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A Polydiacetylenes-Based Sensor for Discriminating Oleic Acid from Stearic Acid and Elaidic Acid

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Oleic acid, a monounsaturated fatty acid, is the most abundant fatty acid in human adipose tissue. As positive health effects, oleic acid may hinder the progression of adrenoleukodystrophy (ALD), which is a fatal disease that affects the brain and adrenal glands and attacks the myelin sheaths of the body, causing symptoms similar to those in multiple sclerosis.[1] Numerous studies indicate that a diet containing rich oleic acid can lower serum cholesterol by diminishing oxidative stress and inflammatory mediators while promoting antioxidant defenses.[2] In addition to being used as an excipient, oleic acid is often used widely as an emulsifying or solubilizing agent in pharmaceuticals and cosmetics.[3] On the other hand, the deleterious effects of oleic acid have been attributed to increases in the permeability of both vascular and alveolar epithelium to solute, caused by changes in membrane fluidity and increases in intracellular calcium concentration.[4] It is not easy to discriminating oleic acid from other fatty acids, such as stearic acid and elaidic acid, owing to their high structural similarity. The common method for detecting oleic acid is gas chromatography, which involves lipid extraction and the conversion of the fatty acids into methyl esters, leading to a time-consuming process.[5] The other method using liquid chromatography-mass spectrometry (LC-MS) afforded the highest sensitivity toward the detection of fatty acid. However, its application has some drawbacks such as low productivity, high cost, and complex analysis process for sample preparation and derivatization.[6] Therefore, it is essential to find a direct and fast method for detecting oleic acid by measure, without chemical pretreatment of samples. Spectral methods (such as fluorimetric and colorimetric methods) in conjunction with suitable probes are preferable approaches for the measurement of the analytes of interest because they are rapidly performed, non-destructive, highly sensitive, suitable for high-throughput screening applications, and can afford real information on the localization and quantity of the targets.[7]

Polydiacetylenes (PDAs), which can be prepared by the UV irradiation of self-assembled diacetylene (DA) supramolecules, have attracted considerable attention in recent years.[8] Upon environmental stimulation, the blue PDAs can undergo a color shift to a red phase with the enhancement of fluorescent intensity at about 560 nm.[8] The unique nature of PDAs has led to the development of a number of chemosensors for various analytes such as DNAs, viruses, anions, metal ions and surfactants.[9] In our previous work, the **IM-**

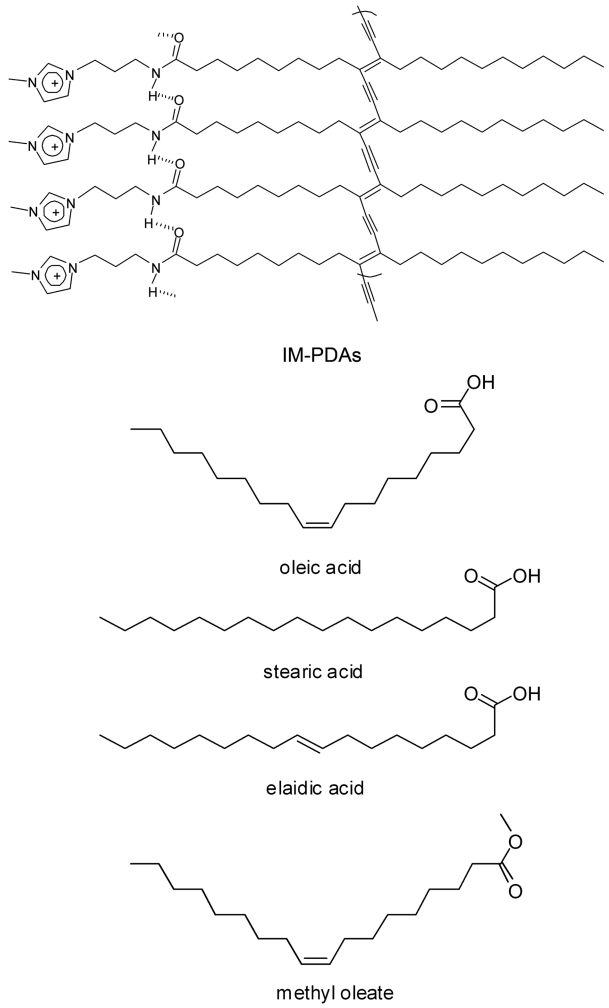

Figure 1. Structures of **IM-PDAs**, oleic acid, stearic acid, elaidic acid and methyl oleate.

[a]These authors equally contributed to this work

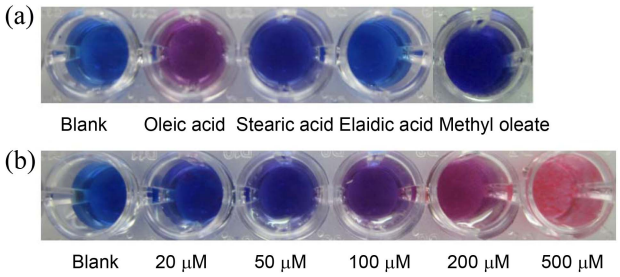

Figure 2. (a) Colorimetric responses of **IM-PDAs** (100 μM) in the presence of various analytes (100 μM) including oleic acid, stearic acid, elaidic acid and methyl oleate, in HEPES buffer (20 mM, pH 7.4); (b) Colorimetric changes of **IM-PDAs** (100 μM) with various amounts of oleic acid.

PDAs containing imidazolium groups at the terminus have shown selective and unique color changes as well as fluorescence changes when exposed to anionic surfactants (Figure 1).[9b] Herein, we further demonstrated the polymer can be used to discriminate oleic acid from stearic acid and elaidic acid through colorimetric changes.

The colorimetric response of the conjugated polymers was examined at room temperature using oleic acid, stearic acid, elaidic acid and methyl oleate. Figure 2(a) shows photographs of **IM-PDAs** (100 μM) in the presence of various analytes (100 μM) in HEPES (20 mM, pH 7.4). Only oleic acid induced color changes from blue to violet. In contrast, no changes were observed with stearic acid, elaidic acid and methyl oleate. Figure 2(b) explains the concentration dependent colorimetric changes of polymer **IM-PDAs** (100 μM) in the presence of various amounts of oleic acid in HEPES (10 mM, pH 7.4). With the concentration of oleic acid increasing from 0-500 μM, **IM-PDAs** underwent the color changes from blue to violet, then to red, and the transformation can be monitored *via* naked-eye.

The oleic acid-induced phase transition of **IM-PDAs** was further monitored by visible absorption spectroscopy. As displayed in Figure 3(a), the addition of oleic acid results in the decrease of the absorption at 620 nm with simultaneous increase of the absorption at 538 nm, indicating this system is a typical blue-to-red transition of the PDA sensors. Figure 3(b) shows the representative percentage colorimetric response values (CR %), calculated for oleic acid from the visible spectra in Figure 3(a). CR is a parameter reflecting the change in the visible spectrum after adding the test analyte to the colorimetric polymers.[10] A linear relationship between CR value and the concentration of oleic acid within the range of 0-20 μM was observed.

Since the blue-to-red transition of the PDAs is accom-

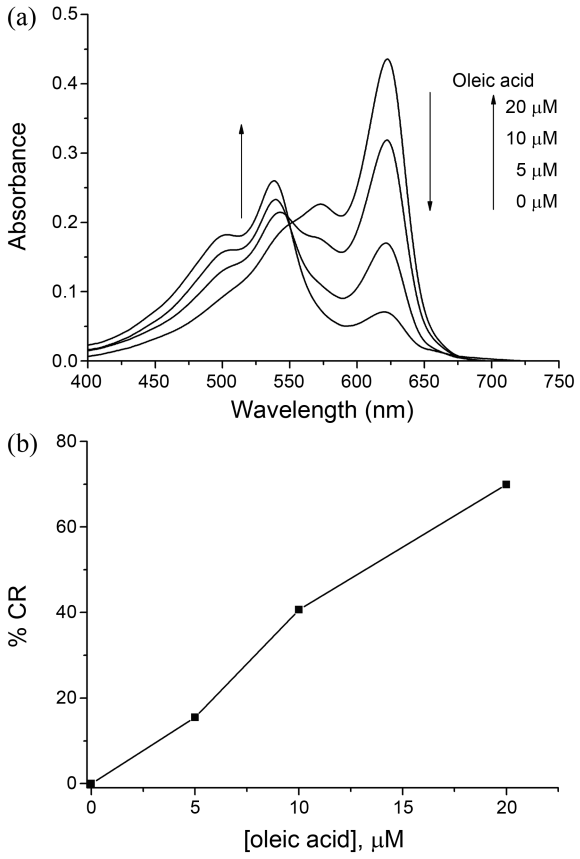

Figure 3. (a) Colorimetric and fluoresent responses of **IM-PDAs** (10 μM) in the presence of oleic acid with various concentrations in HEPES (20 mM, pH 7.4) buffer; (b) Quantitative colorimetric response of **IM-PDAs** (10 μM) in the presence of oleic acid with various concentrations.

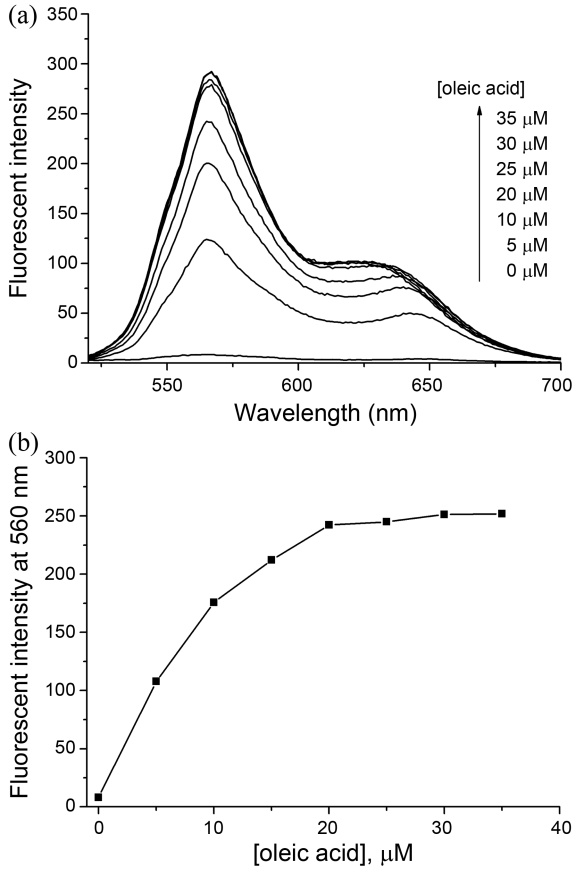

Figure 4. (a) Fluoresent responses of **IM-PDAs** (10 μM) in the presence of various concentrations oleic acid in HEPES (20 mM, pH 7.4) buffer. (Excitation at 485 nm, slit: 5 nm/5 nm); (b) Fluorescent intensity at 560 nm in the presence of various concentrations oleic acid.

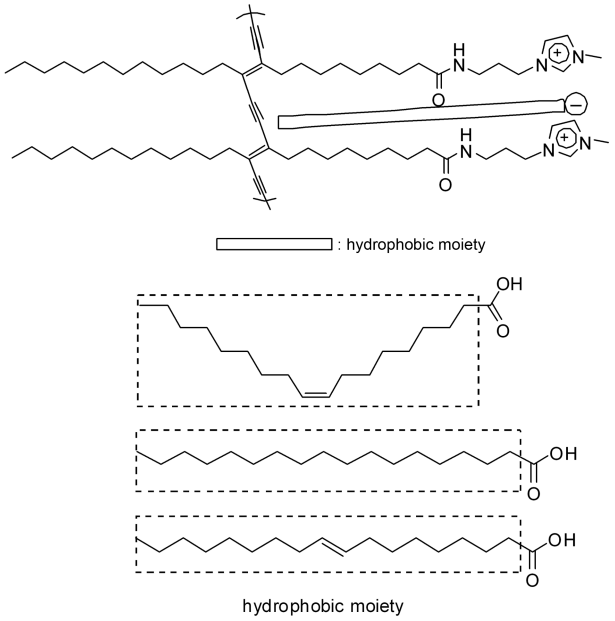

Figure 5. Proposed mechanism of optical responses of **IM-PDAs** induced by oleic acid.

panied by the generation of the fluorescence,[11] the oleic acid-promoted transition was also monitored by fluorescence spectroscopy (Figure 4). As displayed in Figure 4(a), upon the addition of oleic acid, **IM-PDAs** (10 μM) results in a large fluorescent enhancement at about 560 nm when was excited at 485 nm. The fluorescence spectra of **IM-PDAs** showed a gradual increase in the presence of 0-20 μM oleic acid and reach a plateau when the concentration of oleic acid is morn than 20 μM.

The colorimetric and fluorimetric responses of **IM-PDAs** should be speculated to the following mechanism. When oleic acid are added, the disruption of hydrogen bonding and π-π stacking formation can allow the release of the strain energy imposed on the alkyl side chains generated during polymerization.[12] The release of side-chain strain can cause partial distortion of the arrayed p orbitals, which can lead to the observed change in optical properties. Both anionic head group and appropriate long hydrophobic moiety are required. As shown in Figure 2(a), methyl oleate, without anionic head group, did not induce any colorimetric change, indicating the importance of hydrophilic head. Comparing to stearic acid and elaidic acid, the *cis*-form of oleic acid features a shorter hydrophobic moiety, which should be more matched to the formation of **IM-PDAs** (Figure 5). In contrast, in the cases of stearic acid and elaidic acid, longer hydrophobic moieties have negative effect on the interaction between fatty acids and polymer, so that no response was observed.

In conclusion, we have explored the application of **IM-PDAs** as chemosensor system for the detection of oleic acid in aqueous solution. Among the various fatty acids, **IM-PDAs** displayed a selective colorimetric change from blue to red, as well as fluorescence enhancement. Most importantly, the presence of oleic acid can be easily monitored *via* naked-eye detection.

Experimentals

General. Oleic acid, stearic acid, elaidic acid and methyl oleate were purchased from Sigma-Aldrich and used without further purification. The polymer **IM-PDAs** was prepared according to the previous report.[9b] The stock solution of fatty acids was prepared by mixing an appropriate aliquot solid and 1 equiv. NaOH in deionized water, and methyl oleate was dissolved in DMSO.

UV Spectroscopy. Except thermochromic properties experiments, all other measurements were carried out at 25 °C using a 1 cm optical path length cell. To quantify the extent of the blue-to-red or blue-to-yellow color transitions within the polymer, the CR (%) was calculated using the following equation:

$$CR = [(PB_0 - PB_1)/PB_0] \times 100$$

where $PB = A_{blue}/(A_{blue} + A_{red})$, A is the absorbance at either the blue component or the red component in the UV-vis spectrum. PB_0 and PB_1 are pre- and postexposure values, respectively.

Fluorescent Spectroscopy. Stock solutions of **IM-PDAs** (200 μM) were prepared by mixing 2 mL polymers (1 mM) and 8 mL HEPES buffer (0.02 M, pH 7.4). In a typical experiment, test solutions were prepared by placing 150 μL of the stock solution into a test tube, diluting the solution to 3 mL with 0.02 M HEPES (pH 7.4), and adding an appropriate aliquot of oleic acid stock solution. Normally, excitation was at 485 nm. Both the excitation and emission slit widths were 5 nm.

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