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Binding of Pentaammineruthenium(III) to Double-Helical and Single-Stranded DNA

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Recent research into the interaction of square planar Pt(II) antitumor complexes with DNA has indicated that these ions interact preferentially with N-7 guanine sites, which are available in the major grove of DNA [1, 2]. Analogous studies with octahedral metal ions exhibiting *in vitro* biochemical behavior similar to the platinum agents has not been forthcoming. In this communication we report on the interaction of an octahedral metal ion, $(NH_3)_5RuOH_2^{2+}$, with helical and denatured DNA.

The aquo ion was prepared by standard methods [3] from $(NH_3)_5RuCl^{2+}$, which has been shown to exhibit biochemical properties similar to *cis*-$Cl_2(NH_3)_2Pt$ *in vitro*, namely the inhibition of cellular DNA synthesis [4]. In the case of the platinum complex this activity is thought to be initiated by metal coordination to cellular nucleic acids [5]. Direct DNA binding is also suspected as the initial step in DNA synthesis inhibition by the ruthenium species and DNA extracted from cells incubated in the presence of $[Cl(NH_3)_5Ru^{106}]^{2+}$ has been shown to contain radioactivity [7].

Investigations concerning the direct interaction of $(NH_3)_5RuCl^{2+}$ with DNA constituent bases indicate that complexes are formed slowly over a period of days. Reduction of the ion in solution either wholly or in part allows the complexes to form in a matter of minutes [8, 11]. The effect on DNA synthesis inhibition is therefore suspected to occur via a redox mechanism in which a cellular component reduces the Ru(III) to Ru(II) prior to binding. We have shown the feasibility of this mechanism in studies using succinate as a reductant in the presence of rat-liver mitochondria, $(NH_3)_5RuCl^{2+}$ and DNA. In the absence of the mitochondrial redox catalyst, no binding to DNA is observed. In its presence, spectra similar to those reported here for $[(NH_3)_5Ru]$–DNA were observed. The amount of metal–DNA binding was a function of the oxygen content of the solutions. When completely anaerobic conditions were employed, the intensity of the Ru–DNA absorptions was approximately an order of magnitude greater than when air was bubbled through the solutions during the course of the reactions [4, 9].

In the present study the aquo ion was prepared in 0.01 *F* phosphate buffer and syringed into DNA solutions deaerated with argon. The reactions were allowed to proceed for one hour and produced light yellow solutions. Oxidation by O_2 yielded blue to purple solutions. The visible spectra of solutions prepared following this procedure with coiled and heat-denatured DNA are shown in Figures 1 and 2 respectively. The absorption maxima in Figure 1 around

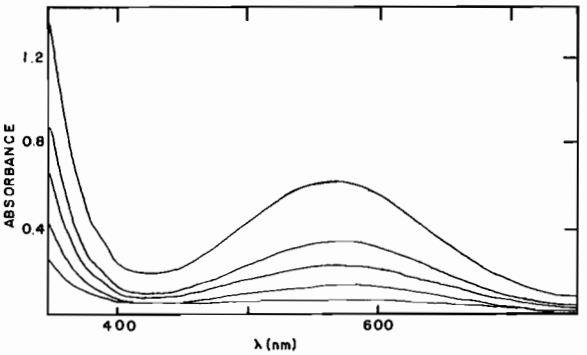

Fig. 1. Visible spectrum of $(NH_3)_5Ru(III)_n$–DNA in phosphate buffer. Guanine concentration is 1.4×10^{-3}. Ru/G ratios are 0.24, 0.48, 0.72, 0.96 and 1.31 with the higher ratios yielding the curves of higher absorbance.

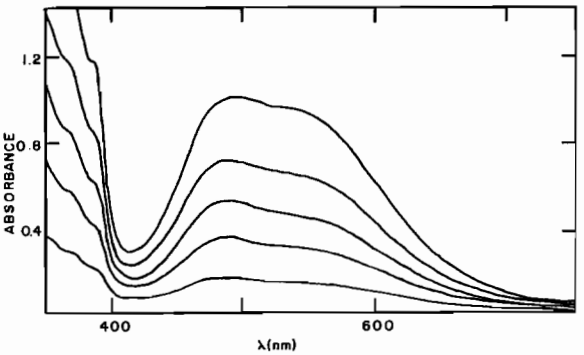

Fig. 2. Visible spectrum of heat-denatured $(NH_3)_5Ru(III)_n$–DNA in phosphate buffer. Guanine concentration is 1.4×10^{-3}. Ru/G ratios are 0.13, 0.28, 0.42, 0.59 and 0.87.

550 nm nearly coincides with that obtained for the 7-[(5′GMP)$(NH_3)_5Ru(III)$] ion [10, 11]. This absorption maximum shifts slightly to higher energy on increasing the Ru/G ratios indicating a small amount of binding to sites other than guanine at the higher ratios. The spectra shown in Figure 2 clearly indicate binding to additional sites in the case of denatured DNA.

Acid hydrolysis of these solutions by warming to 85 °C at pH 1–2 for one hour followed by ion-

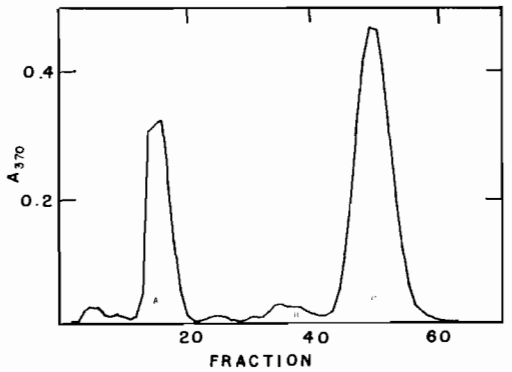

Fig. 3. Absorbance at 370 nm of fractions collected by ion exchange chromatography of hydrolyzed Ru–DNA.

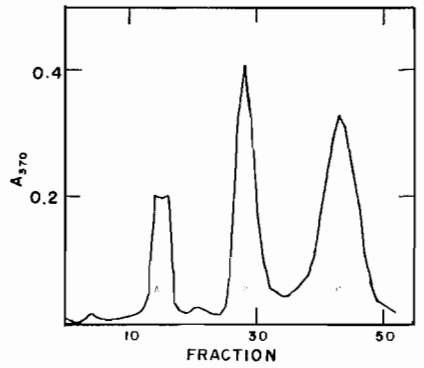

Fig. 4. Absorbance at 370 nm *vs.* fraction number of fractions collected by ion exchange chromatography of hydrolyzed Ru–DNA prepared with heat-denatured DNA.

exchange chromatography on a 1 cm diameter, 6 cm long, Biorex-70 column eluted with an ammonium acetate gradient of 0.2 to 1.5 *F* provided good separation of the purine-containing metal complexes. The absorbances of fractions collected employing this system for coiled and denatured Ru–DNA preparations is seen in Figures 3 and 4 respectively. Component A in these figures arises from the ruthenium starting material. Component C in both figures gives a spectrum identical with that reported for 7-[(Gua)-$(NH_3)_5$Ru(III)] under similar conditions. Component B gives a spectrum identical with that of [Ade$(NH_3)_5$-Ru(III)] [8].

The synthesis of the pentaaminеruthenium(III) complexes of adenosine, cytidine and guanosine is reported elsewhere [8, 10]. Chemical, spectral and crystallographic evidence indicates binding of the metal center to the N-7 of guanosine. Circumstantial chemical evidence indicates coordination at the N-1 of adenosine, but is also consistent with binding to the exocyclic amine [8]. Coordination to the N-7 of adenine residues in DNA under the reaction conditions reported here appears unlikely since this would be expected to occur concurrently with N-7 binding to guanine sites in double-helical DNA. Since binding to adenine residues occurs to a much greater extent in single-stranded DNA, coordination to a site which is blocked in helical DNA can be assumed. Close similarities in the spectra and chemistry of the adenosine and cytidine complexes predicates a similar coordination site for both, *i.e.* the pyrimidine ring nitrogen (N-1 of adenosine, N-3 of cytidine) or, conceivably, the exocyclic amine [8].

The results reported here clearly indicate that N-7 of guanine is the preferred coordination site for the $(NH_3)_5$Ru(II) group in double-helical DNA, but that adenine and cytosine residues also become available on uncoiling. The slight shift in the absorption peak at higher Ru/G ratios in Figure 1 indicates some binding to these additional sites which are normally involved in hydrogen bonding. This in turn suggests some degree of metal-ion-induced uncoiling of DNA.

Acknowledgments

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