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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.

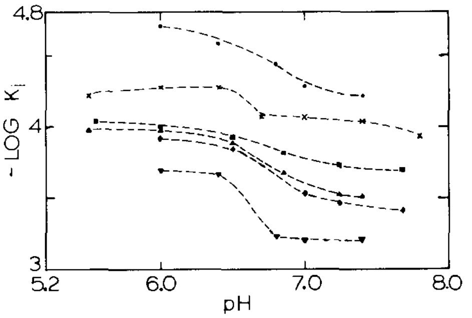

Fig. 1. Dependence of –log Ki as a function of pH for $Cr(H_2O)_4ADP$ (●); $Mg(H_2O)_4ADP$ (X); $Cr(NH_3)_3(H_2O)ADP$ (■); $Co(NH_3)_3H_2OADP$ (▲); $Cr(NH_3)_4ADP$ (◆); and $Co(NH_3)_4ADP$ (▼).

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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