MIXED-METAL RUTHENIUM-PLATINUM POLYAZINE SUPERMOLECULES: SYNTHESIS, CHARACTERIZATION AND EXPLORATION OF DNA BINDING

by

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(ABSTRACT)

The goal of this research was to design, prepare and study a new class of supermolecules coupling ruthenium and platinum, which would display covalent binding to DNA. These supermolecules were modular in design, facilitating the use of coordination chemistry to vary individual components within the design. Drawing upon the well-established efficacy of *cis*-diamminedichloroplatinum(II) (cisplatin) and the DNA-binding properties of select ruthenium polyazine complexes, the approach was to bind the *cis*-$Pt^{II}Cl_2$ active site of cisplatin to ruthenium light absorbers using the dpq and dpb bridging ligands (where dpq = 2,3-bis(2-pyridyl)quinoxaline, dpb = 2,3-bis(2-pyridyl)benzoquinoxaline). These complexes are potentially bifunctional, capable of DNA intercalation through the bridging ligand and covalent binding to DNA through the *cis*-$PtCl_2$ site. The use of the ruthenium(II) center allows these complexes to be cationic and provides for enhanced water-solubility relative to the neutral cisplatin. These ruthenium centers function as chromophores leading to efficient light absorption by the Ru-Pt systems throughout the ultraviolet & visible region of the electromagnetic spectrum.

Synthetic methods were developed to prepare the mixed-metal, bimetallic complexes $[(bpy)_2Ru(BL)PtCl_2](CF_3SO_3)_2$ and $[(phen)_2Ru(BL)PtCl_2](CF_3SO_3)_2$ (where bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline) in high purity and good overall yields. These new systems were characterized using electronic absorption spectroscopy, electrochemistry, and FAB-MS. The DNA-binding ability of these complexes was probed by reaction with linearized plasmid DNA and subsequent analysis by native and denaturing gel electrophoresis. The known DNA binders, cisplatin and *trans*-$\{[PtCl(NH_3)_2]_2(\mu\text{-}H_2N(CH_2)_6NH_2)\}^{2+}$ (1,1/t,t), were examined under equivalent conditions and used as positive controls. Native gel electrophoresis was used to show that these complexes strongly bind DNA, retarding the migration of DNA through the gel in a fashion inversely proportional to the ratio of DNA base pairs (bp) to metal complex (mc). Equivalent studies using the ruthenium monometallic synthons, $[(bpy)_2Ru(BL)](CF_3SO_3)_2$ and $[(phen)_2Ru(BL)](CF_3SO_3)_2$, had no effect on DNA migration, suggesting that the Ru-Pt complexes bind to DNA through the $PtCl_2$ site. Analysis by denaturing gel electrophoresis determined that the Ru-Pt complexes bind to DNA in a fashion similar to cisplatin, forming primarily intrastrand adducts. However, these systems also appear to form interstrand adducts at a 10-fold lower metal concentration than cisplatin.

In addition to affecting the migration rate, the bimetallic complexes also significantly reduced the fluorescence of DNA-intercalated ethidium bromide for the Ru-Pt reacted samples at low-DNA bp: mc ratios. This was not observed for the cisplatin and 1,1/t,t treated samples. This observation was quantitated by gel densitometry. Precipitation of the DNA by cisplatin, 1,1/ t,t and all four Ru-Pt complexes was determined not to be the cause of reduced ethidium bromide fluorescence intensity. Homogenous solution fluorescence quenching studies have revealed that the Ru-Pt complexes quench the emission of ethidium bromide even in the absence of DNA, whereas cisplatin and 1,1/t,t do not.

In order to compare the effects on DNA migration produced by cisplatin, 1,1/t,t and the Ru-Pt complexes, R_f values were calculated. This analysis has revealed that all

four Ru-Pt complexes retard DNA migration to approximately the same degree. Calculation of theoretical DNA migration distances, based upon the molecular weight change of DNA caused by metal-complex binding, have revealed that the observed affect on DNA migration cannot be accounted for by an increase in molecular weight alone. This indicates that changes in charge and three-dimensional shape of the DNA upon binding of the Ru-Pt complexes may also contribute.

The Ru-Pt complexes developed in this work represent a successful attempt to design and produce a new class of supermolecules that will covalently bind to DNA. The design of this molecule, coupling a ruthenium light-absorbing unit to a *cis*-$PtCl_2$ site, has proven to be synthetically achievable and the complexes prepared display easily tunable light absorbing properties and reversible metal and ligand based redox processes. These molecules have been shown to avidly bind DNA through the $PtCl_2$ site. A group of *in vitro* biological assays have been developed to explore and characterize the binding of these molecules to DNA. These molecules therefore have met the goals of this project, and represent a new class of compounds that may find diverse applications in medicine, chemistry, and molecular biology.

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Abbreviations

bpy	2,2′-bipyridine
bp	base pair
CT	charge transfer
C	control
DNA	deoxyribose nucleic acid
dpb	2,3-bis(2-pyridyl)benzoquinoxaline
dppz	dipyrido[3,2-*a*: 2′, 3′-*c*]phenazine
dpq	2,3-bis(2-pyridyl)quinoxaline
HOMO	highest occupied molecular orbital
Kb	kilobase pairs
LC	ligand centered
LF	ligand field
LUMO	lowest unoccupied molecular orbital
mc	metal complex
MLCT	metal to ligand charge transfer
nt	nucleotides
phen	1,10-phenanthroline
py	pyridine
Ru-Pt	ruthenium-platinum complex
S	molecular weight standard

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