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BIOCHEMICAL EFFECTS OF BINDING $[(H_2O)(NH_3)_5Ru^{II}]^{2+}$ AND $[(NH_3)_5Ru^{III}]$ TO DNA

The reaction of $[(H_2O)(NH_3)_5Ru^{II}]^{2+}$ with calf thymus and salmon sperm DNA has been studied over a wide range of transition metal ion concentrations in order to determine the binding sites of the metal ion to the heterocyclic bases. Kinetic studies revealed a biphasic reaction with an initial fairly rapid coordination of the metal ion being followed by slower reactions. Under these conditions a true equilibrium could not be reached; however, it was possible to study metal ion coordination under pseudo-equilibrium conditions after the initial reaction was complete and before subsequent reactions had progressed substantially. For covalently bound Ru levels up to 0.26 Ru per DNA nucleotide, (Ru_{DNA}/P_{DNA}), the predominant binding site on helical DNA is in the major groove at the N-7 of guanine. Spectra of $[(NH_3)_5Ru^{III}]_n$-DNA prepared by incubation of $[(H_2O)(NH_3)_5Ru^{II}]^{2+}$ with DNA (where $[P_{DNA}] = 1.5$ mM and reactant $[Ru^{II}]/[P_{DNA}]$ ratios are in the range 0.1 to 0.3) followed by air oxidation yielded intense charge transfer bands which could only be attributed to $[(NH_3)_5Ru^{III}]$ coordination to N-7 dG sites. HPLC of acid-hydrolyzed samples of $[(NH_3)_5Ru^{III}]_n$-DNA, which had been prepared by this method with helical DNA, also revealed the significant presence of only $[(Gua)(NH_3)_5Ru^{III}]$ for $0.1 < [Ru^{II}]/[P_{DNA}] < 0.5$, which was verified by UV-Vis identification of the isolated chromatographic band. An earlier X-ray molecular structure determination has established the coordination site as the imidazole N-7 of dG, which is exposed in the major groove of the DNA.

At $[Ru^{II}]/[P_{DNA}] \leq 0.5$ T_m values for the DNA decreased linearly with increasing ruthenium concentration and an increase in the intensity of the 565 nm charge transfer band was noted upon melting. The UV and CD spectra of these samples indicated no extensive destacking or alteration in geometry (B family) compared to unsubstituted DNA. At $[Ru^{II}]/[P_{DNA}] > 0.5$ or when single stranded DNA was used, increased absorbance at 530 nm and 480 nm suggested additional binding to the exocyclic amine sites of adenine and cytosine residues. HPLC and individual spectrophotometric identification of the products derived from hydrolysis of these species yielded both $[(Gua)(NH_3)_5Ru^{III}]$ and $[(Ade)(NH_3)_5Ru^{III}]$. Earlier crystallographic, spectroscopic and electrochemical studies have established the adenosine binding site of $[(NH_3)_5Ru^{III}]$ to be the exocyclic amine (N-6). Coordination to the exocyclic amines of adenine and cytosine, is indicative of double helix disruption since these amines are involved in hydrogen bonding on the interior of B-DNA. A highly metallated ($Ru_{DNA}/P_{DNA} = 0.26$) DNA sample found to be rapidly sedimenting and unable to electrophorese into an agarose gel appeared to have undergone counterion induced collapse, which is a phenomenon that has not been previously demonstrated for DNA covalently coordinated by a transition metal ion.

Agarose gel electrophoresis of superhelical PBR322 plasmid DNA after exposure to various amine complexes of $[(NH_3)_5Ru^{III}]$ in the presence of a reductant and air generally revealed moderately efficient cleavage of the DNA, presumably due to the generation of hydroxyl radical via Fenton's chemistry. However, similar studies involving $[(NH_3)_5Ru^{III}]$ directly coordinated to the DNA showed no strand cutting. Polyacrylamide gel electrophoresis of a 381 bp, 3'-^{32}P-labeled fragment of PBR322 plasmid DNA containing low levels of bound $[(NH_3)_5Ru^{III}]$ further indicated negligible DNA cutting by the coordinated metal ion.

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SYNERGISM IN THE METAL ION PROMOTED HYDROLYSIS OF ADENOSINE 5'-TRIPHOSPHATE

The enzyme-catalyzed transfers of phosphoryl or nucleotidyl groups depend on the presence of divalent metal ions, and the various stages of information transfer in genetic processes, DNA replication, RNA synthesis, and protein synthesis all require metal ions [1]. Hence, it is not surprising that the metal ion promoted dephosphorylation of nucleoside 5'-triphosphates, especially of adenosine 5'-triphosphate (ATP^{4-}), has long been recognized [2]. The simplest of these processes is the transfer of a phosphoryl group to water, and some insight into the mechanism of this reaction with ATP has been gained [3,4].

Many enzyme-nucleotide systems involve two (or more) metal ions [1]. Therefore, we are attempting to elucidate the influence of different metal ion mixtures on the dephosphorylation rate of ATP [5]. So far, the initial rates of dephosphorylation (*i.e.*, $v_o = d[PO_4]/dt$) of ATP (10^{-3} M) in the mixed metal ion systems $Cu^{2+}/ATP/Mg^{2+}$, Ni^{2+} or Zn^{2+} with the ratio 1:1:5 have been measured at pH 5.5 and compared with the binary ATP/M^{2+} 1:6 systems (see Table).

The remarkable result is that addition of Mg^{2+} to a Cu^{2+}/ATP 1:1 system accelerates the dephosphorylation rate significantly more than the same amounts of Mg^{2+} accelerate the reaction in a Mg^{2+}/ATP 1:1 system. The same synergism, based also on the Cu^{2+}/ATP 1:1 system is observed with Ni^{2+}, but not with Zn^{2+}. We attribute this observation, based on previous results [4], to the formation of a pre-reactive complex in the Cu^{2+}/ATP 1:1 system, *i.e.* of a $[Cu(ATP)]_2^{4-}$ dimer which involves purine-stacking and a Cu^{2+}/N-7 interaction; the inherent reactivity in this dimer may be triggered by the addition of Mg^{2+} or Ni^{2+}. Furthermore, we suggest that in the Zn^{2+}/ATP 1:1 system a corresponding pre-reactive state is formed and that therefore no synergism with Cu^{2+} is observed.

Table

Evidence for Synergistic Effects in the Dephosphorylation of ATP (10^{-3} M) at pH 5.5 by Mixed Metal Ion Systems ($I = 0.1$, $NaClO_4$; 50°C). The Effect of the Second Metal Ion on M^{2+}/ATP 1:1 Systems is Expressed by Rate Enhancement Factors (= REF)[a]. All Initial Rates are Given as $v_0 \times 10^8$ M s^{-1}

Effect of	system 1:1	system 1:1:5	v_o for 1:1	v_o for 1:1:5	REF [a]
Mg^{2+}	Mg/ATP		0.096		
		Mg/ATP/Mg		0.13	1.4
	Cu/ATP		2.5		
		Cu/ATP/Mg		9.7	4
Ni^{2+}	Ni/ATP		0.075		
		Ni/ATP/Ni		0.18	2.4
	Cu/ATP		2.5		
		Cu/ATP/Ni		9.5	4
Zn^{2+}	Zn/ATP		0.15		
		Zn/ATP/Zn		0.92	6
	Cu/ATP		2.5		
		Cu/ATP/Zn		14	6

a) REF = v_o of a 1:1:5 system divided by v_o of the corresponding 1:1 system; *e.g.*, 0.13/0.096 = 1.4 or 9.7/2.5 = 4. The rate data are summarized from several of our studies [3-7].

In accord with this interpretation are the results of preliminary experiments which suggest that there is also a very pronounced synergism in $Zn^{2+}/ATP/Mg^{2+}$ systems at pH 7.5. This is interesting because, *e.g.*, DNA and RNA polymerase [1] contain tightly bound Zn^{2+} and use nucleoside 5'-triphosphates as substrates, but for activity the enzymes require in addition Mg^{2+}.

ACKNOWLEDGEMENTS

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

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