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Immobilization and Orientation of
Photosystem I Reaction Centers on Solid Surfaces

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Immobilization and Orientation of Photosystem I Reaction Centers on Solid Surfaces

Final Report

SUMMARY

The overall objective of this project was to test the potential for immobilization and orientation of Photosystem I reaction center protein on solid surfaces. In order to maximize the resources available for this work, bleomycin complexes were used as a test substrate. The reaction of $[(\text{H}_2\text{O})(\text{NH}_3)_5\text{Ru}^{\text{II}}]^{2+}$ with bleomycin forms at least two stable products following oxidation to the Ru(III) analog. Spectroscopic and electrochemical measurements indicate monodentate binding of $[(\text{NH}_3)_5\text{Ru}^{\text{III}}]$ to the imidazole and pyrimidine moieties, with coordination to the latter involving the exocyclic amine nitrogen. DNA cleavage studies show the complexes to be ineffective in DNA strand scission.

EXPERIMENTAL WORK

Synthesis

Bleomycin sulfate was dissolved in 4 ml of water and a 3-fold excess of $[(\text{H}_2\text{O})(\text{NH}_3)_5\text{Ru}^{\text{II}}](\text{PF}_6)_2$ added to give $[\text{Ru}] = 9 \text{ mM}$. (The $[(\text{H}_2\text{O})(\text{NH}_3)_5\text{Ru}^{\text{II}}](\text{PF}_6)_2$ was prepared by Kostic's modification of the method of Kuehn¹, which was made available by private communication from N. Kostic, Iowa State University. This typically involves dissolution of 250 mg of $[\text{Cl}(\text{NH}_3)_5\text{Ru}^{\text{III}}]\text{Cl}_2$ in 0.1 M CF_3COOH , deaeration by argon bubbling, and reduction over zinc amalgam for 1 h at 5°C. The solution is then anaerobically transferred into an argon-purged solution containing 2 g of NH_4PH_2 in 5 ml of water. The resulting precipitate is quickly filtered, washed with ethanol and suction dried (before storing in a vacuum desiccator.) Oxidation of the Ru-bleomycin adduct was effected by oxygen bubbling for about 1 h, during which the solution turned from yellow to pink. The complex was then concentrated in a stirred ultrafiltration cell to remove most of the free ruthenium. The remaining mixture was separated on a Sephadex CM-25 column (2 x 10 cm), which had previously been equilibrated with 50 mM Tris buffer at pH 7.2 at room temperature. The column was eluted with a linear gradient of 0.1–0.6 M NaCl in the Tris buffer. The yellow $[(\text{OH})(\text{NH}_3)_5\text{Ru}^{\text{III}}]^{2+}$ eluted first followed by two pink bands, which eluted similarly to and in the same relative abundances as bleomycin- α_2 and bleomycin- β_2 respectively, with that containing the bleomycin- α_2 complex eluting first and being about three times as large as the following band. Alternatively, the reaction mixture was loaded onto a 0.5 x 5 cm column of Biorex-70 constructed in a Pasteur pipette and eluted with 0.1–0.5 M NaCl or ammonium formate. Elution patterns were similar on both columns, however, the Biorex column afforded tighter, better resolved and more easily discernible bands. All subsequent experiments were performed in solution on the bleomycin- α_2 fraction. Whenever possible, characterizations were made on samples in NaCl solution soon after elution.

Physical characterization

UV-visible spectra were taken in 0.1M NaCl, 50 mM Tris buffer at pH 7.2 solution on a Cary-119 recording spectrophotometer at room temperature. Ruthenium concentrations were determined by flame atomic absorption spectrophotometry, using a Varian Techtron AA-5 spectrophotometer. Determinations of pK_a values were made from plots of absorbance values at 370 nm and 470 nm vs. pH fitted to the equation: $pH = pK_a - \ln [A_s - A]/(A - A_s)$, where A_s is the absorbance of the deprotonated form of the complex, A_s the absorbance of the protonated species, and A is the absorbance at the given pH. Cyclic, square-wave and differential pulse voltammetric experiments were carried out on a BAS-100 electrochemical system or on a rapid-response potentiostat controlled by an IBM-XT microcomputer using a BAS platinum or glassy carbon working electrode, Ag/AgCl reference electrode and platinum wire auxiliary electrode in 0.1 M NaCl solution. HPLC was carried out on a Waters HPLC with a Hewlett-Packard 8450 UV-Vis spectrophotometer detector and a 7225 graphics plotter fitted with a Vydac C₁₈ reverse phase column eluted with a linear (0-40%) gradient of 0.1% trifluoroacetic acid (TFA) in acetonitrile mixed with 0.1% TFA in water. ¹H-NMR was carried out in D₂O at pH 5.5 on a Bruker 500 MHz NMR.

Results

Spectra

The reaction of $[(H_2O)(NH_3)_5Ru^{II}]^{2+}$ with bleomycin yields a pink material, which can be separated from the starting compounds and side products by ion-exchange chromatography. Control reactions run in water without the ligand also oxidized to give pink to dark red solutions ($\lambda_{max} = 370$ and 535 nm), which probably consist of ruthenium-red or other μ -oxo ammineruthenium species. Formation of these highly-charged side products was minimized in buffered reactions and they could be easily separated from the desired product, since they remained at the top of Biorex-70 ion-exchange columns and eluted only with difficulty from CM-Sephadex.

Heating Ru-bleomycin in strong HCl causes dissociation of the ruthenium to yield, $[Cl(NH_3)_5Ru]Cl_2$, which was isolated as a solid and identified by its electronic spectrum², indicating that the five ammine ligands remain intact and that the metal ion is held to the antibiotic in a monodentate fashion. At neutral pH and 0°C these complexes usually exhibit no observable spectroscopic change for periods of up to 3 months.

The electronic spectra of the Ru-bleomycin- α_2 fraction at pH 8.2 and pH 2.75 are shown in Fig. 1. Ru/bleomycin ratios estimated from atomic absorption determinations of Ru concentrations and spectrophotometric determination of bleomycin were found to be 1:1 ($\pm 10\%$). Spectrophotometric titrations of these solutions revealed significant absorption changes around 470 nm at relatively low pH and around 370 nm at relatively high pH. Analysis of these changes in absorbance plotted against pH led to pK_a values of approx. 3.7 and 6.7. When other metal ions, such as Fe(II), Co(II), Ni(II) and Zn(II), were added to solutions of Ru-bleomycin, little or no change in the spectra was observed.

Fig. 1. UV-visible spectra of $[(\text{NH}_3)_5\text{Ru}^{\text{III}}]\text{-bleomycin-}a_2$ in aqueous solution at pH 2.75 and pH 8.2. Absorbance scale ranges from 0 to 1.0 absorbance units.

References

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