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Lipopolysaccharide Identification with Functionalized Polydiacetylene Liposome Sensors

Marianne Rangin and Amit Basu*

Department of Chemistry, Brown University, Providence, Rhode Island 02912

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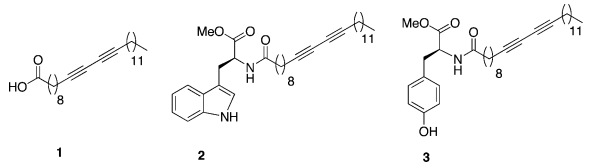
Lipopolysaccharides (LPS) are complex glycolipids embedded within the outer membrane of Gram negative bacteria.[1] Each cell contains over 2 million copies of LPS.[1b] LPS consists of a conserved lipidated disaccharide, known as Lipid A, which is attached to a core oligosaccharide fragment. The core region is extended by additional glycosylation to provide the O-specific antigen or polysaccharide region. The identity of these terminal sugars vary for different bacterial species and serotypes. Sensors which are capable of detecting and identifying different types of LPS can be used to develop devices for bacterial diagnostics.

Polymerized liposomes prepared from 1,3-diacetylenic lipids have found wide application as sensors.[2] The polydiacetylene functions as the signaling component of the sensor, while selective recognition is provided by including lipids that have been modified with a receptor for the analyte of interest. Receptor/analyte combinations that have been successfully analyzed using these liposomes include antibody/antigen, ionophore/cation, glycolipid/lectin, and glycolipid/bacteria pairs.[3] The cross-linked liposomes have a deep blue color due to the extensively conjugated polymer backbone. Binding of the analyte to the embedded receptor causes the liposomes to change color from blue to red.

Our interest in developing a general approach for LPS recognition led us to consider an alternative strategy for employing polydiacetylene sensors based on the principle of the electronic tongue.[4] In an electronic tongue, there is no unique receptor that is selective for a given analyte. Instead, one exposes the analyte to an array of receptors, several of which may interact with the analyte in differing degrees. Selectivity in this scenario is derived from the unique combination of array sites that interact with a given analyte, giving rise to a diagnostic pattern, or fingerprint, for that analyte. Moreover, a single array can be used to generate a different fingerprint for multiple analytes. In this communication, we report the generation of a set of unique fingerprints using diacetylene liposomes. These fingerprints were used to identify and detect LPS from five strains of Gram negative bacteria.



1 2 3

We selected the lipids **2** and **3** as recognition elements for LPS on the basis of the observation that carbohydrate binding proteins frequently use tryptophan and tyrosine residues to make contacts with sugars.[5] Liposomes were prepared by suspending a mixture of either diacetylene **2** or **3** (5 mol %) and **1** (95 mol %) in water, followed by sonication, cooling, and polymerization, according to established protocols.[3d] An aliquot of LPS was added to the liposome solution at room temperature, and the color change of the liposomes from blue to red was quantified by calculating the colorimetric response (CR, eq 1). The CR is derived from the change in the ratio of absorbances at 640 and 550 nm in the absence (B_o) and presence (B_{lps}) of LPS.[2] A representative visible spectrum, along with an image of the liposome solution with and without LPS, is shown in Figure 1.

$$CR = \frac{B_o - B_{lps}}{B_o} \times 100\% \quad (1)$$

$$\text{where } B_o = \frac{A_{640}}{A_{640} + A_{550}} \text{ and } B_{lps} = \frac{A_{640}}{A_{640} + A_{550}}$$

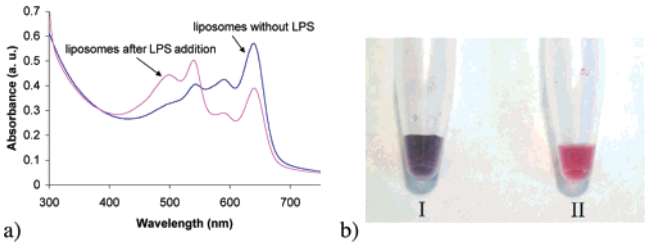


Figure 1. (a) Representative spectrum of polydiacetylene liposome solution in the presence and absence of LPS. (b) I: liposome solution without LPS; II: liposome solution after LPS addition.

The experiment was also conducted at a higher temperature (35 °C) to provide a new set of CR values. We also examined the sensor response in the presence of two additives—the detergent sodium dodecyl sulfate (SDS) and the metal chelating agent ethylenediamine tetraacetic acid (EDTA). LPS aggregates in solution, and the presence of a detergent could potentially affect the aggregation state of LPS and affect the CR.[6] Likewise, EDTA was used to sequester any divalent cations interacting with the phosphate groups present in LPS in an effort to see if this would also alter LPS clustering and influence the CR.[7]

Figure 2A shows the CR values obtained for LPS from *Escherichia coli* O26:B6. In the absence of additives, the CR values at room temperature from the tyrosine liposomes are always greater than those from the tryptophan liposomes. When the binding experiment is carried out at 35 °C, both sensors provide higher CR values. However, the presence of SDS or EDTA affords low CR values with both liposomes. This set of eight CR values constitutes an eight-membered data array which defines a diagnostic fingerprint for *E. coli* O26:B6. Fingerprints for four other LPS were generated in a similar manner, and are shown in graphsB−E of Figure 2.

The CR values vary from 0 to 55% depending on the LPS source organism and the experimental conditions. A single CR value from one experiment is typically insufficient to identify a particular LPS, since many experiments provide the same CR values for different LPS. For example, the CR values at room temperature alone do not allow one to differentiate between LPS from *E. coli* O26:B6

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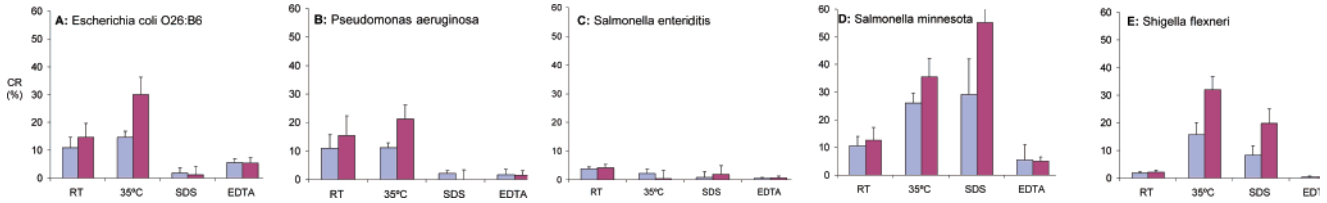

Figure 2. CR values obtained upon exposure of the liposome array to LPS from different Gram negative bacteria [(blue) - Trp; (red) - Tyr)]. All values are the average of at least four experiments: [liposome] ≈ 0.6 mM, [LPS] ≈ 2.2 mg/mL, [SDS] = 2 mM, [EDTA] = 1 mM. See Supporting Information for details.

and *Pseudomonas aeruginosa*, or even *Salmonella minnesota*. Similarly, the CR values obtained at 35 °C with LPS from *E. coli* O26:B6 and *Shigella flexneri* are also almost identical, and it would not be possible to identify these two LPS using only these CR values. A quick comparison of the fingerprints for *E. coli* and *P. aeruginosa* (Figure 2, A and B) indicates two very similar patterns. A closer inspection reveals subtle differences in both the relative and absolute magnitudes of the CR values. The response from the *E. coli* LPS in the presence of EDTA is consistently higher than the CR values obtained in the presence of SDS. In contrast, for *P. aeruginosa*, the EDTA and SDS responses are very similar. The present array also allows us to distinguish between two different serogroups of *Salmonella*, since LPS from only one of the serogroups (*minnesota*) generates a significant colorimetric response.[8] Thus, when considered *in toto*, the complete fingerprint is uniquely diagnostic for a specific LPS in the data set.

Most significantly, the fingerprints obtained from this set of five LPS are unique enough to be able to identify all five lipopolysaccharides unambiguously in a blind test.[9] In this experiment, the sensors were exposed to the five LPS types shown in Figure 2 to generate a set of CR values. An impartial observer was able to identify each LPS by comparing the set of CR values obtained with the unknown sample to the five fingerprints shown in Figure 2.

This set of fingerprints is not without its limitations. For example, a clearer distinction between the fingerprints for *E. coli* and *P. aeruginosa* would reduce the possibility for a misassignment. Likewise, a definitive fingerprint for *Salmonella enteriditis* would be preferable to the null result observed with the current data array. Additionally, as the number of potential analytes increases, a larger array, along with pattern recognition algorithms, will be required for uniquely identifying a particular analyte.

In summary, we have shown that LPS from five different organisms can be identified on the basis of their differential interactions with polydiacetylene liposomes which have been functionalized with amino acids.[10] These proof-of-concept experiments suggest a novel approach for developing sensors for specific strains of bacteria. Future efforts are focused on extending this approach to carry out LPS detection directly on intact bacteria and the preparation of immobilized arrays of liposomes. The results of these studies will be reported in due course.

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Supporting Information Available: Detailed experimental procedures for lipid synthesis, liposome preparation, binding studies, along with compound spectra and CR values for the blind tests (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Control experiments indicate that a fully intact lipopolysaccharide fragment is required to elicit a response. The fingerprint of the intact LPS from *E. coli* O127:B8 was compared to the responses from the detoxified form of that LPS, which lacks the lipid moieties at the terminus. The CR values from the detoxified form of the LPS were uniformly lower than those from the intact LPS, suggesting that the lipid component of LPS is important for the liposome−LPS interaction. However, the lipid portion alone does not account for a significant component of the response, since exposure of the liposomes to Lipid A does not afford high CR values. Thus, the lipid anchor and the polysaccharide are both required for a colorimetric response to be observed. (See Supporting Information for data.)
(9) Data for the blind test is found in the Supporting Information.
(10) The presence of the amino acid side chains in **2** or **3** is required for specific interactions with the LPS, as liposomes composed of only **1** exhibit much lower CR values and poor specificity. Additionally, decreasing the net negative charge of the liposomes by the inclusion of a neutral filler lipid increases the CR values while decreasing specificity. These observations suggest that both electrostatic and aromatic carbohydrate interactions play a role in the association of the LPS with the liposomes.

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