LETTER

Sugar–Phosphole Oxide Conjugates as "Turn-on" Luminescent Sensors for Lectins

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ABSTRACT A mixture of sugar–phosphole oxide conjugates and lectins in a buffer solution displays an intense blue emission, thereby acting as a "turn-on" fluorescent sensor for lectins as they form aggregates.

KEYWORDS: biosensor • lectin • fluorescent • glycoconjugates • aggregation • phosphorus

Carbohydrate–protein interactions, which are found in interactions of proteins, viruses, and bacteria, are among the most important events or mechanisms in biological systems (1). A series of carbohydrate-binding proteins, lectins, existing on cell surfaces mediate the initial recognition processes in biological systems by interaction with saccharide receptors. In this context, the detection of proteins is an important issue and there is a high demand for the development of a rapid, simple, sensitive, and selective sensing of lectins. The optical sensing of lectins is particularly relevant. Indeed, a number of optical sensors for lectins have been developed using different principles in the sensor design (2–4). Among them, the conventional fluorescence sensors are usually based on fluorescence quenching, "turn-off", upon interactions with lectins.

In this report, the design and synthesis of sugar–phosphole oxide conjugates **1** as fluorescent "turn-on"-type sensors for lectins is detailed (Figure 1a). This study demonstrates a simple, effective, and sensitive biosensor for detection of lectins via fluorescence enhancement.

Recently, we have found that phosphole oxide cored dendrimers do not show emission in solution but produce intense emissions in the aggregated or crystal states (5, 6). This unique feature is ascribed to an aggregation-induced emission (AIE), first reported by Tang et al., which is rationalized by intramolecular rotation being restricted in the aggregated states (7, 8). Our synthetic strategy for a fluorescent sensor for lectins is based on AIE-active materials with saccharide groups (Figure 1a). Because sugar–lectin interactions involved in the molecular recognition process are typically found in aggregated structures, phosphole oxides integrated with sugars are envisioned to display an intense emission, i.e., "turn-on" (9), when an aggregated structure forms through the multivalent sugar–lectin interactions (Figure 1b).

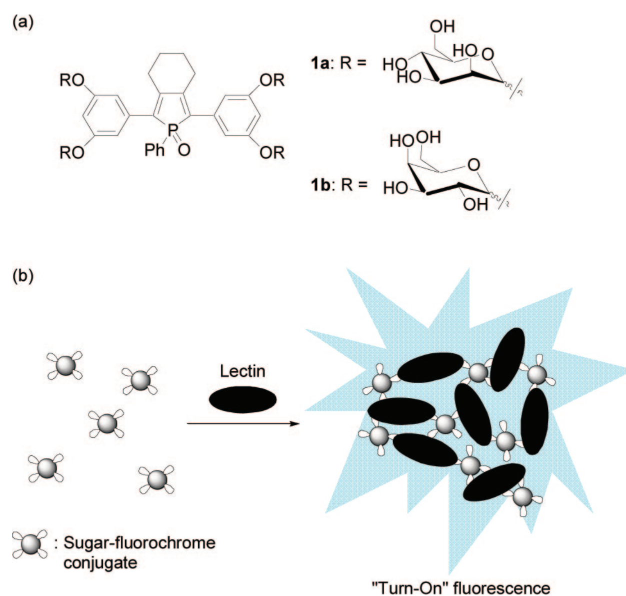

FIGURE 1. (a) Chemical structures of sugar-modified phosphole oxides 1a,b. (b) Schematic representation of a "turn-on" fluorescent sensor for lectins using an AIE-active material. A mixture of sugar-modified phosphole oxide and lectin displays an intense blue emission, thereby acting as a "turn-on" fluorescent sensor for lectins.

The α-mannopyranoside derivative **1a** was synthesized by the reaction of 2,5-bis(3,5-dihydroxyphenyl)-substituted phosphole oxide **3** and acetyl-protected D-mannopyranoside **4a** followed by deprotection, as shown in Scheme 1 (see the Supporting Information for details) (10, 11). α-Galactopyranoside congener **1b** was also synthesized similarly. Importantly, **1a**,**b** are water soluble, which is usually required for biosensing in aqueous media.

In the sensing studies, the interaction of **1** with concanavalin A (Con A), which is a well-studied lectin from *Canavalia ensiformis* (Jack bean) (12), is used as an example. Con A exists as a tetramer at neutral pH and is known to selectively recognize α-mannopyranoside and α-glucopyranoside at its four binding sites located about 6.5 nm apart on one side of the protein. As designed, an aqueous solution of **1a** is practically nonluminescent. When Con A is added to an aqueous solution of **1a** in a buffer solution (pH 7.6),

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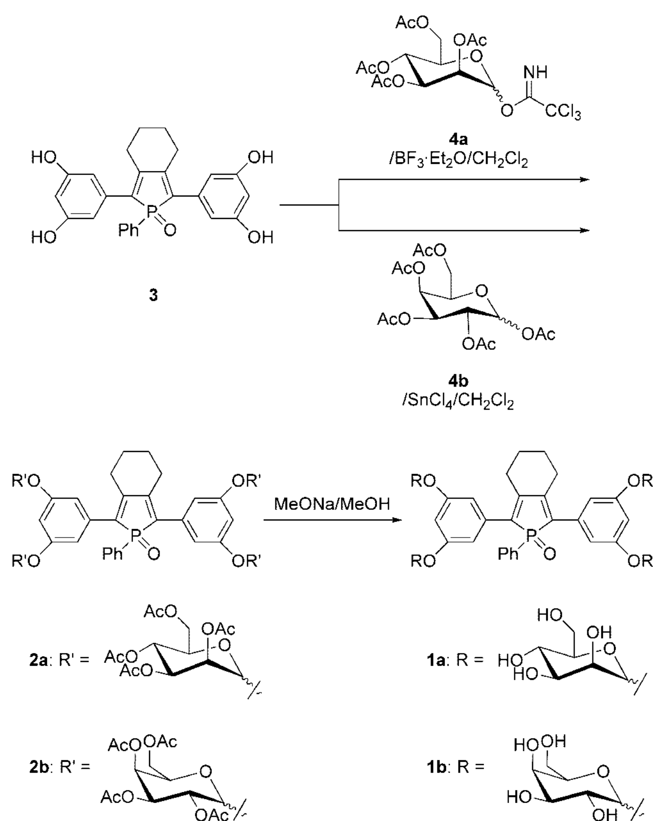
Scheme 1. Synthesis of Sugar–Phosphole Oxide Conjugates 1a,b



the mixture instantly exhibits an intense emission under irradiation with UV light (Figure 2a,b). The increase in the fluorescence intensity was rapid to reach a final value within a few seconds (Figure 2c), while its rate was somewhat dependent on the concentration of Con A. At the same time, a similar progressive increase in turbidity monitored at 500 nm upon addition of Con A is also attended (Figure 1S in the Supporting Information) (13, 14). The observed spectral feature indicates that Con A triggers aggregation through its complexation with **1a**, appending α-mannopyranoside units at its periphery, which in turn induces enhancement of emission, as expected. The fluorescence titration shows that the optimal signal is reached when the molar ratio of Con A to **1a** is approximately 1:2. Because **1a** is too small to bind to multiple binding sites on tetrameric Con A, only the two sugars of the conjugate can be bound at a time, promoting extensive aggregation and precipitation (15). Indeed, after filtration through a 0.45 μm pore size membrane, the residual solution showed no emission (Figure S2 in the Supporting Information). The binding constant (K_a) based on the change in the fluorescence intensity as a function of Con A content is calculated to be 1.5×10^5 M^{-1}, which is higher than that of α-mannopyranose alone (16). An excess amount of mannan (17) was added to this mixture as an inhibitor against **1a** (Figure 2c and Figure S3 in the Supporting Information). The solution returned to being nonluminescent, suggesting the disaggregation of the **1a**–Con A aggregate having taken place.

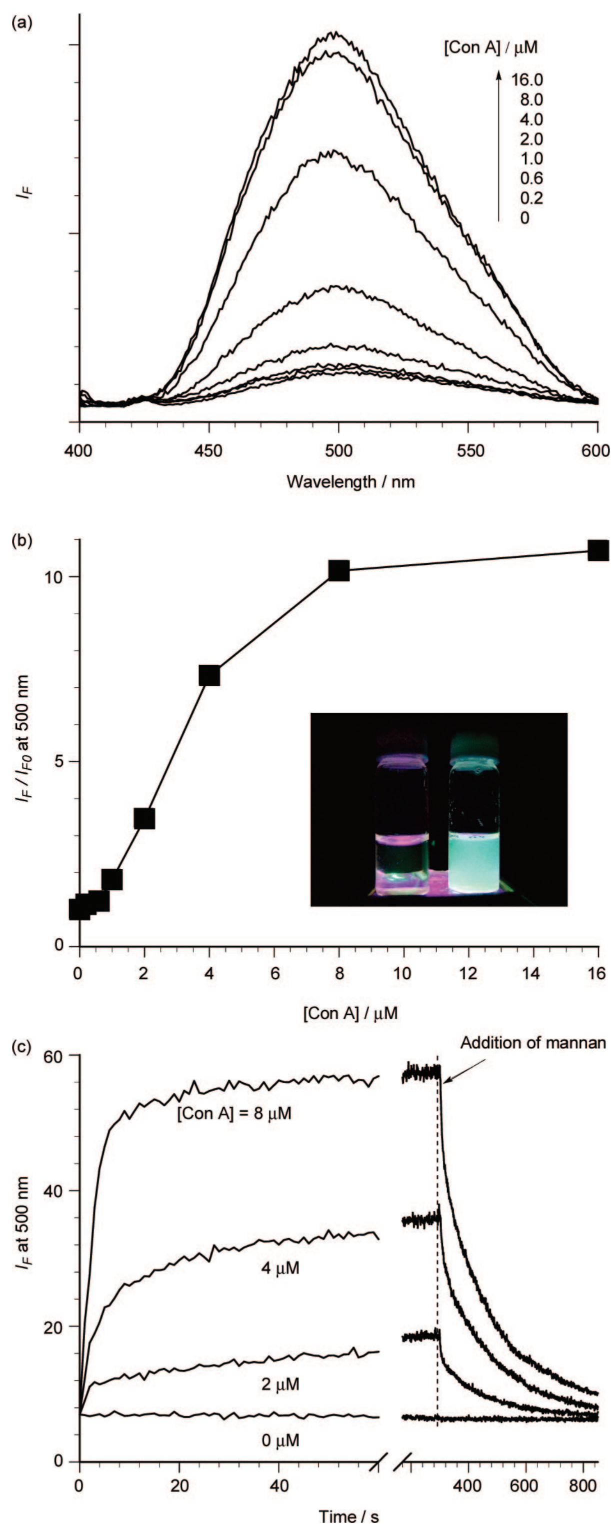


FIGURE 2. (a) Change in fluorescence spectra of 1a (19.2 μM) upon addition of Con A in a buffer solution (Ex = 370 nm). (b) Change in fluorescence intensity (I_F) of 1a with addition of Con A relative to the value in its absence (I_{F0}) monitored at 500 nm (Ex = 370 nm). The inset shows photographs of 1a (left) and its complex with Con A (right) in a buffer solution under irradiation with UV light (365 nm). (c) Fluorescence intensity (I_F) changes monitored at 500 nm on addition of Con A to 1a (17.8 μM) in a buffer solution (Ex = 370 nm). A large excess of mannan ([mannan] = 0.5 mg/mL) as an inhibitor was added at 5 min. All measurements were performed in a buffer solution (10 mM Tris–HCl, 1 mg/mL $CaCl_2$, 1 mg/mL $MnCl_2$, pH 7.6).

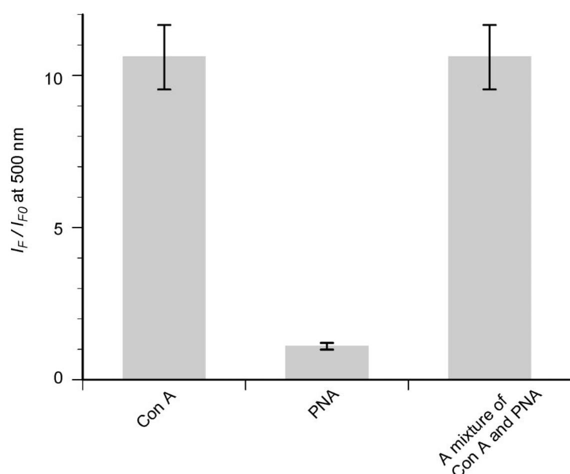


FIGURE 3. Fluorescence response patterns (I_F/I_{F0}, the fluorescence intensity (I_F) relative to the initial fluorescence intensity of the sensor element (I_{F0}), monitored at 500 nm) of the sugar-modified 1a sensor against the lection analytes. All measurements were performed in a buffer solution ([1a] = 19.2 μM, [Con A] = 8.0 μM in 10 mM Tris−HCl, 1 mg/mL $CaCl_2$, 1 mg/mL $MnCl_2$, pH 7.6; [1a] = 20.2 μM, [PNA] = 8.0 μM in 10 mM phosphate buffer, pH 7.4; [1a] = 18.6 μM, [Con A] = 8.1 μM, [PNA] = 7.9 μM in 10 mM Tris−HCl, 1 mg/mL $CaCl_2$, 1 mg/mL $MnCl_2$, pH 7.6).

A control experiment using **1a** and peanut agglutinin (PNA), a galactose-binding lectin from *Arachis hypogaea* (18), has verified the selectivity to mannose-binding lectins, showing no increase in the fluorescence (Figure 3 and Figure S4 in the Supporting Information) (19); therefore, binding of **1a** does not occur. On the other hand, α-galactopyranoside-modified **1b** serves as a sensor for PNA, as confirmed by its response through an intense fluorescence (20). However, **1b** does not sense Con A (Figure S5 in the Supporting Information).

Further, mannose-modified **1a** has the ability to sense Con A even in the presence of other proteins in a mixture. For example, when **1a** is mixed with an equivalent amount of Con A and PNA in a buffer solution, the mixture instantly displays an intense emission (Figure S7 in the Supporting Information). The fluorescence pattern is almost the same as that observed in the case of Con A alone, as shown in Figure 3.

In conclusion, we have demonstrated the design and synthesis of new "turn-on" fluorescent sensors **1** for lectins. When lectins are added to the glycoconjugates, they specifically form aggregates, exhibiting an intense blue emission. This sensor assay is feasible for lectins that are multivalent or are multivalently presented. Further experiments are required to demonstrate the practical usefulness of the fluorescence turn-on assay. In particular, the assay must be tested in a more complex environment, i.e., biological relevant fluids, for their ability to detect species present at very low concentrations. However, this study would introduce a principle to design sensitive fluorescent sensors for potential applications in the fields of chemical, biological, medical, and environmental science (21). Further study is currently in progress.

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Supporting Information Available: Text and figures giving experimental procedures and fluorescence spectral changes of **1a** with Con A/mannan, **1a** or **1b** with PNA, and **1a** with a mixture of Con A and PNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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