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Synthesis of two cyanine dyes as potential artificial antennas for the bacterial photosynthetic Reaction Center

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ABSTRACT:

Particular attention has been recently devoted to the development of biohybrid photoconverters based on the bacterial Reaction Center (RC) of Rhodobacter sphaeroides. This highly efficient photoenzyme has a conversion yield close to unit that makes it extremely appealing in the field of artificial photosynthesis. Isolated RCs suffer of a limited absorption cross-section in the visible spectral region that limits their applicative employment. Here we report the synthesis of two heptamethine cyanine molecules, whose photophysical properties make them potentially suitable as light harvesting antennas for the RC.

INTRODUCTION:

Artificial molecular systems capable of converting solar energy into electrical energy have attracted great interest by scientific community in recent years. However, the major issues related to the application of these systems in solar energy conversion consist in their high structural complexity and difficult synthetic preparation, as well as in their limited photoconversion efficiencies and lifetimes of charge separated states. These issues can be conversely overcome focusing the attention on biohybrid photoconverters that are based on the biological components that regulate the mechanism of solar energy conversion in photosynthetic organisms [1-7]. Indeed, Nature has optimized, in billions of years of evolution, highly efficient biological photoconverters[8], among which the Reaction Center of the photosynthetic bacterium *Rhodobacter sphaeroides* [9].

The RC is the photochemical core of the bacterial photosynthetic apparatus and it converts, with a nearly unitary efficiency, the light harvested by the proteins called light harvesting complexes LH1 and LH2, into charge separated states and biochemical energy[10, 11]. RC based devices have been already proposed for solar energy conversion [12, 13] and biosensing [14, 15]. The absorption cross-section of the native RC extracted from the bacterial strain R26 used in this work is maximum in the near infrared region (see Figure 1) and very limited in the visible range, where the sun reaches the maximum irradiance. To work around this limitation, the pristine isolated protein needs to be ameliorated in its ability to efficiently harvest visible light by covalently linking *ad-hoc* synthesized molecules able to improve enzyme cross-section[16-22].

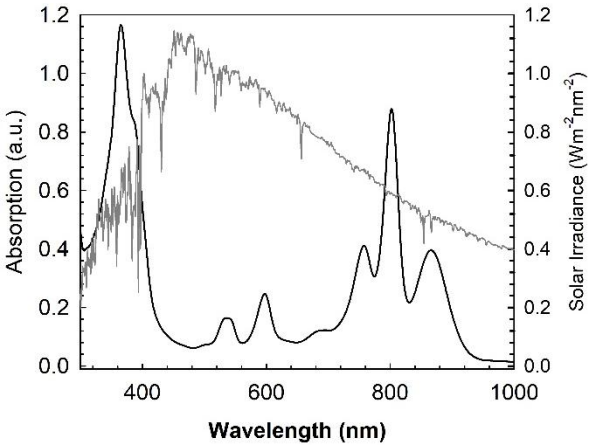

Figure 1. The optical spectra of the bacterial photosynthetic reaction center (black) and of the average solar irradiance (grey).

The organic molecules appointed to act as efficient light harvesting antennas for RC must fulfil specific photophysical and chemical requirements. Firstly, they must have intense visible light (400-700 nm) absorption and efficient NIR emission in correspondence of the spectral region where the RC absorption is maximum (750-900 nm)[16]. Moreover, a strong Stokes shift is desired for the antennas, to avoid self-absorption phenomena that would prevent the energy transfer of the harvested photons to the photoenzyme[16, 19]. The antennas must be also structurally unhindered and should be anchored to the RC surface by an innocent flexible arm[23, 24]. Finally, they must be soluble in aqueous-detergent media, since after the isolation from bacteria, the RC is stable in water only when it is surrounded by a toroid of detergent molecules.

Previously published syntheses of photoactive organic materials[25, 26], were used to design and synthesize a series of aryleneethynylene (AE) antennas that were bioconjugated to the reaction center demonstrating the possibility to enhance the photoconversion efficiency of the enzyme working upon both monochromatic and white light excitation[20, 27]. The aryleneethynylene molecules reported so far, though, suffer from limited absorption cross-section and extinction coefficient in the visible range, limited emission quantum yield in the near infrared spectral region and poor aqueous solubility.

With the aim to further enhance the photoconversion efficiency of RC in the visible range of excitation, the attention was shifted to the class of antenna molecules bearing an heptamethine cyanine backbone. In particular, two commercially available cyanine molecules were selected, namely IR-780 and IR-820. IR-780 and IR-820 bear

indolium and sulphonate charged groups respectively, ensuring their good solubility in aqueous media. In particular, we have synthesized the Cy-3 and Cy-4 cyanines by a nucleophilic substitution of the central chlorine atom in IR-820 and IR-780 with 4-(methylamino)butanoic acid. This reaction allows the molecules to be functionalized with a flexible alkyl spacer that would reduce their sterical hindrance on the protein surface and a pending carboxyl group suitable for their bioconjugation to the protein.

The preliminary optical characterization of Cy-3 and Cy-4 let suggest these molecules as promising artificial antennas for RC, showing a broad and intense light absorption maximum in the visible range.

RESULTS AND DISCUSSION:

Cy-3 and Cy-4 were synthesized by the reaction of IR-820 and IR-780 with 4-(methylamino)butanoic acid carried out in dry *N*,*N*-dimethylformamide and in the presence of triethylamine as the base activating the nucleophile (Figure 2).

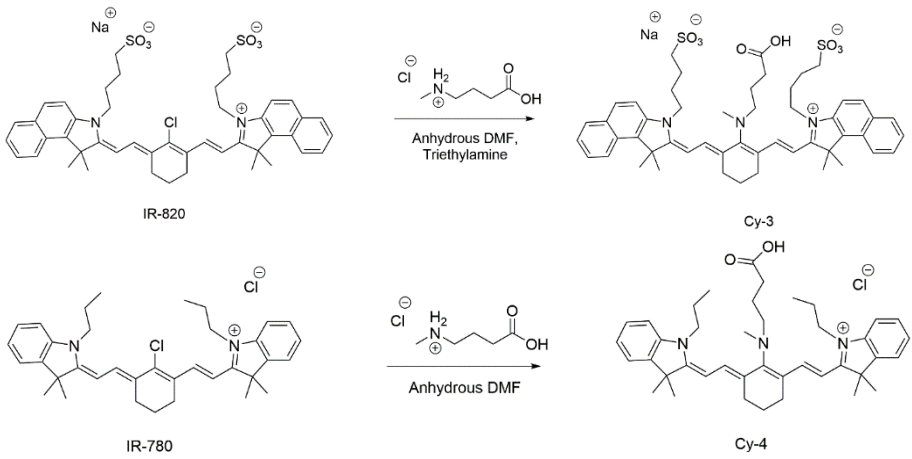

Figure 2. Synthesis of Cy-3 and Cy-4

IR-780 and the resulting Cy-4 have a less extended π-conjugation versus IR-820 and Cy-3, due to the presence of indolium with respect to the more conjugated benzoindolium moieties. This difference also influences the absorption wavelengths of molecules.

A more extended π-conjugation indeed promotes HOMO-LUMO transitions through lowering the energy gap between molecular orbital energy levels. This evidence is supported by the higher wavelength (820 nm) of IR-820 absorption maximum versus that (780 nm) of IR-780. Upon nucleophilic substitution of chlorine atom in IR-820 and IR-780, a significant blue-shift of absorption was observed for both Cy-3 and Cy-4 products with respect to the relative precursors. This result is in accordance with what reported by Peng *et al.* for the product of a reaction of cyclohexylamine with another heptamethine cyanine molecule, probably due to an excited-state intramolecular charge transfer between the amino donor and the cyanine acceptor units[28]. The absorption spectra of both molecular dyes and their mixtures with RC are shown in Figure 3.

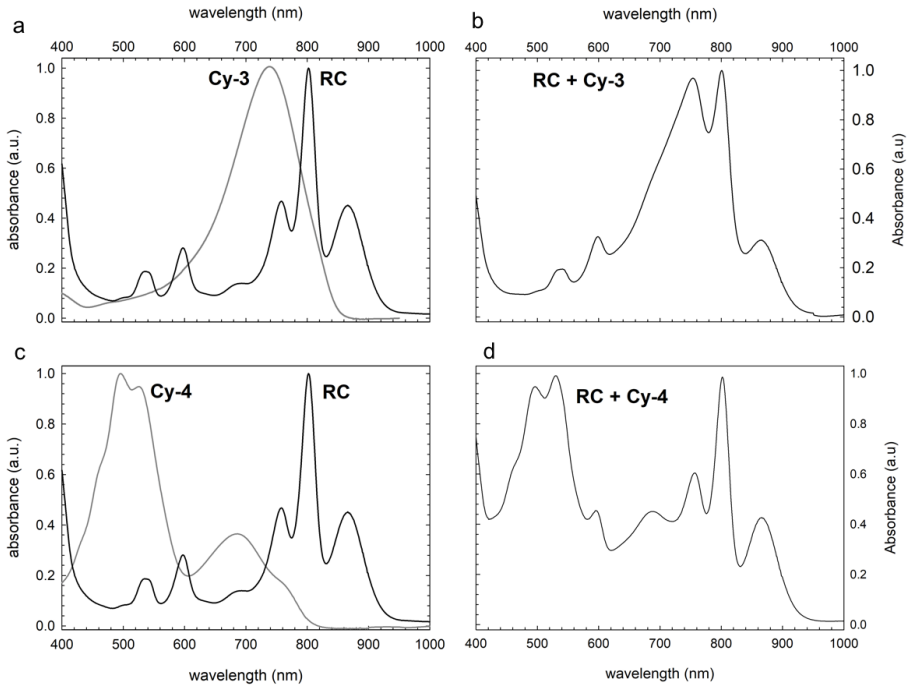

Figure 3. Overlapped absorption spectra of RC and (a) Cy-3 in Tris-HCl 20 mM (0.03% Triton X-100), (c) Cy-4 in Tris-HCl 20 mM (3% Triton X-100). Absorption spectra of (b) a mixture of Cy-3 and RC (Tris-HCl 20 mM, 0.03% Triton X-100), (d) a mixture of Cy-4 and RC (Tris-HCl 20 mM, 3% Triton X-100). Cy-RC molar ratio of both mixtures: 5:1.

Cy-3 was diluted in Tris-HCl buffer 20 mM at pH 8.5 in the presence of Triton X-100 0.03%. Conversely, a higher concentration of Triton X-100 (3%) was necessary for Cy-4 to increase its solubility in the aqueous medium. As shown in Figure 2, the absorption spectrum of Cy-3 is broad, ranging from 500 to 850 nm, with peak at 740 nm. Two absorption bands are instead observed for Cy-4, with peaks at 498, 525 and 686 nm respectively.

MATERIALS AND METHODS:

All reagents and solvents were purchased from Sigma Aldrich and were directly used without any further purification procedure. ^{1}H NMR spectra were recorded by an Agilent 500 spectrometer at 500 MHz, using the peaks at δ = 3.31 ppm and 4.78 ppm of CD_3OD residual protons and CD_2Cl_2 at δ=5.32 ppm as internal standards. UV-Vis-NIR absorption spectra were recorded with a Cary-5000 spectrophotometer, using 1 cm width quartz cuvettes.

Synthesis and characterization of Cy-3

In a 25 mL three-necked round bottom flask, 4-(N-methyl)-butanoic acid hydrochloride (0.272 g, 1.77 mmol) and triethylamine (357 µL, 3.53 mmol) were suspended in anhydrous DMF (7 mL) under a nitrogen atmosphere. The mixture was

vigorously stirred at 80°C for 1 hour and, then IR-820 (0.150 g, 17.7 mmol) was added under a nitrogen flow. The mixture was stirred and after 12 hours, the formation of a blue product was observed by thin layer chromatography using silica aluminum sheets. The mixture was cooled to room temperature, and the product was precipitated as a blue solid in cold acetone and then collected on filter paper in a Büchner funnel. Finally, Cy-3 was isolated in 27% yield by chromatography in a CombiFlash Rf+ C-18 reverse phase column using a mixture of water and methanol in 6:4 volume ratio as the eluent.

^{1}H NMR: 8.27-8.14 ppm (m, 2 H), 8.02-7.88 ppm (m, 4 H), 7.81 ppm (d, J=13.4 Hz, 2 H), 7.65-7.50 ppm (m, 4 H), 7.47-7.37 ppm (m, 2 H), 6.05 ppm (d, J=13 Hz, 2 H), 4.19 ppm (s, 4 H), 3.93-3.76 ppm (m, 2 H), 3.55-3.43 ppm (m, 3 H), 2.96-2.84 ppm (m, 4 H), 2.21-2.09 ppm (m, 2 H), 2.08-1.82 ppm (m, 23 H), 1.41-1.19 ppm (m, 5 H).

Cy-4 synthesis and characterization

In a 25 mL three-necked round bottom flask, 4-(N-methyl)-butanoic acid hydrochloride (0.347 g, 2.25 mmol) and triethylamine (455 µL, 4.50 mmol) were suspended in anhydrous DMF (7 mL) under a nitrogen atmosphere. The mixture was vigorously stirred at 80°C for 1 hour and, then IR-780 (0.150 g, 22.5 mmol) was added under a nitrogen flow. The mixture was stirred for 4 hours and the reaction progress was monitored via thin layer chromatography TLC evidencing the formation of a blue product. After cooling to room temperature, the product was extracted with dichloromethane (25 mL) and the organic phase was washed with water (5 x 25 mL) to remove traces of DMF. After drying with anhydrous Na_2SO_4, the solvent was removed at reduced pressure. A blue solid was obtained and isolated in 7% yield by CombiFlash Rf+ C-18 reverse phase column chromatography with a mixture of water and acetonitrile in 6:4 as the mobile phase.

^{1}H NMR: 7.56 ppm (d, J=13.3 Hz, 2 H), 7.44-7.31 ppm (m, 4 H), 7.17-7.11 ppm (m, 2 H), 6.98 ppm (d, J=7.9 Hz, 2 H), 5.79 ppm (d, J=13.4 Hz, 2 H), 3.93-3.80 ppm (m, 2 H), 2.50 ppm (t, J=7.2 Hz, 2 H), 1.86 ppm (m, 2 H), 1.66 ppm (s, 27 H), 1.31 ppm (s, 2 H), 1.06 ppm (t, J=7.4 Hz, 6 H).

CONCLUSIONS:

Two new organic dyes have been synthesized with the aim to investigate in the future their suitability as light harvesting antennas for the photosynthetic reaction center. Both molecules have been obtained by nucleophilic substitution of the chlorine central atom of commercially available IR-820 and IR-780 cyanines with the amino group of 4-(methylamino)butanoic acid. The resulting Cy-3 and Cy-4 products are soluble in water/detergent solutions and show broad absorption spectrum in the visible region, thus fulfilling some requirements of organic molecules designed to be used as RC light harvesting antennas. The photoluminescence properties of Cy-3 and Cy-4 will be further investigated, together with the suitable strategies for their covalent bioconjugation to the RC.

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