PAMAM dendrimers conjugated with antimicrobial peptides

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Abstract

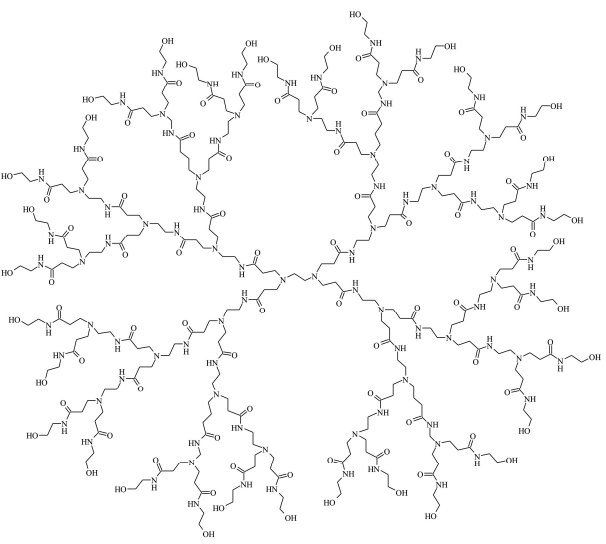
Antimicrobial peptides (AMPs) are potential next-generation antibacterial agents, less prone to bacterial resistance. Dendrimer-based delivery systems such as polyamidoamine (PAMAM), offer new routes to improve and control drug delivery. In order to address the particularities of antimicrobial therapies, we have produced stable, non-covalent PAMAM-AMP conjugates. The binding characteristics of antimicrobial peptides with PAMAM dendrimers were studied in aqueous solution at physiological pH, using fluorescence spectroscopy. The loading efficacy of antimicrobial peptides renders the PAMAM-AMP conjugates as potential candidates for controlled AMPs delivery.

P2 and P6 are AMPs with proven antibacterial activity against gram-positive (*S.aureus*) and gram-negative (*E.coli*) strains

AMPs	Sequence
P6	HRWWRWWRR
P2	RRWHRWWRR

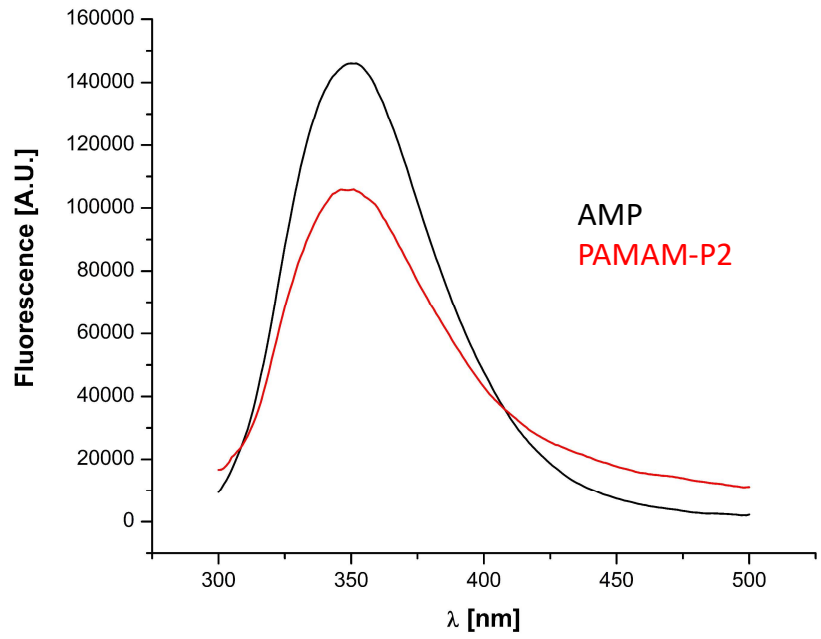
Antimicrobial peptides (AMPs) are small cationic peptides, positively charged at physiological pH, due to their high content in arginine (R), histidine (H) or lysine (K), which can interact either electrostatically or can form hydrogen bonds with the negatively charged phospholipids of bacterial cell membrane. The hydrophobic character is given by tryptophan(W), which inserts easily in bacterial lipid bilayers causing disturbance in the lipid packing.

(G4) PAMAM with -OH surface groups



Poly(amidoamine) PAMAM dendrimers are repetitively branched molecules around a central ethylenediamine core, where the number of ramifications determines the number of generations (G0 to G11). The PAMAM dendrimers exhibit on the surface carboxy, amino or hydroxy groups, and present large internal cavities, able to entrap guest molecules and facilitate the transport of encapsulated compounds. The dendrimers show complete solubility in water and are able to act as drug carriers, vehicle of biological materials and antimicrobial agents.

Fluorescence profiles of free and (G4)PAMAM-OH conjugated P2 peptide

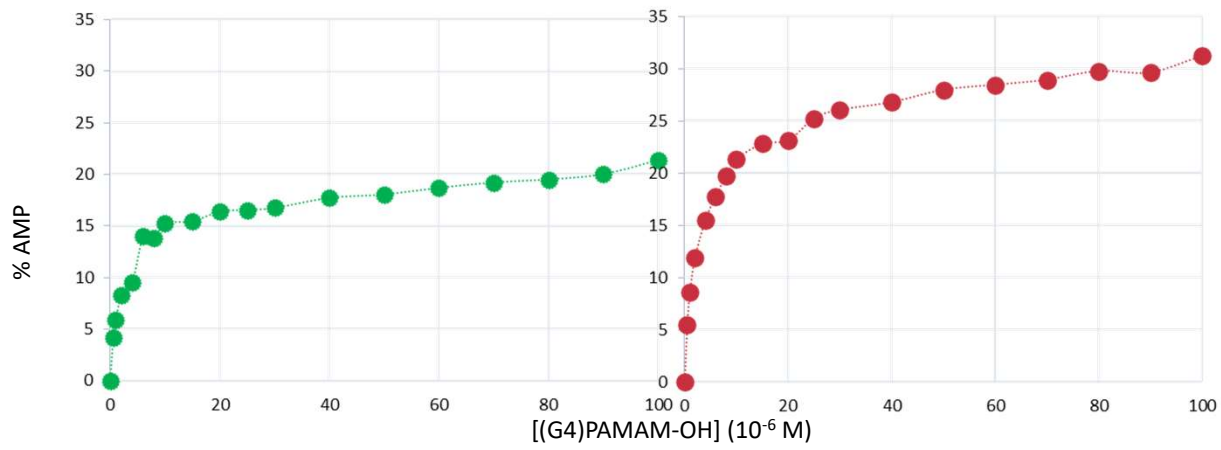


The quenching of intrinsic tryptophan fluorescence results from changes in the local environment polarity experienced by tryptophan residues upon the addition of (G4)PAMAM-OH dendrimer molecules.

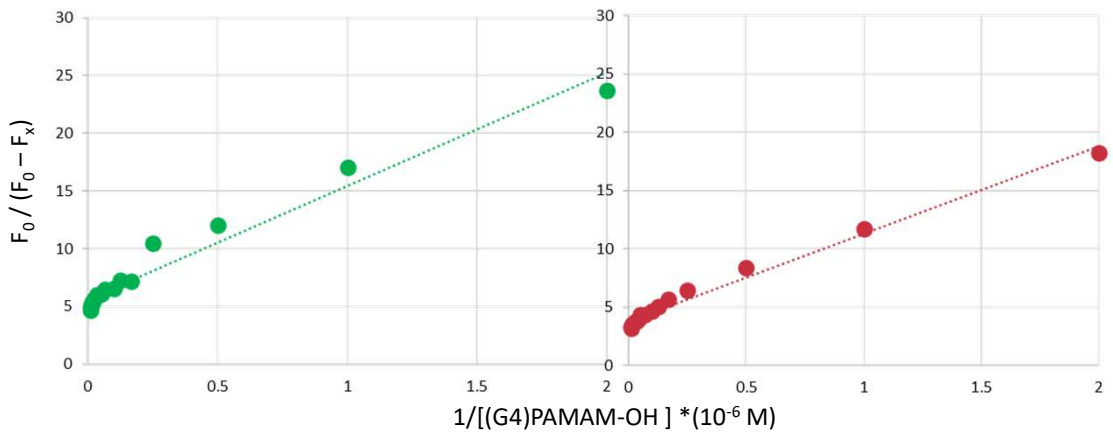
Non-covalent conjugation of AMPs with (G4) PAMAM-OH

(G4)PAMAM-P6 **(G4)PAMAM-P2**

A. Loading efficacy of AMPs into (G4)PAMAM-OH



B. Stern-Volmer linear plots of AMPs fluorescence quenching by (G4)PAMAM-OH



A.

% AMP conjugated with (G4) PAMAM-OH:

$\% AMP = (F_0 - F_x) * 100 / F_0$

(G4)PAMAM-P6 = 20% P6 loading efficacy

(G4)PAMAM-P2 = 30% P2 loading efficacy

B.

Binding constants were calculated from the modified Stern-Volmer Equation:

$F_0 / (F_0 - F) = 1/f + 1/K * 1/[Q]$

(G4) PAMAM-P6: $K (M^{-1}) = 5,8 * 10^5$

(G4) PAMAM-P2: $K (M^{-1}) = 4,97 * 10^5$

Legend

F_0: AMP fluorescence

F_x: (G4)PAMAM-AMP fluorescence

[Q]: (G4)PAMAM-OH molar concentration

K: binding constant

Conclusions: Two antimicrobial peptides with proven antibacterial activity bind generation 4 PAMAM dendrimers, with loading efficacies of 20 and respectively 30%, as a result of hydrogen bond formation and electrostatic interactions. PAMAM-AMP conjugates may represent a starting point for a novel strategy in controlled antimicrobial peptides delivery.

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