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Phospholipid-Induced Aggregation and Anthracene Excimer Formation

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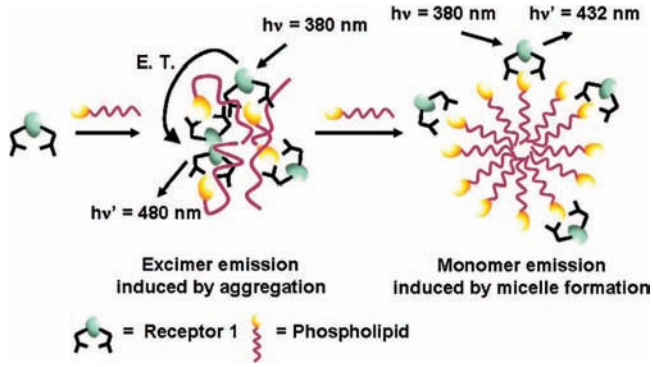
ABSTRACT



The zinc complex of anthryl bis(dipicolylamine) (1) aggregates upon binding with long-chain aliphatic phosphates and displays anthracene excimer fluorescence, which provides a new strategy toward detection of the biologically important lysophosphatidic acid in aqueous solution.

Lysophosphatidic acid (LysoPA) is a phospholipid that influences many biochemical processes, including stimulation of cell growth, promotion of platelet aggregation, and induction of cancer cell invasion.[1] LysoPA is found at a significant level (approximately 10 μM) in ovarian cancer cells[2] and can be a useful marker for diagnosis of ovarian cancer.[3] In this context, a method of effective detection of LysoPA is highly desirable. While fluorescent chemosensors have the general advantages of high sensitivity, high throughput, and real-time screening, examples of LysoPA chemosensors are rare.[4] In particular, one that can selectively distinguish LysoPA from phosphate ions in aqueous solution remains to be developed.

We report herein that the anthryl bis(dipicolylamine)−zinc complex **1** previously reported by Hamachi and co-workers displays an unusual and selective fluorescence response to LysoPA in aqueous buffer solutions through a concentration-dependent formation of aggregates and fluorescent anthracene excimers. Complex **1** has been shown to display an off−on fluorescence response to phosphorylated molecules[5] and phosphatidylserine-containing membranes,[6] which can be ascribed to the modulation of the photoinduced electron

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transfer (PET) from the amino group to the antrhacene fluorophore. However, anthracene excimer emission of **1** has been elusive in these studies. In view of the fact that anthracene excimer vs monomer emission would allow a more favorable ratiometric detection of LysoPA and that the observation of anthracene excimer emission in solutions is rare,[7] it is desirable to understand the origin of LysoPA-induced excimer formation for **1**.

At the early stage when incremental amounts of LysoPA are added to a solution of **1** in HEPES buffer, the fluorescence maximum shows red shifts by 30−50 nm along with decreased fluorescence intensity (Figure 1a). However, when

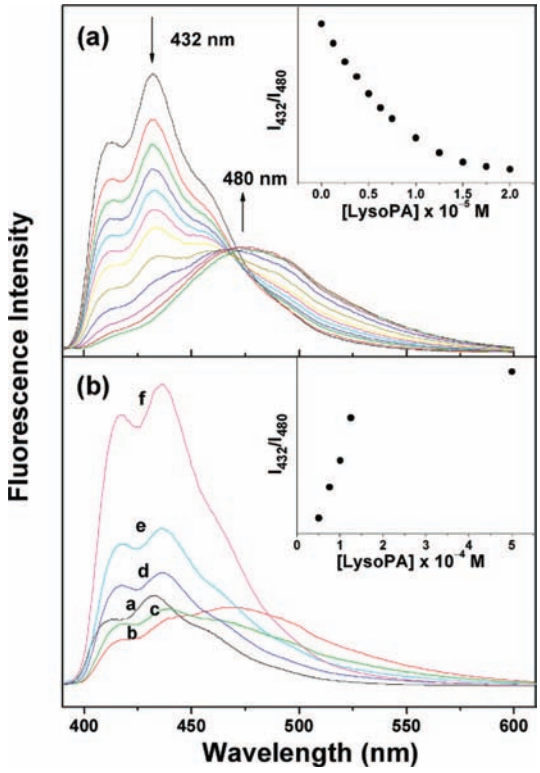
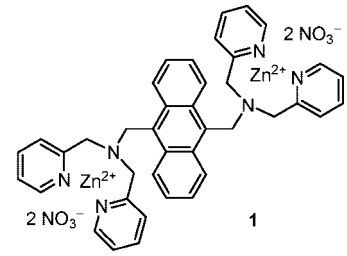
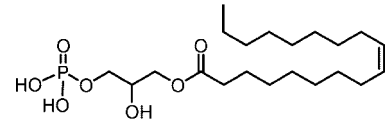


Figure 1. Fluorescence titration spectra of **1** (5 μM) with incremental addition of LysoPA (as the sodium salt) (a) up to 4.0 equiv and (b) up to 100 equiv (spectra a−f are with 0, 10, 15, 20, 25, and 100 equiv of LysoPA, respectively) in HEPES buffer (10 mM, pH 7.22). The excitation wavelength was 380 nm. The inset shows the ratio of the 432- and 480-nm fluorescence intensity (I_{432}/I_{480}) during titration.

the addition of LysoPA exceeds 10 equiv, the fluorescence change is reversed, i.e., the fluorecence maximum shifts back to 437 nm and the fluorescence intensity is recovered and even enhanced (Figure 1b). To our knowledge, such a two-stage and analyte concentration-dependent "reversible" fluorescence sensing behavior is unprecedented.

To understand the distinct fluorescence sensing behavior for **1** to LysoPA vs phosphoryl peptides,[6] we have systematically investigated the fluorescence behavior of **1** in the presence of phosphate ion (PO_4^{3-}) and aliphatic phosphates of different chain lengths, including hexyl phosphate ($C_6H_{13}OPO_3H_2$), octyl phosphate ($C_8H_{17}OPO_3H_2$), decyl phosphate ($C_{10}H_{21}OPO_3H_2$), dodecyl phosphate ($C_{12}H_{25}OPO_3H_2$), and tetradecyl phosphate ($C_{14}H_{29}OPO_3H_2$). As shown in Figure 2, the fluorescence titration spectra





Lysophosphatidic acid (LysoPA)

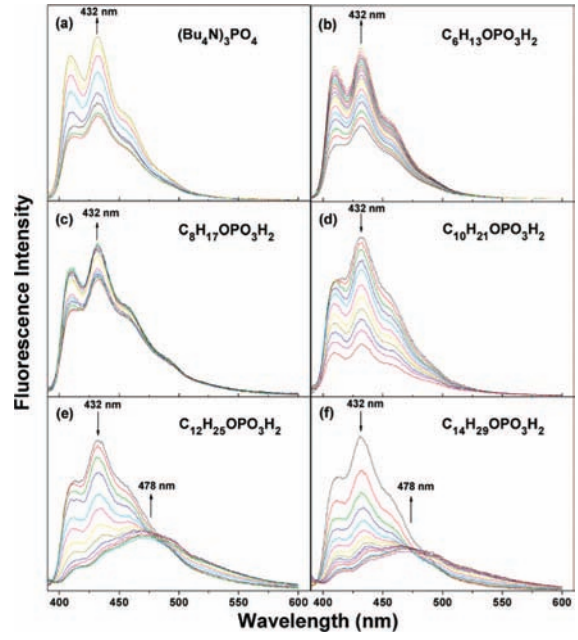


Figure 2. Fluorescence titration of receptor **1** (5 μM) in HEPES buffer (pH 7.22) by incremental addition of (a) $(Bu_4N)_3PO_4$, (b) $C_6H_{13}OPO_3H_2$, (c) $C_8H_{17}OPO_3H_2$, (d) $C_{10}H_{21}OPO_3H_2$, (e) $C_{12}H_{25}OPO_3H_2$, and (f) $C_{14}H_{29}OPO_3H_2$, up to 5 equiv in each case. The excitation wavelength was 380 nm.

highly depend on the length of the *n*-alkyl group. Whereas PO_4^{3-}, hexyl phosphate, and octyl phosphate enhance the fluorescence without changing the wavelength of peak maximum, decyl phospate simply induces fluorescence quenching,[8] and the long-chain dodecyl and tetradecyl phosphates lead to a new and structureless emission band at ~480 nm, similar to that observed in the case of LysoPA. Accordingly, the formation of the 480-nm emission band is

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more likely associated with the behavior of its aliphatic chains. It should also be noted that the phenomenon of "fluorescence reversal" is also observed for both dodecyl and tetradecyl phosphates, albeit in much higher concentrations than that for LysoPA. The corresponding spectra are supplied as Supporting Information.

It is known that aliphatic phosphates can undergo self-assembly in aqueous media to form micelle by aggregation of the hydrophobic hydrocarbon chains,[9] and thus expose their polar phosphate groups to the aqueous environment. While such a micelle might not be formed when LysoPA is at low concentration,[10] the interactions of LysoPA with **1** could induce the formation of aggregates with a nonspecific structure, which is responsible for the new emission band at 480 nm attributed to the excimer of the anthracene fluorophore. Formation of micelle-type aggregation becomes possible with increasing concentration of LysoPA,[10] and the chance of short contact between molecules of **1** is thus reduced. This phenomena might account for the "reversed" fluorescence changes (Figure 1b), and a schematic drawing is shown in the vignette of the abstract. Indeed, the concentration required for the second-stage fluorescence reversal is in the order dodecyl phosphate (~10 mM) > tetradecyl phosphate (~2.5 mM) > LysoPA (~0.075 mM) and comparable to but somewhat lower than their critical micelle concentrations (cmc).[10] It appears that the cmc value of these phosphates is reduced in the presence of **1**.

The aggregate formation for **1** in the presence of LysoPA, dodecylphosphate, and tetradecylphosphoste is consistent with the broadening and red-shift of their absorption and excitation titration spectra. The typical spectra are provided by the case of LysoPA as shown in Figure 3a. The elevation of absorption in the long-wavelength region (>420 nm) might reflect the formation of aggregates in the buffer solution.[11] In comparison, no such phenomena occur in the corresponding spectra with hexylphosphate (Figure 3b). Evidently, the antrhacene excimer results from the ground-state preassociated species (i.e., a static excimer).

The proposed fluroescence sensing mechanism can also account for the observed changes in the fluorescence decay times for **1** in the presence of LysoPA as compared with its free form (Table 1).[12] In the absence of LysoPA, there are two distinct fluorescence lifetimes, 6.6 (88%) and 16.4 ns (12%), for **1** in HEPES solutions. The major and shorter-lived component could be attributed to a loose complex,

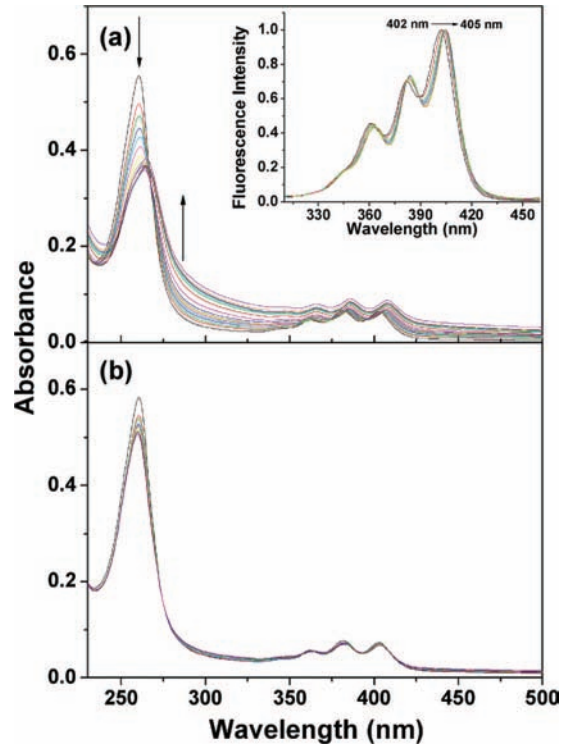

Figure 3. UV−vis titration of receptor **1** (10 μM) in HEPES buffer (pH 7.22) by incremental addition of (a) LysoPA (as the sodium salt, up to 5 equiv) and (b) $C_6H_{13}OPO_3H_2$ (up to 5 equiv). The inset shows the normalized excitation spectra of receptor **1** (5 μM) in HEPES buffer (pH 7.22) upon incremental addition of LysoPA (up to 5 equiv). The emission wavelength was monitored at 480 nm.

Table 1. Fluorescence Lifetime and Composition in Complexation of Receptor **1** with LysoPA[a]

substrate	τ_1 (ns); composition	τ_2 (ns); composition	τ_3 (ns); composition
1	6.6; 88%	16.4; 12%	
1+ 1.5equivofLysoPA	1.7; 16%	11.6; 51%	41.8; 33%
1+ 10equivofLysoPA	1.4; 14%	4.3; 23%	60.5; 63%
1+ 20equivofLysoPA	2.7; 36%	7.7; 39%	58.1; 25%
1+100equivofLysoPA	4.4; 0.4%	14.8; 99.6%	

[a] Measurements were conducted in HEPES buffer (pH 7.22). The excitation wavelength was 380 nm. The complexation on addition of LysoPA was monitored at 480-nm fluorescence.

where the PET process is incompletely inhibited. In the presence of 1.5 equiv of LysoPA, the decay curve at 480 nm is deconvoluted to three lifetimes (Table 1). The long-lived component (41.8 ns, 33%) can be ascribed to the anthracene excimer due to the forbidden nature of optical transition.[13] The other two lifetimes are no longer than that of the unbound **1** and thus they are more likely from the anthracene monomer. Since energy transfer from the excited anthracene monomer to anthracene dimer or unexpected impurities is facile in aggregrates, the observation of short-lived components (1.7 ns) is not unexpected. In a condition

(8) The fluorescence quenching observed in Figure 2d could be attributed to the formation of aggregates, in which the anthracene chromophores are not sandwiched to form excimers but close enough to have significant dipole−dipole interactions (i.e., H-type aggregates, see: Cornil, J.; Beljonne, D.; Calbert, J.-P.; Brédas, J.-L. *Adv. Mater.* **2001**, *13*, 1053−1067). Aggregate formation for **1** in the presence of decylphosphate is supported by the broadened absorption spectra (see the Supporting Information for details).

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(10) The critical micelle concentrations (cmc) for LysoPA, dodecyl phosphate, and tetradecyl phosphate are 0.35, 56, and 13 mM, respectively, in water and 0.28, 42, and 9 mM, respectively, in the HEPES buffer (pH 7.22), which were determined by the previously reported fluorescence method (see: Dominguez, A.; Fernández, A.; González, N.; Iglesias, E.; Montenegro, L. *J. Chem. Edu.* **1997**, *74*, 1227−1231).

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with 10 equiv of LysoPA, the population of excimer increases (63%) and the monomer lifetimes are further shortened, presumably due to the more facile energy transfer to the dimers. However, the excimer population drops to only 25% with 20 equiv of LysoPA, and it becomes negligible with 100 equiv of LysoPA. The observed lifetimes for the latter case are similar to the that of the unbound **1**, but the shorter-lived component, which corresponds to a loose complex, is less than 1%. In other words, the LysoPA-bound **1** strengthens the dipicolylamime−Zn(II) interactions and thus reduces the PET process. This is consistent with the arguments of Hamachi and co-workers for **1** with phosphorylated molecules,[5] and accounts for the fluorescence behavior for **1** with PO_4^{3-} and short-chain alkyl phosphates (Figure 2a,c).

Although the conventional evaluation of binding constants cannot be directly applied to the aggregate formation, comparison of the intensity changes at 432-nm fluorescence against the concentration of alkyl phosphates might be informative. Nonlinear least-squares fitting of the curves (see the Supporting Information) suggests the apparent "binding constants" of 6.2×10^4, 4.0×10^4, 3.3×10^5, 9.0×10^5, and 6.1×10^5 M^{-1} for $C_6H_{13}OPO_3H_2$, $C_{10}H_{21}OPO_3H_2$, $C_{12}H_{25}OPO_3H_2$, $C_{14}H_{29}OPO_3H_2$, and LysoPA, respectively. The phosphates bearing long chains (12 and 14 carbons) appeared to exhibit about 10-fold higher affinity toward the zinc complex. This enhanced binding might reflect certain multivalent effects[14] contributed by aggregation of the long-chain phosphates.

The concept of aggregation-based fluorescence sensing has been recently demonstrated in several systems. For example, aggregation of dye molecules can be induced by large aromatic hydrocarbons,[15] cations,[16] and humidity.[17] Dependent on analytes, a functional polymer can be induced to form varied aggregates to display analyte-dependent multistate responses. Such induced aggregation of poly(thiophene) has been used to identify structurally similar amines.[18] The polyanionic poly(*p*-phenylene ethynylene) has been utilized to detect the natural polyamines through aggregation-enhanced exciton migration.[19] In addition to organic molecules, several functionalized metallic nanoparticles also act as colorimetric chemosensors through the aggregation mechanism.[20]

In summary, we have uncovered that aggregation-induced excimer formation is a novel fluorescence sensing mode for the detection of biologically important LysoPA in aqueous solution. The aggregation behavior relies on the hydrophobic aliphatic chains in LysoPA, and the excimer emission results from excitation of the ground-state preassociated anthracene dimer or clusters. Our study may provide a protocol for the development of aggregation-based fluorescence sensing.

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Supporting Information Available: Synthetic procedures, UV−vis titration, fluorescence titration, photokinetics studies, and NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) The fluorescence lifetimes of anthracene excimers strongly depend on the environment, solvent polarity, and the substituents. Lifetimes of 52−91 ns have recently been reported for the excimer of a 9,10-dimethylene-substituted anthracene chromophore in a cyclodextrin-derived bianthracene system in water (see: Zhang, X.; Sasaki, K.; Kuroda, Y. *J. Org. Chem.* **2006**, *71*, 4872−4877).

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