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ScienceDirect
International Journal of Pharmaceutics 342 (2007) 49–61
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Analysis of Quil A–phospholipid mixtures using drift spectroscopy

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Received 7 December 2006; received in revised form 24 April 2007; accepted 29 April 2007
Available online 6 May 2007

Abstract

The aim of this study was to investigate molecular interactions between Quil A and phosphatidylcholine in the solid state using diffuse reflectance infrared Fourier-transform spectroscopy (DRIFTS). Analysis of the interactions was characterized on the different regions of phosphatidylcholine: hydrophobic chain, interfacial and headgroup regions. The spectra of the hydrocarbon region of phosphatidylcholine alone compared to that for the binary mixture of Quil A and phosphatidylcholine were similar. These findings suggest that Quil A did not cause conformational disorder of the fatty acyl chains of the phospholipid. In contrast, a shift in the wavenumber of the choline group and a broad band in this moiety indicate a modification of the phospholipid in the headgroup region due to interaction between Quil A and phosphatidylcholine. These results suggest possibly ionic interactions between the negatively charged glucuronic acid moiety of the Quil A molecule with the positively charged choline group. The findings could also be the result of conformational changes in the choline group because of the intercalation of sugar moieties in Quil A between the choline and phosphate groups due to hydrogen bonding. Shift of wavenumbers to lower values on the carbonyl group was observed suggesting hydrogen bonding between Quil A and phosphatidylcholine. The difference in degrees of wavenumber shift (choline > phosphate > carbonyl group) and observed broad bands indicated that Quil A preferentially interacted with phosphatidylcholine on the hydrophilic headgroup. Cholesterol influenced such interactions at relatively high concentration (60%, w/w).

Keywords: Quil A; Phosphatidylcholine; Cholesterol; Infra-red spectroscopy

1. Introduction

Lipid-based systems such as liposomes and solid lipid particles or implants are of great interest in the drug delivery research as a means of providing sustained or targeted release of incorporated drugs (Singh and O'Hagan, 2002; Kersten and Crommelin, 2003; El-Aneed, 2004; Demana et al., 2005). In the last two decades, lipid systems containing Quil A have become an important class of delivery systems for protein and antigen delivery in the immunological field (Morein et al., 1984; Barr and Mitchell, 1996; Myschik et al., 2006a,b). The Quil A molecule belongs to a class of saponins that are isolated from the cortex of the South American tree *Quillaja saponaria* Molina (Dalsgaard, 1974; Höglund et al., 1989; Kensil, 1996). The chemical structure of Quil A is a complex mixture of many structurally related bisdesmosidic triterpenoid glycosides (Higuchi and Komori, 1987; Kensil et al., 1991). The different components of Quil A, however, differ mainly in the composition of their sugar moieties (Higuchi and Komori (1987)). Quillaja saponins have a five-ringed quillaic acid backbone with two carbohydrate chains, consisting of two to five sugar units, attached to the 3′ and 28′ carbons of the quillaic acid molecule (Higuchi and Komori, 1987; Ronnberg et al., 1997). Therefore, Quil A consists of hydrophobic aglycone triterpene and hydrophilic regions due to sugar moieties with multiple hydroxyl groups. This structure results in Quil A behaving as a surfactant forming micelles in water at a concentration of 0.03% (Özel et al., 1989).

Quillaja saponins are potent adjuvants that have been used in veterinary vaccines since the 1970's (Horzinek and Mussgay, 1971; Höglund et al., 1989; Barr and Mitchell, 1996). However,

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doi:10.1016/j.ijpharm.2007.04.030

this type of saponins is able to interact with cholesterol in biological membranes as a surfactant, with subsequent disruption of the bilayer and the formation of pores (Glauert et al., 1962). The resulting haemolysis limits the use of non-purified Quil A fractions in human medicine (Kensil, 1996; Barr et al., 1998). The toxicity of Quil A, however, is much reduced if it is bound in the colloidal particles such as immune-stimulating complexes (ISCOMs) or ring-like micelles because the appearance of free Quil A is largely avoided (Kersten, 1990; Sjölander et al., 1998; Morein and Bengtsson, 1999; Demana et al., 2004a). Colloidal particles such as ring-like micelles were found to be less toxic compared to ISCOMs despite the fact that the micelles contained relatively high amounts of Quil A (Demana et al., 2004a). Highly purified Quil A fractions with much reduced toxicity have been reported in the literature (Ronnberg et al., 1995) and human clinical trials on the vaccines containing Quil A fractions have now been conducted (Kersten and Crommelin, 2003; Sanders et al., 2005).

The complexation of Quil A with model and natural lipid membranes has been studied mainly by negative staining transmission electron microscopy (TEM) (Glauert et al., 1962; Özel et al., 1989; Kersten et al., 1991; Demana et al., 2004b; Myschik et al., 2006a,b). Recent studies have revealed that depending on the mass ratios of Quil A, cholesterol and phospholipid, different colloidal structures such as ISCOM matrices, lamellae (hexagonal array of ring-like micelles), ring-like micelles, worm-like micelles, and lipidic/layered structures are formed in aqueous media (Demana et al., 2004b). However, the optimum mass ratios of Quil A, cholesterol and phospholipid for the formation of these colloidal structures are dependent on the preparation method (Demana et al., 2004c; Lendemans et al., 2005a; Myschik et al., 2006a; Pham et al., 2006).

Apart from mainly TEM investigation into the interaction of Quil A with phospholipids, there are few other analytical techniques that have been used to characterize these systems (Myschik et al., 2006a,b). Moreover, there has been hardly any research done on the solid state characterization of Quil A–lipid systems. Attempts to investigate Quil A–lipid systems in the solid state have begun recently (Demana et al., 2005). Differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD) and scanning electron microscopy (SEM) were the analytical techniques used to characterize such systems (Demana et al., 2005). Despite these attempts, specific molecular interactions between Quil A and lipids used such as phosphatidylcholine however, could not be provided. Understanding molecular interactions of Quil A–lipid solid mixtures is very important because such mixtures can be used in the form of pellets or implants as antigen controlled or sustained delivery systems (Demana et al., 2005; Myschik et al., 2006b). It has been demonstrated that increasing amount of cholesterol in an ISCOM pellet resulted in a release of modified albumin in a controlled manner (Demana et al., 2005).

Despite this growing interest in the potential of solid ISCOMs or other related Quil A–lipid mixtures for use as controlled antigen delivery systems (Demana et al., 2005; Myschik et al., 2006b), there has been no work reported in the literature on the specific molecular interactions of Quil A with phospholipids especially in the solid state. Therefore, the aim of this study was to investigate molecular interactions between Quil A and phosphatidylcholine with or without cholesterol in the solid state using diffuse reflectance Fourier-infra red spectroscopy (DRIFTS). Diffuse reflectance sampling, although not without problems, seems to offer some advantages when compared to other solid-state sampling methods for infrared spectroscopy (Dijiba et al., 2005). DRIFTS is suited for analysis of this type of investigation as it does not rely on externally added probes that may influence membrane properties, and it gives information on different parts of the lipid molecule simultaneously (Taylor and Smith, 1980; Mantsch and McElhaney, 1991; Brandenburg et al., 1999; Popova and Hincha, 2003). We have therefore been able to investigate Quil A–phospholipid interactions at the levels of the carbonyl, the phosphate, and the choline groups, in addition to the fatty acyl chains.

2. Methods

2.1. Materials

Quil A was purchased from Superfos Biosector, Denmark. Cholesterol (purity approx. 99%) and L-α-phosphatidylcholine from egg yolk (purity approx. 99%) were purchased from Sigma Co., USA. Distilled deionised water having a conductivity of less than 0.1 μS (Milli-Q Water system, Millipore, Massachusetts, USA) was used throughout the study. All other chemicals and solvents were of at least analytical grade. The chemical structures of Quil A, cholesterol and phosphatidylcholine are shown in Fig. 1.

2.2. Preparation of lipid powder mixtures

Powders were prepared by either freeze-drying an aqueous dispersion of the lipids formed by hydrating a dried thin lipid film with an aqueous solution of Quil A or by physical mixing of lipid powders as described before (Demana et al., 2005). Table 1 summarizes compositions of the lipid powders investigated. Compositions were selected based on the pseudo-ternary phase diagram of aqueous mixtures of Quil A, cholesterol and phosphatidylcholine (Demana et al., 2004b). Based on the pseudo-ternary phase diagram, the selected mass ratios of lipids and corresponding formulations predominantly yield lipidic/layered structures, liposomes, ISCOMs, lamellae (hexagonal array of ring-like micelles) and worm-like micelles upon hydration (for formulations A–E, respectively) (Demana et al., 2004b). All formulations were prepared in triplicate.

Table 1
Composition of the lipid powder mixtures used in the study (w/w, %)

Formulation	Quil A	Cholesterol	Phosphatidylcholine
A	70	–	30
B	–	60	40
C	40	20	40
D	30	60	10
E	70	30	–