Spring 5-15-2010

Catalytic Decomposition of Peroxynitrite and Superoxide by Nafion Films Modified with Iron and Manganese Porphyrin

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Abstract

Nafion films alone and modified with inert electrolytes and metalloporphyrins were tested in their effectiveness of decomposition of aqueous peroxynitrite (PN) and superoxide. Films that were modified with a cationic manganese porphyrin, which has been shown to be responsible for catalytic decomposition of superoxide ion, were found to be most effective. Peroxynitrite was generated in solution by the decomposition of 3-morpholinosydnonimime (SIN-1) that generates nitric oxide and superoxide ion, which rapidly combine to form peroxynitrite. The tyrosine analogue 4-hydroxyphenylacetic (4-HPA) was used to trap the PN in pH 7.00 PBS buffer and was its nitrated product was detected by observing the change in visible absorbance at pH 10. The Nafion films modified with manganese porphyrin were active in superoxide quenching and the therefore blocking of peroxynitrite formation throughout a five week testing period. These experiments offer new strategies for disrupting superoxide and peroxynitrite in vivo.

Introduction

The human body is a complex biological system that recognizes foreign objects and diseases. It will engulf and destroy these intruders to the extent possible. This creates a problem for doctors who want to surgically insert an implant into the human body. Not only does the implant have to survive the human body's initial defense, an important part of its success is that the exterior of the implant be durable and biocompatible. The implant needs to resist or reduce

the inflammatory responses of the body and its tissues. Scientists have tried several materials that mimic the natural cell membrane (discussed later), but these do not last. In some cases the coating must also have specific transport or sensing properties for the implant, creating additional requirements for the surface material. If a better membrane could be found, the implants could be more successful. Scientists are also working on improving drugs and other treatments that deal with inflammatory diseases such as arthritis and Crohn's disease.

Immune response involves nitric oxide (NO) which is produced by phagocytes such as macrophages, neutriphils, and monocytes. Nitric oxide, a reactive free radical with one unpaired electron, is a signal molecule formed in the body that has many functions. It is known to react with the superoxide anion O_2^-, which is also a reactive free radical and oxidizing agent that is over-produced during oxidative stress. The two rapidly combine forming peroxynitrite ($ONOO^-$), which is very reactive anion that can decompose to form other free radicals (Holm). It has been shown that peroxynitrites are capable of directly oxidizing and nitrating lipids, proteins, and nucleic acids. Peroxynitrite is important in the inflammation process and is naturally formed from nitric oxide and superoxide which are both in high concentrations under conditions of oxidative stress. It also may be responsible for cell death in many inflammatory diseases such as arthritis and sepsis (Wang). It has been shown to induce colon and ileum inflammation, and edema (Susuki), and much of the damage associated with Alzheimer's disease (Kopal).

There are several different origins of nitric oxides, some forms are made in the body naturally and some are induced. The form that is induced is iNOS, and it is the form that is currently under study. Nitric oxides are produced by inflammatory cytokines and are involved in osteoporosis, arthritis, and aseptic loosening of implants (Stea). Despite further studies and innovations, aseptic loosening and osteolysis (bone growing away from the implant causing

loosening) occurs frequently in hip implants, and studies have shown that nitric oxides are linked to this problem (Puskas). The body's self-defense includes: A.) macrophages that try to destroy foreign material but fail to digest the implants and consequently cause chronic inflammation, B.) cytokines that deposit collagen around the implant to separate it from the tissue, and C.) restriction of blood flow to the implant (Hetrick).

Epoxy-polyurethane has been shown to improve the durability and structural strength of the implant membrane in mice (Bazung). It has also been shown that titanium oxide can inhibit the reactivity of peroxynitrite in vitro and in mice (Suzuki). Coating implants with certain materials has been and still is being tested to see if they can survive longer inside the body. Nafion, a teflon-crosslined perfluoronated sulfonate polymer, widely used in fuel cells, has also been studied extensively as a membrane coating for implantable glucose sensors that would be beneficial in the treatment of diabetes, since it is a solid electrolyte, capable of transporting cations and small neutral molecules as needed for the sensor. It has relatively good biocompatibility and function for these sensors and has been shown to withstand the tough environment of the body for limited periods of time. Nafion has shown to withstand many acids and oxides that usually cause inflammation and degradation of the membrane, but it loses its characteristics as calcium ions collect in the anionically charged pores of its structure leading to cracking of the polymer (Mercado).

Many scientists have proved that metalloporphyrins are successful decomposition catalysts of PN. In vitro and in the body, two substances that are known to be PN decomposition catalysts are the water-soluble porphyrins FeTMPyP and FeTPPS. They have shown to be neuroprotective in rats with cerebral ischemia (Dhar). Other agents can be used to react with PN and other oxidizing agents stoichiometrically rather than catalytically, requiring replacement

with periodic dosages. Thiols, for example, have been used since they are thought to remove very reactive hydroxyl radicals from the environment, so that nitric oxides do not become the highly reactive and damaging peroxynitrites. When added with the nitric oxides, the damaging effects were eradicated (Kopal). Two other substances used to block the harmful effects of peroxynitrites are penicillamine, a scavenger of peroxynitrite, and tempol, a catalytic scavenger of peroxynitrite (Singh).

When doing experiments in vitro, nitric oxide and peroxynitrites from the body are usually not used since they decompose rapidly. To mimic their formation in the body, peroxynitrite must be generated at low concentration at a relatively constant rate. To accomplish this, a reagent known as SIN-1 (3-Morpholinosydnonimine) is used because it slowly decomposes in air to make peroxynitrite by forming both NO and the superoxide anion, which react to form peroxynitrite. Peroxynitrite's effects are also modeled chemically by using a reagent similar to the tyrosine peptide, since PN is known to nitrate this peptide very quickly in its damage to proteins. This capturing reagent, 4-hydroxyphenylacetic acid (4-HPA), shows a measurable color change upon nitration. The SIN-1 and 4-HPA, therefore make a convenient chemical model system for measuring the persistence of peroxynitrite in the presence of a potential implant surface. Indeed, the presence of peroxynitrite in the body is usually assessed by the nitration of tyrosine in proteins (Holm). If there is damage done by PN, nitrated tyrosine will be present and if there is no damage, only normal tyrosine will be present.

In previous experiments, Nafion was examined in its ability to disrupt the formation and to decompose peroxynitrite with this in vitro model based on SIN-1 and 4-HPA. The Nafion was used alone and modified with inert electrolytes and the catalytic metalloporphyrins FeTMpyP

and MnTMpyP. My experiments involved examination of the longevity of the effectiveness of MnTMpyP in this system.

Materials and Methods

Nafion was soaked in aqueous manganese porphyrin and air dried. Solutions of PBS (pH 7.00), 0.001 mol 4-HPA (Sigma Aldrich 98%), and Sigma Aldrich 0.010 mol of SIN-1 anhydrous stock were made at different volumes; 2mL, 3mL, and 6mL. At each volume, dry SIN-1 and 4-HPA were combined and PBS was added until the desired volume was reached. The 6 mL solution contained 0.913 mg 4-HPA and 12.4 mg SIN-1. The 3 mL solution had 0.456 mg of 4-HPA and 6.20 mg of SIN-1. The 2 mL solution had 0.304 mg of 4-HPA and 4.13 mg SIN-1. A pipette was used to place 1.0 mL of each sample into separate vials. For the second and third solutions, a control was set up without Nafion film. Three controls were set up for the first solution. A hole punch sized (0.5027 mm^2) presoaked Nafion film was then placed in the each of the rest of the vials. The vials were sealed, labeled, and placed in a 37^0C incubator. In two days, a control vial and a vial with presoaked Nafion film from the 6 mL volume were analyzed using a UV/VIS Ocean Optics Spectrophotometer. Each sample was buffered to pH 10.00, with a total of 2 mL of solution being added to the UV/VIS cuvette. Spectral analysis was detected and examined. At one, two, and three weeks the same procedures were used to evaluate a control vial and a vial with Nafion from the 6 mL volume again. At the end of five weeks, the 6 mL, 3 mL, and 2 mL volumes were analyzed. Spectra were taken for a control vial and a vial containing presoaked Nafion at each volume.

Results

At the end of five weeks, there was a visual difference between control vials and vials with presoaked Nafion. The control vials had a deep yellow color in appearance, while the vials with presoaked Nafion had more of a clear appearance, showing no change in color. The change in color represents the nitration of 4-HPA. The nitration is further analyzed with the use of UV/VIS. A UV/VIS Ocean Optics Spectrophotometer was used to find the absorbance and wavelength values of each vial. Peroxynitrite shows up on the spectra between around 410 to 430nm, so absorbance was measured around these wavelengths (Figures 1 and 2).

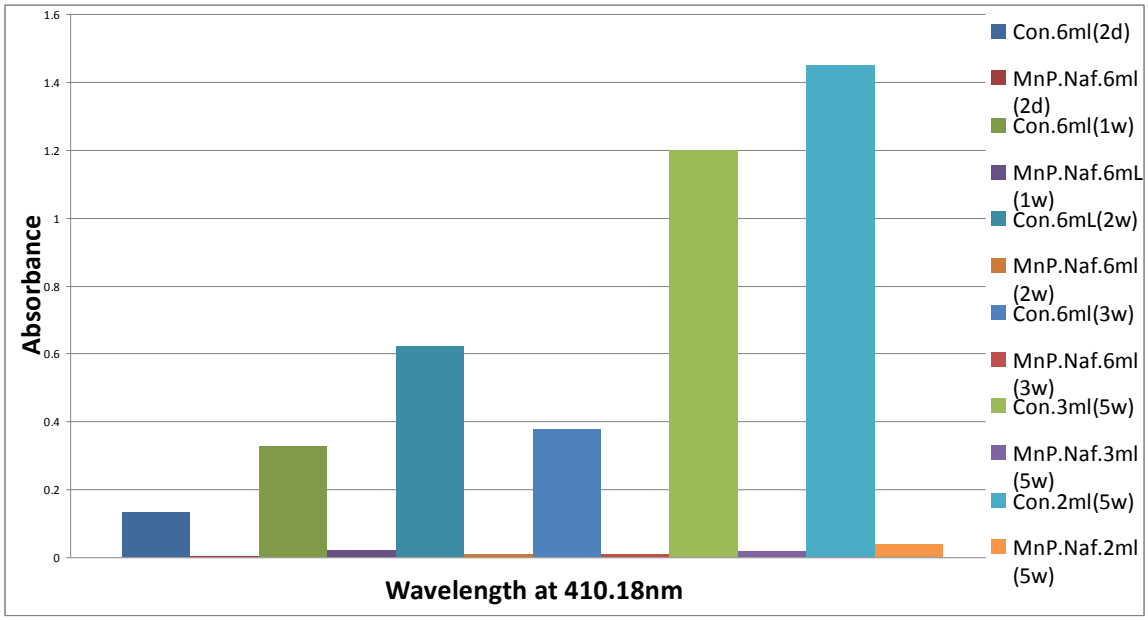

Figure 1: Bar graph showing the absorbance values at a wavelength of 410.18nm. The bar graph shows the blockage of PN by presoaked Nafion at different concentrations. The vials with presoaked Nafion show a very low absorbance, while control samples show high absorbance due to nitration of 4-HPA.

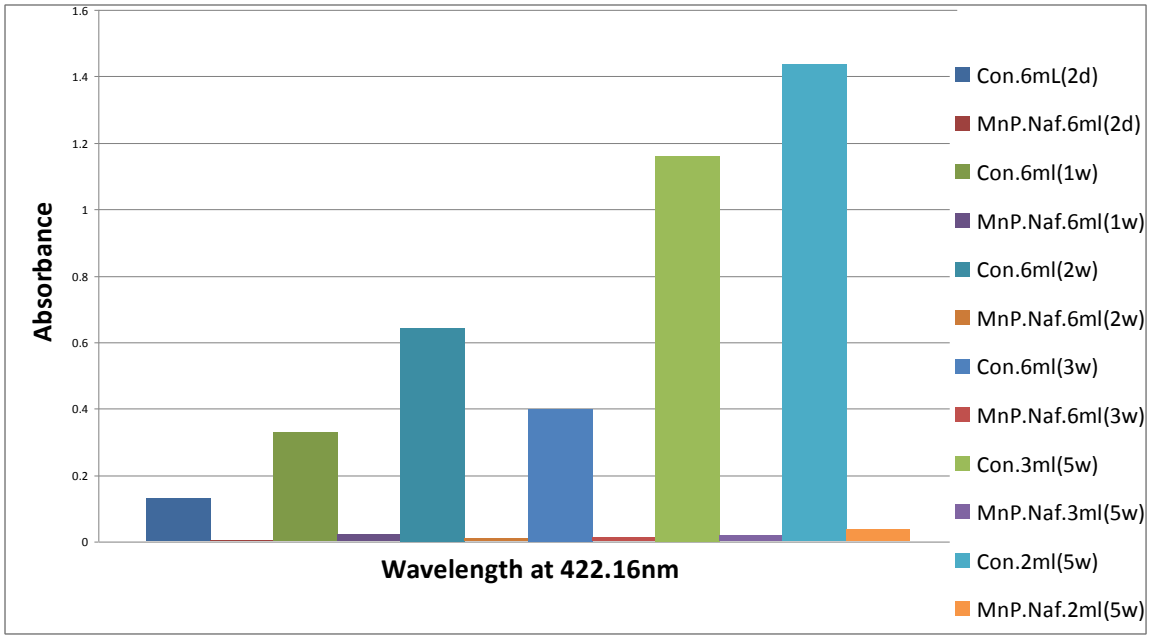

Figure 2: Bar graph showing the absorbance values at a wavelength of 422.16nm. The bar graph shows the blockage of PN by presoaked Nafion at different concentrations. The vials with presoaked Nafion show a very low absorbance, while control samples show high absorbance due to nitration of 4-HPA.

Conclusions

The change in color of only the control vials shows that the presoaked Nafion was successful in blocking PN formation. The UV/VIS spectra also support this. The bar graphs show the blockage of PN by presoaked Nafion at different concentrations. The vials with presoaked Nafion show a very low absorbance at the wavelengths where PN is found, while control samples show high absorbance due to nitration of 4-HPA. The blockage of PN by presoaked Nafion was shown at all time intervals, up until the last analysis at 5 weeks. Since part of the current problem with films is its durability, it is significant that it was successful for the duration of the experiment. This modification of Nafion could be further tested in vivo.

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