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Incorporation of the bacterial reaction centre into dendrimersomes

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ABSTRACT

For the first time the ability of the first generation dendrimer belonging to the family of polyester-benzylether, (3,5)12G1-PE-BMPA-$(OH)_4$, to form dendrimersomes is presented together with their capability to reconstitute the integral membrane protein complex called Reaction Centre (RC) purified from the photosynthetic bacterium *Rhodobacter sphaeroides*. Size, polydispersity and time stability of the empty and protein containing dendrimersomes are presented together with the photochemical activity of the guest protein. The RC presence appears to strongly enhance the self-assembly properties of the Janus dendrimer, leading to the formation of proteo-dendrimersomes showing a photochemical activity similar to that found for RC in solution. The interaction of the embedded RC with reduced *cyt*-c has also been investigated, indicating that the incorporation of the protein is vectorial (almost 90% of the guest protein faces the dendrimerosomes exterior with its *cyt*-c docking site) at variance with lecithin liposomes where the reconstitution is essentially statistical.

1. Introduction

In contrast with other amphiphile molecules, Janus dendrimers instead of a polar head and a hydrophobic tail(s) possess two surfaces of marked different affinity with water [1] (from this analogy with the ancient two faced Roman god, Janus, their name). Janus dendrimers are obtained by linking two chemically distinct dendridic building blocks, thereby breaking the approximately spherical symmetry characterizing most dendrimers. The resulting molecules show extraordinary self-assembly properties which span from micelles, disks, bicontinuous cubic phases to tubular vesicles [2]. Among them, note has to be paid to the vesicular structure called dendrimersomes. These are supramolecular aggregates that mimic the phospholipidic bilayer structure of biological membranes, being characterized by a bimolecular layer separating an inner water compartment from a continuous aqueous bulk. The thickness of the resulting interface has been shown [2] to be of the same order of magnitude of a typical phospholipid bilayer (5–8 nm depending on the Janus dendrimer monomer used), thus suitable for the incorporation of pore-forming antimicrobial peptides, such as melittin [2]. No attempt has yet been made to test this feature with integral membrane proteins whose activity strictly depends on the presence of an organized water|oil|water interface. Integral membrane proteins play a critical role in a conspicuous number of cell functions, *e.g.* selective transport across the membrane and energy transduction in respiration and photosynthesis. In photosynthetic bacteria, the primary event of photosynthesis is accomplished in an integral membrane protein complex called the Reaction Center (RC), which spans the intracytoplasmic membrane and catalyzes the light-induced charge separation across the membrane dielectric. Here we report the first attempt to reconstitute RC into dendrimersomes.

In the RC of *Rhodobacter sphaeroides* the absorption of a photon promotes the primary electron donor, a bacteriochlorophyll dimer (P), to its excited state. An electron is consequently transferred through a molecule of bacteriopheophytin to a first ubiquinone electron acceptor (Q_A, which is located in a hydrophobic pocket of the protein) and subsequently to a secondary ubiquinone molecule (Q_B, which binds a relatively polar protein domain) [3]. When an electron donor, such as reduced cytochrome c_2 (*cyt*-c_2) is available to P^+, the absorption of a second photon yields a dihydroquinone at the Q_B site, as Q_B^- accepts a second electron with the concomitant binding of two protons onto the quinone. The resulting ubiquinol

Abbreviations: (3,5)12G1-PE-BMPA-$(OH)_4$, Benzoic acid, 3,5-bis(dodecyloxy)-2,2-bis[[3-hydroxy-2-(hydroxymethyl)-2-methyl-2-oxopropoxy]methyl]-1,3-propanediyl ester.

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