Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Sodium selective ion channel formation in living cell membranes by polyamidoamine dendrimer

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ARTICLE INFO

Article history:
Received 16 January 2013
Received in revised form 4 April 2013
Accepted 8 April 2013
Available online xxxx

Keywords:
Cationic PAMAM dendrimer
Excitable membrane
Sodium permeability
Channel formation
Nanoscale mechanism
Functional toxicity

ABSTRACT

Polyamidoamine (PAMAM) dendrimers are highly charged hyperbranched protein-like polymers that are known to interact with cell membranes. In order to disclose the mechanisms of dendrimer–membrane interaction, we monitored the effect of PAMAM generation five (G5) dendrimer on the membrane permeability of living neuronal cells followed by exploring the underlying structural changes with infrared-visible sum frequency vibrational spectroscopy (SVFS), small angle X-ray scattering (SAXS) and transmission electron microscopy (TEM). G5 dendrimers were demonstrated to irreversibly increase the membrane permeability of neurons that could be blocked in low-[Na^+], but not in low-[Ca^{2+}] media suggesting the formation of specific Na^+ permeable channels. SFVS measurements on silica supported DPPG–DPPC bilayers suggested G5-specific trans-polarization of the membrane. SAXS data and freeze-fracture TEM imaging of self-organized DPPC vesicle systems demonstrated disruption of DPPC vesicle layers by G5 through polar interactions between G5 terminal amino groups and the anionic head groups of DPPC. We propose a nanoscale mechanism by which G5 incorporates into the membrane through multiple polar interactions that disrupt proximate membrane bilayer and shape a unique hydrophilic Na^+ ion permeable channel around the dendrimer. In addition, we tested whether these artificial Na^+ channels can be exploited as antibiotic tools. We showed that G5 quickly arrest the growth of resistant bacterial strains below 10 μg/ml concentration, while they show no detrimental effect on red blood cell viability, offering the chance for the development of new generation anti-resistant antibiotics.

1. Introduction

Polyamidoamine (PAMAM) dendrimers are nanosized, hyperbranched polymers, widely studied for biomedical applications as nanocarriers in many areas including brain-targeted drug delivery and gene therapy [1,2]. Several lines of experimental and *in silico* evidence suggested direct interaction of dendrimers and model membranes, although the molecular mechanisms remained elusive [3–6]. Solid-state NMR [7] and atomic force microscopy [3,8] data as well as coarse-grained molecular dynamics simulation [6] indicated that NH_2 functionalized PAMAM generation 5 (G5-NH_2) dendrimer may incorporate into model membrane and participate in pore formation. NMR data also showed atomic-resolution details about the dendrimer–lipid interactions indicating that the lipid tails were rigidified by the presence of G5-NH_2 in the hydrophobic core of the lipid bilayer [7,9]. Suggested models accounting for membrane disruption considered hydrophobic interaction driven insertion of acyl chains into the G5 interior [7]. In addition to structural studies, cytotoxic effects of G5-NH_2 but not COOH functionalized PAMAM generation 4.5 (G4.5) dendrimer have been reported [3,6,10]. Moreover, it was shown that G5-NH_2 causes persistent depolarization of neuronal membrane resulting in cell death in brain tissue [11]. Better understanding of the mechanisms underlying interactions of cationic and anionic dendrimers with the membrane of living cells is necessary for the development and safety control of dendrimer-based biomedical applications.

This study focuses on the characterization of dendrimer-related ion transport processes in native plasmamembrane of living cells. We also address the nanoscale mechanism by which the characteristic cationic nanoparticle G5-NH_2 can be incorporated into the membrane. Our results are arranged as follows: i) whole-cell measurements on membrane resistance, capacitance and currents in brain tissue in

Abbreviations: PAMAM, polyamidoamine dendrimer; SAXS, small angle X-ray scattering; TEM, freeze-fracture transmission electron microscopy; SFVS, infrared-visible sum frequency vibrational spectroscopy; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DPPG, 1,2-dipalmitoyl-sn-glycero-3-phospho-(1'-rac-glycerol); MIC, minimum inhibitory concentration

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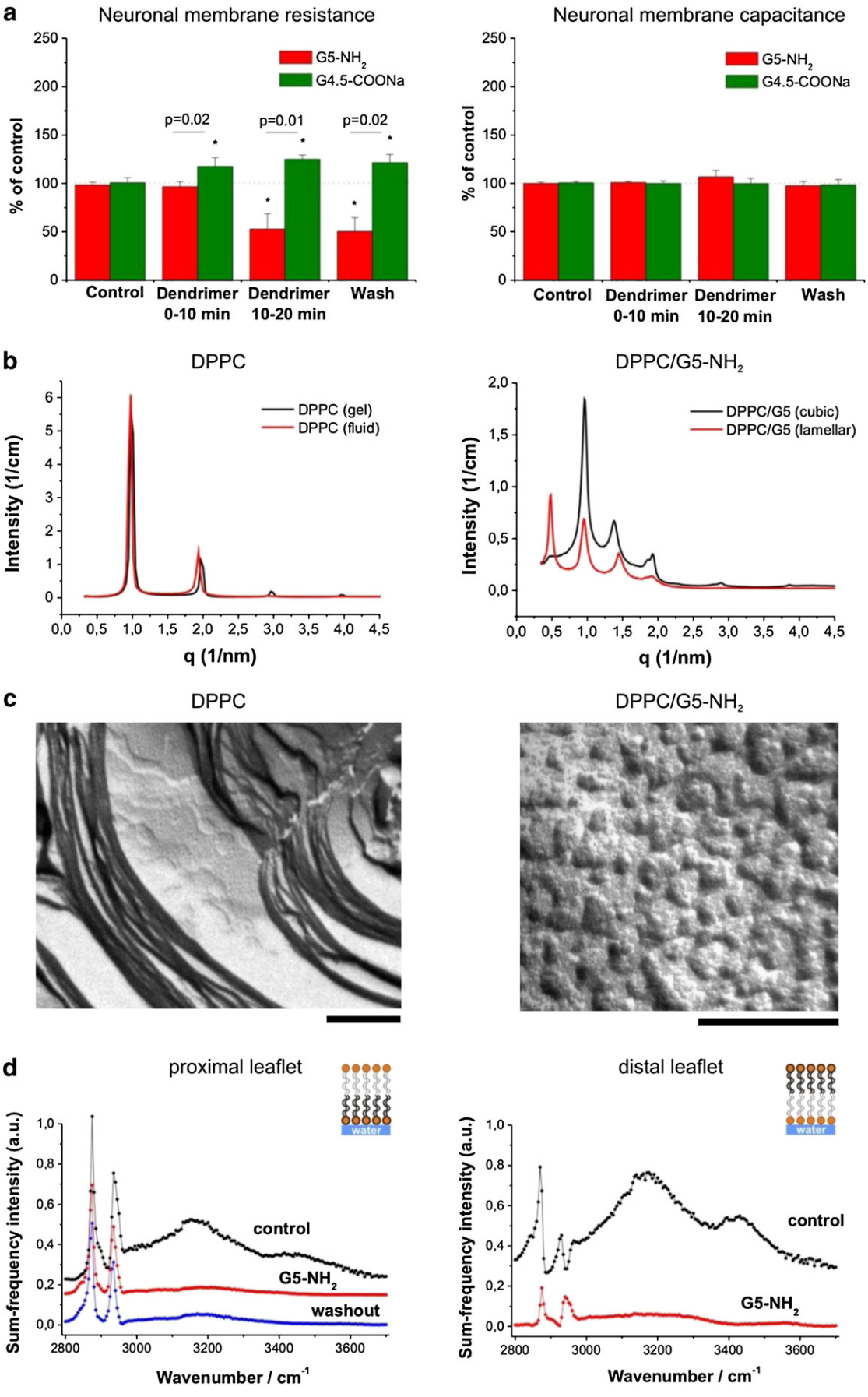
0005-2736/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbamem.2013.04.004



Please cite this article as: G. Nyitrai, et al., Sodium selective ion channel formation in living cell membranes by polyamidoamine dendrimer, Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbamem.2013.04.004