Physico-chemical studies of interactions of water soluble brominated porphyrins with DNA

Physico-chemical studies of interactions of water soluble brominated porphyrins with DNA

Lisa M. Rogers, Shivaraj Yellappa and Francis D'Souza

Department of Chemistry, Wichita State University, Wichita, Kansas 67260, U.S.A.

1. Introduction

Many researchers today have been focusing their energies and resources on developing new methods for cancer detection and cancer therapy that targets cancer cells with minimal damage to healthy cells and virus inhibition. The advantages of developing sensors and nontoxic, highly selective anticancer reagents for electrochemical methods include, among others, rapid detection, sensitivity and low production costs and availability of extremely small electrodes. As electrode technology advances, this field of science becomes more attractive for detection and treatment of various diseases. Researchers are being challenged to develop innovative compounds that can be used in conjunction with electrochemistry to combat these diseases. The compounds that our research group is studying, derivatives of cationic metalloporphyrins (metal = manganese, iron and cobalt), have a natural affinity for DNA and can induce oxidative cleavage when "activated" via reducing agents (ascorbate or superoxide anion) or electrochemical reduction in the presence of oxygen[1]. Electrochemical activation of the metalloporphyrins is advantageous since it does not involve the addition of chemical reagents which contaminate the solution.

We are currently examining the interaction, binding and electrochemically induced oxidative cleavage of various types of synthetic DNA by electron deficient, water soluble, β-halogenated metalloporphyrins utilizing electroanalytical and spectroscopic techniques. One of the primary goals of this research is the electrochemically induced cleavage and subsequent detection of DNA by these brominated metalloporphyrins in solution.

2. Experiment, Results, Discussion, and Significance

Cationic metalloporphyrins have an intrinsic affinity for DNA and induce oxidative cleavage of DNA. Positively charged (cationic) metalloporphyrins such as *meso*-tetrakis(N-methylpyridinium-4-yl)porphyrin and its metal derivatives, (TMPyP)M, bind and cleave DNA more efficiently than neutral or negatively charged porphyrins. These types of compounds are water soluble and bulky enough to hinder them from binding between the base pairs of DNA (where they are virtually inaccessible to the electrode surface). Since

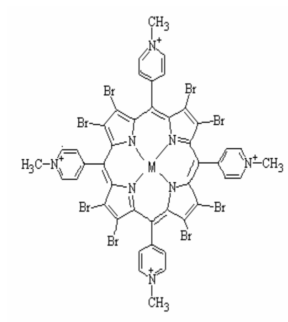

Fig.1. Structure of the compounds, brominated (TMPyP)M, synthesized for this study.

halogens, such as bromine, tend to draw electron density to them, addition of bromine (see Figure 1) should make the porphyrin ring more positive thereby enhancing intrinsic affinity for DNA. The puckering of the brominated porphyrin ring discourages stacking along the phosphate backbone of DNA which makes it much easier to access the metal center of the porphyrin for electrochemical oxidation/reduction (i.e., the metal is not buried within the complex). Stacking of the porphyrins is undesirable since it would inhibit the ability of the electrode to electrochemically activate the metal center of the porphyrin bound directly to the DNA.

Monitoring the changes in the absorption of electromagnetic radiation (ultraviolet and visibleregions) of each of the brominated porphyrins as aliquots of ctDNA was added confirmed the interaction that we had anticipated. As illustrated in Figure 2 for $(Br_8TMPyP)Co$, a reduction in the intensity was

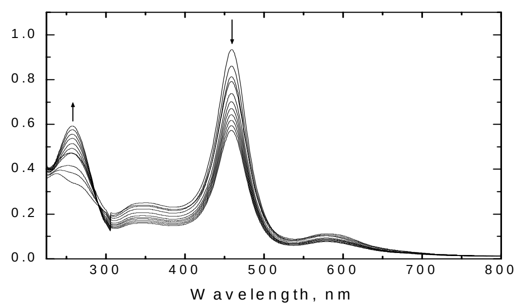

Figure 2. Optical absorption spectral changes observed during the addition of calf thymus DNA to a solution of $(Br_8TMPyP)Co$ in aqueous phosphate buffer at pH = 6.9 at room temperature. Ionic strength was held constant at $\mu = 0.2$ M.

observed as increasing concentrations of ctDNA were added. The spectra of the other brominated porphyrins exhibited similar behavior. In a few instances, a shift to longer wavelengths (lower energy) of the maximum absorbance peak was observed. Isosbestic points (points where every spectrum crosses) were also seen; evidence that only two principle species are present, i.e. bound and unbound porphyrin. These spectral changes, along with information gathered from control experiments are indicative of porphyrin-DNA association. Preliminary apparent binding constants that were calculated from the spectra confirmed that the β-pyrrole brominated porphyrins interact much more efficiently than the analogous (TMPyP)M nonbrominated compounds. Further studies involving the binding of these compounds to polynucleotides and plasmid DNA are in progress.

Preliminary electrochemical studies have been performed using the nonbrominated analogs of these porphyrins (because of time and cost constraints) to confirm the interaction of the porphyrin with DNA and allow us to optimize the conditions (such as pH and ionic strength) for the electrochemical cleavage and detection of DNA.

The electrochemical techniques used involve the measurement of current as the potential of the electrode is varied. The current is proportional to the concentration of the electrochemically active species and the potential either provides electrons to this species (reduction) or accepts electrons from it (oxidation).

The DNA binding to metalloporphyrins resulted in diminished currents for both the first oxidation and the first reduction because of a reduced diffusion of the porphyrin-DNA complex (figure not shown). A shift in the redox potential values was also observed, the magnitude of which was dependent on the oxidation state of the metal (a shift of ~100 mV was observed for Co^{3+} (oxidized species) upon binding to DNA while this shift is < 20 mV for Co^{+} (reduced species)). These results suggest a higher binding ability of porphyrins with metal ions in higher oxidation states, a novel property that could be probed easily by electrochemical techniques. Other experiments using DNA modified glassy carbon electrodes soaked in a solution containing FeTMPyP, CoTMPyP or MnTMPyP were performed. An initial scan of the electrodes in the absence of oxygen confirmed the presence of the metalloporphyrins but did not detect cleavage of the DNA on the electrode. However, once oxygen was bubbled into the solution and another scan was performed (the potential at which the metalloporphyrins were reduced was applied before the potential at which the oxidation of the DNA bases that were exposed from the induced cleavage occurs [2]), peaks corresponding to the exposed bases of DNA were detected. This confirmed the electrochemical induced cleavage and subsequent detection of the damaged DNA was indeed possible.

3. Conclusions

We have demonstrated that the brominated porphyrins interact with ctDNA as we had anticipated. Spectral changes confirmed interaction between the porphyrins and ctDNA and analysis of the data obtained confirmed the brominated porphyrins interacted much more proficiently than the nonbrominated porphyrins. Electrochemical studies using DNA/metalloporphyrin modified glassy carbon electrodes in the presence of oxygen confirmed that electrochemical reduction of the metalloporphyrins caused cleavage of the DNA and this cleavage could be detected with the same electrode. Further studies using various methods are currently being performed.

4. Acknowledgements

The Department of Education is gratefully acknowledged for the GAANN fellowship.

[1] M.Rodriguez, T.Kodadek, M.Torres, and A.J.Bard, Bioconj.Chem. 1, 123(1990).

[2] A.M.Oliveira-Brett, J.A.P.Piedade, L.A.Silva and V.C.Diculescu, Anal.Biochem. 332, 321(2004).