

Effect of polymeric surfactants on the release of encapsulated marker from large unilamellar vesicles

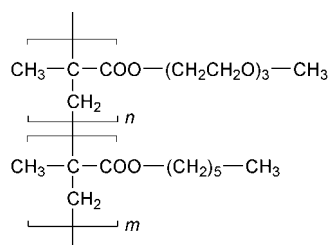
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Large unilamellar vesicles (LUV) of lecithin, radioactively labeled in the membranes and in the aqueous interior, were used as membrane models to measure the stabilizing effect of different polymeric surfactants on the membrane integrity upon addition of sodium cholate as a detergent. Parameters for the membrane damage were the release of encapsulated [^3H]inulin, indicating larger membrane defects and the release of [^{14}C]dipalmitoylphosphatidylcholine, representing membrane dissolution. The polymeric surfactants were block copolymers of the type



$$67 < n < 134 \text{ and } 0 < m < 88$$

with triethylene glycol monomethyl ether and hexyl alcohol as hydrophilic and lipophilic side-chains coupled to poly(methacrylic acid) by ester bonds. All polymers had a molecular weight of 30 000 and differed in the mass ratio of hydrophilic to lipophilic parts: 100/0 (A), 90/10 (B), 60/40 (C) and 50/50 (D). It was found that the addition of these compounds in a concentration range from the critical micellar concentration (c.m.c.) to 100 times the c.m.c. to liposomes had no measurable membrane-destabilizing effect, in contrast to monomeric surfactants of low molecular weight such as cholate. Furthermore, the more lipophilic polymers C and D even show a more pronounced membrane-stabilizing effect than the hydrophilic compounds against cholate attack.

Introduction

Different grades of copolymers with triethylene glycol monomethyl ether and hexyl alcohol as hydrophilic and lipophilic side-chains coupled to poly(methacrylic acid) by the ester backbone are able to solubilize insoluble drugs and should be further studied with respect to possible use in the parenteral administration of lipophilic drugs. In this work, they were tested for their interaction with membranes. Liposomes were used as model membrane and we studied the effect of these polymeric surfactants on the release of encapsulated markers. On the one hand, we estimated the membrane-damaging properties of the polymers themselves. On the other, we studied their membrane-stabilizing effect against monomeric surfactants such as sodium cholate, which are known to produce transient membrane pores even at concentrations below their critical micellar concentration (c.m.c.).¹

Materials and methods

Liposome preparation

Large unilamellar vesicles (LUV) of homogeneous size were prepared by using the method of fast and controlled dialysis of mixed detergent-lipid micelles.² Egg lecithin (a gift from Lipoid, Ludwigshafen, Germany) and [^{14}C]dipalmitoyl-

phosphatidylcholine ([^{14}C]DPPC) (NEN Chemicals, Dreieich, Germany) were dissolved together with sodium cholate (Sigma Chemical, St. Louis, MO, USA) in methanol. Solvent was completely removed under reduced pressure and the dry lipid-detergent film was dissolved in HEPES buffer (10 mmol L⁻¹ HEPES, 150 mmol L⁻¹ NaCl, pH adjusted to 7.4) to give a final lipid concentration of 17 mmol L⁻¹. The mixed micelle solution was dialyzed for at least 24 h at room temperature against a continuous flow of HEPES buffer, using a commercially available dialysis apparatus (Lipoprep; Diachema, Langnau, Switzerland) and a highly permeable dialysis membrane with a cut-off of 10 kDa (Diachema). Entrapment of ^3H -labeled inulin was performed by adding radioactive marker to the buffer used to dissolve the dry lipid-detergent mixture. Free inulin was separated from encapsulated on Sepharose 4B-CL.

Polymer synthesis and characterization

The four different copolymers were synthesised by anionic polymerization³ in tetrahydrofuran and diphenylethylene and with 1,1-diphenyllithium as initiator. The mean molecular weight of each polymer was 30 000 within a narrow distribution. The c.m.c. values of the polymers were determined by the tensiometer method and were found to be 0.26, 0.33, 0.83 and 1.06 μM for 100/0, 90/10, 60/40 and 50/50, respectively, where

the first number represents the mass % of the hydrophilic part of the polymer (triethylene glycol monomethyl ether methacrylate) and the second number the mass % of the lipophilic part of the polymer (hexylmethacrylate).

Vesicle stability

A total of 1.0 ml of liposome suspension containing LUVs with a lipid concentration of $500 \mu\text{mol L}^{-1}$ and various block copolymer concentrations was incubated for 15 min and was then ultracentrifuged ($140\,000 g$, 210 min, 20°C). The release of radioactively labeled inulin and DPPC from vesicles was determined after mixing 0.1 mL of the supernatants with 2.0 mL of scintillation fluid (Ultima Gold, Packard-Canberra). All measurements were performed in duplicate. The results, which did not differ by more than 10%, were averaged.

Effect of block copolymers on cholate-induced inulin release from liposomes

These experiments were carried out to show the stabilizing effect of different block copolymers against cholate-induced membrane damage. The vesicle stability was measured as shown above after preincubation of the liposomes with a particular polymer concentration for 15 min and then incubated with cholate of different concentrations for 10 min before ultracentrifugation.

Results and discussion

The release of [^3H]inulin and [^{14}C]DPPC from liposomes induced by the four different polymers up to concentrations of 100 times the c.m.c. was in any case less than 3% and was therefore negligible (results not shown).

In previous studies, we showed that upon addition of cholate, larger membrane defects are formed which results in the release of inulin (M_r 5000). The onset of defect formation occurred within a few seconds after cholate addition and the defects re-sealed within a few minutes.⁴ Our present studies show that the formation of these transient defects is suppressed after preincubation of block copolymer 60/40 with liposomes (Fig. 1). At the c.m.c., the polymer reduced the inulin release by approximately 15% in the cholate concentration range 2.7–3.5 mmol L^{-1} . At 10 times the c.m.c., the stabilizing effect was approximately 25%. The stabilizing effect of the more lipophilic block copolymer 50/50 was even more pronounced. It was found to be approximately 25% at the c.m.c. of the polymer at comparable cholate concentrations (data not shown) and was the same at 10 times the c.m.c. The homopolymer (100/0) and of block copolymer 90/10 had a

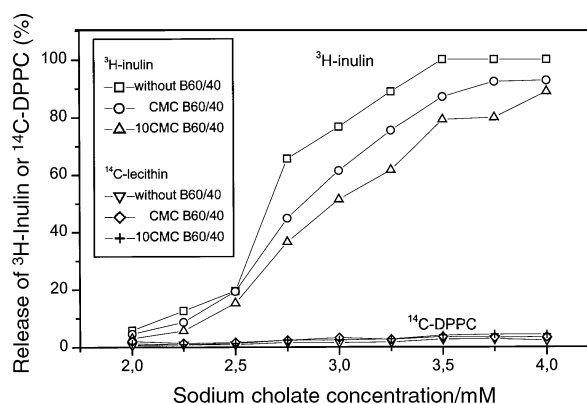


Fig. 1 Effect of block copolymer 60/40 on cholate-induced inulin release from liposomes. Radioactively double-labeled egg-lecithin liposomes ($[^{14}\text{C}]$ DPPC as membrane label, [^3H]inulin as label for encapsulated hydrophilic material of M_r 5000) were incubated with increasing cholate concentrations or preincubated with $0.83 \mu\text{M}$ (*i.e.*, c.m.c.) or 10 times the c.m.c. of block copolymer 60/40 before cholate addition. After ultracentrifugation, the release of labels was measured in the supernatants.

much smaller effect on cholate-induced inulin release from liposomes. It was in both cases less than 10% even at 10 times the c.m.c.

The conclusion of our studies is that block copolymers of triethylene glycol monomethyl ether methacrylate and hexyl methacrylate are able to solubilize lipophilic drugs without inducing any membrane damage up to 100 times the c.m.c. This makes these polymers candidates for further studies on their suitability as excipients for parenteral use. Furthermore, they even stabilize liposomes as models for biomembranes against defect formation. Stabilization is more pronounced for copolymers with larger lipophilic parts, suggesting that a prerequisite for the stabilizing effect is their unsymmetric adsorption on the outer membrane monolayer or even an insertion into the membrane similar to the cytoskeleton of cellular plasma membranes.

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