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Short note

Filamentous Virus Oriented Pyrene Excimer Emission and Its Efficient Energy Transfer

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Dedicated to professor Chen-Ho Tung on the occasion of his 80th Birthday.

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ABSTRACT

We present here that pyrene derivative can be arranged in close proximity using M13 virus as framework through click reaction. The observation of excimer emissions of pyrene was a direct evidence for the proper spatial arrangements of chromophores in the architecture, in which both ground-state and excited-state electronic interactions of pyrenes were facilitated. Efficient energy transfer could be induced through the electrostatic interactions between the M13 backbone and the positively charged rhodamine 6G. The investigation of chromophore-modified M13 and its energy transfer process would be beneficial for exploiting the structures of various natural virus and artificially self-assembled virus-like particles.

1. Introduction

Viruses belong to a fascinating class of natural supramolecular structures, composed of multiple highly ordered coat proteins (CPs) structures [1]. Because of their advantages including simple/economic production, well-defined structural features, unique shapes and sizes, genetic programmability and robust chemistries, viruses have been widely used as functional nanomaterial for various electrical [2–4], chemical [5] and optical applications [6–9]. For example, tobacco mosaic virus (TMV) [10–12] and DNA [13,14] have been used as frameworks for the generation of ordered assemblies of a large variety of chromophores. Extended multi-chromophore arrays have electronic properties that are attractive for use in optical and electronic devices, as well as for diagnostic applications [5–14]. Filamentous M13 bacteriophage, a naturally occurring nanowire-like virus, is a non-toxic monodisperse bionanoparticle with 6.6 nm in diameter and 880 nm in length. M13 can be easily replicated and amplified in bulk in gram negative bacteria. M13 bacteriophage is composed of a circular, single-stranded DNA that is encapsulated by approximately 2700 copies of the major coat protein p8 and approximately 5 copies of each of their minor coat proteins (p3, p6, p7, and p9) at the end of the virus. Ours and others work have shown that M13 coat protein could be modified by bioconjugation strategy in a very simple way [8,15–16]. The conjugation sites could be the exposed N-termini and the Lys residue on the viral surface. The calculated average distances between two neighboring N-termini at the exterior surface of M13 are 3.2 nm and 2.4 nm respectively [17]. Thus, it is highly probable that such close distance would enable the strong interaction between the neighboring pigments attached on the surface of the M13, if the flexible N-terminus allow the pigments to have considerable freedom for their orientation. However, the exploitation of a pigment probe of coat protein structure on M13 bacteriophage are yet to be done.

Excimer formation by pyrene is a well-known process in solution and a useful tool for structural studies in biochemistry and molecular biology as a probe of proximity [18–22]. The broad long-wavelength emissions of pyrene excimer (around 450–500 nm) appears as a result of the formation of a stacked excited dimer between an excited pyrene and a nearby ground-state pyrene. Pyrene in an excimer are co-planar to some extent and are less than 4 Å apart. Pyrene excimer emission has been widely investigated as a potential probe of DNA and RNA secondary and tertiary structure [18,19,22]. On the other hand, the efficient transfer of the excitation energy to the reaction center or acceptor due to the spatial organization of chromophores has been the longstanding goal in

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artificial photosynthesis [23]. Thus, the investigation of the energy transfer process for the chromophore-modified M13 would be a good probe to indicate such spatial arrangements of chromophores on virus. In this context, we present the artificial self-assembled system consisting of M13 scaffolds, intercalated pyrene donor dyes and a rhodamine 6G (R6G) acceptor anchored to the scaffolds through electrostatic attraction, investigating the pigment-pigment interactions and Förster resonance energy transfer (FRET) modulated by the M13 architecture (Scheme 1).

2. Experimental section

2.1. General

All reagents were used as received. Bacteriophage M13 was prepared by infecting *Escherichia coli* as reported.[1c] Absorption spectra and emission spectra were determined through a U-3900 UV–Vis spectrophotometer (HITACHI, Japan) and F-4600 fluorescence spectrophotometer (HITACHI, Japan). Circular dichroism spectra were obtained at 25 °C through a Jasco-J-815 circular dichroism spectropolarimeter. Bio-Rad SDS-PAGE system was applied to confirm the success of chemical conjugation. The concentration of unmodified virus was measured by absorbance at 269 nm; 0.1 mg mL^{-1} of M13 gives a standard absorbance of 0.384. The molecular weight of a single p8 subunit of wild-type M13 is 5238 Daltons. Dialysis tubing of 300 000 molecular weight cutoff (MWCO) biotech was purchased from Spectrum Laboratories. TEM analyses were carried out by depositing 20 μL aliquots of each sample at a concentration of 0.1 mg mL^{-1} onto 100-mesh carbon-coated grids for 2 min. The grids were then stained with 20 uL of 2% uranyl acetate and viewed with a JEM-2100 (JEOL, Japan) TEM electron microscope. Atomic force microscopy (AFM) images were obtained with a Bruker multimode8 in tapping mode under ambient conditions using etched silicon cantilever tip.

2.2. MALDI-TOF MS of M13 p8 subunit

Virus was denatured by adding guanidine hydrochloride (6 μL, 6 M) to the sample (24 μL) and mixing for 5 min at room temperature. Denatured proteins were spotted onto a MALDI plate using Millipore ZipTip$_{\mu\text{-C18}}$ tips to remove salts and assist the binding of protein to the sinapic acid matrix. The spots were analyzed by a Bruker Ultra-Flex I TOF/TOF mass spectrometer. MS-grade 2, 5-dihyroxybenzoic acid with 0.1% TFA was used as the matrix.

2.3. M13 bioconjugation protocol

2.3.1. The preparation of M13-Alkyne

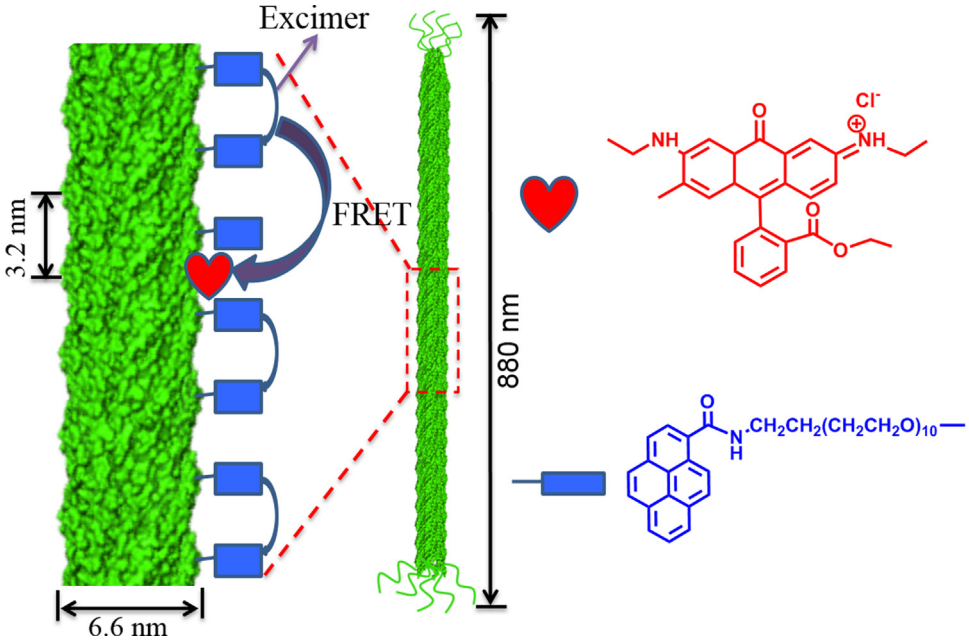
4 mL of M13 (c = 3.98 mg mL^{-1}) was added to 4 mL of PBS buffer solution (10 mM, pH = 7.38) to keep the concentration of M13 around 2 mg mL^{-1}. Then 135 mg of excessive alkyne-NHS ester in 2 mL of DMSO was added slowly to the above M13 aqueous solution in ice-bath. The resulted mixture was stirred slowly at ambient temperature overnight, centrifuged to remove the precipitate and then dialyzed for 3 times. The final volume was around 8 mL. As a result, the concentration of the M13-Alkyne was around 2 mg mL^{-1}.

2.4. The preparation of M13-Pyrene

Pyrene-PEG$_{10}$-N$_3$ was prepared as shown in supporting information. 1 mg mL^{-1} of M13-Alkyne with 2.8 mg mL^{-1} of pyrene-PEG$_{10}$-N$_3$ in a mixed solvent of 20 vt% DMSO and 80 vt% 1 M tris-base buffer (pH = 7.8). 10 uL of $CuSO_4$ (100 mM) and 10 uL of NaAsc (200 mM) was added. The reaction was allowed to stir at room temperature for 1 day, dialyzed for 3 times. The final volume was measured. The concentration of M13-Pyrene was based on pyrene dye concentration connected on the virus, which is determined from the absorption spectrum and the molar coefficient extinction of the pyrene-PEG$_{10}$-N$_3$ (3.41×10^4 mol^{-1} L cm^{-1} at 340 nm, 3.20×10^4 mol^{-1} L cm^{-1} at 268 nm). The concentration of M13 virus for M13-Pyrene was determined from the absorption intensity at 268 nm in which the contribution of pyrene at 268 nm has been excluded.

3. Results and discussion

To introduce chromophores on the surface of M13, opportunities are offered by the chemical modifications of abundant amino



Scheme 1. Schematic representation of the formation of the pyrene excimer emission on the pyrene modified M13 and its efficient energy transfer to rhodamine 6G.

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