DNA as a catalyst and catalytic template for the racemisation of metal tris-phenanthroline complexes [‡]

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$[Fe(1,10\text{-phenanthroline})_3]^{2+}$ is known to racemise in solution and DNA is known to shift the equilibrium point of the Δ/Λ-$[Fe(1,10\text{-phenanthroline})_3]^{2+}$ mixture from 50:50 to a position favouring a slight excess of the Δ enantiomer. In this paper it is shown that DNA catalyses the racemisation reaction, presumably by providing vibrational energy to species that bind in a less favoured mode for that enantiomer. The racemisation of $[Co(1,10\text{-phenanthroline})_3]^{3+}$, by way of contrast, is not enhanced by DNA, contrary to literature claims, unless $[Co(1,10\text{-phenanthroline})_3]^{2+}$ is also added to the solution. In this case, the DNA acts as a template to facilitate the Co(II) ↔ Co(III) electron transfer. Racemisation occurs while the $[Co(1,10\text{-phenanthroline})_3]^{n+}$ is in the labile Co(II) form.

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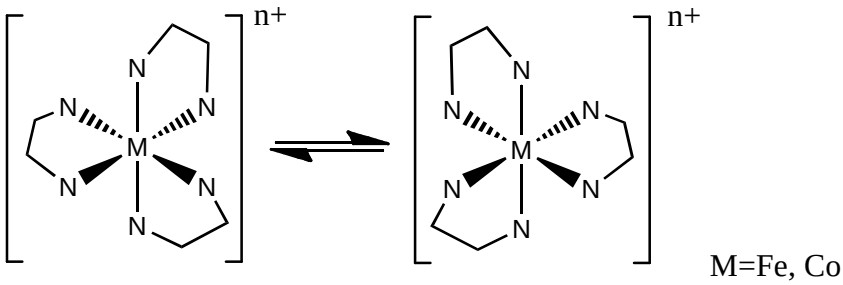
Introduction

The presence of DNA has long been known to shift the position of the Δ/Λ equilibrium of [Fe(1,10-phenanthroline)$_3$]$^{2+}$ in favour of the Δ enantiomer on the timescale of minutes to hours.[1,2] There is also a suggestion in the literature that [Co(1,10-phenanthroline)$_3$]$^{3+}$ rapidly racemises over a period of minutes,[3] despite the well accepted stability of Co(III) d^6 amine systems, and there is some suggestion that DNA affects this rate. We therefore chose to investigate both these racemisation reactions in the presence and absence of DNA in order to determine what if any role DNA plays in the kinetics.



Results and Discussion

[Fe(1,10-phenanthroline)$_3$]$^{2+}$: DNA catalyses a reaction

The results of the kinetic experiments with a range of DNA:Δ and DNA:Λ ratios for [Fe(1,10-phenanthroline)$_3$]$^{2+}$ are given in Table 1. All *CD* traces (not shown) fitted to a single exponential decay, the rate of change in the *CD* signal that is given in the final column is the constant in the exponent. For the simple case of free solution racemisation, the rate constant for the, *e.g.*, Δ→ Λ reaction is half this constant as shown below. The error range quoted shows the scatter between repeat runs. The rate of racemisation in the DNA-free systems at 20ºC and 25ºC, assuming a simple Arrhenius behaviour, gives an activation energy of ~ 40 kJ mol^{-1}. Mudasir *et al.* recently reported a rate constant for the racemisation of the Λ enantiomer at room temperature (stated to be approximately 25°C) to be (0.72±0.05)×10^{-3} s^{-1}.[4] Their value was estimated from two runs where points were measured at intervals (rather than the trace monitored continuously as in our experiments). Assuming their data has been correctly analyzed, in order to be consistent with our data, the room temperature in their experiments must have been above 25° (~28°C) or their error has been underestimated.

The first observations to be made from our data are that the end point of the kinetics is the same for given concentrations of metal complex and DNA independent of which enantiomer is added initially, however, the value of the end point *CD* signal varies as a function of the DNA concentration. This variation is due to the DNA shifting the positions of the Δ/Λ equilibrium (the end point is when the equilibrium is established) as previously observed by Härd and Nordén [1,2] and also the perturbation of the *CD* of the cations on binding to DNA. The final observed *CD* signal at 545 nm is always positive. As the unbound metal complex will be a 50:50 mix of the two enantiomers at the conclusion of the kinetics, and so have no net *CD*, this means that the bound metal complexes must always have a net positive *CD*. Two factors might contribute to this, a Pfeiffer-effect shift in the equilibrium between the two enantiomers to give an excess of one enantiomer [1,2] and/or a positive *CD* signal being induced into the transition metal complex transitions due to their interaction with the DNA.[5]

To try to understand this behaviour at a molecular level it is helpful to consider the binding modes that have been established [5,6,7] for $[Ru(1,10\text{-phenanthroline})_3]^{2+}$ (which does not racemise in solution) since previous experience suggests that any information on binding modes determined for ruthenium complexes can be transfered to the iron (II) complex with the same ligands since the charge and steric effects are almost identical. At the low ligand loadings, the Δ enantiomer is predominantly a minor groove binder with either one or two chelates pointing into the groove whereas the Λ enantiomer is 90% in a major groove mode with a chelate partially inserted between two base pairs and a lower equilibrium binding constant than the Δ enantiomer. When its preferred DNA binding modes have been fully occupied then each enantiomer occupies less favoured modes and the Λ enantiomer on average binds more strongly.[5,7] Whether the site preference is perturbed by the presence of the other enantiomer is not clear, though the racemate induced *CD* spectra [5] suggest the binding modes are not changed when the other enantiomer is present. However, the two enantiomers must reduce each other's binding constants since the racemate binds more weakly to DNA than either enantiomer.[5] For $[Ru(1,10\text{-phenanthroline})_3]^{2+}$ above ~5:1 DNA:Δ or DNA:Λ ratios the most favoured sites are occupied. Thus in our experiments with $[Fe(1,10\text{-phenanthroline})_3]^{2+}$, except at the beginning of the 2.5:1 runs, it is reasonable to conclude that each enantiomer occupies its preferred site at all times. This means that we therefore have conditions where the Δ enantiomer binds more strongly than the Λ enantiomer, so the positive final *CD* could simply be due to the Pfeiffer effect causing an excess of Δ.

The other observation to be taken from Table 1 is that the racemisation rates of both enantiomers speed up when DNA is present but to differing extents. At high DNA concentrations, where the Δ enantiomer has a stronger DNA binding constant, [5] the rate of racemisation of this enantiomer is the faster, whereas at lower DNA concentrations, where the Λ enantiomer binds more strongly to DNA this enantiomer's rate is more enhanced.

The rate enhancements observed in the presence of DNA might be due to the shift of the equilibrium position of the reaction or might be due to the DNA facilitating the reaction and providing a slightly lower energy pathway for racemisation. In order to determine which of these is occurring, let us assume that the latter is not the case, in other words, the DNA-bound molecules do not rearrange (or do so at the free solution rate), and deduce the consequences. We are thus, assuming that the presence of the DNA affects only the concentration of free molecules in solution that can rearrange and plays no direct role in the racemisation reaction acting only to change the end point of the reaction due the differing percentages of the two bound enantiomers.

Racemisation reactions probed using *CD* spectroscopy measure the rate of change of the *CD* signal (rather than the actual disappearance of one reactant or appearance of one product). To determine the rate of change of the metal complex concentration in the presence of DNA we need to determine the concentrations of each species present as a function of time and multiply by the appropriate Δε values and pathlengths (Beer Lambert Analogue). In addition to assuming that the DNA-bound metal complexes cannot racemise, let us also assume that the bound and free forms are in fast equilibrium compared with the rate of racemisation (a reasonable assumption even if the on-rate is diffusion controlled as the off-rate will be at worst in the microsecond domain given the binding constants of references [5,7]). This is schematically illustrated as in Equation (1)

$$\begin{array}{ccc} x + S_f & \xleftrightarrow{K_x} & X \\ k_1 \downarrow \uparrow k_1 & & \\ y + S_f & \xleftrightarrow{K_y} & Y \end{array} \qquad (1)$$

where x is the free concentration of the enantiomer that begins in excess, X is its bound concentration, k_1 is the rate constant for the conversion of one enantiomer to the other in free solution, K_x is the equilibrium binding association constant for species x with DNA, and the y symbols relate to the other enantiomer.

Because the DNA binding is in fast equilibrium, the rate of change of x and y may be written:

$$\frac{dx}{dt} = k_1(-x + y)$$
$$\frac{dy}{dt} = k_1(x - y) \quad (2)$$

or in matrix notation

$$\frac{d\mathbf{c}}{dt} = \boldsymbol{\Lambda}\mathbf{c}$$
$$\text{where } \mathbf{c} = \begin{pmatrix} x \\ y \end{pmatrix} \text{ and } \boldsymbol{\Lambda} = k_1 \begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix} \quad (3)$$

Solving Equation (3) gives

$$x(t) = \frac{x_o}{2}(1 + e^{-2k_1 t})$$
$$y(t) = \frac{x_o}{2}(1 - e^{-2k_1 t}) \quad (4)$$

where x_o is the starting concentration of x.

The *CD* signal as a function of time may be written

$$CD(t) = \Delta\varepsilon_x(x(t) - y(t)) + \Delta\varepsilon_X X(t) + \Delta\varepsilon_Y Y(t) K_y \quad (5)$$

where $\Delta\varepsilon_x$ is the *CD* extinction coefficient for the unbound enantiomer that is in excess at the beginning of the reaction, $\Delta\varepsilon_X$ is the *CD* extinction coefficient for the x when it is bound to DNA *etc.* Since

$$K_x = \frac{X(t)}{x(t) S_f(t)} \quad (6)$$

where $S_f(t)$ is the concentration of free sites on DNA, from Equations (4) and (5) we may write

$$CD(t) = \Delta\varepsilon_x(x_o e^{-2k_1 t}) + \Delta\varepsilon_X K_x(\frac{x_o}{2} + \frac{x_o}{2} e^{-2k_1 t}) S_f(t) + \Delta\varepsilon_Y K_y(\frac{x_o}{2} - \frac{x_o}{2} e^{-2k_1 t}) S_f(t) \quad (7)$$

which is the *CD* signal as a function of time *if the enantiomers may only racemise in free solution.*

The two sources of time dependence in Equation (7) are the single exponential decay terms with constant $-2k_1$ and $S_f(t)$. The latter can be deemed approximately time independent since the binding constants of the two enantiomers are similar. (At the high DNA:metal complex ratios, by analogy with the $[Ru(1,10\text{-phenanthroline})_3]^{2+}$, 99% of the metal complex will be bound and 1% free for both enantiomers so the amount of free DNA is large and not varying much during the reaction. At the low DNA:metal complex ratios very little of the DNA is unoccupied throughout the reaction. So in both cases, $S_f(t)$ is approximately constant in any one run.) Thus if racemisation occurs only for free metal complexes (as required for Equation (7)), then the rate of decay of the *CD* signal is $-2k_1$ for all DNA concentrations. Table 1 summarizes how the rate of decay of the *CD* signal *changes*

with DNA concentration; we therefore must conclude that both enantiomers also rearrange when bound to the DNA and Equation (7) is not valid. In other words *the DNA acts as a catalyst for this reaction, slightly lowering the activation energy compared with the free-solution value.*

One can speculate as to how the DNA achieves its catalytic effect. The most likely possibility is that the metal complexes will be moving on and off the DNA and if one enantiomer hits the DNA in one of its less favoured binding positions (*e.g.* the wrong groove) then during its time on the DNA it is encouraged to bind more strongly by changing its handedness. The natural breathing motions of the DNA would help provide the energy required to break the metal-ligand bond (as required for metal *tris* chelate racemisation reactions with a few exception such as dithiocarbamate complexes [8,9]) and the DNA will encourage the formation and net accumulation of the desired product for that DNA binding mode.

$[Co(1,10\text{-phenanthroline})_3]^{3+}$: DNA as a catalytic template

Following literature claims of racemisation of $[Co(1,10\text{-phenanthroline})_3]^{3+}$ [3] in the presence of DNA on a timescale too fast for NMR experiments, we attempted to characterize the kinetics of this process. However, we found no evidence of racemisation for the Co(III) complex prepared as described in the methods section either with or without DNA in the solution. We concluded that the previously observed effect must have been due to trace amounts of the labile Co(II) complex remaining in solution — our oxidation conditions involved chlorine gas. Electron transfer occurs readily between the Co(II) and Co(III) *tris*-phenanthroline complexes and the Co(II) form is labile and racemises readily. The net effect of this is the racemisation of the Co(III) complex. We therefore probed the kinetics of the racemisation of $[Co(1,10\text{-phenanthroline})_3]^{3+}$ in the presence of $[Co(1,10\text{-phenanthroline})_3]^{2+}$ and DNA.

The racemisation of the Co(III) complex proceeds slowly in the presence of the Co(II) complex with a half life of the order of hours (depending on reagent concentrations) rather than minutes or seconds. In a series of experiments at widely varying concentrations of the two metal complexes and DNA, we observed that the DNA causes a significant increase in the rate of racemisation of the Co(III) complex in the presence of the Co(II) complex. In other words the DNA enhances the catalytic activity of the Co(II) complex taking it to half lives of a few seconds (at high enough concentrations, see Table 2). As with the $[Fe(1,10\text{-phenanthroline})_3]^{3+}$, the rate of change of *CD* as a function of time in an individual run follows a simple exponential decay, however, the

dependence of the rate on the concentration of each reagent is not straightforward as illustrated by a data set for the Λ-enantiomer in Table 2. These observations can be understood when it is realised that the DNA is forming a template to facilitate the electron transfer reaction between two cobalt *tris*-phenanthroline complexes of different oxidation states. The DNA may also be playing a more active role in the electron transfer. Increased concentrations of the cobalt complexes make such encounters on the DNA more likely. If *e.g.* Co(III) is in excess, however, it will inhibit Co(II) binding and decrease the effect. Similarly, decreasing the DNA concentration increases the likelihood of an encounter between the reacting species molecules until the same saturation of occurs.

In conclusion, we have shown that DNA catalyses the racemisation reaction of both Δ- and Λ-$[Fe(1,10\text{-phenanthroline})_3]^{2+}$ but has no effect on the stability of Δ- and Λ-$[Co(1,10\text{-phenanthroline})_3]^{3+}$. However, in the presence of (±)-$[Co(1,10\text{-phenanthroline})_3]^{2+}$ DNA increases the effectiveness of the Co(II) complex catalysis of the racemisation of both Δ- and Λ-$[Co(1,10\text{-phenanthroline})_3]^{3+}$ by acting as a template to enhance the rate of electron transfer between the labile Co(II) and stable Co(III) species. The concentration dependence of this process is complicated and maximized for DNA concentrations that encourage Co(II) and Co(III) to be bound together on the DNA. Too high a concentration of DNA reduces the nearest neighbour interaction of the cobalt complexes and too high a concentration of one complex relative to the other means that complexes of different oxidation state are less likely to meet. The Co(II) with its intrinsically weaker binding constant is particularly likely to be displaced.

Thus we have an example of a DNA catalyzed reaction where the DNA does not chemically take part but does provide some energy (~ 40 kJ mol^{-1}) to help the rearrangement and another reaction where DNA is a catalytic template.

Experimental Section

The Δ and Λ enantiomers of $[Fe(1,10\text{-phenanthroline})_3]^{2+}$ were produced from cold (just above 0°C) 0.025 M ferroin solution (Aldrich) by adding potassium antimonyl tartrate (also at 0°C) in a 2:1 molar ratio. $NaClO_4$ (Aldrich) was added to the filtrate and the resulting crystals were collected. The L enantiomer of antimonly tartrate (Aldrich) was used to produce the Λ-enantiomer of $[Fe(1,10\text{-phenanthroline})_3]^{2+}$. The D enantiomer of antimonly tartrate, used to produce the Δ-enantiomer of $[Fe(1,10\text{-phenanthroline})_3]^{2+}$, was made by mixing D-tartaric acid and KOH and then

adding Sb_2O_3 all in equimolar ratios. The solution was either boiled until all the Sb_2O_3 appeared to have dissolved or, for better yield, heated under reflux for 2 days. The solution was filtered and the filtrate left to evapourate to dryness producing D-potassium antimonyl tartrate. [Fe(1,10-phenanthroline)$_3$]$^{2+}$ enantiomeric purity was determined using the literature values of $\varepsilon_{510\ nm}$ = 11200 mol^{-1} dm^3 cm^{-1} and $\Delta\varepsilon_{545\ nm}$ = 20.0 mol^{-1} dm^3 cm^{-1} for the Δ enantiomer.[10] The Δ enantiomer proved to be 90% enantiomerically pure and the Λ enantiomer 103% (suggesting the literature value for $\Delta\varepsilon$ is an underestimate).

The racemisation kinetics of [Fe(1,10-phenanthroline)$_3$]$^{2+}$ were monitored at 20°C at 545 nm in the presence and absence of calf thymus DNA (Sigma, highly lyophilised sodium salt) using a Jasco J-720 spectropolarimeter. Concentrations in DNA base were determined using $\varepsilon_{258\ nm}$ = 6600 mol^{-1} dm^3 cm^{-1}.[11] All runs could be fitted with a simple single exponential decay (using Kaleidagraph) and the results are reported in Table 1 in terms of the decay constants of the *CD* signal. All runs were repeated at least once. To ensure that the solutions did not racemise during preparation the [Fe(1,10-phenanthroline)$_3$]$^{2+}$ was kept cold until mixed with the DNA solutions. In fact, the apparent first order nature of the kinetics (even under stopped flow conditions where data collection started after tens of ms (data not shown)) meant this precaution proved to be unnecessary.

Λ-[Co(1,10-phenanthroline)$_3$]$^{2+}$ was synthesized according to reference [12] analogously to the [Fe(1,10-phenanthroline)$_3$]$^{2+}$ case. To produce the Co(III) complexes, the least soluble antimonly tartrate salt was isolated and then oxidized with chlorine gas to give yellow crystals of resolved [Co(1,10-phenanthroline)$_3$]$^{3+}$. As discussed below it proved crucial to fully oxidize the Co(II) at this stage. The Δ enantiomer was prepared analogously to the Λ enantiomer using the potassium antimonly D-tartrate as for the Fe complexes. This method gave a >90% yield and higher enantiomeric purity than the Lee method (17% yield).[12] Alternative literature methods for producing enantiomerically pure Co(III) *tris*-chelates are more complicated and require expensive reagents.[13] [Co(1,10-phenanthroline)$_3$]$^{3+}$ enantiomeric purity was determined using the literature values of $\varepsilon_{273\ nm}$ = 68000 mol^{-1} dm^3 cm^{-1} and $\Delta\varepsilon_{281\ nm}$ = 222.0 mol^{-1} dm^3 cm^{-1} for the Δ enantiomer.[14] The Δ enantiomer was 90% enantiomerically pure and the Λ enantiomer 94%.

The racemisation kinetics of [Co(1,10-phenanthroline)$_3$]$^{3+}$ was monitored using a Jasco J-720 at 379 nm in a 1 cm cuvette or at 307 nm in a 0.8 mm cuvette with a Biologique SFM-3 stopped-flow system attached between the sample compartment and the photomultiplier tube. In the stopped flow system: the light beam was focused with a single lens;[15] data were collected with a band width of 2

nm, response time of 1 s; and the samples were loaded as follows: syringe 1: Co(III) sample, syringe 2: premixed Co(II) sample and DNA, and syringe 3.

Table 1 Rate of change of *CD* signals for both enantiomers of $[Fe(1,10\text{-phenanthroline})_3]^{2+}$ in aqueous solution with and without calf thymus DNA. The columns give the following data: (1) DNA concentration, (2) Δ- or (3) Λ-$[Fe(1,10\text{-phenanthroline})_3]^{2+}$ concentrations, (4) DNA:ligand ratio, (5) final *CD* reading in millidegrees, and (6) the single exponential decay constant for the *CD* signal as a function of time. All data were collected at 20°C except for the final row. The (±#) indicates the variation between identical runs.

[DNA]/μM	[Δ]/μM	[Λ]/μM	Ratio	Final *CD*/mdeg	$k\times1000/s^{-1}$
0	121		0:∞	0	0.43 (±0.005)
301	121		2.5:1	6	0.53 (±0.01)
608	121		5:1	11	0.78 (±0.01)
1210	121		10:1	22	0.95 (±0.01)
2540	121		21:1	31	1.44 (±0.005)
0		112	0:∞	0	0.43 (±0.005)
280		112	2.5:1	4	0.63 (±0.01)
560		112	5:1	10	0.76 (±0.01)
1120		112	10:1	21	0.83 (±0.005)
2352		112	21:1	29	1.05 (±0.01)
0 (25°C)		112		0	0.56 (±0.01)

Table 2 Rate of change of *CD* signals for both enantiomers of Λ-$[Co(1,10\text{-phenanthroline})_3]^{3+}$ in aqueous solution in the presence of (±)-$[Co(1,10\text{-phenanthroline})_3]^{2+}$ and calf thymus DNA. The columns give the following data: (1) DNA concentration, (2) Λ-$[Co(1,10\text{-phenanthroline})_3]^{2+}$ concentrations, (3) (±)-$[Co(1,10\text{-phenanthroline})_3]^{2+}$ concentration, (4) DNA:Co(III) ligand ratio, (5) the single exponential decay constant for the *CD* signal as a function of time. All data were collected at 20°C. (–) in column 5 indicates too slow a rate to measure with the stopped flow system. The +3 charged Co(III) complex binds much more strongly to DNA than does the Co(II) complex, hence its smaller concentrations.

[DNA]/μM	[Λ-Co(III)]/μM	[±-Co(II)]/μM	DNA:Co(III)	$k\times100/s^{-1}$
790	69	500	11.4:1	18
790	69	1000	11.4:1	23
790	69	1500	11.4:1	20
790	56	250	14:1	22

790	111	250	7.1:1	21
790	166	250	4.8:1	17
790	222	250	3.6:1	11
790	277	250	2.9:1	12
160	56	450	2.9:1	14
160	111	450	1.4:1	9
160	166	450	0.96:1	7
160	222	450	0.72:1	6.5
160	277	450	0.58:1	5
160	56	250	2.9:1	10
160	111	250	1.4:1	8
160	166	250	0.96:1	6
160	222	250	0.72:1	5
160	277	250	0.58:1	3.5

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