Colloids and Surfaces B: Biointerfaces 127 (2015) 122–129

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Adsorption and transport of charged vs. neutral hydrophobic molecules at the membrane of murine erythroleukemia (MEL) cells

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ARTICLE INFO

Article history:
Received 3 October 2014
Received in revised form 7 January 2015
Accepted 8 January 2015
Available online 19 January 2015

Keywords:
Molecular adsorption on membranes
Molecular uptake
Murine erythroleukemia
Friend virus/cell
Hydrophobic interactions
Second harmonic generation

ABSTRACT

The adsorption and transport of hydrophobic molecules at the membrane surface of pre- and post-DMSO induced differentiated murine erythroleukemia (MEL) cells were examined by time- and wavelength-resolved second harmonic light scattering. Two medium (<600 Da) hydrophobic molecules, cationic malachite green (MG) and neutral bromocresol purple (BCP), were investigated. While it was observed that the MG cation adsorbs onto the surface of the MEL cell, neutral BCP does not. It is suggested that an electrostatic interaction between the opposite charges of the cation and the MEL cell surface is the primary driving force for adsorption. Comparisons of adsorption density and free energy, measured at different pH and cell morphology, indicate that the interaction is predominantly through sialic acid carboxyl groups. MG cation adsorption densities have been determined as $(0.6 \pm 0.3) \times 10^6$ μm^{-2} on the surface of undifferentiated MEL cells, and $(1.8 \pm 0.5) \times 10^7$ μm^{-2} on differentiated MEL cells, while the deduced adsorption free energies are effectively identical (ca. -10.9 ± 0.1 and -10.8 ± 0.1 kcal mol^{-1}, respectively). The measured MG densities indicate that the total number of surface carboxyl groups is largely conserved following differentiation, and therefore the density of carboxylic groups is much larger on the differentiated cell surface than the undifferentiated one. Finally, in contrast to synthetic liposomes and bacterial membranes, surface adsorbed MG cations are unable to traverse the MEL cell membrane.

1. Introduction

The transport of small atomic ions across the red blood cell (RBC) membrane have been well characterized, and a vast knowledge base of ion channels in the RBC membrane have been established [1–5]. For RBCs, molecular interactions at the cell membrane surface are critical to many problems including the stabilization of the RBC. For example, it has been observed that block copolymers such as glycocalyx adsorbed on the RBC membrane can prevent settling and aggregation of RBCs [6,7]. Similarly, aggregation of RBCs is prevented by the net negative charge distribution of the cell surface, which is determined collectively by all charge bearing functional groups on (or close to) the cell surface. Information such as surface charge densities, and properties pertaining to molecular adsorption at the cell membrane, are thus critical for understanding cell/cell interactions, which are important for defining conditions for blood storage without coagulation.

Characterization of molecular interactions with cell membranes also provides useful information for pharmaceutical applications. Drug molecules or drug carriers often suffer from the limitation that they are quickly removed from the target, where critical drug concentrations need to be sustained for drug efficacy. It has been proposed that hitchhiking on the surface of RBCs could prolong the circulating lifetime of a variety of drug molecules [8,9]. Understanding molecular interactions with the RBC membrane may provide useful insights for the development of efficient drug delivery systems.

Numerous methods including: electrophoresis [10], fluorescence [11], surface plasmon resonance [12], and Fourier transform infrared spectroscopy [13] have been used to investigate molecular interactions at the surfaces of biological membranes. Studies span the binding of small ions [10], the association of polymer particles as drug carriers [8,9], and the adsorption of peptides and proteins [11,14]. However, rather than probing the actual cells/tissues of interest, the majority of such studies have been performed on biomimetic model membrane systems (e.g., liposomes and supported lipid membranes) [15,16]. Typically, either centrifugation or other indirect assays are used to infer surface adsorbed vs. bulk quantities [15].

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http://dx.doi.org/10.1016/j.colsurfb.2015.01.014
0927-7765/

It has previously been demonstrated that the nonlinear optical technique, second harmonic generation (SHG), can be used to study time-resolved molecular adsorption onto and transport across membranes of living biological cells [17,18]. In this approach, an SHG signal is detected at twice the frequency (i.e., 2ω) of an incident fundamental light source of frequency ω. SHG is induced through dipole allowed polarizability, which does not exist in molecules exhibiting inversion symmetry (i.e., centrosymmetric). For molecules that are noncentrosymmetric, each individual molecule has non-zero second-order polarizability, and hence is capable of producing SHG. For an ensemble of such molecules in a solution, the nonlinear polarization has a net zero sum due to the random isotropic orientation. However, if these molecules adsorb onto a surface or interface (e.g., a cell membrane), they can adopt similar anisotropic orientations. The nonlinear polarizations from surface adsorbed molecules with similar orientation constructively interfere and yield a net positive SHG response. Subsequently, the SHG response detected from a solution containing both cells and SHG-active molecules originates solely from the molecules adsorbed onto the cell membranes.

Following the first demonstration of the detection of SHG from dye molecules adsorbed on micro-colloidal particles by Eisenthal and coworkers [19], SHG has since proven to be an effective technique for quantitative characterization of molecular adsorption in various colloidal systems. In addition to probing adsorption and transport across living cell membranes [17,18,20], SHG has been applied to other biologically relevant systems including liposomes [21–25], neurons [26], and collagen networks [27,28].

In the 1950s, Charlotte Friend discovered a virus in the spleens of Swiss mice which induced erythroleukemia (i.e., cancer of the blood) [29,30]. Infected spleens exhibited a malignant overproduction of mutated progenitor erythrocytes (i.e., immature blood cells), which were shown to be developmentally arrested at a stage morphologically similar to normal developing basophilic erythroblasts [29–31]. Later, Friend and colleagues observed that these malignant murine erythroleukemia (MEL) cells, also known as Friend cells, could be chemically induced to continuing differentiation along the normal erythrocyte developmental line by incubating them in a polar organic solvent (e.g., dimethyl sulfoxide, DMSO). DMSO induced differentiated MEL cells were shown to have a morphology similar, though not identical, to an orthochromatic normoblast [32]. Subsequently, MEL cells were quickly established as model systems for studying the erythroid differentiation process leading to a mature RBC, as well as for characterizing the associated genetic mechanisms underlying leukemia [33–37].

It has been established that the most significant changes during the chemically induced differentiation of the MEL cell include a reduction of cell size, condensation (but not extrusion) of the nucleus, reduction of membrane fluidity, variation of protein composition, and the onset of hemoglobin synthesis [30,35,38,39]. These studies, on the other hand, have shown that there are only minor changes to the cell membrane composition. In this way, the MEL cell surface serves as a reasonable model system for studying molecular interactions at the surface of blood cells.

Similar to the mature RBC, the membranes of both pre- and post-differentiated MEL cells exhibit an asymmetric phospholipid distribution. Specifically, the outer leaflet of the membrane is composed of choline-containing head groups (i.e., 47% phosphatidyl choline (PC) and 42% sphingomyelin (SM)) and 11% phosphatidyl ethanolamine (PE), which are all zwitterionic, and therefore yield a net *neutral* charge distribution [40]. Despite this, the outer membrane still exhibits a net anionic charge due to sialic acid carboxyl groups which extend out from the surface of the membrane. Conversely, while the inner leaflet of the membrane contains zwitterionic phospholipids (i.e., 44% PE, 14% PC, 9% SM), it also contains a significant fraction of acidic phospholipids (i.e., 30% phosphatidyl serine (PS), 2% phosphatic acid (PA), 1% phosphatidyl inositol (PI)), all of which are anionic and therefore yield a net *negative* charge distribution [40]. Further, both MEL and RBCs possess an inner skeletal protein network which is bound to the inner leaflet of the membrane, and is suggested to interact with the membrane via an electrostatic interaction [41].

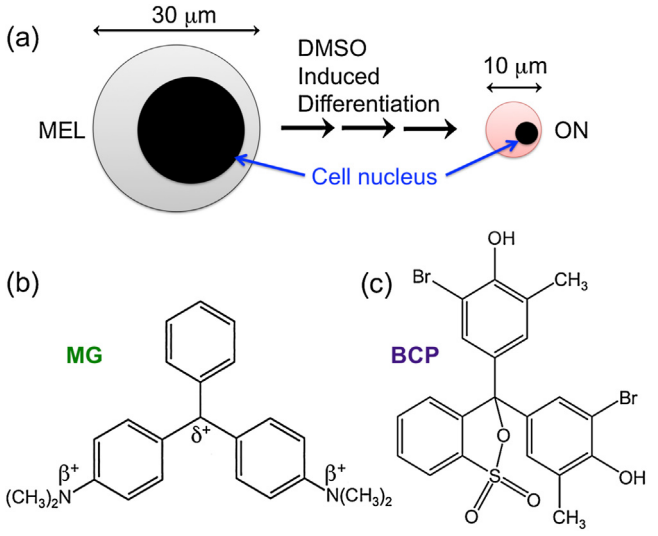

Fig. 1. Cellular and molecular samples used in this study. (a) Cartoon representation of MEL cells before and after DMSO induced differentiation to a morphology similar to an orthochromatic normoblast (ON) stage of normal erythrocytes. Molecular structures of the triphenylmethanes, (b) cationic malachite green and, (c) neutral bromocresol purple.

In this report we demonstrate the use of time-resolved SHG for probing interactions of hydrophobic molecules with the surface of pre- and post-DMSO differentiated MEL cells. In contrast to mature RBCs, both pre- and post-differentiated MEL cells are spherical due to the orientation of the skeletal protein network (Fig. 1). The spherical shape eliminates any possible defects or fluctuations that irregular shapes may cause, and enables a straightforward analysis of the SHG signal. Moreover, the pre- and post-DMSO differentiated MEL cells have average volumes of 1200 μm^3 and 500 μm^3, respectively [42], suggesting that the majority of the SHG scattering can be detected in the experimentally expedient forward-propagation direction. In what follows, we examine the interactions of a pair of medium sized hydrophobic triphenylmethane dyes: cationic malachite green (MG, Fig. 1b) and neutral bromocresol purple (BCP, Fig. 1c), with the membrane surface of pre- and post-differentiated MEL cells. Specifically, it is recognized that membrane association with various biomolecules is governed by a complex interplay of hydrophobic and electrostatic interactions. MG and BCP are hydrophobic molecules with and without charge, respectively. Therefore, both dyes serve as model systems capable of providing a general understanding of molecular interactions with cell surfaces.

2. Materials and methods

MEL cells, provided by Gerd Blobel of The Children's Hospital of Philadelphia, were grown at 37 °C in 5% CO_2 in complete Dulbecco's modified Eagle's medium, supplemented with: 10% fetal bovine serum, penicillin G (100 units/mL), streptomycin (100 μg/mL), 1% L-glutamine, and 1% Na-Pyruvate. Cell density was determined using a Bürker counting chamber. After eliminating culture medium by centrifugation, cells were re-suspended in either neutral or acidic (i.e., pH = 4.5) water. Additionally, as depicted in Fig. 1a, a portion of the MEL cells were incubated with 1% DMSO (over a period of 4 days) to induce committed differentiation