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Covalent Functionalization of Fullerene C_{60} with Polyethyleneimine †

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Abstract

The rise of antimicrobial resistance requires urgent therapeutic strategies. We report the synthesis and characterization of a C_{60} fullerene derivative functionalized with polyethyleneimine (C_{60}-PEI), designed as a photosensitizer (PS) for antimicrobial photodynamic therapy. The conjugate was prepared in *N*,*N*-dimethylformamide with triethylamine and purified by aqueous dialysis. Spectroscopic studies confirmed characteristic bands of substituted fullerenes in the UV-visible region. Photodynamic assays showed that C_{60}-PEI generates singlet oxygen. These findings indicate that PEI improves C_{60} solubility in polar media and supports its potential as a photodynamic antimicrobial agent.

Keywords: fullerene C_{60}; polyethyleneimine; photosensitizer

1. Introduction

The increase in bacterial resistance to commonly used drugs threatens to bring an end to the "Antibiotic Era." As a consequence, infections that were once treatable now cause greater morbidity and may even become life-threatening, including in hospitals [1]. Therefore, it is essential to develop new drugs and antibiotic methodologies [2]. In this context, antimicrobial photodynamic therapy (PDI) emerges, which is based on the selective accumulation of a photosensitizer (PS) in microbial cells. Subsequent illumination with visible light activates the PS and causes lethal damage to cellular macromolecules [3,4]. The activation of the PS in the presence of $O_2(^3\Sigma_g^-)$ can generate reactive oxygen species (ROS) through two pathways: type I (free radical formation) and type II (singlet oxygen $O_2(^1\Delta_g)$ generation) [5,6]. Both processes may coexist and depend on the PS, the substrate, and the medium.

Several families of PSs have been evaluated for microbial inactivation [7]. Among them, fullerene C_{60} derivatives have attracted particular interest [8]. These compounds offer relevant advantages: they are photostable, absorb in the visible region of the spectrum, and produce ROS through both photochemical pathways. However, their low solubility in polar solvents promotes aggregation in aqueous and biological media, which limits their photoactivity. The development of covalent functionalization strategies for C_{60} has enabled the incorporation of chemical groups capable of improving its biological activity [8–10]. Moreover, the inclusion of polycationic fragments in the PS structure

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increases its ability to interact with bacterial membranes, enhancing microbial inactivation. In this context, polyethylenimine (PEI) is an aliphatic polycationic polymer characterized by the presence of primary, secondary, and tertiary amino groups [11]. Its high density of protonable amino groups at physiological pH confers positive charges, which facilitate interaction with microbial membranes [12,13]. Therefore, in this work, a new C_{60}-PEI conjugate was synthesized as a promising photodynamic agent.

2. Materials and Methods

2.1. Equipment and Chemical Agents

All reagents were obtained from Sigma-Aldrich (Milwaukee, WI, USA) and used without further purification. Thin-layer chromatography (TLC) plates coated with silica gel (250 μm) were purchased from Analtech (Newark, DE, USA). Absorption spectra were carried out on a Shimadzu UV-2401PC spectrometer (Shimadzu Corporation, Tokyo, Japan).

2.2. Synthesis of C_{60} Fullerene Derivatives

C_{60} fullerene functionalized with PEI (1:3 ratio) was synthesized in the presence of triethylamine (TEA), using *N*,*N*-dimethylformamide (DMF) as the solvent at 60 °C under an Ar atmosphere for 72 h. The reaction was stirred at room temperature. The progress of the reaction was monitored by TLC (silica gel, toluene) until the complete disappearance of the C_{60} fullerene reactant was observed. The product was purified by dialysis in aqueous medium, yielding the final C_{60}-PEI conjugate [12].

2.3. Spectroscopic Studies

The UV-visible absorption properties of fullerene synthesized C_{60}-PEI and the C_{60} reference were determined in homogeneous medium [12].

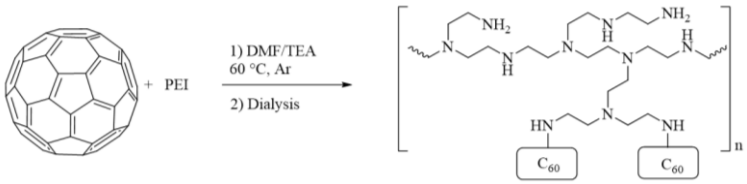
2.4. $O_2(^1\Delta_g)$ Production

In the presence of the PSs, the photodecomposition of 9,10-dimethylanthracene (DMA) and 2′-(anthracene-9,10-diyl)bis (methylmalonate) tetrasodium salt (ABMM) was studied in DMF and water (λ_{irr} = 450–700 nm). Anthracene derivatives deactivate $O_2(^1\Delta_g)$ exclusively through chemical reaction; therefore, spectroscopic monitoring of their photodecomposition allowed indirect estimation of the quantum yield of $O_2(^1\Delta_g)$ formation (Φ_Δ) for C_{60}-PEI in both solvents, using fullerene C_{60} and 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin ($TPPS^{4-}$) as reference molecules in DMF and water, respectively [12,14].

3. Results and Discussion

3.1. Synthesis of C_{60} Fullerene Derivatives

C_{60}-PEI derivative was synthesized from the reaction between C_{60} fullerene and PEI using DMF as solvent and in the presence of TEA (Scheme 1). The reaction mixture was kept under stirring at 60 °C and subsequently purified by dialysis in aqueous solution, yielding the C_{60}-PEI conjugate as the final product [12]. This methodology improved the solubility of C_{60} fullerene through its covalent linkage to PEI, generating a PS with potential properties for application in polar media. Moreover, the incorporation of PEI could promote its interaction with the biological membrane, enhancing the photodynamic action.



Scheme 1. Synthesis of C_{60}-PEI.

3.2. Spectroscopic Studies

Spectroscopic studies of C_{60}-PEI were carried out and compared with reference C_{60} fullerene using DMF and water as solvents (Figure 1). C_{60} fullerene exhibited a broad absorption range extending up to approximately 710 nm, with intense absorption bands in the UV region (<400 nm) in DMF. In contrast, the conjugate showed weaker absorption bands in the 300–800 nm range, which are characteristic of substituted C_{60} fullerenes (Figure 1) [8,12]. The absorption spectrum of C_{60}-PEI in water confirms the presence of PEI in the fullerene structure.

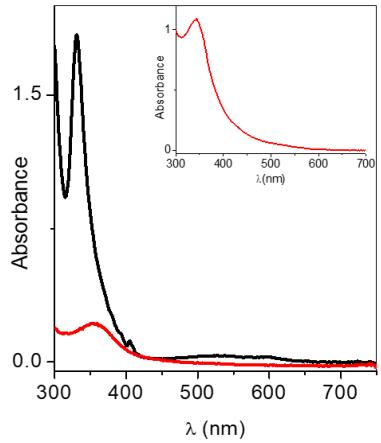


Figure 1. Absorption spectra of C_{60}-PEI (red solid line) and C_{60} (black solid line) in DMF. Inset: absorption spectra of C_{60}-PEI in water.

3.3. $O_2(^1\Delta_g)$ Production

The photodynamic properties of the fullerene derivative were evaluated in DMF and water using anthracene derivatives (DMA and ABMM) [12,13]. The photooxidation of DMA and ABMM in the presence of C_{60}-PEI under aerobic irradiation (λ_{irr} = 450–700 nm) followed pseudo-first-order kinetics (Figure 2). From the observed rate constants (k_{obs}), the $O_2(^1\Delta_g)$ quantum yields (Φ_Δ) for C_{60}-PEI were determined, yielding values of 0.15 in DMF and 0.70 in water. The high singlet oxygen production of the PS in water compared to the organic medium could indicate, in addition to considerable photodynamic activity, an association between the cationic PS and the anionic substrate ABMM [14]. Nevertheless, these results demonstrate that the conjugate exhibits photodynamic activity in both organic and aqueous media, suggesting that the incorporation of the polymer improves the solubility of C_{60} fullerene in polar systems.

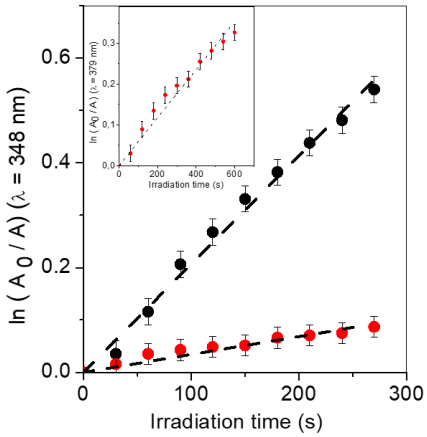


Figure 2. First-order plots for the photooxidation of DMA photosensitized by C_{60} (●) and C_{60}-PEI (●) in DMF. Inset: First-order plots for the photooxidation of ABMM photosensitized by C_{60}-PEI (●) in water. λ_{irr} = 450–700 nm.

4. Conclusions

A C_{60} fullerene derivative modified with a polymer precursor of positive charges was synthesized to be used as a potential PS for the photodynamic inactivation of microorganisms. Absorption spectroscopic studies indicated that the conjugate exhibits a characteristic spectrum of substituted fullerenes and that the presence of PEI allows the solubilization of fullerene in water. Likewise, photodynamic studies confirmed that C_{60}-PEI generates $O_2(^1\Delta_g)$ in both organic and aqueous media. Taken together, the results suggest that C_{60}-PEI possesses suitable properties for application as a photodynamic agent in microbial inactivation.

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