Absence of chiral discrimination in the interaction of tris(diphenylphenanthroline)ruthenium(II) with DNA

Hye-Kyung Kim, Per Lincoln, Bengt Nordén and Eimer Tuite*

Department of Physical Chemistry, Chalmers University of Technology, S-412 96 Göteborg, Sweden

Δ- and Λ-$[Ru(dpphen)_3]^{2+}$ (dpphen = 4,7-diphenyl-1,10-phenanthroline) bind non-intercalatively to B-form DNA and, unlike other octahedral metal complexes, show insignificant enantioselectivity in their interaction.

Octahedral tris-diimine metal complexes possess two enantiomers having right (Δ)- and left (Λ)-handed propeller forms which might be intuitively anticipated to interact favourably with hosts having the same helical chirality, *e.g.* the right (B)- and left (Z)-handed forms of DNA, respectively. Indeed, most of these complexes have been found to interact to some degree stereoselectively with DNA.[1,2] The most dramatic reported example of such behaviour is that of $[Ru(dpphen)_3]^{2+}$, (dpphen = 4,7-diphenyl-1,10-phenanthroline) for which it has been claimed that the Δ-enantiomer interacts specifically with B-form DNA and the Λ-enantiomer specifically with Z-form DNA.[3–5] It was also suggested that this compound might bind to DNA by intercalation, perhaps with a single phenyl ring of one ligand threading through the helix to lie in the opposite grove.[6] However, the hydrophobicity and limited solubility of the salts of this complex which have been examined (Cl^-, ClO_4^-, PF_6^-) necessitated the addition of an organic solvent (10% Me_2SO in the cited studies) which could potentially have perturbed the DNA structure and influenced the findings.

In order to study the interaction of $[Ru(dpphen)_3]^{2+}$ with DNA in purely aqueous medium, we have prepared the diacetate salt of the complex, which is more soluble than those previously examined.† The experiments have been carried out in acetate buffer which minimizes precipitation problems and allows spectroscopic measurements in aqueous solution.

Interaction with DNA is demonstrated by changes in the absorption spectrum of the complex when calf thymus (CT) DNA is present [Fig. 1]. The MLCT (metal-to-ligand charge transfer) band in the visible region experiences a red-shift, a change of band shape and hyperchromism, and the changes are of equal magnitude for both enantiomers. This contrasts with the behaviour of related metal complexes such as the externally binding $[Ru(bpy)_3]^{2+}$,[6] the non-intercalating $[Ru(phen)_3]^{2+}$,[7,8]

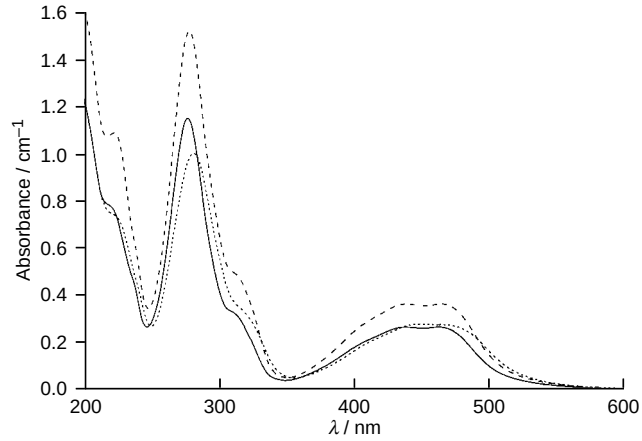
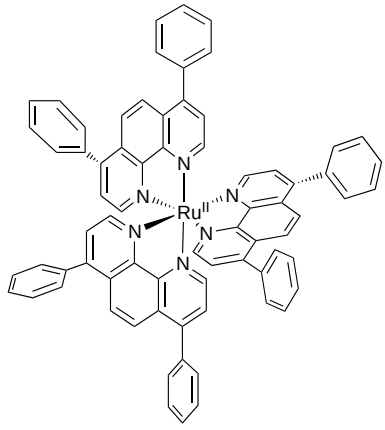
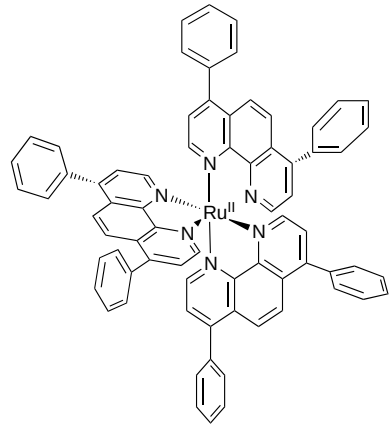


Fig. 1 Absorption spectra of Δ-$[Ru(dpphen)_3]^{2+}$ (10 μM) in 5 mM sodium acetate buffer (pH 6.0) (—) and 50% ethanol (---) and, after subtraction of the DNA spectrum, in the presence of CT DNA (500 μM) (···). Identical changes were observed for Λ-$[Ru(dpphen)_3]^{2+}$.

and the intercalating $[Ru(phen)_2(dppz)]^{2+}$:[9] in these cases, the red-shifts in the MLCT band in the presence of DNA are accompanied by hypochromism. In order to further investigate the cause of the unusual spectral changes for $[Ru(dpphen)_3]^{2+}$, which are suggestive of a de-aggregation process, the effect of adding ethanol to an aqueous solution of the complex was examined.‡ In 50% ethanol [Fig. 1], *ca.* 33% hyperchromism, without substantial red-shifts or changes of band-shape, was observed over the entire spectrum for each enantiomer which likely results from de-aggregation of the hydrophobic metal complex, since addition of the same quantity of ethanol to a solution of the much less hydrophobic $[Ru(phen)_3]^{2+}$ (dichloride salt in the same buffer) has only a very slight hyperchromic effect (*ca.* 5%, data not shown). In the presence of DNA, it is likely that the observed spectral changes result from a combination of hyperchromism due to de-aggregation, and a



Λ-$[Ru(dpphen)_3]^{2+}$



Δ-$[Ru(dpphen)_3]^{2+}$

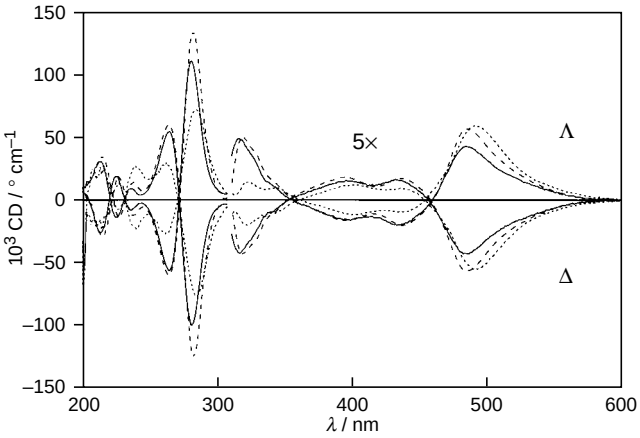


Fig. 2 CD spectra of $[Ru(dpphen)_3]^{2+}$ enantiomers (10 μM) in 5 mM sodium acetate buffer (pH 6.0) (—) and 50% ethanol (---) and, after subtraction of the DNA spectrum, in the presence of CT DNA (500 μM) (···)

red-shift, altered band shape and hypochromism owing to the interaction with the nucleic acid (features which are quite marked in the UV region after subtraction of the DNA spectrum, assuming the latter is unaltered). The similarity of the absorption changes for Δ- and Λ-$[Ru(dpphen)_3]^{2+}$ indicates similar binding modes and affinities for both enantiomers.

In order to probe for enantioselectivity in the interaction of $[Ru(dpphen)_3]^{2+}$ with B-DNA, the circular dichroism (CD) of the complex was measured in the absence and presence of CT DNA. The Δ- and Λ-enantiomers have intrinsic CD spectra which are mirror images of each other. In the presence of DNA [Fig. 2], almost identical CD changes of equal magnitude but opposite sign were observed for the two enantiomers both in the VIS and, after subtraction of the DNA spectrum, in the UV. The CD changes due to addition of ethanol [Fig. 2] are not identical to the changes caused by DNA, so DNA binding does induce CD in addition to changes that accompany de-aggregation. Although changes of CD indicate interaction of the complex with DNA, it is not possible to interpret these changes in absolute structural terms. However, for other related metal complexes the interaction with DNA generally produces CD changes that are non-symmetric,[8,9] even for the external binder $[Ru(bpy)_3]^{2+}$. Hence, the CD spectroscopy indicates that both enantiomers interact in a similar manner with B-form DNA. This conclusion was supported by dialysis experiments of the racemate *vs*. CT DNA in which no enantioselectivity could be detected.

Linear dichroism (LD) spectroscopy has proven to be an excellent discriminator between different modes of binding of small molecules to DNA through comparison of the average orientation of the DNA bases with that of known transition moments in the molecule.[10] When $[Ru(dpphen)_3]^{2+}$ (25 μM) is added to a concentrated solution of CT DNA (500 μM), the linear dichroism observed for the metal complex in the visible region is extremely small (for Λ at *ca*. 420 nm, LD^r = +0.023 normalized to perfect orientation), even by comparison with the non-intercalating $[Ru(bpy)_3]^{2+}$ complex (for Λ at *ca*. 420 nm, LD^r = +0.261). This suggests that although $[Ru(dpphen)_3]^{2+}$ interacts with DNA, the orientation of the bound complex is very random which is not consistent with an intercalative geometry of the bound ligand. There is, however, a notable decrease of LD magnitude in the DNA band in the presence of $[Ru(dpphen)_3]^{2+}$ which indicates that the metal complex has a sufficiently intimate interaction with DNA to decrease its orientation.§ The DNA orientation in the presence of either Δ- or Λ-$[Ru(dpphen)_3]^{2+}$ is about 60% that of free DNA. This is a further indication of the similarity of binding of Δ- and Λ-$[Ru(dpphen)_3]^{2+}$ to B-DNA. Other metal complexes have also been found to influence DNA orientation[8,9,12] but unlike $[Ru(dpphen)_3]^{2+}$ the enantiomers usually have significantly different effects: for $[Ru(phen)_3]^{2+}$ which binds enantioselectively under similar conditions, the Δ-enantiomer causes a 50% reduction in orientation while the Λ-enantiomer affects orientation only slightly.[8]

Our conclusions are that $[Ru(dpphen)_3]^{2+}$ is an extremely hydrophobic molecule of low solubility that neither intercalates nor binds stereoselectively to B-form DNA.

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Footnotes and References

* E-mail: etuite@phc.chalmers.se

† Resolution of racemic $[Ru(dpphen)_3]^{2+}$ was accomplished by repetitive recrystallization of its arsenyl salt from ethanol–water [arsenyltartrate of L-(+)-tartaric acid gave the Δ-enantiomer and D-(−) gave Λ]. The complex was converted to its acetate salt by dissolution of the arsenyltartrate salt in hot acetic acid and subsequent precipitation with saturated aqueous sodium acetate. The enantiomeric purities were estimated to be > 95%.

‡ At concentrations < *ca*. 20 μM there was no precipitation of $[Ru(dpphen)_3]^{2+}$. Light-scattering experiments did not indicate the formation of large aggregates (detection limit *ca*. 40 nm) and no residual absorbance was observed above 600 nm which provided a qualitative indication of light scattering for concentrations above 50 μM.

§ The LD^r of added methylene blue[11] at low binding ratio was used as an internal orientation reference.[9,12]

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