Channel Protein-Containing Liposomes as Delivery Vehicles for the Controlled Release of Drugs-Optimization of the Lipid Composition

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CHANNEL PROTEIN-CONTAINING LIPOSOMES AS DELIVERY VEHICLES FOR THE CONTROLLED RELEASE OF DRUGS-OPTIMIZATION OF THE LIPID COMPOSITION

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Summary

In the design of liposomal drug formulations containing a controllable channel protein (MscL), the lipid composition is dictated in part by this membrane protein. This work addresses the question whether therapeutically optimal lipid compositions (phospholipid with high T_m/cholesterol/PEG) are compatible with channel activity. As a result of optimization, MscL-proteoliposomes have been prepared that rapidly release encapsulated model drug (calcein), as well as the chosen cytostatic drug, cisplatin.

Introduction

The mechanosensitive protein of large conductance (MscL) from *E.coli*, normally gated by the application of tension to the lipid bilayer, stays fully functional when purified and reconstituted in liposomes [1]. The signal leading to activation of the channel can, however, be changed from tension to, e.g., a drop in pH by altering the MscL via mutation and chemical modification. We have shown, for example, that the mutation of glycine at the 22nd position into cystein (G22C mutant) enables targeting of charged and pH-sensitive chemical groups to MscL. A lower pH (e.g., in solid tumors) can be used as a trigger for activation of the MscL after its reconstitution in the drug-containing liposomes leading to induced release of the drug.

In the present work, the charge-induced gating of G22C by MTSET has been used to demonstrate the activity of MscL after reconstitution in proteoliposomes. The attachment of this positively charged reagent to the cystein of G22C causes MscL to gate spontaneously. Previously, we were succesful in reconstitution of overexpressed and purified MscL in liposomes consisting of both, neutral unsaturated lipids, like DOPC, DOPE and mixtures of neutral and negatively charged lipids, like DOPC/DOPS, DOPC/DOPG. However, it remained to be seen whether we could functionally reconstitute MscL in the therapeutically optimal lipid composition, typically represented by mixtures of a high transition temperature lipid, cholesterol and polyethyleneglycol (PEG). In addition, important parameters, like the protein-to-lipid ratio, lipid concentration, size of proteoliposomes and storage conditions have been optimized.

Experimental methods

Reconstitution: The detergent-mediated reconstitution of G22C mutant of the MscL into liposomes of different lipid compositions included titration of liposomes with Triton X-100 (or octylglucoside), incubation with MscL, addition of either the fluorescent marker (calcein) or cytostatic (cisplatin) and the removal of detergent by BioBeads SM-2. Unencapsulated drug was separated on Sephadex G50 column.

Release of drug: The in vitro release profile of a model drug (fluorescent calcein) from G22C-containing liposomes was estimated in the calcein efflux assay after activation with MTSET ([2-(trimethylammonium)ethyl] methanethiosulfonate). The percentage release of a fluorescent drug was calculated from the dequenching of calcein fluorescence.

The release profile of cisplatin was determined after the separation of the released drug on PD 10 column (1×10 cm) and detection with ICP-AES.

The size of MscL-liposomes after extrusion through the filters of different pore size was determined by using dynamic light scattering.

Results

Reconstitution: MscL could be successfully reconstituted in the phospholipids with low transition temperature (fluid lipid bilayer), with the best activity obtained in DOPC, DOPG and less in POPC and eggPC. MscL-liposomes consisting of lipids with high transition temperature, like DMPC, DPPC, SM and DSPC, failed in preserving the channel gating activity. About 20 to 30 mol% cholesterol and up to 10 mol% DSPE-PEG 2000 could be incorporated in MscL-proteoliposomes without decreasing the channel activity. Thus, the best working lipid composition was the one consisting of DOPC/CHOL/PEG 2000 at a molar ratio of 70:20:10.

Cisplatin was entrapped in the optimized MscL-proteoliposomes at a drug-to-lipid ratio of about 4 to 8 (μg/mg total lipid).

Release of drug: The optimized DOPC/CHOL/PEG 2000 proteoliposomes released about 80% of encapsulated calcein and about 45% of cisplatin under the charge-inducing activation of MscL with MTSET. The limited release of cisplatin might be explained by the possible interaction of this cytostatic with the channel protein.

The protein-to-lipid ratio: For immunogenicity reasons, the amount of the reconstituted MscL should be kept as low as posssible. We were able to reduce the MscL-to-lipid ratio from 1:30 to 1:120 by optimizing the lipid concentration used in the reconstitution while retaining full functionality.

Size: By extrusion of reconstituted MscL-liposomes through 200 nm filter, a homogenous population of particles of about 150 nm size was prepared. The extrusion of MscL-liposomes through 50 nm filter resulted in decreased channel protein activity.

Conclusions

We have demonstrated the controlled release of a model drug, as well as a real drug from channel-containing liposomes. Liposomes with MscL labelled with a pH-sensitive group will provide a useful tool for the delivery of drugs at target sites with a lowered pH value, such as solid tumors or sites of inflammation. The preparation of such MscL-containing liposomes is now in progress.

References

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THERMOSENSITIVE AND BIODEGRADABLE POLYMERIC MICELLES WITH TRANSIENT STABILITY

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Summary

A novel class of thermosensitive and biodegradable polymers, poly(*N*-(2-hydroxypropyl) methacrylamide lactate) (poly(HPMAm-lactate)), was synthesized. The cloud points of the polymers in aqueous solution were between 10 and 65 °C, depending on the copolymer composition. Block copolymers of poly(HPMAm-dilactate) with poly(ethylene glycol) (pHPMAmDL-*b*-PEG) formed polymeric micelles with a size around 50 nm in aqueous solution and showed transient stability due to the hydrolysis of lactate side chains, demonstrating the potential applicability of these systems for controlled drug delivery.

Introduction

Thermosensitive polymers with a lower critical solution temperature (LCST) are presently under investigation for biomedical and pharmaceutical applications [1,2]. These polymers are soluble in aqueous solution below the cloud point (CP), but precipitate above this temperature due to the dehydration of the polymer chains.

For biomedical and pharmaceutical applications of thermosensitive polymers, it is important to have possibilities to control the CP around body temperature. Furthermore, polymers whose CP increase from below to above body temperature in time are very attractive materials, because, e.g., the controlled release of drugs without thermal treatment is feasible using such polymers [3].

The aim of this study is to develop a novel class of thermosensitive and biodegradable polymeric micelles that show controlled destabilization based on the increase of their critical micelle temperature (CMT) in time.

Experimental methods

Poly(HPMAm-monolactate) (pHPMAmML), poly(HPMAm-dilactate) (pHPMAmDL) as well as their copolymers (p(HPMAmML-*co*-HPMAmDL)) were synthesized by radical polymerisation from the corresponding monomers. p(HPMAm-dilactate)-*b*-PEG block copolymers (pHPMAmDL-*b*-PEG) were synthesized by radical polymerisation using HPMAm-dilactate as monomer and methoxy-PEG-ABCPA (4,4-azobis(4-cyanopentanoic acid) as macroinitiator. The products were characterized by ^{1}H NMR (solvent: $CDCl_3$) and gel permeation chromatography (GPC).

The CP and the CMT of the polymer solution were determined with static light scattering (SLS). Onsets on the *x*-axis, obtained by extrapolation of the intensity–temperature curves to intensity zero were considered as the CP and the CMT. The critical micelle concentration (CMC) of block copolymers was determined using pyrene as a fluorescence probe. These measurements were performed in isotonic 120 mM ammonium acetate buffer (pH = 5.0, to minimize hydrolysis of lactate ester side groups) unless mentioned.

Micelles of block copolymers were formed by quickly heating an aqueous polymer solution from 0 °C (below CMT) to 50 °C (above CMT) [4]. The size of micelles was determined by dynamic light scattering (DLS) at 37 °C. The destabilization of polymeric micelles at 37 °C and at different pH's was evaluated by DLS and SLS.

Results and dscussion

Five poly(HPMAm-lactate) polymers (Fig. 1) with different monomer compositions were synthesized and their LCST properties were evaluated. Interestingly, all these polymers showed LCST behaviour, namely, reversible turbidity changes by heating and cooling.