Voltammetric studies of the interaction of tris(1,10-phenanthroline)cobalt(III) with DNA
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significant g-anisotropy for both bridgehead hydrogens (Table I). Together with the absence of
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fits in Figure
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2Bl
rather than the analogous carbon-centered 2A state for the
ring-closed parent oxirane cation,19 the change in the state ordering
made by using the modified Bloch equations for a two-jump
model,23 and the interconversion rate constants used to obtain the
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groups for the rigid-limit spectrum in plot a, while the coa-
exchange between 2H, and 2Hb and between 2H, and 2H,. The inten-
140-K spectrum in Figure
1
2.

Thus, the 140-K spectrum in Figure 1 corresponds to the 13
line groups for the rigid-limit spectrum in plot a, while the coa-
lescence spectrum at 160 K fits the reconstruction in plot c of line
components whose resonances remain unaltered by exchange (plots
a and b). Simulations of the temperature-dependent spectra were
made by using the modified Bloch equations for a two-jump
model,29 and the interconversion rate constants used to obtain the
fits in Figure 1 obeyed an Arrhenius relation with
A = 5.6 \times 10^{12}
s^{-1} and
E_a = 3.66 \text{ kcal mol}^{-1}. These parameters are very similar to those reported for ring inversion in the cyclohexyl radical25 and
the cyclohexanesemidione radical anion.26

An obvious corollary is that dynamic effects should be absent in
the rigid cation of syn-sesquinorbornene oxide (2). In

agreement, its ESR spectrum showed no temperature dependence
and consisted of a quintet from coupling to the four equivalent
bridgehead hydrogens (Table I). Together with the absence of
significant g-anisotropy for both 1 and 2, the detection of angular-
dependent couplings for hydrogens in \( \beta \) positions with respect
to the oxirane ring carbons strongly supports the assignment to
carbon-centered rather than oxygen-centered cations. Although
high-level ab initio MO calculations predict a \( ^2B_1 \) ground state
rather than the analogous carbon-centered \( ^2A_1 \) state for the
ring-closed parent oxirane cation,19 the change in the state ordering
on going to these rigid species is attributable to the effect of

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Voltammetric Studies of the Interaction of Tris(1,10-phenanthroline)cobalt(III) with DNA

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We report here how the changes in the cyclic voltammetric (CV) behavior of tris(1,10-phenanthroline)cobalt(III), Co-
(phen)\(^{3+} \), in an aqueous medium upon addition of DNA can be
used to probe the interaction between these species. Coordination complexes of 1,10-phenanthroline and 4,7-diphenyl-1,10-
phenanthroline with Ru(II) and Co(III),12 and other metal chelates26 that intercalate between the stacked base pairs of native

C. V.; Barton, J. K.; Turro, N. J. J. Am. Chem. Soc. 1985, 107, 5518. (d)
(2) (a) Barton, J. K.; Dannenberg, J. J.; Raphael, A. L. J. Am. Chem.
DNA, have been actively investigated as probes of DNA structure in solution and as stereoselective or conformation-specific agents for the photoactivatable cleavage of DNA. The nature of the interaction between these metallointercalation agents and DNA has primarily been studied by spectroscopic and X-ray crystallographic methods. While electrochemical investigations of DNA, via the reduction of the purine and pyrimidine bases, have been carried out, to our knowledge, no such studies of metallointercalation agents in the presence of double-stranded DNA have been published. Electrochemical studies of transition-metal complexes, e.g., of phen, have been extensive, and the effect of ligand concentration on potential can be used to determine formation constants. Thus electrochemical investigations of metal–DNA interactions can provide a useful complement to spectroscopic methods, e.g., for nonabsorbing species, and yield, as shown below, information about interactions with both the reduced and oxidized form of the metal.

Typical CV behavior of Co(phen)$_3^{3+}$ in the absence (curve 1) and presence (curve 2) of calf thymus DNA is shown in Figure 1.

Figure 1. Cyclic voltammograms of (A) 0.12 mM Co(phen)$_3^{3+}$ (1) in the absence ($E_{pc} = 0.095 V$, $E_{pa} = 0.160 V$) and (2) in the presence of DNA (5.3 mM nucleotide phosphate) ($E_{pc} = 0.115 V$, $E_{pa} = 0.175 V$) and (B) 0.10 mM Co(phen)$_3^{3+} + 0.11$ mM Mo(CN)$_6^{4-}$ (1) in the absence and (2) in the presence of 4.8 mM nucleotide phosphate. Supporting electrolyte, 50 mM NaCl, 5 mM Tris, pH 7.1. Sweep rate, 100 mV/s. Glassy carbon-working electrode (0.07 cm$^2$). All potentials reported vs saturated calomel electrode (SCE).

The addition of an excess of DNA causes the peak currents of the CV waves for reduction of Co(phen)$_3^{3+}$ to the 2+ form and anodic wave on the reverse scan to diminish considerably. The cathodic peak current, $ip$, shown in curve A2, decreased to ca. 40% of that in the absence of DNA. Additionally, the peak potentials, $E_{pc}$ and $E_{pa}$, both shifted to more positive values, equivalent to a shift in the formal potential of the Co(phen)$_3^{3+}/^{2+}$ couple, $E^0$ (taken as the average of $E_{pc}$ and $E_{pa}$), of 17 mV.

Figure 1. CV peak current for total Co(III) reduction for $3.02 \times 10^{-5}$ mol nucleotide phosphate titrated with 1.0 mM Co(phen)$_3^{3+}$. Initial solution volume 5 cm$^3$. Other conditions as in Figure 1. Solid curve represents the results for best fit parameters as given in text. Straight lines are the limiting slopes.

To show that the decrease in $ip$ is due to binding of Co(phen)$_3^{3+}$ to the large, slowly diffusing DNA and not to an increase in solution viscosity, we performed CV experiments on a mixture of Co(phen)$_3^{3+}$ (0.1 mM), which intercalates between the DNA base pairs and Mo(CN)$_6^{4-}$ (0.11 mM), which, because of its negative charge, should not interact with DNA. In the absence of DNA (Figure 1b), well-defined waves for the free Mo(CN)$_6^{4-}$ ($E^0 = 0.51 V$) and Co(phen)$_3^{3+}$ ($E^0 = 0.13 V$) couples are evident (curve B1). Upon addition of an excess of nucleotide phosphate (curve B2), $E^0$ and $ip$ changed as described previously (curve A). The $E^0$ of Mo(CN)$_6^{4-}$ was essentially unaffected by the addition of DNA, and the ratio of the cathodic peak currents, corrected for the background current, which increased upon DNA addition, before and after DNA addition was ca. 0.90. Thus, the small perturbation in the Mo(CN)$_6^{4-}$ waves by the

(12) Calf thymus DNA (Sigma Chemical Co.) was purified by phenol extraction (Maniatis, T.; Fritsch, E. F.; Sanbrook, J. Molecular Cloning; Cold Spring Harbor Laboratory, 1982).

DNA can be attributed to an increase in solution viscosity, while the larger changes in both $E^0$ and $I_C$ for Co(phen)$_2^{3+}$ are attributed to interactions with the DNA duplex.

CV experiments were carried out in which the ratio of DNA to Co(phen)$_2^{3+}$ was varied. The ratios are reported in terms of $R = [\text{nucleotide phosphate}]/[\text{cobalt(III)}]$. At $R = 0$, the diffusion coefficient of the free Co(phen)$_2^{3+}$, $D_0$, was obtained from the $I_C/2e^{-t/2}$ data ($5 \leq e \leq 200$ mV/s) as $3.6 \pm 0.8 \times 10^{-5}$ cm$^2$/s. At $R = 304.5$, the apparent diffusion coefficient of the bound metal complex, $D_B$, obtained by differential pulse voltammetry, was $3.1 \pm 1.6 \times 10^{-5}$ cm$^2$/s.

A titration of 30.2 nmol nucleotide phosphate with Co(phen)$_2^{3+}$ while measuring the total cathodic peak current ($I_T$) as a function of umol metal chelate added (C-T) or (R) gave the results shown in Figure 2. Two limiting regions are found. At large $R$ the current is attributed primarily to Co(phen)$_2^{3+}$ intercalated to DNA (characterized by a concentration, $C_B$, and $D_B$), while at very small $R$ the main contribution to $I_T$ is free Co(phen)$_2^{3+}$ in solution (concentration, $C_T$, and $D_T$). The total current at any $R$ is

$$I_T = B[D]^{1/2}C_T + D[2C_T]$$

where $B = 2.69 \times 10^5$ n$^{-1/2}$ A$^{-1/2}$ for a CV of a Nernstian wave at 25$^\circ$C. $C_T$ is related to the total metal added, $C_T$, with the assumption of control by the equilibrium binding of the chelate by DNA by

$$C_T = [b - (b^2 - 2K(C_T[NP])^{1/2})/2K]$$

where $K$ is the intrinsic binding constant of the +3 species, $n_i$ is the number of pairs required per Co(phen)$_2^{3+}$, and $[NP]$ is the nucleotide phosphate concentration. Equation 2 is valid for noncooperative, nonspecific binding with the existence of one type of discrete binding site. An analogous treatment could be used for a more complicated type of binding interaction.17

The small viscosity changes that occur upon addition of the metal ion solution are also neglected. A regression analysis of the data in Figure 2 to eq 1 and 2 yield the following: $K = 5.8 \times 10^5$ M$^{-1}$, $n_i = 6.7$ base pairs, $D_T = 4.1 \times 10^{-6}$ cm$^2$/s, $D_B = 1.2 \times 10^{-6}$ cm$^2$/s. The value for $n_i$ found here is somewhat larger than that reported for Ru(phen)$_2^{2+}$ ($n_i = 4$ bp). $K$ is close to that for the Ru(phen)$_2^{2+}$ ($K = 6.2 \times 10^5$ M$^{-1}$). The positive 40-mV shift in the peak potential for bound complex compared to free Co(phen)$_2^{3+}$ unequivocally shows the +2 species (binding constant, $K$) is bound more strongly than the +3 species with $K'/K = 4.8$; $K' = 2.8 \times 10^4$ M$^{-1}$. This stronger binding of +2 might be explained by the importance of hydrophobic interactions, in addition to electrostatic ones.14 Similar effects have been found, for example, in the interaction of viologens and metal chelates with micelles18 and perfluorosulfonated (Nafion) films.19

The results given here demonstrate that rather straightforward electrochemical methods can be employed to characterize the intercalative interaction between a metal complex or other electroactive species and DNA to yield estimates of the binding constants and binding site sizes. The electrochemical oxidation

and reduction of selected bound species on DNA can also be carried out and in favorable circumstances may allow chemical changes in the DNA, e.g., strand scission.

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2-Deoxy-2-fluoroglucosides: A Novel Class of Mechanism-Based Glucosidase Inhibitors

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Glucosidase inhibitors are of interest in the treatment of diabetes and obesity due to their potential in controlling blood glucose levels.1 Currently available glucosidase inhibitors include the noncovalent, naturally occurring inhibitors such as acarbose2 and nortijirimycin3 and covalent, mechanism-based inhibitors such as the conduritol epoxides4 and glucosylmethyltriazenes.5 This paper describes a novel mechanism-based glucosidase inhibitor based on a strategy which has not previously been exploited for this class of enzymes.

The enzymic hydrolysis of glucosidases likely proceeds through a glucosyl enzyme intermediate via oxocarbonium ion-like transition states as shown in Scheme 1.6 Therefore substitution of an electronegative fluorine atom for a hydroxyl group adjacent to the reaction center, at C-2, should destabilize these transition states and decrease both the rates of glycosylation ($k_1$) and deglycosylation ($k_2$). Indeed, we have synthesized several 2-deoxy-2-fluoroglucosides and glucosyl phosphates and found them to be very slow substrates for their respective glucosidases or glucosyl transferases, with $k_2$ values generally similar to those for the normal substrate. A similar approach has been employed previously7 in studies of terpene biosynthetic enzymes where reaction proceeds via carbocationic intermediates.

The incorporation of a highly reactive leaving group as the aglycone into such deactivated substrates might increase the glycosylation rate sufficiently to permit trapping of the 2-deoxy-2-fluoroglucosyl enzyme intermediate, therefore inhibiting the enzyme in a temporary covalent fashion. We describe here the synthesis8 and testing of such an inhibitor, 2,4-dinitrophenyl

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8. Hydrolysis of 3,4,6-tri-Dacetyl-2-deoxy-2-fluoro-D-glucopyranosyl bromide6 afforded the anomeric mixture of protected hemiacetals. Treatment of this mixture with 1-fluoro-2-4-dinitrobenzene in the presence of DABCO10 gave a mixture of the $\alpha$- and $\beta$-dinitrophenyl glycoside peracetates which was separated by fractional crystallization. Deprotection (NaOMe/MeOH) of the $\beta$-anomer afforded crystalline 1. Satisfactory spectral and analytical data were obtained for all compounds.

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