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Structural stability of SoPIP2;1 aquaporin under reconstitution in polymersomes

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ABSTRACT

In the present paper we show the preparation of polymersomes using ABA triblock copolymers with poly(dimethylsiloxane) and poly(2-methyloxazoline) blocks with the incorporation of SoPIP2;1 aquaporin in their structure. The presence of the protein in the polymersome membrane was demonstrated using imaging techniques, and the conformation of the protein into the membranes was assessed using Raman spectroscopy and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). The results suggest the presence of SoPIP2;1 aquaporin in a functional form which could be used for the preparation of Aquaporin-based membranes for water purification via reverse osmosis without the needed of liposome or lipidic-based reconstitution media.

1. Introduction

Functional protein reconstitution in polymeric membranes represents an emerging research area with a promising potential in a wide variety of applications such as in biosensing and membrane separation technologies among others. Biomimetic membranes based on lipid compounds have been used for many years in membrane protein reconstitution but important problems relying on the inherent instability of the lipid membranes, protein denaturation and the low protein density in the reconstituted membrane have impeded their wide-spread use for these applications. In the last years, to solve these issues new strategies has turned the attention into amphiphilic polymers, as these can be used successfully to prepare biomimetic membranes with higher chemical and mechanical stability than lipidic ones. Currently, polymeric membranes can be used to mimic biological membranes and incorporate functional membrane proteins and peptides with many successful examples being available in recent literature [1–8]. Additionally, synthetic channels cannot only be incorporated in lipid membranes but also in polymeric ones [9,10].

The use of water channels like aquaporins have found their way into polymeric nanofiltration membranes, and the preparation of aquaporin-based membranes is currently a very active research area with promising applications in water purification via reverse osmosis [11–14]. Recent reviews summarize the current status of aquaporin-based membranes looking at their application in the water purification business [12,13]. Among the different fabrication strategies, aquaporins embedded in vesicles, forming proteoliposomes or proteopolymersomes, are being incorporated into a polymeric matrix to form membranes for water purification [15–20]. Despite the advances in membrane preparation using aquaporins, the type block copolymers that can be used for a functional reconstitution is very limited only to some polymer motifs.

A recent work by Kuang et al. [21] has shown that a light-driven proton pump was spontaneously reconstituted in amphiphilic block copolymer membranes in a frozen state. This result suggests that the fluidity often regarded as an essential condition for the membrane protein functionality is not a necessary condition when using block copolymers as an alternative to lipids. Additionally, even membranes-forming polymer blocks in entangled states have shown to be suitable for functional membrane proteorhodopsin reconstitution as shown by Hua et al. [6]. Therefore, necessary or desirable conditions widely accepted to get membrane protein functionality in lipid-based membranes may not be necessary for functional membrane protein reconstitution in amphiphilic block copolymer analogues.

Focusing our attention on aquaporin-based membranes, functional assays are the natural step to show the suitability of an aquaporin-polymer system for intended water filtration application. Many of related

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works are application-driven and more fundamental aspects like the membrane protein structure in the block copolymer membrane are left behind on the workflow. More fundamental works on the membrane protein structure in amphiphilic polymeric membranes is needed to address the underlying mechanisms of the protein reconstitution process in order to design the research in a more rational approach. The protein conformation can give valuable information about the suitability of the membrane protein-membrane polymer system for these applications as well as to avoid unnecessary and time consuming functional assays.

In previous papers, we investigated the stability of a triblock copolymer with a large hydrophobic block comprised of 60 blocks of poly(dimethylsiloxane), that was successfully used for functional gramicidin A reconstitution [8,22]. In the current paper, we will investigate the reconstitution of aquaporin into polymersomes with ABA triblock-copolymer membranes based on poly(dimethyloxazoline) and poly(dimethylsiloxane) blocks with a particular focus on the structural stability of the channel-forming protein after reconstitution into the polymeric membrane.

2. Materials and methods

2.1. Materials

Triblock copolymers consisting of a middle block of 60 units of poly(-dimethylsiloxane) (PDMS) and two 7 unit side blocks of poly(2-methyloxazoline) (PMOXA) carrying methacrylate end groups with a total molecular weight (MW) of 5800 g/mol ($PMOXA_7$-$PDMS_{60}$-$PMOXA_7$) (in short, ABA) were obtained from BioCure (USA). All other additional chemicals were purchased from different commercial sources and were of reagent grade and used without further purification.

SoPIP2;1 aquaporin was obtained in solution from a stock concentration of 10–15 mg/ml in 10 mM phosphate buffer, pH 7.5, NaCl 150 mM (PBS) with additional 10% glycerol and 1% octyl-β-D-glucopyranoside (OG) following the procedure from Plasencia et al. [23]. SoPIP2;1 is one of the major integral proteins in spinach leaf plasma membranes. The folding patterns for SoPIP2;1 in detergent and lipids as well as its thermal stability and secondary and tertiary structure was previously characterized [23]. Structural and functional analysis of SoPIP2;1 mutants were investigated by Nyblom et al. [24].

2.2. Sample preparation

A few microliters (2 to 3) of SoPIP2;1 stock solution were added in a stock solution of polymersomes, mixed for a few minutes and left in stock for one day. The sample was used freshly after preparation. The polymer concentration used in our experiments was ca. 10 mg in 1.5 ml of distilled water similarly to previous work on polymersome deposition onto solid supports [25]. In our current preparation protocol we allowed the sample to dry in an analogous manner to previous works [26]. The same final stock solution was used to perform all the analysis of this work.

2.3. Dynamic light scattering (DLS)

DLS experiments were performed using a Malvern Zetasizer Instrument (Nano ZS) to determine the presence of polymersomes in the stock solution. Experiments were performed at room temperature. DLS correlation data were analyzed by the constrained regularized method CONTIN to obtain the distribution decay rate. The hydrodynamic radius was obtained from the diffusion coefficient using the Stokes-Einstein equation, assuming that the solvent viscosity is that of pure water.

2.4. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra of aquaporin inserted into the polymeric membrane were determined by using a FTIR spectrometer Varian 670-R equipped with a horizontal ZnS ATR accessory. The spectra were obtained at a resolution of 2 cm^{-1} and generally from 500 to 700 scans were accumulated to get a reasonable signal-to-noise ratio. Several samples were prepared under the same conditions and measured in order to extract and average final spectrum. The FT-IR spectrum of the polymer membrane in the absence of the protein was also recorded as a control using the same accessory and instrument conditions, and substracted from the final protein-polymer membrane spectrum with the criterion that the latter to be featureless at about 3600 and between 2200 and 1800 cm^{-1} [26]. Baseline correction was carried out in the range 1700–1450 cm^{-1} to get amide I and II bands. The number, position and width of component bands were estimated by performing Fourier self-deconvolution and second-derivative processes to the protein infrared amide I band. Based on these parameters, a curve-fitting process based on a least-square fitting program was carried out by using the ATR Varian Resolution Pro software to get the best Gaussian-shaped curves, which fit the original protein spectrum. Plot fits were performed until the simulated curve matched the experimental one with the minimal standard error. After identifying the individual bands corresponding to each secondary structure, the percentages of each secondary structure component were calculated by the area of their respective component bands [27].

2.5. Raman spectroscopy

Raman spectra were performed with a micro-Raman Renishaw Via Reflex system. The spectrograph used high-resolution gratings (1200 or 1800 g mm^{-1}) with additional bandpass-filter optics. The laser excitation energy employed was 785 nm (diode). All measurements were made with the help of a Raman microscope system in backscattering geometry using a 50× objective. The laser power was 400 mW. Acquisition times for spectra collection in extended mode, using the Renishaw continuous grating mode, were 20 s. Background and aquaporin-free polymer membrane spectra were substracted. Spectrum displayed in the Figure was the average of more than three individual spectra taken at different locations in the polymer film.

Conformational analysis of the SoPIP2;1 aquaporin was performed by analyzing the asymmetric amide I band in the Raman spectra. The amide I band (1590–1720 cm-1) was fitted with the Wire 3.0 Raman software through a routine based on the Levenberg-Marquardt non-linear least-squares method. This band was fitted assuming four symmetrical components corresponding to the different structural conformations the protein may adopt. These peaks were selected in the amide I region with a 30 cm^{-1} bandwidth at half-height. Gaussian functions were employed and the baseline was assumed to be linear. In addition to the described four-component fit, we also tested three- and five-component fits. Comparisons of the $\chi 2$ values and the residuals of the fits were used as criteria for assessing the quality of fits.

2.6. Transmission electron microscopy (TEM)

Suspensions (a drop) of aqueous block copolymer solutions at different concentrations were applied to an electron microscope copper grid, blotted, washed, negatively stained with 2% (w/v) of phosphotungstic acid, washed and evaporated under air. After drying, electron micrographs of the sample were obtained with a Phillips CM-12 electron microscope operating at 120 kV.

2.7. Atomic force microscopy (AFM)

Tapping Mode AFM in air was performed on a MultimodeTM SPM (Nanoscope IIIa, Digital Instruments). Samples were deposited on