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Photophysical Study of a New Rhodium-Aminopyrene Complex as a Model for a Long-Lifetime Fluorescent DNA Probe

Lisa C. Franklin
McNair Scholar

Stephanie A. Schaertel, Ph.D.
Faculty Mentor

ABSTRACT:
Tri-l-aminopyrenetrichlororhodium (III) (APR), a new rhodium-aminopyrene complex with potential as a fluorescent DNA probe, has been synthesized. Fluorescence studies were done using steady-state fluorescence and nanosecond time-resolved fluorescence lifetime studies. This complex has a relatively long fluorescence lifetime in methanol. The lifetimes vary from 4 to 6 nanoseconds (ns) with various solvents and pHs. The lifetime variation and frequency-domain characteristics of the complex are explored in terms of the perturbation of the 1-aminopyrene electronic structure caused by complexation to a metal. The complex has been found to be promising as a DNA probe because of its water solubility, long fluorescent lifetime, and potential for binding site selectivity.

Introduction
Syntheses of metal-ligand complexes that have potential to be used as fluorescent DNA probes are on the cutting edge of research because of interest in DNA structure and dynamics. Jackie Barton's group at the California Institute of Technology has synthesized rhodium and ruthenium complexes that show binding site selectivity. Her research team developed Rh(phen)$_2$ (phen = 1, 10 phenanthroline), a complex that binds to DNA via intercalation of its phenanthroline ligands (Barton, 1990). The interesting characteristic of this complex is that it fluoresces only when its ligands are inserted between the base pairs of DNA and not when in aqueous solution. Her team also synthesized several other complexes which exhibit binding site selectivity. For example, her team developed A-Rh (phen)$_2$ phi$^+$ (phi = 9,10-phenanthrenequinone diimine), and enantiomers of this complex which act as sequence-dependent structural probes (Campisi, 1994).

The binding site selectivity depends upon the local variation in structure at the specific binding site. Her group also discovered that there is selectivity in binding with respect to the major and minor groove. These shape-selective probes are useful in helping to understand the propeller motion of DNA in solution.

We have previously developed a new rhodium-amine complex, 1-aminopyrenetrichlororhodium (III) (APR, shown in Figure 1) that has potential as a long-lifetime fluorescent DNA probe (Franklin 1997). This complex is slightly soluble in water and has a relatively long-lifetime. The synthesis for APR was modeled after the syntheses performed by Dixon, et.al. (1984). Researchers who synthesize rhodium-amine complexes tend to use rhodium trichlorotrihydrate (RhCl$_3$(H$_2$O)) because the waters of hydration make the compound soluble in water, moreover because rhodium metal does not tend to quench fluorescence as do many transition metals (Gollman, 1966; Greenwood, 1984). Rhodium can also act as a Lewis acid, accepting electrons from a nitrogen bound to the ligand. Nitrogen, which bears a lone pair of electrons, is what makes 1-aminopyrene a good ligand (Figure 1). The nitrogen on the 1-aminopyrene can act as a Lewis base, donating its lone pair of electrons to rhodium. 1-aminopyrene also contains aromatic rings, making it a flat planar molecule. This configuration means that it could be possible for the 1-aminopyrene ligands to insert between the base pairs of DNA. 1-aminopyrene also has a long-lifetime and a high fluorescent yield.

Reactions of rhodium-amine complexes tend to proceed under specific conditions. For example, Gillard and others have discovered that reactions of this type must use ethanol not just as a solvent but also as a catalyst (Gillard 1965). Also, Dixon et.al. have always performed these reactions at pH 7 (Dixon, 1984). After careful experimentation, we have also concluded that these reaction conditions need to be met for a successful synthesis (Franklin 1997).
more slowly than fluorescence. Internal conversion is another deactivation process in which a molecule vibrationally relaxes to its ground electronic state. Finally an excited state reaction (not depicted in Figure 2) can also occur.

As mentioned above, steady state and time-resolved fluorescence are used to study the effects of complexing a metal to the ligand. We predicted that the lifetime of the ligand will shorten when it is complexed to a metal center. Lianos et al have shown that substituted pyrenes show fluorescence lifetimes which are shorter than that of the parent molecule pyrene molecule because perturbation of the pyrene symmetry makes the \( S_1 \rightarrow S_0 \) transition more allowed (Lianos 1980). The complexation should perturb the symmetry of the ligand's electronic structure allowing symmetry forbidden transitions to occur more easily. In making these transitions more allowed, easy absorption occurs, thus, easy fluorescence. Since the fluorescence lifetime depends upon the transition between excited electronic states and the ground electronic state, the lifetime should shorten as this transition becomes more allowed.

**Methodology**

The materials used for the syntheses were purchased from Aldrich Chemical Company. The starting materials were used without further purification.

**Synthesis I:**

The synthesis for APR is given in Franklin (1997). Crystals have yet to form under the recrystallization conditions thus far, instead a brown powder forms.

**Synthesis II:**

A new complex has been synthesized following the same procedure for the synthesis of APR except that no HCl was added. 0.400g of AP was dissolved in 20 ml ethanol with constant stirring. To this solution was added 0.100g of RhCl₃·H₂O dissolved in 5 ml E-pure water. After mixing for 5 minutes, the pH was adjusted to ~7 using 6M NH₃. The reaction mixture was refluxed for two hours and the product was collected through vacuum filtration. The product was orange and proper recrystallization conditions have yet to be determined.

**Attempted Syntheses:**

Additional syntheses were tried to remove inner-sphere chlorides. One method called for vigorously stirring Rh(1-aminopyrene)Cl, in concentrated ammonium hydroxide. Another method used Rh(NH₃)₆, Rhodium hexaammine, as a starting material. Again 1-aminopyrene was dissolved in ethanol and to this mixture was added Rh(NH₃)₆. The pH was adjusted to 7 and the mixture was refluxed for two hours. The last attempted synthesis included dissolving some of the complex in a small amount of ethanol and adding silver oxide and water. The mixture refluxed for approximately 2 hours. None of the reactions succeeded in replacing the inner-sphere chlorides and further experiments will be performed to replace or remove the inner-sphere chlorides.

**Steady State Spectroscopy:**

Absorbance spectra were taken using a Perkin Elmer Lambda 6 UV/VIS spectrophotometer and fluorescence spectra were taken on a Perkin Elmer LS 50 fluorometer. The emission spectra were always taken at the fixed excitation wavelength of 337 nm at which our laser apparatus operates. The excitation spectra were taken a fixed emission wavelength subject to where the maximum emission intensity was found to be.
**Fluorescence Lifetime Measurements:**

Figure 3 represents a view of the laser apparatus. An LSI pulsed nitrogen laser was used to excite the molecules. The laser operated at a wavelength of 337 nm with a pulse of approximately 3 nanoseconds (ns) in width. The laser pulse was left unfocused because the fluorescence signal was strong. The Pyrex sample cell was placed in a black box to minimize the light coming from the outside environment. A low pass filter was also used to reduce the effects of outside light. A monochromator and a Hamamatsu 1P28 photomultiplier tube (PMT) were connected to the black box. There was a slit in the black box to allow fluorescent light to enter the monochromator/PMT system. The fluorescent light was focused onto the slit of the black box with a lens (focal length of 4 cm). A cutoff filter was placed in front of the slit to help minimize the amount of scattered laser light reaching the PMT. This filter had a cut-off of approximately 380 nanometers (nm). The PMT was connected to a Lecroy 9310 digital oscilloscope. The oscilloscope was triggered by reflecting a portion of the laser pulse off the beam splitter onto a photodiode trigger.

**Computer Modeling Studies:**

Modeling was done on a Silicon Graphics workstation with the Cerius II program from Molecular Simulations Incorporated. Energy calculations were done using a classical force field method.

**Results and Discussion**

**Characterization of New Complex**

The new complex synthesized was found to be orange in color. It decomposes at 189°C. Solubility tests show it to be more soluble in the solvents of intermediate polarity and in water than APR. This property makes it more promising as a DNA probe. Proper recrystallization conditions are still being determined, and elemental analysis will follow. Studies of this new complex will be continued in the future.

**Characterization of APR**

APR is a red complex that decomposes at 238°C. APR is soluble in most solvents of intermediate polarity such as methanol, ethanol, and acetone. It is also soluble in water as a micromolar solution. Although APR is slightly water soluble, it would be more soluble in water if the inner-sphere chlorides were outer-sphere and therefore replaced by another ligand. In this case, APR would be ionic and able to form ion-dipole interactions with the surrounding water. This complex would be more useful for DNA studies if it were more water soluble because of the water-based environment of DNA. Therefore experiments will continue in order to synthesize a more water soluble complex. Recent attempts to synthesize a more water soluble complex are described in the Methodology section of this paper.

**Table 1. Elemental Analysis of APR**

<table>
<thead>
<tr>
<th>Elements</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
<th>%Rh</th>
<th>%Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted</td>
<td>66.95</td>
<td>3.86</td>
<td>4.88</td>
<td>11.95</td>
<td>12.35</td>
</tr>
<tr>
<td>Obtained</td>
<td>67.21</td>
<td>3.70</td>
<td>4.94</td>
<td>12.08</td>
<td>10.31</td>
</tr>
</tbody>
</table>

Note. *Elemental Analysis performed by Desert Analysis, Tucson, AZ.*
**Absorbance Spectroscopy**

The absorbance spectra of 1-aminopyrene and APR are shown in Figure 4. The absorbance spectrum of APR reveals some absorbance for the complex between 420 nm and 550 nm that was not apparent in the spectrum for 1-aminopyrene. This increase in absorption could be due to the disturbance of symmetry caused by the complexation to a metal. Previous workers have assigned the long wavelength peak of the aminopyrene spectrum centered at 350 nm as two overlapping peaks corresponding to the $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ states (Lianos, 1980). The short wavelength peak corresponds to the $S_0 \rightarrow S_1$ transition. Unlike its parent molecule, pyrene, whose $S_0 \rightarrow S_1$ transition is very weak for reasons of symmetry (Nakajima, 1971), 1-aminopyrene shows a strong absorbance at long wavelengths (Lianos, 1980). This long wavelength absorption is due to the amine substituent that removes the totally symmetric nature of pyrene ground state. It is therefore reasonable to expect that complexes of 1-aminopyrene would also have long wavelength absorption and that complexation to a metal would make the forbidden transitions of 1-aminopyrene more allowed.

**Fluorescence Spectroscopy**

Figure 5 exhibits the excitation spectrum of the complex compared to that of 1-aminopyrene. The spectra are very similar in shape although the relative peak height intensities differ. The emission spectra shown in Figure 6 show that complexation to a metal does not have a profound effect on the 1-aminopyrene emission spectrum.

**Time-Resolved Lifetime Studies**

For reasons mentioned previously, it was understood that APR's fluorescent lifetime might be shorter than that of 1-aminopyrene. Excessive lifetime shortening would result in lifetimes that are no longer accessible with a simple nitrogen laser system but would require a more costly picosecond system. In addition, large scale DNA motions which are meant to be probed with fluorescent probes occur on longer timescales, so lifetime shortening is not advantageous. The results shown in Table 2 and 3 indicate that there is little or no lifetime shortening upon complexation of 1-aminopyrene to a rhodium metal center. For example, in degassed methanol at pH 9, the 1-aminopyrene lifetime is $6 \pm 1$ ns and the APR lifetime was $5 \pm 1$ ns. Although there may be a slight lifetime shortening, these values overlap within error bars. It is encouraging that complexation does not appear to lead to lifetime regimes which are too short for experimental convenience.
Table 2. Lifetimes of 1-Aminopyrene

<table>
<thead>
<tr>
<th>Solvent</th>
<th>pH</th>
<th>Lifetimes emission λ 440 nm</th>
<th>Lifetimes (Literature)</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol (nondegassed)</td>
<td>9</td>
<td>4 ± 1 ns</td>
<td>5.68 ns* (Sarpal, 1992, 1993)</td>
<td></td>
</tr>
<tr>
<td>Methanol (degassed)</td>
<td>9</td>
<td>6 ± 1 ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (nondegassed)</td>
<td>9</td>
<td>4 ± 1 ns</td>
<td>6.95 ns*◊ (Sarpal, 1992, 1993) and 5.20 ns* (Hite, 1986)</td>
<td></td>
</tr>
<tr>
<td>Water (degassed)</td>
<td>9</td>
<td>4 ± 1 ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water w/ 10% Methanol</td>
<td>7</td>
<td>6 ± 1 ns</td>
<td>5.40 ± 0.50** ns (Shizuka, 1981)</td>
<td></td>
</tr>
</tbody>
</table>

Note. *These literature references do not state whether or not the sample was degassed. ◊ This reference does not state the pH at which the sample was analyzed or how the sample was dissolved in water. ** This sample was dissolved in a 10% water/methanol mixture; the pH was stated to be 7.

Table 3. Lifetimes of APR

<table>
<thead>
<tr>
<th>Solvent</th>
<th>pH</th>
<th>Lifetimes emission λ 440 nm</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol (nondegassed)</td>
<td>7.69</td>
<td>4 ± 1 ns</td>
<td></td>
</tr>
<tr>
<td>Methanol (degassed)</td>
<td>9.26</td>
<td>5 ± 1 ns</td>
<td></td>
</tr>
<tr>
<td>Water (nondegassed)</td>
<td>7.69</td>
<td>6 ± 1 ns</td>
<td></td>
</tr>
<tr>
<td>Water (degassed)</td>
<td>9.26</td>
<td>5 ± 1 ns</td>
<td></td>
</tr>
<tr>
<td>Water w/ 10% Methanol</td>
<td>7</td>
<td>4 ± 1 ns</td>
<td>5.47 * 10⁻³ M</td>
</tr>
</tbody>
</table>
SGI Modeling Results:
Energy calculations using a classical force field method found that the facial isomer is less energetically favorable than the meridional isomer. We had predicted that the meridional structure would be the more energetically favorable because two of the aminopyrene ligands are at 180° and the other is 90° from the other two. On the other hand, in the facial isomer the aminopyrenes are all 90° away from each other.

In addition, a modeling study was done in which APR was allowed to approach a rigid DNA β-helix in order to observe possible binding site geometries. It was found that the APR could intercalate its ligand when the complex rested in the DNA major groove; however, minor groove binding was sterically blocked.

Conclusion
The new complex synthesized, tri-1-aminopyrenetrichlororhodium (III) (APR) displays promise as a long-lifetime fluorescent DNA probe. Many of its properties have been found to be useful for a potential probe of DNA, specifically, its water solubility, relatively long fluorescent lifetime, and molecular geometry. Its molecular geometry has been shown via computer modeling studies to make the molecule selective for major groove binding. A second, more water soluble complex has been synthesized and remains to be studied.

Acknowledgments
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