JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Peptide Functionalized Polydiacetylene Liposomes Act as a Fluorescent Turn-On Sensor for Bacterial Lipopolysaccharide

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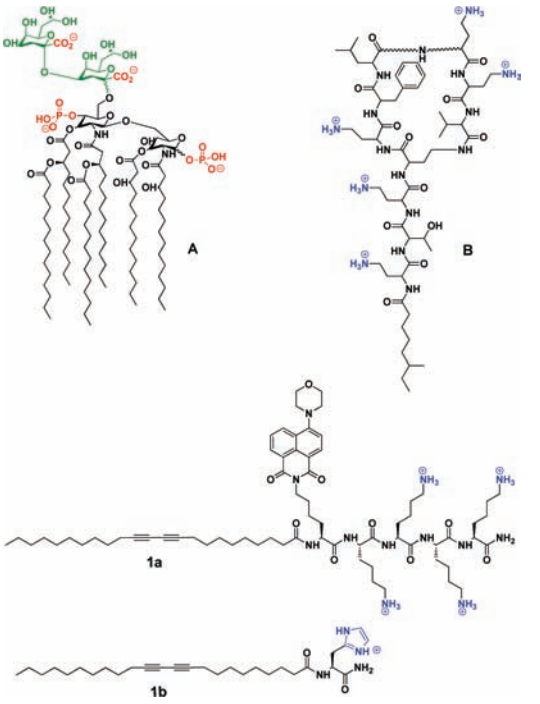
Supporting Information

ABSTRACT: Mixed polydiacetylene (PDA) liposomes functionalized on their surface with a fluorescent pentalysine peptide derivative and histidine in a ratio of 1:9 can identify bacterial lipopolysaccharide (LPS). Upon photopolymerization of the self-assembled liposomes the initial fluorescence of the peptide-diacetylene amphiphiles is quenched. Interaction with LPS in aqueous solution or on the surface of *E. coli* DH5α restores the fluorescence. This increase in fluorescence is selective for LPS relative to other negatively charged analytes including nucleotides and ctDNA. This simple turn-on fluorescent sensor allows detecting LPS even at low micromolar concentrations.

Lipopolysaccharide (LPS, also known as bacterial endotoxin) is the main constituent of the outer membrane of gram negative bacteria and mainly responsible for the integrity of the membrane and its low permeability, protecting the bacteria also in part from chemical attacks by antibiotics.[1] Although LPS can have beneficial effects at low concentrations (stimulating the immune response for example), it is toxic at higher concentrations and can lead to septic shock and even death.[2] The development of specific sensors for LPS is therefore a challenging field of current research. Even though a large variety of fluorescent or colorimetric chemical sensors for different biomedical analytes (including e.g. nucleotides,[3] anionic sugars,[4] proteins,[5] and even microorganisms[6]) were developed in recent years, only a very few synthetic receptors for LPS are known. The first reported example was based on amino acid functionalized polydiacetylene liposomes which changed their color from blue to red after addition of LPS.[7] However, this color change required rather high concentrations of LPS (ca. 100 μM). LPS detection down to micromolar concentrations was possible with a peptide hairpin beacon derived from the LPS-binding domain of the LPS receptor CD14 using a FRET effect.[8] Electrochemical sensing of LPS which was immobilized in self-assembled alkanethiol monolayers on gold electrodes was also reported.[9] Phosphate binding by a thiourea host was also employed for developing an artificial host for LPS.[10] The most recent sensor for LPS reported so far used lipophilic pyrene derivatives which formed excimers when they interact with LPS.[11] The formation of the excimer caused a shift of the fluorescence from the typical emission of a pyrene monomer at 370−430 nm to the emission of the excimer at 486 nm. Given the importance of LPS the lack of easy to use and sensitive chemical sensors of LPS is surprising.

Scheme 1. Structure of Lipid A (A), Polymyxin B (B), and the Two Peptide Amphiphiles 1a and 1b[a]



[a] Complementary charges are shown in red (negative) and blue (positive).

LPS consists of a polysaccharide attached to a lipid part (called lipid A) which anchors LPS into the membrane. LPS is highly negatively charged due to two phosphorylated glucosamine sugars in the Lipid A part and two 2-keto-3-deoxyoctanoate units connecting it to the polysaccharide (Scheme 1). It also contains at least six fatty acid chains making the overall molecule amphiphilic. Our idea was to take the naturally occurring antibiotic polymyxin B (PMB)[12] as a lead to designing a new fluorescent sensor for LPS. PMB is a cyclic peptide derivative with five positively charged L-*R*-γ-diaminobutyric acid residues connected to an alkyl chain. The five ammonium cations electrostatically bind to the negative charges of LPS whereas the alkyl chains anchor PMB within the Lipid A part increasing

Received: May 2, 2011
Published: May 26, 2011

dx.doi.org/10.1021/ja204013u | *J. Am. Chem. Soc.* 2011, 133, 9720–9723

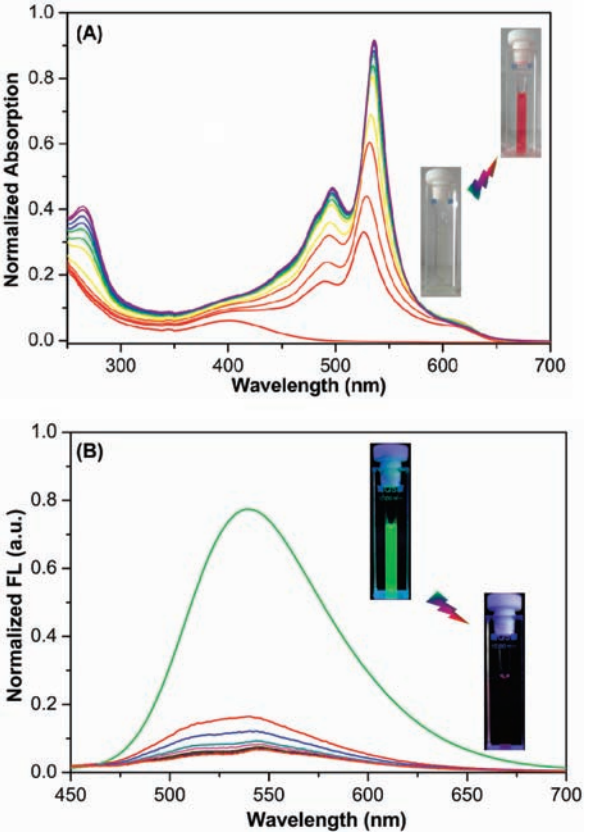


Figure 1. Normalized absorption (A) and emission (B) of a mixture of **1a**/**1b** in a molar ratio of 1/9 (total monomer concentration = 10.0 μM, 25 °C) as a function of irradiation time with UV-light (254 nm, *ca.* 3.0 mW/cm^2 for 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 min) in 10.0 mM DMSO/TBS (v/v = 1/4, pH = 7.4). The insets show the color and fluorescence changes of the solution.

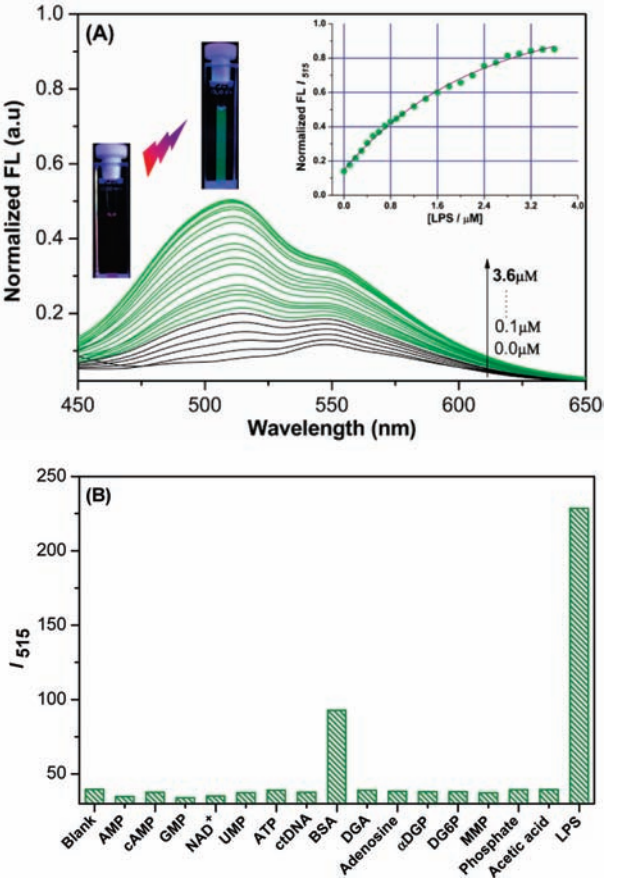


Figure 2. (A) Fluorescent emission spectra (λ_{ex} = 400 nm) from a titration of the PDA liposomes (molar ratio **1a**/**1b** = 1/9, total monomer concentration = 10.0 μM, 25 °C) in 10.0 mM DMSO/TBS (v/v = 1/4, pH = 7.4). The insets show the normalized fluorescence intensity at 515 nm vs the concentration of LPS (0−3.6 μM) and the fluorescence turn-on. (B) The selectivity of the increase of fluorescence upon addition of various biologically important species (at a concentration of 5.0 μM). Only LPS (and to a much lesser extent also the protein bovine serum albumin) gives rise to a significant increase in fluorescence.

the overall affinity for LPS. Our group has a long-standing expertise in the development of receptors and sensors for anionic biomolecules such as amino acids,[13] peptides,[14] sugars,[15] and nucleotides[16] using ion pair interactions. We therefore now wanted to design a structurally simpler analogue of PMB making use of similar binding interactions (ion pairing and hydrophobic interactions), but we also wanted to introduce an additional fluorophore. This fluorophore hopefully would allow us to sense LPS due to spectroscopic changes imposed by the complexation.

We have synthesized the two diacetylene amphiphiles **1a** and **1b** using solid-phase microwave-assisted peptide synthesis (for details, see Supporting Information (SI)). In **1a** a pentalysine oligopeptide is connected to 10,12-tricosadiyonic acid. We furthermore attached a naphthalic acid fluorophore to the N-terminal lysine. The other four lysine residues are unmodified and thus carry positive charges at physiological pH. In **1b** a histidine was connected to the tricosadiyonic acid instead of the pentalysine peptide. Both amphiphiles self-assemble in aqueous solution into vesicles. Due to the diacetylene unit, irradiation with UV light polymerized the aggregates forming a conjugated polydiacetylene liposome.[17] Such PDA liposomes have found widespread use as colorimetric sensors in recent years.[18,19] Normally one obtains a nonfluorescent 'blue-form' of the PDA liposomes, which changes into a weakly fluorescent 'red form' upon binding of analytes to its surface. In our case however irradiation of the highly fluorescent self-assembled monomer **1a** in a 1:9 mixture with **1b** led to a complete quenching of the fluorescence of the naphthalic acid unit. This quenching is caused by an energy transfer from the napthalic acid fluorophore (emission maximum at 540 nm) to the cross-linked polymer (absorption maximum at 536 nm). This absorption is not present in the monomers **1a** and **1b** but only in the cross-linked polymer (see SI) and might be due to mechanical stress induced onto the backbone due to the bulky head groups of the monomers.[18b] Polymerization studies with different ratios of **1a** and **1b** showed that the ratio of 1:9 is optimal to ensure complete fluorescence quenching (SI Figure S3). Hence, upon polymerization the colorless, highly fluorescent mixture of the self-assembled monomers turns into a nonfluorescent red solution of the cross-linked PDA liposomes (Figure 1). Dynamic light scattering in solution and atomic force microscopy on mica confirmed the presence of spherical self-assembled liposomes (SI Figures S4 and S5).

Binding of LPS to the polymerized PDA liposomes (10.0 μM) restored the fluorescence (Figure 2) even at submicromolar concentrations of LPS to approximately 48% of the original value. This fluorescence response is highly selective for LPS.

dx.doi.org/10.1021/ja204013u |*J. Am. Chem. Soc.* 2011, 133, 9720–9723

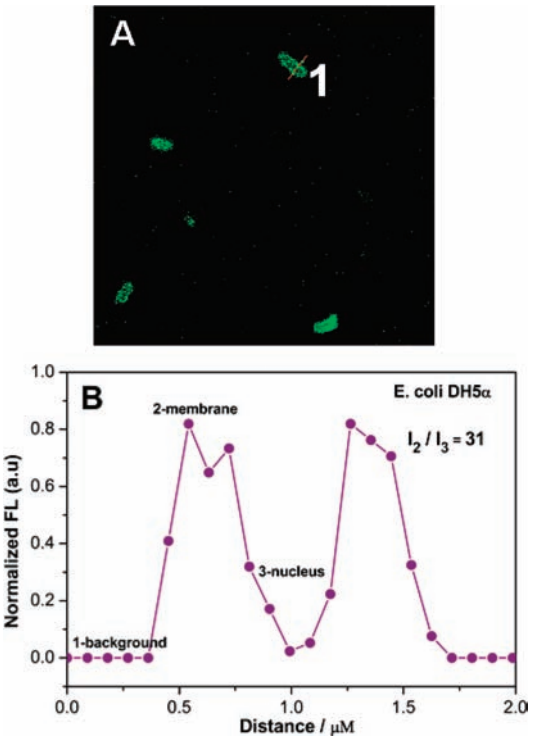


Figure 3. Confocal luminescence image (A) and luminescence intensity profile (B) across the line shown in (A) for *E. coli* DH5α after incubation with the PDA liposomes (40.0 μM) for 12 h at 37 °C (λ_{ex} = 405 nm). The large membrane-to-nucleus ratio (I_2/I_3) indicates that the fluorescence stems mainly from the membrane of the *E. coli* DH5α.

Other biological relevant species such as nucleotides, anionic sugars, or ctDNA do not give any detectable increase in fluorescence at all at a concentration of 5.0 μM. The only exception is the protein BSA (bovine serum albumine) which also leads to a fluorescence increase but significantly smaller than that for LPS (only 40% compared to LPS). Hence, our liposomes function as a highly selective fluorescence turn-on sensor for LPS. A Stern−Volmer analysis of the fluorescence increase provided a binding constant of $K = 1.5 \times 10^6$ M^{-1}. DLS showed that the interaction of the liposomes with LPS causes a significant increase in size of the aggregates present in solution from 18 to 48 nm in diameter (Figure S4).

We then tested whether our functionalized PDA liposomes are also capable of interacting with LPS on the surface of bacteria. Therefore, freshly diluted *E. coli* (strain DH5α, OD = 0.982, $C = 4.91 \times 10^8$ cfu/mL) were incubated in the presence of the PDA liposomes at a concentration of 40.0 μM (TBS buffer, pH = 7.4, at 37 °C for ca. 12 h). Even at such low concentrations a significant increase in fluorescence was observed (SI Figure S8). These results were confirmed by confocal laser scanning microscopy which showed a significant fluorescence coming from the membrane of *E. coli* DH5α after incubation with the PDA liposomes (Figure 3). The large membrane to nucleus ratio of >30 indicated that the fluorescence stems mainly from the membrane of the cell. The control experiment showed that the PDA liposomes are not toxic to either bacteria or human cells (at least up to concentrations of 40 μM; see SI).

In conclusion, we have presented the first fluorescent turn-on sensor for the detection of bacterial lipopolysaccharide (LPS) at micromolar concentrations in water. The sensing is based on fluorescence changes of polydiacetylene liposomes which are functionalized on their surface with a naphthalic acid modified pentalysine peptide and histidine in a ratio of 1/9. Upon polymerization the initial fluorescence of the naphthalic acid fluorophore is quenched due to an energy transfer to the conjugated polymer. Binding of LPS to the PDA liposomes restores the fluorescence. This fluorescence turn-on is selective for LPS compared to other anionic biological substrates and even allows fluorescence staining of the membrane of *E. coli* bacteria. We are currently exploring the selectivity of our sensing system on other bacteria, and we are testing whether the sensor can be further improved by using tailor-made anion binding sites instead of simple lysines on the surface of the PDA liposomes. These results will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information. Details of the syntheses, spectroscopic characterization, UV−vis and fluorescent spectra, DLS, AFM, and CLSM experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

J.W. thanks the Alexander von Humboldt Foundation for a postdoctoral fellowship. We also thank Dr. Ying Zou (Fudan University) and Dr. Meng Xiao Yu (University of Texas at Dallas, US) for help with the confocal luminescence imaging.

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