Colloids and Surfaces B: Biointerfaces 146 (2016) 716–721

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Photo-triggered release from liposomes without membrane solubilization, based on binding to poly(vinyl alcohol) carrying a malachite green moiety

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ARTICLE INFO

Article history:
Received 25 January 2016
Received in revised form 31 May 2016
Accepted 6 July 2016
Available online 7 July 2016

Keywords:
Liposome
Release
Malachite green
Copolymer
Photoionization
Binding

ABSTRACT

When working with liposomes analogous to cell membranes, it is important to develop substrates that can regulate interactions with the liposome surface in response to light. We achieved a photo-triggered release from liposomes by using a copolymer of poly(vinyl alcohol) carrying a malachite green moiety (PVAMG). Although PVAMG is a neutral polymer under dark conditions, it is photoionized upon exposure to UV light, resulting in the formation of a cationic site for binding to liposomes with a negatively charged surface. Under UV irradiation, PVAMG showed effective interaction with liposomes, releasing the encapsulated compound; however, this release was negligible under dark conditions. The poly(vinyl alcohol) moiety of PVAMG played an important role in the photo-triggered release. This release was caused by membrane destabilization without lipid solubilization. We also investigated different aspects of liposome/PVAMG interactions, including PVAMG-induced fusion between the liposomes and the change in the liposome morphologies.

1. Introduction

Since liposomes are analogous to cell membranes, the interaction of liposomes with macromolecules helps in understanding the interaction of the external surface of cells with drugs or drug delivery systems loaded with bioactive compounds. Ideally, the cell membrane should not interact with drugs or drug delivery systems until they have been transported to the target cells by endocytosis, or until external perturbations are applied. Numerous attempts have been made to regulate the interaction of liposomes with macromolecules [1–10]. A promising approach is to introduce specialized groups on the macromolecules that interact with the liposomes [4–10]. Ideal drug delivery systems contain interacting groups that can respond to environmental stimuli such as pH, light, and temperature; hence, we have designed poly(vinyl alcohol) carrying a malachite green moiety (PVAMG) that undergoes photoionization (Fig. 1) [11]. In this design, UV irradiation leads to the formation of a cationic site on the malachite green moiety, a phenomenon not observed under dark conditions. We used liposomes with a negative zeta potential as simple cell model system. With such liposomes, the cationic sites of photo-irradiated PVAMG promote adsorption on the liposome surface. Therefore, PVAMG is expected to interact with liposomes when irradiated. Light enables spatial and temporal control, thus allowing PVAMG to act on a targeted area of the liposome membrane. One possible outcome of the photo-triggered adsorption of PVAMG is the destabilization of the liposome membrane. Drug molecules are required to penetrate a destabilized domain on the cell membrane, which in turn necessitates targeted destabilization of the liposome membrane. Walde and coworkers investigated the interaction of oligoarginines with anionic vesicles, and indicated that the bilayer destabilization correlates well with the cellular uptake efficiency [12]. Since solubilization of the cell membrane results in cytotoxicity, cellular uptake of drugs in the targeted domain without any solubilization is desired for ideal drug delivery systems. In this regard, investigations have been conducted on liposomes containing photoresponsive compounds such as azobenzene, stilbene, spiropyrane amphiphiles [13–15], and photo-polymerizable lipids [16,17], wherein the lipid membrane is destabilized by light. However, in those cases, specific liposomes with photoresponsive compounds had to be prepared, which rendered such liposomes unsuitable models for the cell membrane. Photosensitive proton sources such as 3,3′-dicarboxydiphenyliodonium salts allow for efficient destabilization of the phosphatidylcholine bilayer by adsorption of the hydrophobic polyelectrolyte, but result in vesicle-to-micelle transition, and consequently, complete solubilization of

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http://dx.doi.org/10.1016/j.colsurfb.2016.07.018
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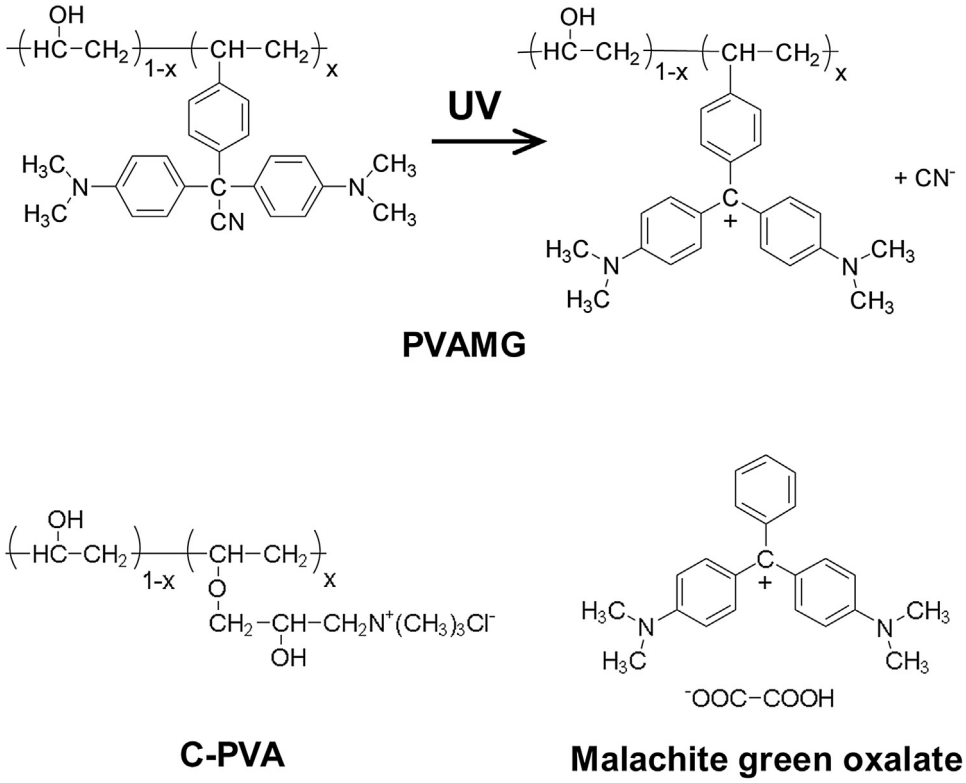

Fig. 1. Chemical structures of binding compounds used in this study.

the membrane [18]. Coumarin-modified poly(vinyl alcohol) caused photo-triggered release of content encapsulated by the egg yolk phosphatidylcholine liposome, however, the liposome structure was not well defined after irradiation [19]. Therefore, it is necessary to ensure photo-triggered destabilization of phosphatidylcholine membrane without disruption of the liposome structure.

In this research, we studied the interaction of photoionizable PVAMG with egg yolk phosphatidylcholine liposomes. Besides destabilization, polymers have various effects on lipid bilayers, including stabilization [4,20–25], aggregation [3,26], fusion [27–29], and total membrane disruption [30,31]. Hence, we have studied each phenomenon during the interaction of PVAMG with liposomes. By means of zeta potential measurements and fluorescence emission spectroscopy, we confirmed the binding of PVAMG to phosphatidylcholine liposomes under irradiation and examined the photo-triggered release of a liposome-encapsulated compound to evaluate the liposome membrane destabilization. Fluorescence resonance energy transfer (FRET) was used to assay fusion events, and the liposome morphologies were characterized by cryo-transmission electron microscopy (cryo-TEM). Cationic PVA carrying a trimethylammonium moiety (C-PVA, Fig. 1) was synthesized to compare the complexing properties of C-PVA and PVAMG. Malachite green oxalate (Fig. 1), as a monomeric part of ionized PVAMG, was also examined for comparison with PVAMG.

2. Materials and methods

2.1. PVAMG sample

PVAMGs were synthesized as described previously [11]. The composition and molecular weight data of PVAMG copolymers are summarized in Table 1. Because the ionized malachite green moiety in PVAMG is hydroxylated under basic conditions, all the samples were prepared using 0.1 M acetate buffer solution at pH 4.0, unless otherwise noted. Photoionized malachite green gave an absorption peak at around 625 nm [11], and the peak intensity remained constant at pH 4.0 for more than 1 day. The UV light source was a xenon lamp (500 W) equipped with a photoguide tube and a Toshiba UV-D33 S filter. Irradiation was carried out for 35 min. The concentration of the ionized malachite green moiety (MG^+) of PVAMG was determined from the absorbance at 625 nm using calibration curves prepared for malachite green oxalate.

Table 1
Molecular weight and molar fraction data of cationic units of PVAMGs and C-PVA.

copolymer	M_w	M_w/M_n	x
PVAMG **1**	1.5×10^4	1.73	6.0×10^{-4}
PVAMG **2**	1.3×10^5	2.31	7.2×10^{-4}
C-PVA	1.3×10^4–2.3×10^4	–	8.3×10^{-2}

The average molar mass (M_n) and weight-average molar mass (M_w) of PVAMGs are determined by gel permeation chromatography using poly(methyl methacrylate) calibration standards. The M_w of C-PVA is that of unmodified PVA. The molar fraction of the cationic unit in copolymer (x) is estimated by measuring the absorption spectra of PVAMG in a buffer solution after irradiation and the NMR spectra of C-PVA. Average numbers of cationic site per polymer chain are included in Supplementary material (Table S1).

2.2. Synthesis of PVA modified with a cationic group (C-PVA)

Cationic modification of PVA was carried out per a literature procedure [30]. About 2 g of 3-chloro-2-hydroxypropyltrimethylammonium chloride was added to a 100 mL aqueous solution containing 10 g of PVA (M_w = 13000–23000) and 0.2 g of NaOH. PVA was purchased from Aldrich and used as received. After stirring for 22 h at 45 °C, the mixture was neutralized using hydrochloric acid and poured into excess acetone. The precipitate was washed with methanol in a Soxhlet extractor and dried. The cationic group content in the polymer (C-PVA) was determined by NMR analysis in order