INFLUENCE OF DNA ON THE REACTION RATE OF AN ELECTRON TRANSFER PROCESS: A KINETIC STUDY OF THE REACTION BETWEEN $[Co(PYRAZINECARBOXYLATE)(NH_3)_4]^{2+}$ AND $[Fe(CN)_6]^{4-}$ IN AQUEOUS SOLUTIONS IN THE PRESENCE OF DNA

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The kinetics of the electron transfer reaction between tetraammine(pyrazinecarboxylate)cobalt(III), $[Co(pyrazinecarboxylate)(NH_3)_4]^{2+}$, and hexacyanoferrate(II), $[Fe(CN)_6]^{4-}$ was studied in aqueous solutions in the presence of DNA at concentrations 0–$2.28 \cdot 10^{-3}$ mol dm^{-3}. A decrease in the rate constant with increasing DNA concentration was observed. The results are interpreted on the basis of the pseudophase model. The meaning of its parameters for the second-order reaction is discussed.

Keywords: DNA; Electron transfer; Kinetics; Pseudophase model; Restricted geometry conditions; Ferrocyanides; Cobalt complexes; Pyrazinecarboxylates.

Studies of electron transfer reactions are important on their own and, in fact, constitute a classic research field since Marcus established his theoretical treatment[1,2]. In the last few years, these studies have been extended to conditions that imply a restriction in the movement of one or both reactants (*i.e.*, the donor and the acceptor)[3]. These conditions frequently induce drastic changes in the reactivity due to the fact that the properties of the local environment are quite different from those in the bulk of the solvent. The studies of electron transfer in the vicinity of DNA molecules[4] are of interest since DNA can capture positively charged reactants, producing restricted geometry conditions. These studies concern conductor properties of DNA [4], a field closely related to that of molecular electronics[5].

Along another line, an important question in structural biology is to characterise the interaction of DNA with small ligands that may have potential therapeutic effects, acting as sequence recognition agents or as modifiers of DNA properties[6]. At the same time, DNA can modify properties of

ligands and, consequently, change their reactivity. Thus, the studies of DNA/ligand interactions are of interest from both viewpoints.

The interactions of small molecules with DNA depend on a number of factors such as planarity, aromaticity, surface extension of the interacting moiety, electrostatic interactions, *etc.*[7] Separation of different (energetic) contributions of such factors seemed to be of interest. With this idea in mind, we undertook the present work that dealt with the study of changes in the rate of the electron transfer reaction between $[Co(pCOO)(NH_3)_4]^{2+}$ (pCOO = pyrazinecarboxylate) and $[Fe(CN)_6]^{4-}$. This reaction was selected in order to probe the interactions of one of the reactants ($[Co(pCOO)(NH_3)_4]^{2+}$) with DNA. These interactions are assumed to be only (or predominantly) electrostatic in nature, given that this complex does not intercalate between DNA bases. The other reactant ($[Fe(CN)_6]^{4-}$) bears a negative charge; thus, it can be safely assumed that, on average, this complex in a DNA-containing solution is distributed far from the DNA surface. Accordingly, the modified reactivity should arise only as a consequence of the interaction between the cationic reactant and DNA, and can be explained by the pseudophase model[8].

EXPERIMENTAL

Materials

Sodium hexacyanoferrate(II) and Na_2H_2edta (edta = ethylenediaminetetraacetate) were purchased from Fluka and Merck PA., respectively. The cobalt complex $[Co(pCOO)(NH_3)_4](ClO_4)_2$ was prepared by the described method[9] and twice recrystallized. Its visible absorption spectrum showed a single band at λ_{max} = 485–490 nm (ε_{max} = 70.5 mol^{-1} dm^3 cm^{-1}). Calf thymus DNA from Pharmacia was used without further purification[10]. Neither buffer solution nor background electrolyte were added. Thus, as documented by preliminary experiments, as long as the ionic strength of the investigated solutions is kept constant, the addition of the buffer does not modify the kinetic result. The need of a background electrolyte in order to maintain a constant ionic strength in the aqueous phase in contact with DNA, was checked[10] and no change in this parameter was observed. Consequently, the observed variation of the rate constant is only caused by the presence of DNA.

Polynucleotide concentrations were determined spectrophotometrically, considering the molar absorption coefficient of 6 600 mol^{-1} dm^3 cm^{-1} at 258 nm [11].

Water obtained from a Milli-Q water system had conductivity lower than 10^{-6} S cm^{-1}.

Kinetic Measurement

Kinetic runs were carried out in the thermostatted cell compartment of a Grating 722 UV-VIS spectrophotometer. A manual syringe system was used to mix the reactant solutions. The cell temperature was kept constant at 298.2 ± 0.1 K.

The kinetics were followed at 420 nm, the wavelength of maximum absorbance for the hexacyanoferrate(III) ions, produced in the reaction:

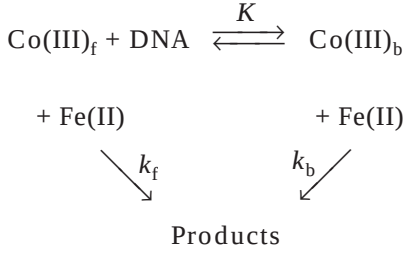
$$[Co(pCOO)(NH_3)_4]^{2+} + [Fe(CN)_6]^{4-} \rightarrow [Co(pCOO)(NH_3)_4]^{+} + [Fe(CN)_6]^{3-}$$

The reactant concentrations were [Co(III)] = $1.7 \cdot 10^{-4}$ mol dm^{-3} and [Fe(II)] = $8.3 \cdot 10^{-4}$ mol dm^{-3}. All solutions were freshly prepared and deaerated by bubbling N_2 through them to avoid hexacyanoferrate(II) oxidation. $Na_2(H_2edta)$ had to be added to prevent precipitation of $Co_3[Fe(CN)_6]_2$ by binding the produced Co^{2+} ions as $[Co(edta)]^{2-}$. The concentration used for this species was $1.7 \cdot 10^{-4}$ mol dm^{-3}. The DNA concentration ranged from $5.4 \cdot 10^{-5}$ to $2.28 \cdot 10^{-3}$ mol dm^{-3}.

Pseudo-first-order rate constants were obtained from the slope of ln ($A_t - A_\infty$) *versus* time, where A_t and A_∞ are the absorbances at time t and when the reaction is completed, respectively. These plots were good straight lines up to, at least, three half-lives.

RESULTS AND DISCUSSION

Table I presents the values of the observed rate constant corresponding to the reaction between $[Co(pCOO)(NH_3)_4]^{2+}$ and $[Fe(CN)_6]^{4-}$ complexes. These data are plotted in Fig. 1. They reveal that the effect of DNA on this reaction is similar to that caused in this type of processes by micelles[12], which can be explained by employing the pseudophase model. According to this model, the reaction takes place through two parallel paths, involving free and associated (with DNA in the present case) states of the cobalt complex, as in Scheme 1.



SCHEME 1

Subscripts f and b denote free and bound states of the cobalt complex; these states are in equilibrium and K is the equilibrium binding constant of the cobalt complex to DNA. The cobalt complex reacts in these states with the iron complex with rate constants k_f and k_b, respectively. According to Scheme 1, in the case where only one of the reactants is distributed between free and bound states, the observed (pseudo-first-order) rate constant is given by Eq. (*1*).

TABLE I
Observed rate constant k_{obs} (in s^{-1}) for the reaction between $[Co(pCOO)(NH_3)_4]^{2+}$ and $[Fe(CN)_6]^{4-}$ at T = 298.2 K and different DNA concentrations

$10^5\ c_{DNA}$, mol dm^{-3}	$10^2\ k_{obs}$, s^{-1}
0	6.70
5.4	5.80
9.1	4.80
11.4	4.50
18.0	3.70
25.0	3.15
36.5	2.70
45.5	2.40
66.0	2.00
91.0	1.60
114.0	1.40
135.0	1.30
155.0	1.10
180.0	0.88
228.0	0.82

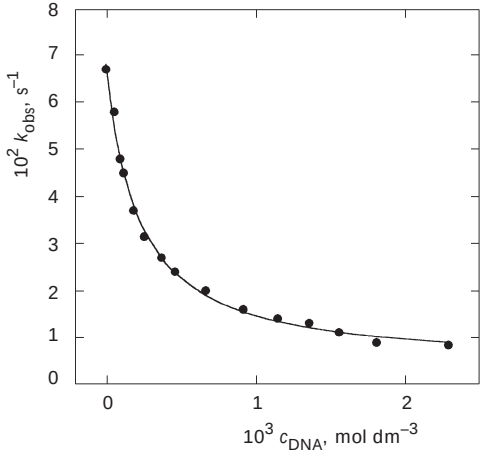


FIG. 1
Plot of k_{obs} (in s^{-1}) *vs* the DNA concentration. Experimental data (●) and values calculated using Eq. (*1*) (——)

$$k_{obs} = \frac{k_f + Kk_b[DNA]}{1 + K[DNA]} \quad (1)$$

In this equation, K is given by Eq. (*2*).

$$K = \frac{[Co(III)]_b}{[Co(III)]_f[DNA]}, \quad (2)$$

where [Co(III)]b and [Co(III)]f are the concentrations of the free and associated cobalt complexes, respectively. The constants k_f and k_b are the pseudo-first-order rate constants that refer to the total concentration of complex $[Fe(CN)_6]^{4-}$, $[Fe(II)]_t$. These constants are related to the second-order rate constants for the free and bound ions, respectively, by Eqs (*3*) and (*4*)[13].

$$k_w = \frac{k_f}{[Fe(II)]_w} \quad (3)$$

$$k_{DNA} = \frac{k_b}{[Fe(II)]_{DNA}} \quad (4)$$

Here k_w and k_{DNA} are the second-order rate constants and $[Fe(II)]_w$ and $[Fe(II)]_{DNA}$ the concentrations of the hexacyanoferrate(II) ion in the aqueous and DNA pseudophases, respectively.

In this case it applies (Eq. (*5*)):

$$[Fe(II)]_w \approx [Fe(II)]_t . \quad (5)$$

On the other hand, if we define a parameter κ as by Eq. (*6*)[13],

$$\kappa = \frac{[Fe(II)]_{DNA}}{[Fe(II)]_w}, \quad (6)$$

it follows that (Eq. (*7*))

$$[Fe(II)]_{DNA} \approx \kappa[Fe(II)]_t . \quad (7)$$

Note that the parameter κ in Eq. (*6*) has a meaning different from that of the parameter *K* in the pseudophase model (see Eq. (*2*)). However, both parameters are related by Eq. (*8*)[13]:

$$K[\mathrm{DNA}] = \kappa\varphi \,, \tag{8}$$

where φ is the volume ratio $\varphi = V_w/V_{DNA}$; V_w and V_{DNA} are the volumes of aqueous and DNA pseudophases, respectively.

The complete set of data in Table I can be fitted to Eq. (*1*) with the following parameters:

$$k_f = k_w[\mathrm{Fe(II)}]_w = 6.8 \cdot 10^{-2}\ \mathrm{s^{-1}}$$

$$k_b = k_{DNA}[\mathrm{Fe(II)}]_{DNA} \approx k_{DNA}\kappa[\mathrm{Fe(II)}]_w = 3.3 \cdot 10^{-3}\ \mathrm{s^{-1}} \tag{9}$$

$$K = 4\,742\ \mathrm{mol^{-1}\ dm^3}\,.$$

According to this result, the rate of the reaction is diminished by the binding of the cobalt complex to DNA due to a low value of $[\mathrm{Fe(II)}]_{DNA}$ (see below). On the other hand, the value of the binding constant K = 4 742 $\mathrm{mol^{-1}\ dm^3}$ is similar to that obtained for this complex in a previous study in DNA solutions[10]. Note that as κ is not known, it is impossible to decode whether the intrinsic reactivity of this process is higher or lower at the DNA surface than in water. However, a clue to this problematics can be obtained as follows. The value of *K* does not differ much from the *K* values for closely related complexes in micellar solutions[12]. In these solutions, the difference of the electric potential between the micellar and aqueous pseudophases lies in the order of 100 mV[14]. Thus, assuming that κ is mainly determined by electrostatic interactions, its value is given by Eq. (*10*):

$$\kappa = \exp -\frac{Z_{Fe}F\phi}{RT} = 1.7 \cdot 10^{-7}\,, \tag{10}$$

where Z_{Fe} is the charge of the complex $[\mathrm{Fe(CN)_6}]^{4-}$, *F* is the Faraday constant and ϕ the surface potential of DNA. From this result and Eq. (*9*) it follows that $k_{DNA} >> k_w$. Thus, the decrease in the rate of the reaction occurring close to DNA molecules is due to a decrease in the concentration

of one of the reactants ($[Fe(CN)_6]^{4-}$) rather than to a decrease in the intrinsic reactivity, as given by the second-order rate constants.

The quality of the fit (using Eq. (*1*)) can be verified by comparing the calculated values of k_{obs}, as given by the curve in Fig. 1, with the measured values. The results of this fit are given in a different way in Fig. 2, where the calculated values of k_{obs} are plotted *versus* the experimental ones. Apparently, a good straight line is obtained (slope = 0.996, r = 0.998).

It is worth pointing out that the results obtained in this work are similar to those obtained by Cusumano *et al.*[7,15] in studies of the influence of DNA on the rate of ligand substitution reactions. In these studies, the authors have explained the observed behaviour taking into account the pseudophase model, *i.e.* the kinetic effect of DNA related to the binding of complexes to the DNA double helix. However, the mechanism of the binding of the complexes to DNA depends on the environment of the polynucleotide helix and on the nature of the complex. In fact, other authors have obtained data showing that the binding constant depends on the DNA concentration, performing the reaction under different conditions[10]. They found that K increases when the [cationic complex]/[DNA] ratio decreases and this fact was explained taking into account anticooperative binding[16] of the cationic complex to DNA.

In conclusion, double-helix DNA inhibits the electron transfer reaction between cationic and anionic complexes and its influence can be explained by the pseudophase model.

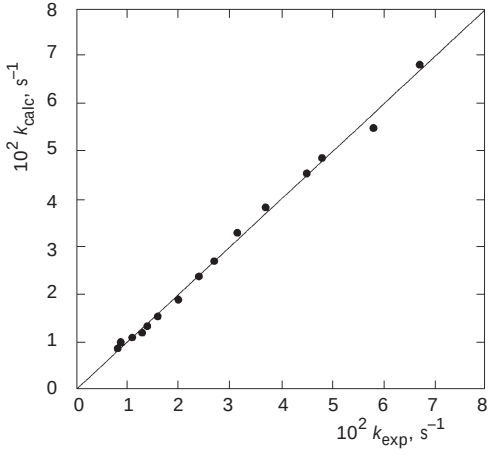


FIG. 2
Plot of k_{calc} *vs* k_{exp} (both in s^{-1})

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