A new nanosensor for detection of *in vivo* hydrogen peroxide

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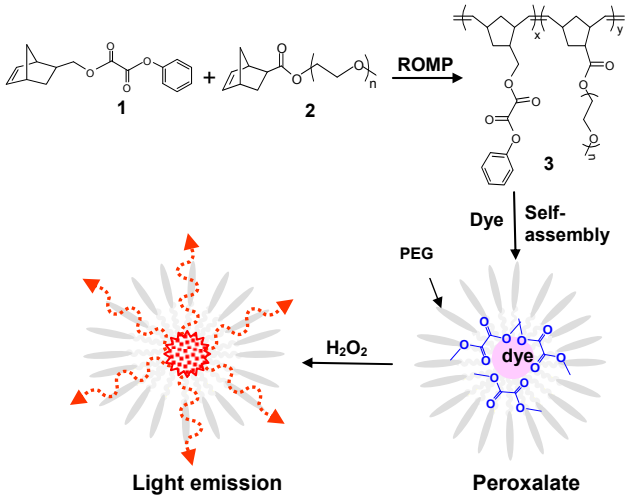
Statement of Purpose: Hydrogen peroxide is an important oxygen metabolite in living systems, but its overproduction is implicated in numerous human diseases[1]. Therefore, there is great interest in developing contrast agents that can image hydrogen peroxide *in vivo*. We reported that peroxalate nanoparticles composed of polymeric peroxalate esters and fluorescent dyes image hydrogen peroxide in the intraperitoneal cavity of mice, after a direct intraperitoneal injection, by performing a three component chemiluminescent reaction between a peroxalate ester, hydrogen peroxide and a fluorescent dye[2]. However, the applications of peroxalate nanoparticles are limited by their large size (550 nm) and hydrophobic surface. The objective of this research is the development of a new chemiluminescent nanosensor for hydrogen peroxide, which has nanomolar sensitivity and physicochemical properties needed for intravenous injections.

Methods: Norbornenylphenyl oxalate (**1**) was synthesized from the reaction of 5-norbornene-2-methanol, phenol and oxylic chloride. Norbornene-ended polyethyleneglycol (PEG) (**2**) was synthesized from the reaction of monomethoxy PEG and 5-norbornene-2-carboxylic acid. Amphiphilic peroxalate copolymer (**3**) was synthesized by ring opening metathesis polymerization (ROMP) of compounds **1** and **2**. Copolymer **3** and fluorescent dye rubrene in acetone was mixed with a large excess of phosphate buffer and vortexed, generating peroxalate micelles which have peroxalate esters and rubrene in their cores and PEG chains on their surface. Chemiluminescence response of peroxalate micelles were investigated using a fluorometer and a luminometer.



Scheme 1. Synthetic strategy of amphiphilic peroxalate copolymer and peroxalate micelles.

Results: Peroxalate micelles were designed to sequester peroxalate esters and fluorescent dyes in their core, enabling the three component peroxalate chemiluminescent reaction to occur in response to hydrogen peroxide. The peroxalate micelles were composed of the amphiphilic peroxalate copolymer **3** and the fluorescent dye rubrene. The copolymer **3** was synthesized by ROMP of compounds **1** and **2** and was determined to have average molecular weight 38,000. **3** had peroxalate esters and PEG chains grafted to their hydrophobic backbone and self-assembled with rubrene in aqueous solutions, generating peroxalate micelles that are in the range of 20 ~ 50 nm and have PEG corona. Their small size and PEG corona may enhance their extravasation into tissues and the circulation time, respectively. The ability of the peroxalate micelles to detect hydrogen peroxide was investigated by measuring their chemiluminescence intensity in the presence of hydrogen peroxide at various concentrations in the range of 0 - 1 μM, which is the speculated intracellular hydrogen peroxide concentration range of 1 ~ 700 nM[3]. Peroxalate micelles have excellent sensitivity to hydrogen peroxide and exhibit a linear correlation between chemiluminescence intensity and hydrogen peroxide concentration. The peroxalate micelles were capable of detecting hydrogen peroxide at concentrations as low as 50 nM, suggesting great potential for the detection of hydrogen peroxide at physiological concentrations.

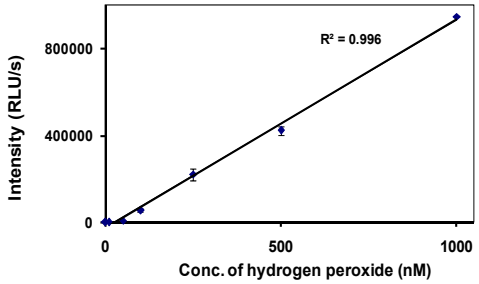


Figure 1. Chemiluminescence response of peroxalate micelles in the presence of hydrogen peroxide. Data are obtained from 1 mL of micelles (1 mg/mL) in response to the addition of increasing concentration of hydrogen peroxide. RLU, relative light intensity. Mean ± s.d., n=3.

Conclusions: We demonstrated that peroxalate micelles composed of amphiphilic peroxalate copolymer and rubrene are capable of detecting physiologically relevant concentrations of hydrogen peroxide through chemiluminescence. We anticipate numerous applications of peroxalate micelles, given their small size, high sensitivity, specificity and biocompatible PEG corona.

References

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(2) Lee, D. et al, *Nature Materials* **2007**, *6*, 765-769.
(3) Miller, E. W.et al, J. *Journal of the American Chemical Society* **2005**, *127*, 16652-16659.