Designing Photoreactive Ruthenium$^{II}$ Complexes for Biomedical Applications

Kevin GARNIR*, Lionel MARCÉLIS, Andrée KIRSCH-DE MESMAEKER, Cécile MOUCHERON

Chimie Organique et Photochimie,
Université libre de Bruxelles, Faculté des Sciences,
Av. Franklin D. Roosevelt 50, 1050 Brussels
Tel.: +32(0)2/650 25 27
Fax: +32(0)2/650 30 18
E-mail: kevin.garnir@ulb.ac.be

The development of therapeutic agents represents one of the main research objectives in the fight against cancer. In this context, the “laboratoire de Chimie Organique et Photochimie” focuses its interest in the design of photoreactive Ruthenium$^{II}$ complexes. The interesting photoreactivity is mainly due to the photooxidizing properties of complexes bearing π-deficient ligands. It has been demonstrated that a photo-induced electron transfer (PET) is indeed possible between a DNA guanine moiety and a Ru$^{II}$ complex containing at least two π-accepting ligands. This primary PET process can lead to the formation of a photoadduct, in which a covalent link is formed between the Ru$^{II}$ complex and a DNA strand. This covalent binding allows the development of gene silencing strategies where two complementary oligonucleotides (ODNs) are irreversibly photocrosslinked.

The present work concerns the preparation and a first photophysical study of new Ru$^{II}$ complexes based on the 2,2'-bipyrazine (bpz) ligand in order to potentially use them as phototherapeutic agents.

Ru$^{II}$ complexes; 2,2'-bipyrazine; photo-induced electron transfer; DNA; DNA photoadducts

Introduction

At the origin of 7.6 million deaths, or almost 13% of the global mortality in 2008 [1], oncological diseases actually represent a major public health problem to be tackled. Since the discovery of Cisplatin by Barnett Rosenberg in 1964 [2] the use of metal-based drugs as antitumor agents became effective in medicine. The search for new metal-based compounds giving rise to a more effective therapeutic action and fewer side effects also developed strongly.

In this framework some polyazaaromatic Ru$^{II}$ complexes appear to be particularly attractive, especially as photo-reagents towards biomolecules such as DNA.[3,4] In contrast to the well known [Ru(bpy)$_3$]$^{2+}$ based on the bpy (2,2'-bipyridine) ligand, these compounds exhibit properties which give rise to a very interesting mode of action towards DNA.

More precisely, by comparaison to the bpy ligand some of the chelated polyazaaromatic ligands carry supplementary unchelated nitrogen atoms that confer to the system a very good π-accepting character. If two or more π-accepting ligands, such as 1,4,5,8-tetraazaphenanthrene (TAP), are chelated to the Ru$^{II}$ centre, the $^{3}$MLCT (Metal to Ligand Charge Transfer) excited state of the resulting complex, populated by visible absorption, is sufficiently oxidizing to extract an electron from rather poor reductants, such as guanine moieties.[5]
Figure 1. Structures of polyazaaromatic ligands (a) 1,4,5,8-tetraazaphenanthrene (b) 2,2'-bipyrazine and (c) 1,10-phenanthroline

The two radical species formed after this photo-induced electron transfer (PET) process can recombine and lead to the formation of a covalent adduct.[6] This photoadduct with a G base can be used as damaging agent of the DNA enzyme machinery. For instance it has been shown that Ru\textsuperscript{II}-TAP complexes inhibit the \textit{in vitro} transcription rate of a plasmid DNA by the RNA polymerase upon visible light illumination.[7]

In order to use this new kind of compounds for biomedical applications, a crucial issue is the selectivity toward tumor cells and specific genes. An original strategy using photoreactive metal complexes tethered to oligonucleotides (ODNs) has been developed by our research group.[8] It combines the selectivity of gene therapy (based on non-covalent association between ODN strands) and the space and time control of the activity by light triggering.[9-11]

This strategy, which relies on a supramolecular recognition followed by the photoreaction described above, is represented in the following scheme.

Figure 2. Schematic representation of the « Sepukku » process. Yellow: non-specific sequence. Red: synthetic Ru\textsuperscript{II}-ODN. Blue: target sequence for the photocrosslinking

More specifically, when a Ru\textsuperscript{II}-ODN conjugate (red) is illuminated in presence of its complementary target sequence containing a G base (blue), the photocrosslinking between the two strands is permitted. In contrast, if the probe sequence (red) does not find its target or is in presence of a wrong or non complementary sequence (yellow) an intramolecular adduct is formed under illumination between the attached Ru\textsuperscript{II}-complex and the guanine moiety of its own probe sequence.[12] This process, named “Seppuku”, corresponds thus to a self-inhibition of the Ru\textsuperscript{II}-ODN conjugate.

The published patent corresponding to the “Seppuku” process[13] was based on results obtained for the well known Ru\textsuperscript{II} complex [Ru(TAP)\textsubscript{2}phen]\textsuperscript{2+}, which is tethered to ODNs via derivatization of the ancillary 1,10-phenanthroline (phen) ligand, named phen’’ (5-((N-ter-butoxycarbonyl)-O-((carboxymethyl)hydroxylamine)glycinamido)-1,10-phenanthroline).[14,15]

Since the TAP ligand is not the only polyazaaromatic derivative which can confer photooxidizing properties to the resulting Ru\textsuperscript{II} complexes it is interesting to study the behavior of other ligand-based complexes. In this context, the 2,2'-bipyrazine (bpz) ligand, which contains also two non-chelated nitrogens, which induce properties similar to those of Ru\textsuperscript{II} TAP complexes. Indeed, it has already been shown that the homoleptic [Ru(bpz)\textsubscript{3}]\textsuperscript{2+} complex is photoreactive towards DNA.[16,17]
The focus of this work is thus the synthesis and the studies of a potentially alternative \( \text{Ru}^{II} \) complex based on the \( \pi \)-accepting 2,2'-bipyrazine (bpz) ligand: \([\text{Ru}(\text{bpz})_2\text{phen}]^{2+}\). Like with the TAP ligands, the presence of two bpz ligands should give rise to remarkable redox properties of the resulting \( \text{Ru}^{II} \) complex. Moreover the 1,10-phenanthroline (phen) ligand should allow functionalization and subsequent tethering to ODNs.

![Figure 3. Structures of the complexes (a) [Ru(TAP)\_2phen]\^{2+} and (b) [Ru(bpz)\_2phen]\^{2+}](image)

This paper reports the results to date obtained with the new \([\text{Ru}(\text{bpz})_2\text{phen}]^{2+}\) and \([\text{Ru}(\text{bpz})_2\text{phen}'']\^{2+}\) (phen'' = functionalized phen) complexes and the comparison with data for \([\text{Ru}(\text{TAP})_2\text{phen}]^{2+}\).

**Results and Discussion**

*Synthesis and characterization*

The complexes \([\text{Ru}(\text{bpz})_2\text{phen}]^{2+}\), \([\text{Ru}(\text{bpz})_2\text{phen}'']\^{2+}\) and \([\text{Ru}(\text{TAP})_2\text{phen}]^{2+}\) have been prepared from a \(\text{Ru}(L)_2\text{Cl}_2\) (\(L = \text{TAP or bpz}\)) precursor and the phen or phen'' ligand according to the methods previously described.[15] The resulting novel \([\text{Ru}(\text{bpz})_2\text{phen}]^{2+}\) complex has been characterized by ES mass spectrometry and \(^{1}H\) NMR spectroscopy (Figure 4). The \(^{1}H\) NMR spectrum reveals the expected symmetry of the complex of interest, and both analyses confirm the formation of the desired complex.

![Figure 4. \(^{1}H\) NMR (300 MHz) spectrum of [Ru(bpz)\_2phen]\^{2+} in CD\_3OD](image)

*Absorption and Emission Spectroscopy*

![Figure 5. Absorption spectra in aqueous solution at room temperature. Black: [Ru(bpz)\_2phen]\^{2+} (~45 \(\mu\)M). Red: [Ru(TAP)\_2phen]\^{2+} (~25 \(\mu\)M)](image)
The spectroscopic properties of \([\text{Ru(bpz)}_2\text{phen}]^{2+}\) in absorption and emission have been examined in aqueous solution at room temperature and compared to those of \([\text{Ru(TAP)}_2\text{phen}]^{2+}\) in the same experimental conditions. The absorption maxima in the UV-Vis spectra are displayed in Table 1. As expected, the new bpz-based complex absorbs in the same wavelength regions that the TAP equivalent complex. The intense absorption bands in the ultraviolet region are attributed to ligand centered (LC) transitions involving \(\pi\) and \(\pi^*\) molecular orbitals, while the visible part of the spectrum corresponds to metal to ligand charge transfer (MLCT) transitions.

The molar extinction coefficients of \([\text{Ru(bpz)}_2\text{phen}]^{2+}\) were determined by atomic absorption from plasma atomization and are much lower for \([\text{Ru(bpz)}_2\text{phen}]^{2+}\) than for \([\text{Ru(TAP)}_2\text{phen}]^{2+}\).

### Table 1. UV-Visible absorption data: \(\lambda_{\text{max}}\) of absorption (±1nm) in aqueous solution and molar extinction coefficients in parentheses.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>UV</th>
<th>Vis</th>
</tr>
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<tbody>
<tr>
<td>([\text{Ru(bpz)}_2\text{phen}]^{2+})</td>
<td>256, 293</td>
<td>420 (6200), 468 (8070)</td>
</tr>
<tr>
<td>([\text{Ru(TAP)}_2\text{phen}]^{2+})</td>
<td>272</td>
<td>412 (16500), 466 (14300)</td>
</tr>
</tbody>
</table>

The emission data (\(\lambda_{\text{max}}\) of emission and luminescence lifetimes) of the complex \([\text{Ru(bpz)}_2\text{phen}]^{2+}\) in aqueous solution are presented in Table 2 and compared to the data for the reference complex \([\text{Ru(TAP)}_2\text{phen}]^{2+}\).

### Table 2. Emission data: \(\lambda_{\text{max}}\) of emission (±1nm) and luminescence lifetimes (±2%) in aqueous solution

<table>
<thead>
<tr>
<th>Solvent</th>
<th>(\lambda_{\text{max}}) / nm</th>
<th>(\tau_{\text{air}}) / ns</th>
<th>(\lambda_{\text{max}}) / nm</th>
<th>(\tau_{\text{air}}) / ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(_2)O</td>
<td>650</td>
<td>365</td>
<td>645</td>
<td>730</td>
</tr>
</tbody>
</table>

The luminescence lifetime is significantly shorter for the bpz Ru\(\text{II}\) complex than for the corresponding TAP complex, but is still long enough for a PET process. This observation can be linked to the less aromatic character and the less rigid structure of the 2,2’-bipyrazine, which induce a faster deexcitation of the excited state.

**The photo-induced electron transfer process with GMP**

According to the Stern-Volmer relation, the record of the emission intensity (I) of the excited complex as a function of the concentration of the quencher (Q), here the guanosine-5’-monophosphate (GMP), allows the determination of the luminescence quenching rate constant, \(k_q\).

\[
\frac{I_0}{I} \propto \frac{[\text{Complex}^*]_0}{[\text{Complex}]_0} = 1 + k_q\tau_0[Q]
\]

*Equation 1. Stern-Volmer relation*
The rate constant of luminescence quenching of [Ru(bpz)₂phen]²⁺ (5.10⁻⁵ M) by GMP at pH 7 is 2.7 .10⁸ M⁻¹s⁻¹. As demonstrated for [Ru(TAP)₂phen]²⁺ (kₚ=9.8 .10⁸ M⁻¹s⁻¹), the luminescence quenching for [Ru(bpz)₂phen]²⁺ is attributed to a PET process from the guanine moiety to the excited complex. In the same way, a luminescence quenching is observed for [Ru(bpz)₂phen]²⁺ in the presence of calf-thymus DNA. Nevertheless those experiments are not sufficient to constitute a proof for the formation of a photoadduct between GMP or G bases and the bpz ligand of the complex. Other experiments are still needed.

**Photocrosslinking with the anchored complex**

As explained before the photoreactive complex [Ru(bpz)₂phen]²⁺ could be tethered to an oligonucleotide with a view to steer the photoreaction toward a specific gene sequence. Thus the functionalized phen derivative, phen’’ (5-((N-ter-butoxycarbonyl)-O-((carboxymethyl)hydroxylamine)glycinamido)-1,10-phenanthroline), was prepared according to an already described method.[14] The different steps for the synthesis of [Ru(bpz)₂phen']²⁺ are shown in Figure 6, along with the anchoring strategy. [Ru(bpz)₂phen’’]²⁺ tethered to the G-containing ODN was characterized by ES Mass spectrometry.

![Figure 6. Preparation of the phenanthroline derivative (5-((N-ter-butoxycarbonyl)-O-((carboxymethyl)hydroxylamine)glycinamido)-1,10-phenanthroline), formation of the corresponding complex [Ru(bpz)₂phen’’]²⁺ and anchoring to ODN.](image)

The first PAGE (Polyacrylamide gel electrophoresis) experiments with G-containing Ru²⁺-ODN conjugates (Ru²⁺-ODN₆) are consistent with the strategy explained before (Figure 2). The observation of photocrosslinking after illumination in presence of the complementary target strand and the presence of the Sepukku intramolecular adduct after illumination alone constitute proves of the capability of the Ru²⁺-bpz complex to form photoadducts with guanine moieties. In comparison to [Ru(TAP)₂phen’’]-ODN, the photoreaction yields seem a bit lower but allow photoadduct formation in a specific way. This is consistent with the previous presented results, indeed if the luminescence lifetime is shorter and the rate constant of luminescence quenching in lower the photoreaction should be less effective.
Conclusion

This contribution gives self-consistent results that indicate the potential of the new [Ru(bpz)2phen]2+ complex for biomedical applications. In addition to the similar absorption and emission properties of [Ru(bpz)2phen]2+ in comparison to [Ru(TAP)2phen]2+, the rate constant of luminescence quenching by GMP is as explained of smaller amplitude. Moreover we demonstrated by gel electrophoresis analysis the formation of photoadduct when the G-containing RuII-bpz ODN conjugate is illuminated either alone (cyclic intramolecular adduct) or in presence of the target ODN (photocrosslinking). This shows that [Ru(bpz)2phen]2+ is capable to give rise to a PET process with G moieties. The experiments also validate the “Seppuku” strategy presented above.

Those different points allow us to conclude that in comparison to the TAP ligand, the 2,2’-bipyrazine (bpz) is also an excellent candidate for photoreactive complexes. Indeed these resulting compounds show similarities with the reference complex, [Ru(TAP)2phen]2+, in the photophysical and photochemical properties and are potential new photo-active agents for biomedical applications.

Moreover as shown with [Ru(TAP)2phen]2+, [Ru(bpz)2phen]2+ could be also efficient for a photo-induced electron transfer with amino acids such as tryptophan and tyrosine.

Références