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A graphitic carbon nitride based fluorescence resonance energy transfer detection of riboflavin

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ABSTRACT

Fluorescence resonance energy transfer (FRET), which occurs between two luminescent chromophores, can greatly improve the selectivity and sensitivity of a fluorescent assay when a ratiometric signaling with the fluorescence enhancement of the acceptor at the expense of the donor is adopted. In this study, a fluorescence ratiometric detection (FRD) of riboflavin (RF) has been made based on FRET, as the strong overlap occurred between the emission spectrum of graphitic carbon nitride (g-C_3N_4) and absorption spectrum of RF, in which g-C_3N_4 acts as the energy donor and RF as the energy acceptor. With increasing concentration of RF, the fluorescence intensity of g-C_3N_4 emission at 444 nm decreased and the fluorescence peak at 523 nm for RF increased regularly, making the fluorescence intensity ratio of 523 nm to 444 nm linearly dependent on the concentration of RF in the range from 0.4 μM to 10 μM, giving a limit of the detection of 170 nM. This method can be used to quantify RF in complex systems such as milk and drink, showing that the novel FRET-based fluorescence ratiometric detection can enable an attractive assay platform for analytes of interest.

1. Introduction

Fluorescence resonance energy transfer (FRET), a nonradiative photophysical process, occurs between an energy donor and an energy acceptor through dipole–dipole interactions [1]. FRET efficiency (E), an elementary parameter to describe the FRET properties, is very sensitive to the spectral overlap between the emission of fluorescent donor and the absorption of the acceptor, the distance and the relative dipole orientation of the partners. The inverse association of the FRET efficiency to the sixth power of the distance between the donor and the acceptor requires that the two molecules are in close proximity. When FRET happens, the energy-transfer signals between donor and acceptor can be measured with low background interference. In such case, FRET between organic donor and acceptor has been applied for analytical purposes [2,3]. However, the platform may still be compromised sometimes by the variations such as pH, temperature, and so forth. Fortunately, a ratiometric strategy could improve the selectivity and sensitivity of a measurement since it can eliminate most or all of the possible background interference by signaling changes of a fluorescent system at two bands exhibiting enhancement of one band at the expense of the other, so it is essential to establish a ratiometric method for target detection based on FRET by introducing some fluorescent donor with relative high PL quantum yield and good FRET efficiency.

Bulk graphitic carbon nitride (g-C_3N_4) has attracted worldwide research interest for its structural similarity to graphene [4]. As the nanosheets can promote the photo-with respect to bulk materials, g-C_3N_4 nanosheets might be used as a potential candidate for the phosphor [5]. Zhang et al. [6] have found two-dimensional carbon nitride nanosheet (CNNS) exfoliated from bulk g-C_3N_4 showing bright blue luminescence. It has been used for bioimaging due to its high PL quantum yield and excellent biocompatibility. Inspired by its inspiring properties, the intrinsic blue fluorescence emission of CNNS has been used for the fluorescent detection of Cu^{2+} [7], GSH [8] and DNA [9].

Riboflavin (RF), also called vitamin B2, is one of the most widely distributed water-soluble vitamins which can be easily absorbed into eukaryotic cells [10]. It serves several vital functions in biological systems such as metabolism of fats, carbohydrates and proteins, and also plays an important role in the human diet. RF deficiency may be associated with various human diseases including fatigue, slowed growth, digestive problems et al. [11]. To

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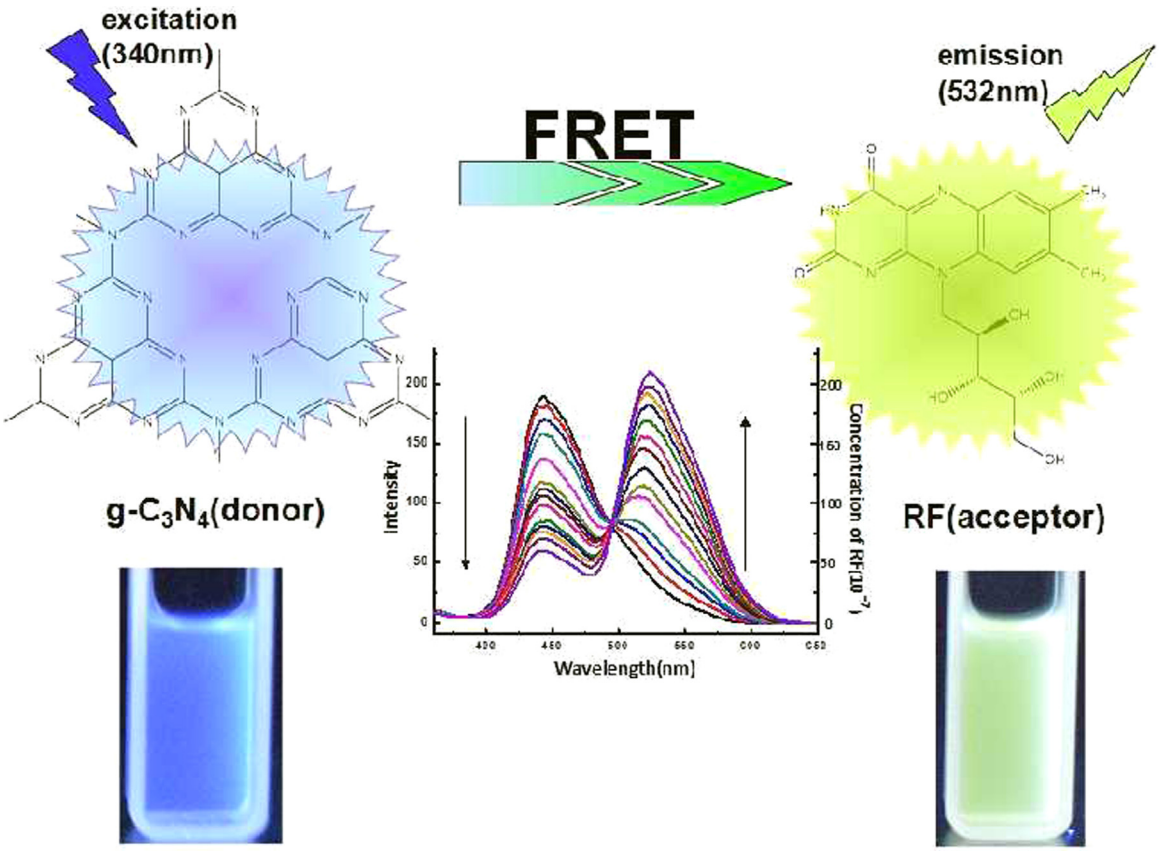

Fig. 1. Schematic representation of FRET based fluorescence ratiometric sensor for RF.

construct an accurate determination method of RF is essential for assessing nutritional metabolic requirements [12]. Up to now, a variety of methods have been proposed for detecting RF, such as high performance liquid chromatography (HPLC) [13], capillary electrophoresis [14], indirect immunoassay [15], electrochemical methods [16] and fluorescence spectroscopy [17]. Compared with other techniques, fluorescence spectroscopy holds significant advantages for its high sensitivity, simplicity, and nondestructive properties. However, it is quite difficult to measure RF in metabolite and foodstuff directly because of the complexity of the matrix and the extremely low concentration of these compounds. The time-consuming pretreatment and separation procedures have to be carried out to lower the matrix effect. Therefore, in this paper, we developed a FRET-based fluorescence ratiometric detection (FRD) of riboflavin. The principle is illustrated in Fig. 1. Because of the presence of active functional groups (e.g., carboxyl and amino groups) on the surface of the nanosheet, RF can be easily adsorbed onto g-C_3N_4 sheet, shortening the distance between g-C_3N_4 sheet and RF, and thus the bright blue fluorescence of g-C_3N_4 nanosheet can significantly quenched due to FRET from g-C_3N_4 to the RF, resulting in enhancement of the fluorescence of RF. Thus, a fluorescence ratiometric detection method could be achieved for sensitive RF detection in complex matrix such as milk and drink.

2. Experimental

2.1. Materials

Melamine and riboflavin were obtained from Shanghai Shenbo Chemical Co., Ltd. (Shanghai, China). Other reagents were of analytical reagent grade. Mili-Q purified water (18.2 MΩ cm) was used to prepare solutions throughout the experiment.

2.2. Apparatus

Transmission electron microscopy (TEM) measurements were obtained from a Tecnai G2 F20 S-TWIN microscopy (FEI, USA). The X-ray photoelectron spectroscopy (XPS) analysis was conducted by an ESCALAB 250 X-ray photoelectron spectrometer (Thermo, USA). The samples for XPS were made by the deposition of a nanocrystal suspension in water on Si substrate. UV–vis–NIR absorption spectra were obtained using a Hitachi U-3600 spectrophotometer. Steady-state fluorescence spectra and fluorescence anisotropy were measured with an F-2500 fluorescence spectrophotometer (Hitachi, Japan) with the nanoparticles dispersed in reagents. Fluorescence lifetimes were measured by an FL-TCSPC fluorescence spectrophotometer (Horiba Jobin YvonInc., France) using a NanoLED laser light source at the respective excitation wavelength of the dyes. The data were fitted by an exponential decay model.

2.3. Synthesis of g-C_3N_4 nanosheet

15 g of melamine was put into a covered crucible and calcined at 550 °C for 2 h in a muffle furnace, with a heating rate of 3 °C/min. Then, the yellow product was collected. Appropriate amount of powder dissolved in purified water was sonicated for 24 h, then the solution was centrifuged and the supernatant was collected for further use.

2.4. Fluorescence ratiometric detection of RF

Different concentrations of RF (0, 0.1, 0.4, 0.7, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 μM) was added to 0.2 mL 0.25 mg/mL g-C_3N_4 solution and diluted with PBS (pH=7.4) to