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Immobilization of histidine-tagged proteins on monodisperse metallochelation liposomes: Preparation and study of their structure

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ABSTRACT

Liposomes represent a biocompatible platform for the construction of self-assembling proteoliposomes using nickel or zinc metallochelation. Potential applications of such structures consist in the development of new biocompatible vaccination nanoparticles and drug delivery nanoparticle systems. Here, we describe the design and construction of a flow-through ultrafiltration cell suitable for the preparation of monodisperse liposomes enabled for metallochelation and, hence, the formation of proteoliposomes. The linkage of the cell with a fast protein liquid chromatography system facilitates automation of the procedure, which fits the criteria for upscaling. Proof-of-concept experiments are performed using a mixture of egg phosphatidyl choline and nickel-chelating lipid DOGS–NTA–Ni (1,2-dioleoyl-*sn*-glycero-3-{[*N*(5-amino-1-carboxypentyl)iminodiacetic acid]succinyl}(nickel salt)) to formulate proteoliposomes with proteins attached by metallochelation, including histidine (His)-tagged recombinant green fluorescent protein and rgp120 (derived from HIV-1 Env). These model proteoliposomes are characterized by gel permeation chromatography and by dynamic light scattering. Transmission electron microscopy and immunogold staining are used to characterize surface-bound proteins, revealing the tendency of rgp120 to form microdomains on liposome surfaces. These microdomains possess a two-dimensional crystal-like structure that is seen more precisely by atomic force microscopy.

Liposomes represent the oldest nanoparticle systems described for applications in biological studies as model membranes and in medicine. Over 44 years, liposomes have been shown to be suitable drug delivery systems for applications ranging from cosmetics and dermatology through to medical applications in antiinfection therapy, anticancer therapy, and veterinary vaccination [1]. Liposome-based vaccines have been around for approximately 30 years, and numerous liposome variants have been developed, some with evident immune-stimulatory properties and attractive safety profiles, hence resulting in registered products on the market or preparations in advanced stages of clinical testing (e.g., hepatitis A, Epaxal).

Liposomes represent almost ideal carrier/delivery systems for the components of synthetic vaccines due to their ready biodegradability and their ability to retain/incorporate a variety of essential vaccine components simultaneously, even components possessing quite different physicochemical properties (different size, hydrophobicity, charge, etc.). Different synthetic vaccine components can be encapsulated within the aqueous cavities of liposomes (if hydrophilic) or associated with liposome bilayers (if at least partially hydrophobic in character). Furthermore, essential components can be attached to either internal or external outer leaflet membrane by electrostatic, covalent, or metallochelation interactions. The most diverse synthetic vaccine components are typically adjuvants needed to provoke innate immune reactions (e.g., monophosphoryl lipid A, CpG oligonucleotides, muramyl dipeptide and analogues). In addition, these can be combined with antigens needed to provoke specific immunity such as soluble or membrane proteins. Finally, the liposome may present ligands to assist functional delivery of antigens and adjuvants to antigen-presenting cells necessary to invoke immunostimulation [2]. The laboratory and industrial procedures for the liposome preparation have been established, and liposomes have been approved by the US Food and Drug Administration for biomedical applications. The potential for the participation

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of liposome-based recombinant vaccines in the human and veterinary vaccine market is very promising [3].

There have been a few reports concerning the metallochelation of recombinant proteins to liposomes. The implementation typically requires an insertion of a metallochelation lipid into the outer leaflet membrane of liposome bilayers that interacts selectively in the presence of metal ions (e.g., nickel ions) with a 4- to 6-amino-acid residue histidine tail (His tag)[1] expressed at the N or C terminus of a recombinant protein of interest such as a putative antigen. The reversible character and high affinity of the metallochelation are very useful for the construction of various self-assembled supramolecular structures useful for the construction of experimental synthetic vaccines [4,5]. Here, we describe the design and construction of a special flow-through stirred ultrafiltration cell linked to a fast protein liquid chromatography (FPLC) system for the automated production of unilamellar highly monodisperse liposomes using the detergent removal method [6]. A simplified procedure for the preparation of mixed lipid–detergent micelles (both containing and not containing nickel-chelating lipids) was developed, and liposomes were formed in the cell during the ultrafiltration step, which guaranteed a sustained and well-defined removal of detergent and facilitated a well-reproducible conversion of the micelles into liposomes. The preparation of monodisperse liposomes is of crucial importance to our investigation of the interaction of recombinant His-tagged proteins with liposomes enabled by metallochelation of the proteins. We also describe the optimization of liposome formulation parameters and processes for the preparation of metallochelating proteoliposomes as well as structural investigations by dynamic light scattering (DLS), *sn*-glycero-3-phosphocholine (GPC), transmission electron microscopy (TEM), atomic force microscopy (AFM), and confocal microscopy.

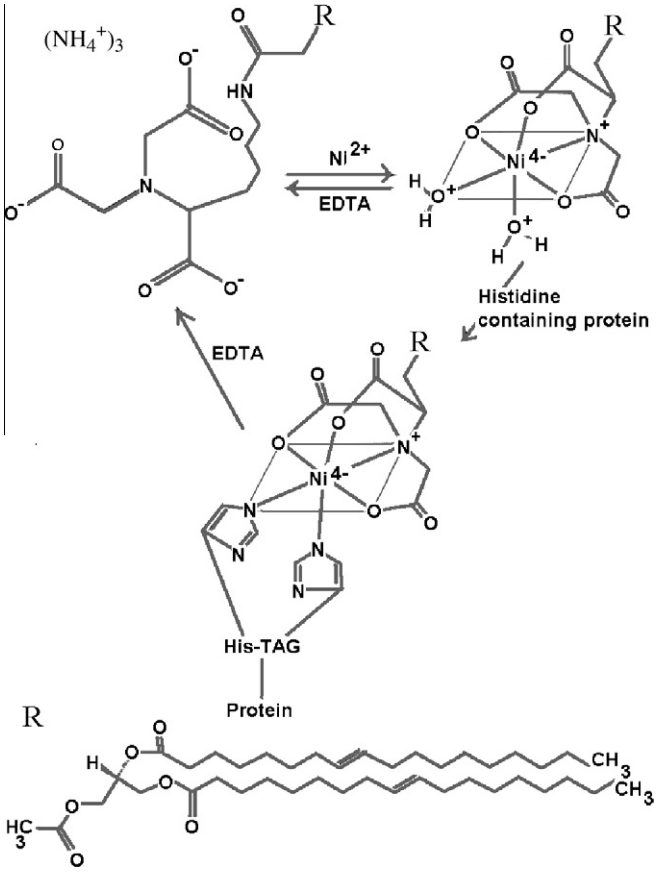

Fig.1. Structural formulas of lipids for metallochelation and illustration of interaction between metallochelation center and His tag of recombinant proteins.

Materials and methods

Materials

Egg phosphatidyl choline (EPC, purity of 99%), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (LR-PE), and 1,2-dioleoyl-*sn*-glycero-3-{[*N*(5-amino-1-carboxypentyl)iminodiacetic acid]succinyl} (nickel salt) (DOGS–NTA–Ni) (Fig. 1) lipids were purchased from Avanti Polar Lipids (Birmingham, AL, USA). A 20-nm membrane filter Anotop 10 and a 0.2-μm membrane filter Anotop 10 LC were purchased from Whatman. A membrane ultrafilter Amicon YM-10 was purchased from Amicon–Millipore. Sodium cholate, tetrahydrofurane, 6-carboxyfluorescein (6-CF), and all other chemicals were purchased from Sigma.

Preparation of micelles

EPC was solubilized in ethanol (600 mg/ml). Sodium cholate was solubilized in appropriate buffer to the final concentration of 20 mM. This solution was filtered through the 0.2-μm membrane filter Anotop 10 LC and the 20-nm membrane filter Anotop 10. The ethanolic solution of EPC and the aqueous detergent solution were mixed at the ratio of 1:30 (v/v) and stirred for 30 min by electromagnetic stirrer at room temperature while light-protected by plastic cap. The initial molar ratio of cholate to lipid was 2:1. The prepared micelles were filtered through 0.2-μm sterile filter, and their size was measured by the DLS instrument NanoSizer NS (Malvern, Worcestershire, UK). The prepared micelles were used immediately or stored overnight in plugged vials at 4 °C for next-day experiments. Due to the generally small particle size of the resulting micelles, the arrangements were done to avoid any interference of contaminating larger particles (unrelated to micelles) at the size measurement. Therefore, all of the solutions were thoroughly filtered and the cuvettes were excessively cleaned.

Preparation of micelles with DOGS–NTA–Ni

DOGS–NTA–Ni lipid (1 mg) was dissolved in 40 μl of tetrahydrofuran (THF), and an appropriate amount of EPC lipid was dissolved in ethanol. Then the solutions were mixed to reach the final ratio of 95 M% EPC and 5 M% DOGS–NTA–Ni. The solution of micelles was prepared by the dispersion of ethanol/THF (66:33, v/v) lipid solution in 20 mM sodium cholate to obtain a desired cholate/lipid ratio.

Ultrafiltration cell

The flow-through stirred ultrafiltration cell was designed to accept 25-mm ultrafiltration membrane discs and withstand the pressure necessary for the ultrafiltration (up to 4 bars). No leakage was found up to 40 bars. It is a limit for the FPLC system that is 10 times higher than the maximal pressure limit of the ultrafiltration membranes (4 bars). The ultrafiltration membrane YM-10 was

[1] *Abbreviations used:* His tag, histidine tail; FPLC, fast protein liquid chromatography; DLS, dynamic light scattering; GPC, *sn*-glycero-3-phosphocholine; TEM, transmission electron microscopy; AFM, atomic force microscopy; EPC, egg phosphatidyl choline; LR-PE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl); DOGS–NTA–Ni, 1,2-dioleoyl-*sn*-glycero-3-{[*N*(5-amino-1-carboxypentyl)iminodiacetic acid]succinyl} (nickel salt); 6-CF, 6-carboxyfluorescein; THF, tetrahydrofuran; PBS, sodium phosphate buffer; rGFP, recombinant green fluorescent protein; MBL, mannan binding lectin; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; UV, ultraviolet; UV/VIS, UV/visible; CSLM, confocal scanning laser microscopy; HPMC, hydroxypropylmethylcellulose; CMC, critical micelle concentration; 2D, two-dimensional; PDI, polydispersity index; Ni–NTA_3–DTDA, Ni-(nitrilotriacetic acid)-ditetradecylamine complex.