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Engineered-membranes: A novel concept for clustering of native lipid bilayers

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ABSTRACT

A strategy for clustering of native lipid membranes is presented. It relies on the formation of complexes between hydrophobic chelators embedded within the lipid bilayer and metal cations in the aqueous phase, capable of binding two (or more) chelators simultaneously Fig. 1. We used this approach with purple membranes containing the light driven proton pump protein bacteriorhodopsin (bR) and showed that patches of purple membranes cluster into mm sized aggregates and that these are stable for months when incubated at 19 °C in the dark. The strategy may be general since four different hydrophobic chelators (1,10-phenanthroline, bathophenanthroline, Phen-C10, and 8-hydroxyquinoline) and various divalent cations (Ni^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, and Cu^{2+}) induced formation of membrane clusters. Moreover, the absolute requirement for a hydrophobic chelator and the appropriate metal cations was demonstrated with light and atomic force microscopy (AFM); the presence of the metal does not appear to affect the functional state of the protein. The potential utility of the approach as an alternative to assembled lipid bilayers is suggested.

1. Introduction

Since the introduction of the fluid mosaic model more than four decades ago [1] as the fundamental architecture of lipid bilayers in living organisms, major efforts were directed toward developing model systems to characterize their chemical, electrical, thermodynamic, and physiological properties. X-ray diffraction and calorimetric studies were first applied to small unilamellar vesicles produced by sonication of large multilamellar liposomes as these provided excellent model systems for biomembranes [2].

In the early 1980s, however, a novel approach for the production of lipid bilayers on solid supports (e.g., glass, quartz, and silicon) was introduced [3]. These so-called *supported phospholipid bilayers* are stable, possess planar geometry, and thus allowed direct measurement of lateral diffusion of membrane components by fluorescence recovery after bleaching (FRAP) [4] and single particle tracking (SPT) [5]. Additional applications of these supported bilayers include the study of the following: (i) secondary structure and orientation of membrane proteins and lipophilic peptides [6], (ii) interactions between helices in signaling proteins [7], (iii) kinetics of ligand binding to membrane embedded receptors [8], (iv) protein induced vesicle fusion in viruses [9], (v) exocytosis [10], and (vi) as biosensors for different target molecules [11–13].

We postulated that the possibility of creating natural biomembranes with large surface area has the potential of providing a native environment for membrane proteins while at the same time allowing an acceptable signal-to-noise ratio for spectroscopic studies since large numbers of proteins could be accommodated. Therefore, as a first step toward this goal, we sought to develop a specific and mild process for interbilayer clustering that would preserve the integrity of embedded integral membrane proteins.

The clustering process depicted in Fig. 1A relies on the formation of strong complexes between hydrophobic chelators embedded within the membrane and transition metal ions capable of binding at least two chelators.

We speculated that hydrophobic chelators would partition into the membrane, thereby transforming it into what we term an *Engineered-membrane*. This construct, in turn, would assemble with other *Engineered-membranes* only in the presence of the appropriate metal cations.

Using several different [metal: chelator] combinations, the properties of the proposed clustering process, as applied to purple membranes containing the light driven proton pump, bacteriorhodopsin (bR) from *Halobacterium salinarum* [14,15], are described. Clearly, the ideal clustering process would not significantly alter the membrane organization and would thus preserve the bR

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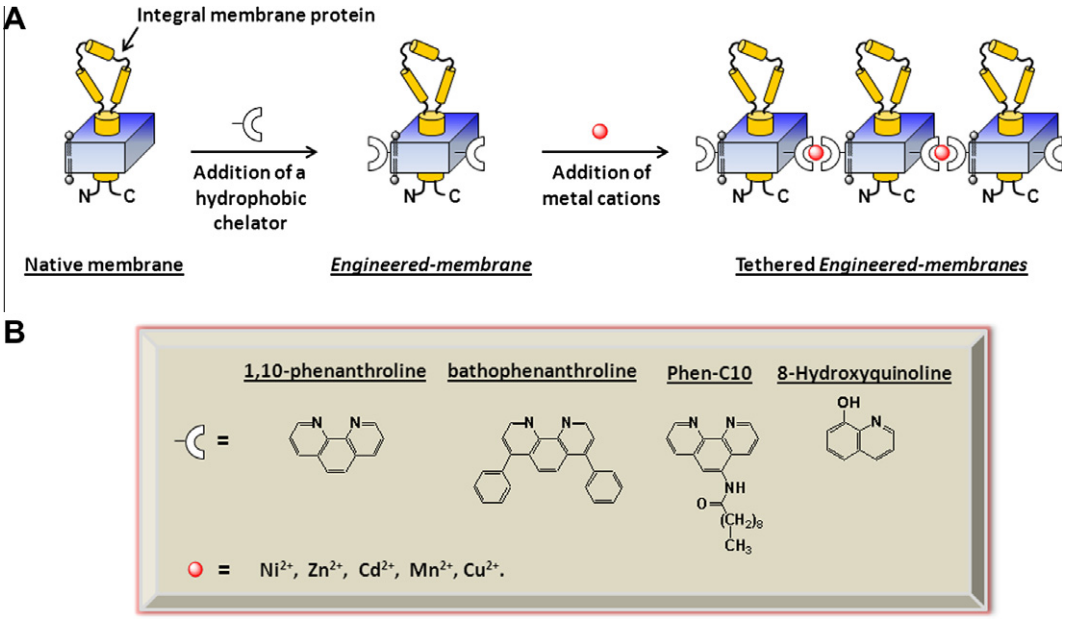

Fig. 1. Illustration depicting the formation of clusters of *Engineered-membranes*. (A) Native membranes are transformed into the corresponding *Engineered-membranes* by the addition of a hydrophobic chelator. Further addition of metal cations, capable of binding up to three chelators simultaneously, induces membrane assembly. (B) Chemical structures of the hydrophobic chelators and metals studied.

protein in its native environment and functional state. Our method satisfies these requirements.

2. Materials and methods

2.1. Materials

Bathophenathroline, 1,10-phenanthroline, 1,10-phenanthroline-5-amine, PEG-6000, NaCl, $NiBr_2$ $ZnCl_2$, $CdCl_2$, $MnCl_2$, and $CaCl_2$ were obtained from Sigma–Aldrich (St. Louis, MO).

2.2. Synthesis of a N-(1,10-phenanthrolin-5-yl)decanamide (Phen-C10)

Phen-C10 was synthesized according to Patchornik et al. [16].

2.3. Preparation of purple membranes

H. salinarum was grown from the S9 strain, and purple membranes containing bacteriorhodopsin were isolated as previously described [17].

2.4. Transforming purple membranes into the corresponding Engineered-membranes

Freshly prepared purple membranes (3.5 μl, OD = 6.6) were first diluted in 6 μl of double distilled water (DDW), which was followed by the addition of 0.5 μl of either 20 mM 1,10, phenanthroline, 20 mM bathophenanthroline or 20 mM Phen C-10 (all in methanol). The slow chelator addition was performed with constant vortexing. The resulting mixture was incubated for 15 min at 4 °C in the dark prior to the addition of divalent cations.

2.5. Engineered-membranes containing 8-hydroxyquinoline

The identical protocol that was used with the phenanthroline derivatives was utilized to transform purple membranes into *Engineered-membranes* containing the hydrophobic chelator 8-hydroxyquinoline. Thus, the slow addition of 0.5 μl of 20 mM 8-hydroxyquinoline (in methanol) to the membrane suspension was followed accordingly by 15 min incubation at 4 °C in the dark.

2.6. Clustered Engineered-membranes with or without a precipitant

Engineered-membranes were clustered on siliconized cover slides (Hampton Research, Aliso Viejo, CA) by the addition of 1 μl of the prepared *Engineered-membranes* to a medium containing: 1 μl of either 20 mM $NiBr_2$, $ZnCl_2$, $CdCl_2$, $FeSO_4$ or $MnCl_2$ in DDW and 2 μl of 9% PEG-6000 in 200 mM NaCl. The resulting mixture was incubated in VDX™ plates (Hampton Research, Aliso Viejo, CA) and equilibrated against 9% PEG-6000 in 200 mM NaCl at 19 °C in the dark. An identical protocol was employed for clustering of *Engineered-membranes* in the absence of the precipitant, PEG-6000.

2.7. The effect of EDTA or catechol on the clustering process

1 μl of 500 mM EDTA (pH 7.5) or 500 mM catechol (DDW) was added to samples containing 4 μl of clustered *Engineered-membranes*. Incubation in VDX™ plates was as described above.

3. Methods

3.1. Light microscopy

Images of hanging drops were obtained using an Olympus CX-40 light microscope equipped with an Olympus U-TV1X-2 digital camera.

3.2. Absorption spectroscopy of clustered purple membranes

Absorption measurements of native and *Engineered-PM* were performed using the HP 8453 UV–Vis spectrophotometer.

3.3. Atomic force microscopy (AFM) measurements

The samples for AFM imaging were prepared on freshly-cleaved mica. A suspension (8 μl) containing purple membranes with or