A New Strategy for Neurochemical Photodelivery: Metal−Ligand Heterolytic Cleavage

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Over the past few years, the uncaging of biomolecules using phototriggers has been established as a promising technique in biological sciences, especially in the field of neurophysiology.[1]

In most cases, the communication between neurons is achieved by means of the release and detection of molecules called neurotransmitters.[2] Thus, the controlled delivery of either these molecules, their analogues, or other substances that promote a neuronal response is a powerful tool for the determination of circuitry in a neuronal tissue.

A number of biomolecules, including neurocompounds, have been caged to produce molecules that can be phototriggered.[3] The basic approach involves the modification of the molecule by introducing a group that can be cleaved by the absorption of light, thus releasing the bioactive compound. This strategy requires breaking a σ covalent bond, which involves the use of UV ($\sim$300 nm) photons. This type of radiation may induce cellular damage and requires expensive optical equipment.

To lower the energy needed for the biomolecule release, the use of coordination metal compounds in which the biomolecule acts as a ligand is an interesting possibility. There are many known complexes that undergo heterolytic photocleavage using low-energy light.[4] Although some chelate compounds have been used as caged metal ions (Ca^{2+}, La^{3+}, etc.),[5] the release of the radical NO from nitrosyl complexes[6] is the sole example of the use of the photochemical properties of coordination compounds for this purposes.

In this work, we present a new strategy for designing phototriggers based in transition-metal complexes in which the bioactive compound is released using visible light pulses. The photorelease of 4-aminopyridine (4AP), a widely used neurocompound that blocks certain K^+ channels,[7] promoting depolarization and increasing neuron activity, is achieved from a ruthenium polypyridyl complex. Its synthesis, characterization, and biological activity are presented in this work, and some inherent advantages of this new approach are discussed.

$[Ru(bpy)_2(4AP)_2]Cl_2$ (bpy = 2,2′ bipyridine) was obtained by the reaction of $Ru(bpy)_2Cl_2$ in water with excess of 4AP and purified by recrystalization (One hundred and fifty-nine milligrams of Ru-$(bpy)_2Cl_2$ were suspended in 7 mL of water at 85 °C under N_2. After dissolution, 66 mg of 4AP was added, and the solution was heated for 20 min. The compound was precipitated by addition of NH_4PF_6, washed, and dried. The red solid was dissolved in acetone and reprecipitated with tetraethylammonium chloride. Yield: 79%.) UV−vis spectra in water were obtained with a HP 8453 diode array spectrophotometer. RMN ^1H spectra were done using a Bruker 500 MHz equipment. CV measurements were performed with a PAR 273A potentiostat. The irradiation of the samples was effected by means of a pulsed Xe lamp, (pulse energy $\sim$0.5 J), with a low-pass filter at 480 nm. Irradiation using a 473-nm DPSS laser gave similar results.
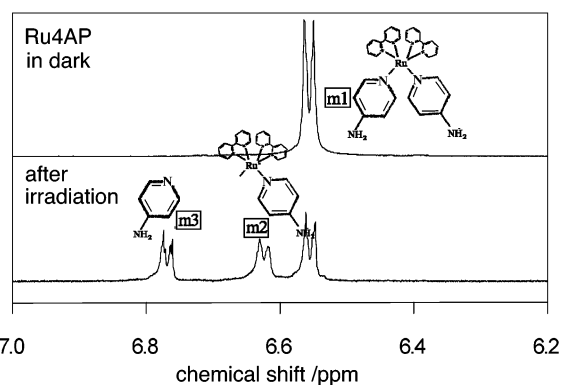

Figure 1. Partial ^1H NMR spectra of $RU4AP_2$, showing the signals corresponding to the 4AP meta hydrogens. *m1*: in $[Ru(bpy)_2(4AP)_2]^{2+}$ *m2*: in $[Ru(bpy)_2(H_2O)(4AP)]^{2+}$, and *m3*: in free ligand 4AP.

The compound $[Ru(bpy)_2(4AP)_2]Cl_2$ ($RU4AP_2$) is very soluble in water and stable in the dark, while undergoing decomposition under irradiation with visible light in its MLCT band, centered at 489 nm. (In CH_3CN solution, the absorption band is red-shifted to 492 nm, consistently with the lower polarity of the solvent, despite a previous characterization that reported 450 nm. However, light exposure of CH_3CN solutions produced a yellow compound with absorption maximum at 450 nm that possibly corresponds to the previously misinterpreted assignments for this compound.[8] This photoproduct is probably the complex $[Ru(bpy)_2(4AP)(CH_3CN)]^{2+}$.) Several ruthenium polypyridyl complexes present this behavior.[9] Although at pH 7 the spectrum of the irradiated complex is very similar to that of the original complex, a diminished shoulder at 470 nm becomes evident. To determine the nature of the photoreaction, NMR spectra were taken before and after irradiation with visible light. Figure 1 shows the signal assigned to the meta hydrogens $RU4AP_2$ (m1). After irradiation this signal decreases, and two new ones appear at lower fields: one corresponding to the free ligand (m3), and the other to the aquo-4AP complex (m2), indicating photorelease of the 4AP. These two latter signals integrated for 0.30 and 0.27 of the initial signal, which corresponds to photoreaction of 60%.

The redox potential of the couple Ru^{III}/Ru^{II} for $RU4AP_2$ measured in water is $E = 0.76$ V vs Ag/AgCl, which is consistent with the higher basicity of 4AP compared with that of pyridine. Thus, the redox and the photochemistry of this compound is in total agreement with previous results corresponding to the $Ru(bpy)_2XY$ family, X and Y being monodentate ligands.[10]

The photoactivity of these compounds has been explained in terms of a reaction pathway that involves the transition between the MLCT state to a lower-energy d−d state, which promotes ligand release. There is a direct correspondence between the energy of the MLCT transition and the quantum yield of the photoreaction.[9]
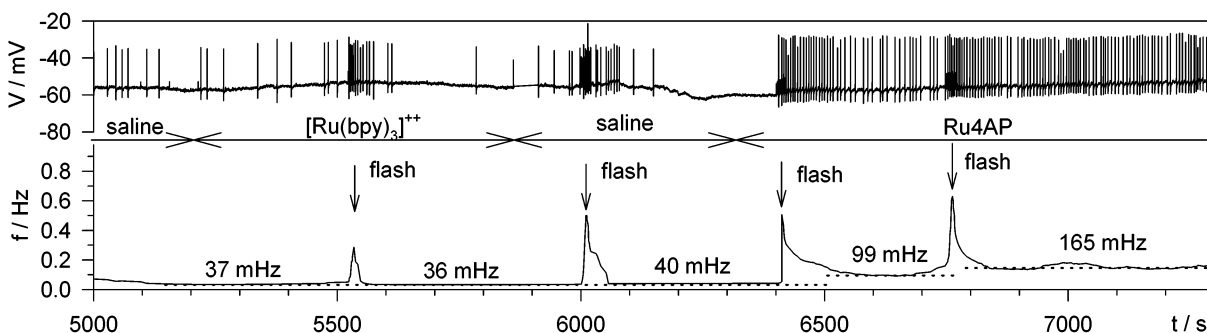
Figure 2. (Top) Action potentials (spikes) recorded in a leech neuron for pure saline and two ruthenium complexes solutions. (Bottom) Frequency of the spikes. Arrows indicate irradiation with Xe flashlamp. (Middle) Composition of the extracellular medium.

For $[Ru(bpy)_2Py_2]^{2+}$, the photoreaction yield is about $\phi_{PR} = 0.4$. Since $RU4AP_2$ presents a red-shifted band, a lower photoreaction yield is expected. Preliminary data allow us to estimate $\phi_{PR} \cong 0.02$ at 473 nm.

To establish the potential usefulness of this compound in real neurophysiological experiments, we have built a standard setup for intracellular voltage measurements. The medicinal leech *Hirudo medicinalis* was used as model. This invertebrate has a central nerve cord with several ganglia, each one containing about 400 neurons arranged in a known pattern.[11] An entire ganglion was mounted on a dish. The transmembrane potential for a single cell in the ganglion was recorded by inserting inside the neuron a glass micropipet with a micrometer-sized end, filled with saturated aqueous KCl that acts as a luggin bridge for a Ag/AgCl electrode. Another Ag/AgCl electrode was used as reference. The signal was taken with an AM-System 1600 amplifier, and the entire setup was covered with a Faraday cage. A 12-bit A/D acquisition card was used to digitize the data using an ad-hoc program written in QuickBasic.

Low Ca^{2+}-high Mg^{2+} saline solution (concentrations in mM: NaCl 102, KCl 4, $CaCl_2$ 1, $MgCl_2$ 10, Tris base 5.4; pH adjusted to 7.4.) was perfused through the dish. The Ru complexes and the free ligand 4AP were injected in the mainstream at controlled times. A pulsed Xe lamp located under the dish was used to irradiate the solution. UV light was removed using a band-pass filter at 500 nm (see Supporting Information).

Figure 2 shows the behavior of the membrane potential recorded at one of the so-called Retzius (Rz) cells in the ganglion. The upper graph shows the raw data, presenting periods of rest potential and very fast spikes (action potentials), produced by the changes in membrane ion permeabilities.[2] The lower graph shows the instantaneous spiking frequency at each time. After impaling the cell with an electrode, many experiments were done on the same cell to ensure reproducibility. After 5000 seconds, the cell shows low activity, as can be seen at the left of the graph. At $t = 5200$ s, $\sim$100 μM $Ru(bpy)_3Cl_2$ is added to the saline solution, without important changes in activity. Three hundred seconds later a light flash is directed to the ganglion. The sudden increase in the frequency of the action potentials is mainly due to the temperature pulse,[12] but after a short time the activity decreases to the basal level. After washing by perfusion, further irradiation with a pulse shows a very similar pattern ($t = 6000$ s). At $t = 6250$s, $\sim$100 μM $RU4AP_2$ is added to the saline, and the activity remains unchanged; however, after a new light flash ($t = 6400$ s), sudden activity is recorded, and it remains high after 300 s. A second light pulse at 6750 s promotes an even higher activity, which only decreases after cleaning perfusion with pure saline (not shown).

A similar frequency increase occurs when free 4AP is perfused onto the ganglion, showing that the release of 4AP is the cause of this maintained frequency increase (see Supporting Information). Calibration of the cell activity using solutions of 4AP showed that in each irradiation, $10-15$ μM of 4AP were released from $RU4AP_2$ during the previous experiments. Neither toxicity nor deleterious effects were observed on the neuron during the experiments.

In conclusion, we have shown that we can stimulate the neuron response using a coordination compound to photorealease a neurochemical. This strategy presents several important advantages compared with those gained from the use of organic analogues. The metal-organic bond is normally weaker than a covalent σ bond and therefore can be broken using a lower-energy irradiation. Moreover, since the coordination sphere of the metal can be changed by means of ligand replacement or modification, the absorption bands can be tuned to allow different colors to deliver different biomolecules. Redox potential, size, lipophilicity, charge, and almost any chemical property can be tuned by means of ligand modification. To take advantage of these possibilities, further research is being carried out.

Supporting Information Available: NMR, CV, and UV$-$vis spectra of $Ru4AP_2$ before and after irradiation, spectrum of the band-pass filter and records of neurons in the presence of free 4AP (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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