Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Controlled Release 106 (2005) 146–153

journal of controlled release

www.elsevier.com/locate/jconrel

Cytosolic delivery of macromolecules 4. Head group-dependent membrane permeabilization by pH-sensitive detergents

Aravind Asokan, Moo J. Cho*

Division of Drug Delivery and Disposition, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States

Received 7 February 2005; accepted 14 April 2005
Available online 24 June 2005

Abstract

Three tertiary amine-based detergents with zero, one, or two hydroxyl groups at various positions in their head group were characterized for their ability to promote the cytosolic delivery of macromolecules. Critical micellar concentrations (CMC) and membrane-bound pKa values of the lipid constructs increased with increasing head group polarity, ranging from 1–5 μM and 5.9 to 6.3, respectively. Fluorescence resonance energy transfer (FRET) and calcein leakage experiments revealed that when the amine group is protonated introduction of –OH moieties to detergent head groups enhanced their ability to interact with and permeabilize anionic, endosome-mimicking vesicles. Different formulations of a diethanolamine-based lipid (DEL) were further evaluated for pH-dependent hemolytic activity and ability to promote cytosolic delivery of macromolecules in vitro. Intact liposomes containing DEL at its maximum limit of incorporation were less efficient than DEL-containing micelles in promoting hemoglobin leakage from human erythrocytes at acidic pH. In HeLa cells, DEL-containing detergent micelles facilitated efficient cytosolic release of endocytosed macromolecules such as fluorescein-labeled dextran of MW 10 kDa. This observation was further corroborated by a functional assay based on antisense-mediated up-regulation of enhanced green fluorescent protein (EGFP). Taken together, our findings emphasize the key role of polar head groups and micellar architecture of pH-sensitive detergents in mediating endosomal permeabilization and the efficient cytosolic delivery of macromolecules.

Keywords: pH-sensitive; Detergent; Micelles; Endosomal permeabilization; Cytosol

1. Introduction

Macromolecules internalized via the endocytic pathway are subsequently routed to lysosomes for enzymatic degradation. Thus, timely permeabilization

* Corresponding author. CB # 7360, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7360, United States. Tel.: +1 919 966 1345; fax: +1 919 966 7778.
E-mail address: m_j_cho@unc.edu (M.J. Cho).

0168-3659/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2005.04.010

of the endosomal membrane is a prerequisite for cytosolic translocation of macromolecular drugs in order to exert their pharmacological effect. Within this framework, our lab and others have focused on exploiting the differences in proton activity between endosomes and the cytosol in the design of pH-sensitive drug carriers [1]. Several studies suggest that pH-responsive amphiphiles, when incorporated into liposomal bilayers, can trigger release of entrapped material and enhance the endosome-to-cytosol transfer of macromolecular drugs [2–5]. Amongst such agents, lysosomotropic detergents are particularly well-suited for in vitro drug delivery to the cytosol due to their ability to selectively permeabilize endosomal/lysosomal membranes [6].

However, the formulation of such pH-sensitive detergents has been hampered by their low limit of incorporation in liposomal bilayers and their propensity to be transferred to biological membranes or serum proteins in circulation in vivo [7–9]. The preparation of stable liposomal formulations containing sufficient levels of detergent to promote triggered release as well as endosomal permeabilization has been particularly challenging. A chemical solution to this problem involves masking the membrane-disrupting activity of single-tailed detergents by cross-linking their polar head groups using cleavable moieties to generate bilayer-favoring detergent dimers [10–13]. Subsequent hydrolysis of the cross-linker in response to specific stimuli such as acidic pH of the endosomal lumen [14] generates detergent monomers that promote lamellar-to-micellar phase transition and membrane permeabilization.

While our long-term goal is to address how to facilitate lamellar-to-micellar transition in liposomes through judicious structural design of pH-sensitive lipids, the current study focuses on single-tailed detergents that will serve as building blocks for future detergent dimer or 'bis-detergent' constructs. Briefly, we have investigated the membrane-permeabilizing potential of three single-tailed, pH-sensitive detergents with identical stearoyl (C_{18}) chains but increasingly polar head groups (Fig. 1). The results obtained herein from lipid mixing and calcein leakage/hemolysis assays, fluorescence microscopy studies, and an antisense functional assay argue for increasing membrane-permeabilizing potential of pH-sensitive detergents with increasing head group polarity. Also discussed is the key role of detergent micelles in permeabilizing the endosomal membrane and promoting the cytosolic delivery of macromolecules.

2. Materials and methods

2.1. Lipids and chemicals

The detergents (bis(2-hydroxyethyl)amino) ethyl stearate (DEL), (2-hydroxy-3-morpholino) propyl stearate (ML1), and (2-morpholino) ethyl stearate (ML2) were synthesized as described previously [8]. Egg phosphatidylcholine (PC), L-α-phosphatidic acid (PA), N-(lissamine rhodamine B sulfonyl)phosphatidylethanolamine (Rh-PE), and N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (NBD-PE) were obtained from Avanti Polar Lipids (Alabaster,

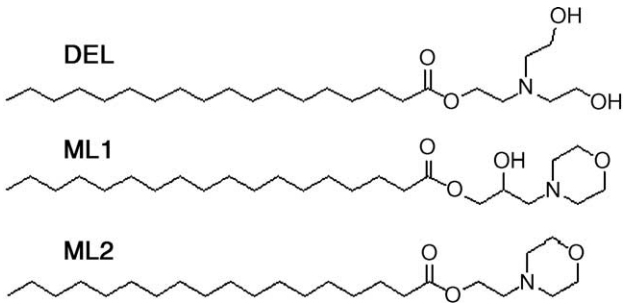

Fig. 1. Structures of pH-sensitive detergents characterized in this study: (bis(2-hydroxyethyl)amino) ethyl stearate (DEL), (2-hydroxy-3-morpholino) propyl stearate (ML1), and (2-morpholino) ethyl stearate (ML2).