self-association tendency of $Zn(ADP)^-$ and $Cd(ADP)^-$ or $Zn(IDP)^-$ and $Cd(IDP)^-$ is much larger than of $Mg(ADP)^-$; this is explained [5] by an increased tendency to form an *inter*molecular metal ion bridge in dimeric stacks, which may

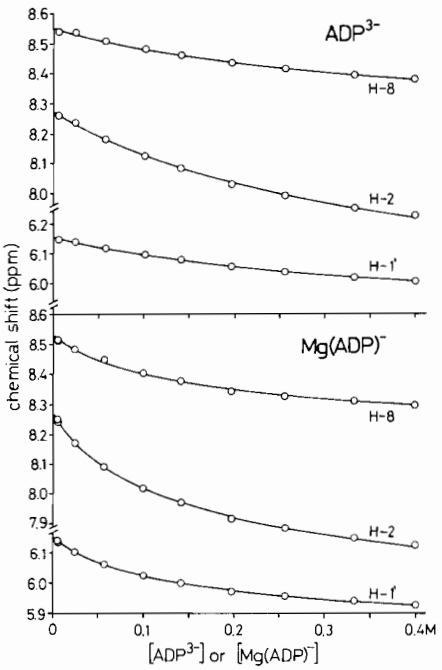


Fig. 1. Variation of the chemical shift for H-2, H-8, and H-1′ of ADP with varying concentrations of ADP^{3-} (pD 8.9) or $Mg(ADP)^-$ (pD 7.5). The spectra were measured on a Bruker FT 90 at 90.025 MHz (D_2O; 27 °C; I = 0.1 to ~1.7, $NaNO_3$). The curves are the computer-calculated best fits of the experimental data, using the indefinite non-cooperative stacking model: $K_{ADP} = 1.8 \pm 0.5\ M^{-1}$ and $K_{Mg(ADP)} = 6.4 \pm 0.9\ M^{-1}$).