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Supramolecular Binding of Cationic Porphyrins on a Filamentous Bacteriophage Template: Toward a Noncovalent Antenna System

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Photosynthesis in green bacteria is accomplished by chlorosomes, which contain bacteriochlorophyll aggregates self-assembled in a protein scaffold.[1] The inter-chromophoric structural orientation and organization results in a high yield of absorption and transfer of light for the ultimate conversion into chemical energy.[2] Nature exploits mainly noncovalent interactions to achieve such amazing efficiency. Synthetic effort for efficient artificial antenna systems mostly relies on time-consuming and low-yielding covalent approaches.[3] Apart from some recent elegant examples,[4] noncovalent strategies have been usually limited by the difficulty in controlling the exact stoichiometry and geometry of the resulting assemblies.

Recently, engineered viral particles have been used as nanoreactors or scaffolds for preparing or ordering various nanostructured materials.[5] Viral capsids offer the advantages of being robust and monodisperse and can exhibit various sizes and shapes. Filamentous bacteriophage M13 is a flexible rod, of 6 nm diameter and 800−2000 nm length, depending on the genome length. The capsid is mainly constituted by ∼2700 copies of an α-helical protein (g8p) arranged in a helical array with a 5-fold symmetry axis around a single-stranded DNA molecule.[6] A series of aspartate and glutamate residues ensure a negative potential on the surface. At least one tryptophan (Trp) residue is buried in the hydrophobic region responsible for packing of the capsid. This kind of bacteriophages can be easily genetically modified and are widely used in phage display technology.[7]

Here we describe a noncovalent approach based on the interaction between the water-soluble cationic *trans*-(*N*-methylpyridinium-4-yl)diphenylporphyrin (*t*-H_2P_{agg}) and M13 and some genetically modified strains (Chart 1). The latter two structurally differ from wild-type M13 (wt) for an insert of 13−15 amino acids in the N-terminal region of g8p, which is exposed to the solvent, bearing one (8M) or three (MP14) Trp residues. We anticipate that, depending on the nature of the mutants, different extent of resonant energy transfer (RET) from the external Trp residues of the capsid to the interacting porphyrins can be evidenced. This is a prerequisite for designing new efficient artificial antenna systems.

The porphyrin *t*-H_2P_{agg} displays a strong tendency to form extended self-aggregates[8] or supramolecular assemblies on nucleic acids and homo-polypeptides.[9] At low phage concentration, bathochromic shifts and large hypochromicity (see Figure SI3) of the Soret band for *t*-H_2P_{agg}, together with a substantial quenching of the fluorescence emission, indicate extensive aggregation of the cationic porphyrin on the negative capsid surface. Upon increasing

Chart 1. Amino Acid Sequence of g8p in wt, 8M, and MP14

M13wt NH_2 - AEG D DPAKAAFDSLQASATEYIGYAWAMVVVIVGATIGIKLFKKFTSKAS - COOH

8M NH_2 - AEG EFDYAWDDFYAMG DPAKAAFDSLQASATEYIGYAWAMVVVIVGATIGIKLFKKFTSKAS - COOH

MP14 NH_2 - AEG EFWEWDWPRIELNIG DPAKAAFDSLQASATEYIGYAWAMVVVIVGATIGIKLFKKFTSKAS - COOH

the phage concentration, the Soret band eventually moves to an intermediate position between the free and the aggregated species. The fluorescence emission sharpens and exhibits a substantial increase in intensity, together with a small bathochromic shift. Slight differences are observable in the position of the absorption and emission bands for the different mutants, in line with the different microenvironments experienced by the porphyrin at the binding site. The interaction between the porphyrin and the chiral template is also supported by the presence of induced circular dichroism (ICD) in the Soret region. In all three cases, even with different intensity and location, weak bisignate features are present, in line with electronically coupled porphyrins.[10] These experimental findings indicate disassembling of the porphyrin self-aggregates and the dispersion of the monomers on the surface of the bacteriophages.

To better understand the role and nature of the various microenvironments, fluorescence quantum yields (ϕ_F) of the porphyrin, upon exciting at three different wavelengths (see Table 1), have been evaluated for the free molecule, in buffered solution or dispersed on the surface of wt phage, 8M and MP14. The quantum yields of *t*-H_2P_{agg} in buffer or interacting with wt (first two rows in Table 1) are independent of the excitation wavelength, as it is commonly observed for a typical fluorophore.[11] The slight increase of ϕ_F when the porphyrin is dispersed on the surface of wt could be ascribed to the N-terminal site of g8p, which protects the fluorophore from quenching by the solvent. On the contrary, in the presence of 8M and MP14, the ϕ_F values exciting at 295 nm are much higher than those observed on exciting at longer wavelength, where the values are very close to those measured with wt.

These results are confirmed by the corrected fluorescence excitation spectra performed on *t*-H_2P_{agg} in the presence of wt, 8M, and MP14 and are reported in Figure 1 (upper graph), together with the corresponding absorption spectra (lower graph).[12] For a simple fluorophore, differences in fluorescence emission intensity depend only on changes in the absorption, and therefore, the corrected fluorescence excitation spectrum overlaps the absorption spectrum. This case holds for *t*-H_2P_{agg} dispersed on M13 surface (Figure 1, black). On the contrary, in the presence of 8M (red) and MP14 (blue), the fluorescence excitation spectra of the porphyrin (upper graph) show an intense band in the UV region that is absent in the corresponding absorption spectra (lower graph). This spectral feature, which is higher for 8M with respect to MP14 (in line with the fluorescence quantum yields), is centered at about 280 nm and can be confidently assigned to Trp absorption. The involvement of

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Table 1. Fluorescence Quantum Yields for *t*-H_2P_{agg} and its Adducts on the Surface of Different Filamentous Phage Mutants (pH 7 in phosphate buffer 10 mM, at 298 K)

	ϕ_F		
	$\lambda_{ex} = 295$ nm	$\lambda_{ex} = 400$ nm	$\lambda_{ex} = 438$ nm
buffer	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
wt	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
8M	0.20 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
MP14	0.14 ± 0.01	0.08 ± 0.01	0.08 ± 0.01

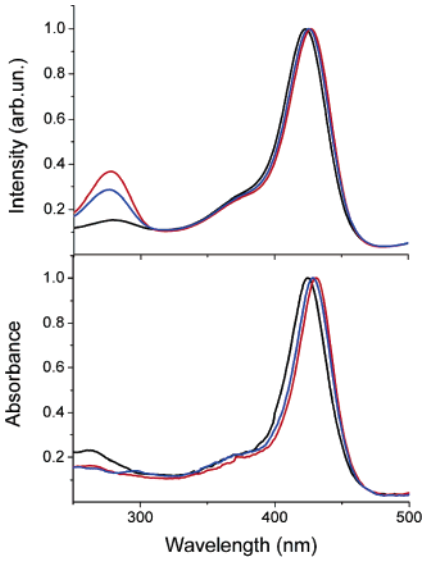

Figure 1. Normalized excitation (A) and UV/vis (B) spectra of the various *t*-H_2P_{agg} adducts on the surface of different filamentous phage: wt (black), MP14 (blue), 8M (red); [*t*-H_2P_{agg}] = 0.5 μM, pH 7 phosphate buffer 10 mM, [phage] = 88 μM, $T = 293$ K, $\lambda_{em} = 666$ nm.

Trp residues exposed on the external surface of the phage is confirmed by the observation that, while wt does not show any effect, the enhancement is well apparent in the case of the two mutants. All the experimental evidence indicates that, in these latter cases, RET from the external Trp residues (donor) to the interacting *t*-H_2P_{agg} (acceptor) occurs.

As it is well-known, RET is strongly related to the Förster radius (R_0), which depends (i) on the donor fluorescence quantum yield in the absence of acceptor, (ii) on the relative orientation in space of the transition dipoles of donor and acceptor, and (iii) on the overlap integral between the donor emission and the acceptor absorption.[11] Notwithstanding, in our system, the overlap between Trp emission and the porphyrin Soret absorption band is not perfect, the very large molar extinction coefficient of *t*-H_2P_{agg} ($\sim 2 \times 10^5$ M^{-1} cm^{-1} at the Soret band) leads to a calculated R_0 value of about 30 Å (see Supporting Information). Such a value is fully consistent with an involvement of the Trp residues present in the N-terminal g8p region of the modified phage particles, and therefore, they are able to transfer the excitation energy to the porphyrin.

Furthermore, the bathochromic shift of the Soret band, observed when 8M and MP14 are used, could be ascribed to the occurrence of stacking interactions between the aromatic moieties of *t*-H_2P_{agg} and Trp residues, with a consequent very short distance between the supposed donor and acceptor.

The lack of knowledge of the wt/mutant ratio of g8p proteins on the capsids of 8M and MP14 (which depends on the two-gene system used for the expression of recombinant proteins[13]), together with the small decrease of both intensity and decay traces in the steady-state and time-resolved fluorescence measurements on Trp emission, does not allow precise quantification of the RET efficiency values (see Supporting Information).

Notwithstanding the limitation of a semiquantitative description, our experimental data show that RET occurs. Furthermore, under our experimental conditions, the ratio of porphyrin to Trp residues is roughly 1:250, so that only a small percentage of these amino acidic residues is actually involved in donor−acceptor coupling. Consequently, we expect that the efficiency could be increased by using phages with a high level of expressed mutations on the capsid. These findings, together with the possibility of a fine-tuning of both the protein scaffold (through a selection of proper peptide sequences from phage libraries) and the porphyrins, open new promising ways toward the design of efficient noncovalent antenna systems.

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Supporting Information Available: Materials and methods. UV/vis, CD, and steady-state and time-resolved fluorescence measurements (Figures SI1−SI7). This material is available free of charge via the Internet at http://pubs.acs.org.

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