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Sensing of Bacterial Endotoxin in Aqueous Solution by Supramolecular Assembly of Pyrene Derivative

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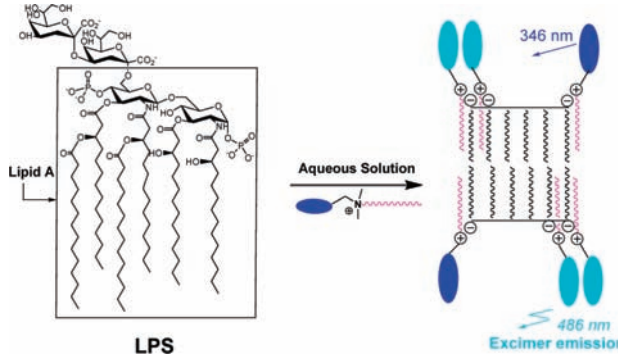
ABSTRACT



***N,N*-Dimethyl-*N*-(pyrenyl-1-methyl) dodecan-1-ammonium has been designed and applied as a fluorescent probe for sensing bacterial endotoxin. Upon assembly with bacterial endotoxin (lipopolysaccharide) in aqueous solution, the probe exhibits a ratiometric and sensitive fluorescence response with a low detection limit (100 nM). Moreover, the probe shows high selectivity for bacterial endotoxin over other related biological species.**

Sepsis and septic shocks cause about 150 000 casualties annually in the United States.[1] These are the results of massive release of a bacterial endotoxin, lipopolysaccharides (LPS), from the cell walls of Gram-negative bacteria.[2,3] Due to the high toxicity of LPS, considerable research has been focused on developing specific LPS detection systems. Currently, enzymatic limulus amebocyte lysate (LAL) assay is the most commonly used method for detection and quantification of LPS in clinical samples. While it is a sensitive method, it is highly susceptible to changes in temperature and pH environments.[4] In addition, other carbohydrate derivatives, such as β-glucans, also react positively in this assay.[4,5] It is thus highly desirable to develop other simple and more selective assays for detection of LPS in complex clinical samples.

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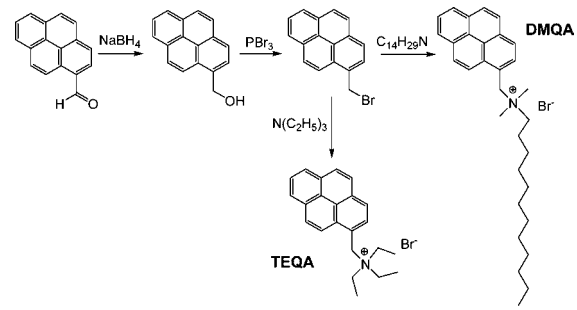
Fluorescent sensors for biological species are receiving considerable attention because of their high selectivity, sensitivity, and simplicity.[6] Various synthetic receptors have been used for detecting biomedical analytes including adenosine-triphosphate (ATP),[6] heparin,[7] protein,[8] and IP3.[9] However, for LPS, there has only been very limited progress.[10] The first synthetic sensor for LPS is a lipid-functionalized polydiacetylene liposome, which was applied to discriminate LPS from different species based on a colorimetric change.[10a] However, the concentration of LPS used in that experiment (around 100 μM) is much higher than the IC_{50} value (10 μM). Another sensor for LPS is based on carboxyltetramethylrhodamine/fluorescein-labeled CD 14 peptide,[4] in which the sensor is expensive and requires a complicated synthetic process. Therefore, we tried to develop a simple, efficient and low-cost sensor that can detect LPS at trace levels.

The primary toxic component of LPS is the lipid A core (see Abstract Graphic). In most LPS molecules, two 2-keto-3-deoxyoctonate units (each carrying carboxylic group) are linked to lipid A.[11] The sugar framework combined with 1 and 4′ phosphate moieties on the lipid A make LPS highly negatively charged. Indeed, LPS has amphiphilic properties as the glucosamine disaccharide core is modified with various long-chain fatty acid ester and amides. As a result, these long fatty chains of LPS would automatically arrange in order in aqueous solution. When the concentration of LPS reaches a certain extent, phospholipids bilayers would be formed in aqueous solution. With the detailed feature of LPS in mind, we designed a fluorescent molecule based on pyrenyl quaternary ammonium for LPS sensing. The synthetic pathway is described in Scheme 1, and experimental details are given in the Supporting Information. Pyrenyl bromide reacts with *N*,*N*-dimethyldodecylamine to yield a quaternary ammonium (**DMQA**) with a long alkyl chain. Such a modified **DMQA** can interact with the highly negatively charged LPS by the electrostatic interaction and hydrophobic

Scheme 1. Synthetic Pathway of Sensor (**DMQA**) and Model Compounds (**TEQA**)



interaction in aqueous solution.[12] We envision that the negatively charged carboxylate and phosphate groups located nearby in LPS molecule could catch two **DMQA** molecules via electrostatic interaction. Meanwhile, the long alkyl chain in **DMQA** could assemble with lipid A by the hydrophobic interaction. In this process, pyrenyl groups from the two **DMQA** molecules would stack onto each other via $\pi-\pi$ interaction. Upon photoexcitation, this molecular ensemble should thus give an enhanced pyrene excimer photoluminescence and applied as fluorescent probe for LPS detection.

UV−Vis spectrum of **DMQA** in HEPES solution displays a characteristic absorption band of pyrene (Figure 1). In the

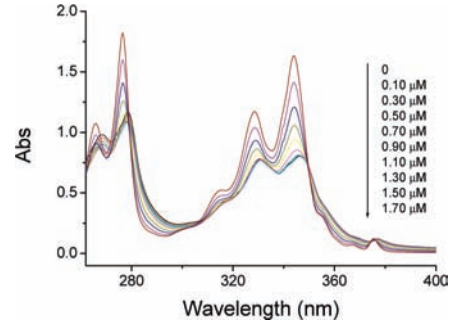


Figure 1. Changes in UV−vis spectra of **DMQA** (8.0 μM) upon addition of LPS in 10.0 mM CH_3OH/HEPES (v/v = 1/6, pH = 7.4).

presence of LPS, band broadening and a small red-shift are observed, which can be attributed to the intermolecular $\pi-\pi$ stacking of two pyrenyl groups in their ground state.[13] Figure 2a shows the corresponding fluorescence spectra of **DMQA** in the presence of LPS. Free **DMQA** exhibited a typical pyrene monomer emission around 370−430 nm. Upon

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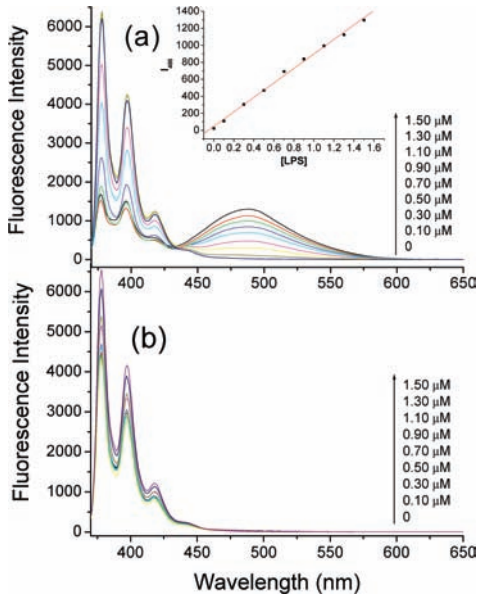

Figure 2. Changes in fluorescence emission spectra of (a) **DMQA** (8.0 μM) and (b) **TEQA** (8.0 μM) upon addition of LPS in CH_3OH/HEPES (v/v = 1/6) buffer solution (10 mM, pH 7.4). Excitation wavelength: 346 nm. (Inset) Variation in fluorescence intensity of **DMQA** (I_{486} upon addition of LPS (0−1.50 μM) in CH_3OH/HEPES solution (v/v = 1/6, 10 mM, pH = 7.4).

addition of LPS, a broad and structureless emission band peaked at about 486 nm builds up at the expense of the monomer emission peak. This spectral change was accompanied with an isoemissive point at 435 nm, indicating coexistence of the two fluorescent species. This attribution is further supported by time-resolved fluorescence decay measurements. The fluorescence lifetime of free **DMQA** is 22.8 ns monitored at 376 nm. However, in the presence of LPS, **DMQA** shows two fluorescence lifetimes (22.8 and 34.9 ns), which demonstrates that the excited molecules have two decay pathways. Moreover, the fluorescence lifetime monitored at 486 nm is 39.5 ns, which is also in favor of the excimer formation in the excited state.

By plotting the fluorescence intensity at 486 nm (I_{486}) versus the LPS concentration, a good linear relationship (I_{486} = 45.60 + 851.91 × [LPS], R = 0.998) was observed with the LPS concentration ranging from 0.10 to 1.50 μM (Figure 2a, inset). The detection limit of the present approach was found to be at least as low as 100 nM. This result is comparable to those obtained from previous sensor based on CD 14 peptide.[4]

To investigate the binding model between **DMQA** and LPS, we also synthesized a model compound (**TEQA**) whose only difference to **DMQA** is the lacking of a long alkyl chain (Scheme 1). As shown in Figure 2b, **TEQA** shows little response to the addition of LPS. It is considered that without the hydrophobic chain, **TEQA** cannot efficiently form an assembly with LPS. LPS is an amphiphilic molecule which would automatically arrange in order in aqueous solution, as shown in Figure 3. LPS will form monolayer or phospholipids in aqueous solution which its phosphate and

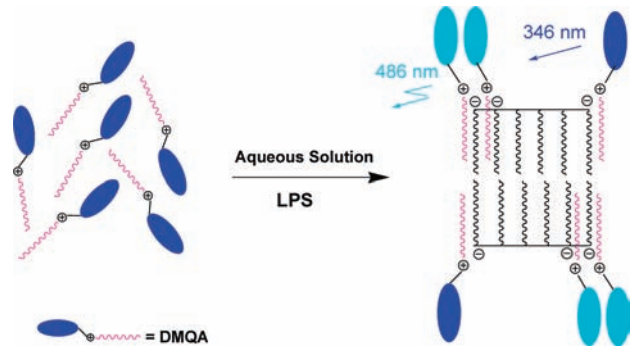

Figure 3. Proposed assembly mechanism between **DMQA** and LPS in aqueous solution.

carboxylate groups locate on the outer surfaces of the phospholipids bilayer.[14] In this circumstance, **DMQA** can easily incorporate into the phospholipids bilayer through hydrophobic effect and approach the negatively charged groups via electrostatic interaction. As a result, the neighboring phosphate and carboxylate groups in a LPS molecule can catch two **DMQA** molecules, resulting in excimer emission. As show in Figure 3, there also exist isolated carboxylate or phosphate groups along edges of the phospholipids bilayer. When a single **DMQA** molecule interacts with such isolated carboxylate or phosphate groups, it cannot form the stacked pyrene structure, which gives the excimer emission.

To further demonstrate the supramolecular assembly mechanism, we measured the fluorescence responses of **DMQA** to LPS in different solvents, and the results are shown in Figure 4. Only in aqueous solution, **DMQA** exhibits a distinct fluorescence response to LPS, while in absolute methanol, it cannot give rise to any excimer emission even at a higher LPS concentrations (5.0 μM). In aqueous solution, when the water fraction reaches 82.5%, addition of LPS can lead to the strongest **DMQA** excimer emission, indicating that the supramolecular assembly is the most efficient at this condition (Figure S8, Supporting Information).

Fluorescence responses of **DMQA** (8.0 μM) to various biological species are shown in Figure 5. The fluorescence intensity of **DMQA** at 486 nm is unchanged in the presence of phosphate, pyrophosphate, or triphosphate adenosine (ATP) even at a higher concentration (5.0 μM), indicating that the **DMQA** probe shows a good selectivity for LPS over other simple anionic phosphates. Moreover, the sensor shows no response to some coexisting proteins in practical samples, such as immunoglobulin and glucose. Phosphatidylcholine, which carries an ammonium end group, an anionic phosphate group, and a long alkyl chain, cannot induce any significant fluorescence enhancement at 486 nm. Furthermore, we also

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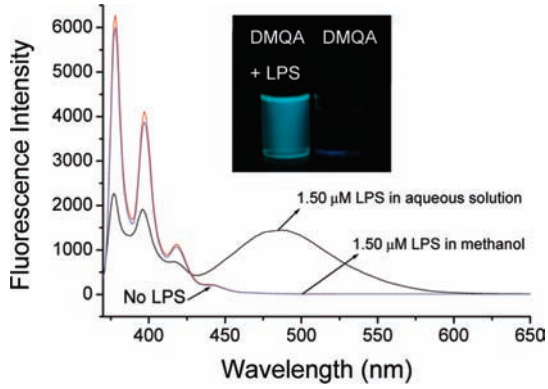

Figure 4. Fluorescence responses of **DMQA** (8.0 μM) to LPS in aqueous solution and absolute methanol. (Inset) Photographs of **DMQA** in the presence of 1.50 μM LPS in CH_3OH/HEPES (v/v = 1/6) solution (10.0 mM, pH = 7.4).

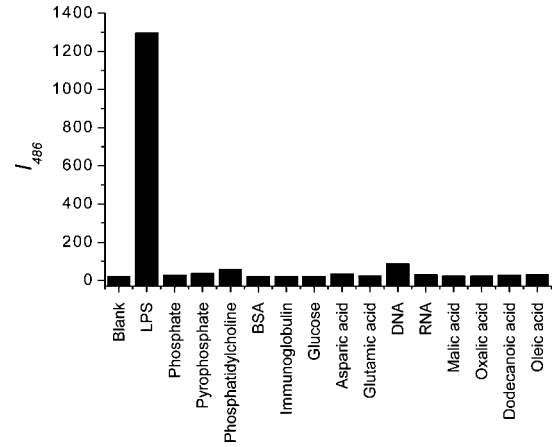

Figure 5. Selectivity of **DMQA** (8.0 μM) to some biologically important species in 10.0 mM CH_3OH/HEPES (v/v = 1/6, pH = 7.4). Pi, PPi, ATP, phosphatidylcholine, BSA, immunoglobulin, glucose, aspartic acid, glutamic acid, DNA, RNA, malic acid, oxalic acid, dodecanoic acid, oleic acid, and LPS were added at 1.50 μM into the sensor solution, respectively. Blank refers to free **DMQA** (8.0 μM) solution in 10.0 mM CH_3OH/HEPES solution (v/v = 1/6, pH = 7.4).

checked responses of the **DMQA** probe to some ordinary fatty acids including dodecanoic acid and oleic acid. Although these fatty acids have long alkyl chain and negatively charged end group, they cannot induce any **DMQA** excimer emission. This can be simply explained by the fact that a single fatty acid cannot effectively bind with two **DMQA** molecules to form the stacked pyrene structure. In addition, fluorescence titration curves of **DMQA** to LPS exhibit an obvious excimer formation even in the presence of other coexisting species, indicating the formation of the supramolecular assembly and a good selectivity to LPS (Figure S9, Supporting Information).

In summary, we have successfully developed a new pyrenyl-derived long-chain quaternary ammonium (**DMQA**), which exhibits a fluorescence response to LPS in aqueous solution. The sensing mechanism is due to the formation of pyrene excimer by supramolecular assembly of **DMQA** with LPS. **DMQA** can be applied for a high sensitive detection of LPS in aqueous solution with a low detection limit (100 nM). This work not only provides a simple method to detect LPS but also opens a new perspective to rationally design sensors via supramolecular assembly.

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Supporting Information Available: Synthetic procedures, charicterization of new compounds, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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