Journal of Molecular Structure: THEOCHEM 901 (2009) 174–185

Contents lists available at ScienceDirect

Journal of Molecular Structure: THEOCHEM

journal homepage: www.elsevier.com/locate/theochem

Molecular dynamics simulation of a mixed lipid emulsion model: Influence of the triglycerides on interfacial phospholipid organization

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ARTICLE INFO

Article history:
Received 11 September 2008
Received in revised form 19 January 2009
Accepted 20 January 2009
Available online 25 January 2009

Keywords:
Lipid emulsion
Soybean oil
Egg lecithin
Molecular dynamics
Parenteral nutrition

ABSTRACT

The lipid droplets, in total parenteral nutrition admixtures, are composed of triglycerides surrounded by a phospholipid monolayer. Physicochemical interactions between lipids and the other components are critical for the emulsion stability. To assess these interactions at the molecular level, we have constructed a model of a mixed phospholipid monolayer at the oil–water interface mimicking the lipid droplet surface. Physico-chemical properties of this model are computed by molecular dynamics simulations, and surface pressure–surface area compression isotherms at the air/water interface were recorded in order to compare experimental data to calculated area values. Both phospholipid and triglyceride phases are mixtures close to the composition of the commercial Ivelip® 20% lipid emulsion. In the studied model, the triglyceride phase is formed of 191 triglyceride molecules of four different formulas and each monolayer is composed of 100 molecules of five different zwitterionic or anionic phospholipids. Mixing of different phospholipids has a strong influence on the molecular area and modifies the hydrogen bonding network within the interface. Comparison of the mixed monolayer to a bilayer with the same phospholipid composition demonstrates that the triglycerides play an important role on the interfacial properties of the system. Phospholipid polar headgroups appear more hydrated, molecular area is larger, and lipid tails more disordered in the simulated lipid emulsion model than in the bilayer. The electrostatic potential, however, is the same. Taken together, these results demonstrate the utility of this mixed monolayer model at the oil–water interface instead of a hydrated bilayer model for studying lipid–cations interactions. These simulations are a starting point for further studies where the role of calcium will be analyzed.

1. Introduction

Total parenteral nutrition (TPN) emulsions are a vital treatment to supply some patients with glucose, lipids, amino acids, electrolytes, trace elements and vitamins. Lipids (triglycerides) are dispersed in the bulk aqueous phase in the form of oil droplets surrounded by a phospholipid monolayer [1], and excess phospholipids are organised in liposomal bilayers [2]. A small proportion of anionic phospholipids in the monolayer surrounding the oil droplet create a negative electrostatic surface potential, stabilising the emulsion [3]. Conversely, the addition of other nutriments, especially cations, has a destabilising effect as the anionic phospholipid/cation interaction neutralises the surface potential and leads to droplet coalescence [4]. An experimental approach using fluorescence probes has demonstrated that the destabilisation mechanisms take place in phospholipid monolayers at the surface of oil droplets [5]. However, experimental studies cannot resolve all the interactions mechanisms at the molecular scale, and molecular dynamics simulation has emerged as a useful tool to complement experimental works for a comprehensive understanding of structure and dynamics of various lipid systems [6,7]. Along with the advancement in computational power, simulations of pure or

Abbreviations: LLL, trilinoleoylglycerols; LLLn, dilinoleoyl-linolenoylglycerol; LLO, dilinoleoyl-oleoylglycerols; MD, molecular dynamics; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, phospholipid; PLL, palmitoyl-dilinoleoylglycerols; PS, phosphatidylserine; POPC, PalmitoylOleoylPhosphatidylCholine; POPE, PalmitoylOleoylPhosphatidylEthanolamine; POPS, PalmitoylOleoylPhosphatidylSerine; POPG, PalmitoylOleoylphosPhatidylGlycerol; POPI, PalmitoylOleoyPhosphatidylInositol; RDF, radial distribution function; TG, triglyceride; TPN, total parenteral nutrition.

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doi:10.1016/j.theochem.2009.01.020

mixed systems became realistic at long time scales (few nanoseconds) [8,9]. The great majority of the published studies focused on reproducing biological membrane properties, often including proteins, ionic channels or other molecules [10–15].

Moreover, the system studied by molecular dynamics is not a biological membrane but an artificial emulsion system analogue to those used in TPN. Once this model is validated, it will be used as a starting point to simulate the interactions between lipids and cations. We chose to model a phospholipid monolayer at a triglyceride–water interface, with a composition close to that of Ivelip® 20%, a commercial lipid emulsion commonly used in TPN.

The main constituents of Ivelip® 20% are egg lecithin and soybean oil. Sometimes egg lecithin and egg phosphatidylcholine (EPC) are used as synonyms in the literature. However, egg lecithin is not just solely composed of PC headgroups, but is a complex natural product containing zwitterionic (73% PC and 16% PE) and anionic phospholipids (0.6%), esterified mainly by saturated palmitoyl (C16:0) and unsaturated oleoyl (C18:1ω9) chains [4,16,17]. The other components are sphingomyelin, lysophosphatidylcholine and cholesterol, but they are in very low quantities. Soybean oil is a mixture of triglycerides with saturated and polyunsaturated long fatty chains.

This paper describes the design and equilibration of a mixed lipid emulsion model during a 100 ns MD simulation. The properties of this system are compared to those of a hydrated mixed bilayer, modelling liposomes, in the aim to understand how the triglycerides modify the phospholipids properties as already reported in previous experimental studies [18–20].

In order to experimentally assess the effect of the nature of the film-forming components on the molecular areas in mixed monolayers, dynamic compressions were performed at the air/water interface.

2. Methods

2.1. Simulated system

To avoid oil/water contact as an artefact of periodic boundary conditions, our system consists of 5 parts: Water/phospholipid monolayer/Triglyceride phase/phospholipid monolayer/Water (Fig. 1).

Each monolayer is generated as a mixture of 100 phospholipid molecules: 81 of palmitoyl-oleoyl phosphatidylcholine (POPC), 16 of palmitoyl-oleoyl ethanolamine (POPE), and one of each of the three anionic phospholipids palmitoyl-oleoyl serine (POPS), palmitoyl-oleoyl glycerol (POPG) and palmitoyl-oleoyl inositol (POPI), leading to a composition close to that of egg lecithin. A C language program was written to create a monolayer composed of several phospholipids with user-defined proportions at random positions. This routine has been interfaced with the GROMACS package [9,21–25]. After minimization of this monolayer, the second leaflet was obtained by rotating and translating the first one.

The triglycerides phase, intercalated between the two monolayers, is composed of four triglycerides representative of the soybean oil [26,27]: 47 molecules of trilinoleoylglycerol (LLL), 48 of dilinoleoyl-linolenoylglycerol (LLLn), 48 of dilinoleoyl-oleoylglycerol (LLO) and 48 of palmitoyl-dilinoleoylglycerol (PLL).

Initial molecule geometries were obtained with the Chem 3D® software. All subsequent simulations were carried out with the GROMACS 3.3.1 software package [22–25] on a dual CPU computer (2 Athlon at 2.8 GHz). The system was solvated with 8134 SPC (Simple Point Charge) water molecules [28] using genbox tool. Six water molecules were replaced by Na^+ to neutralize the two monolayers. Recently, different force fields and water models have been evaluated as for their performance on correctly modelling ion interactions, energies and environments. The conclusion was that the

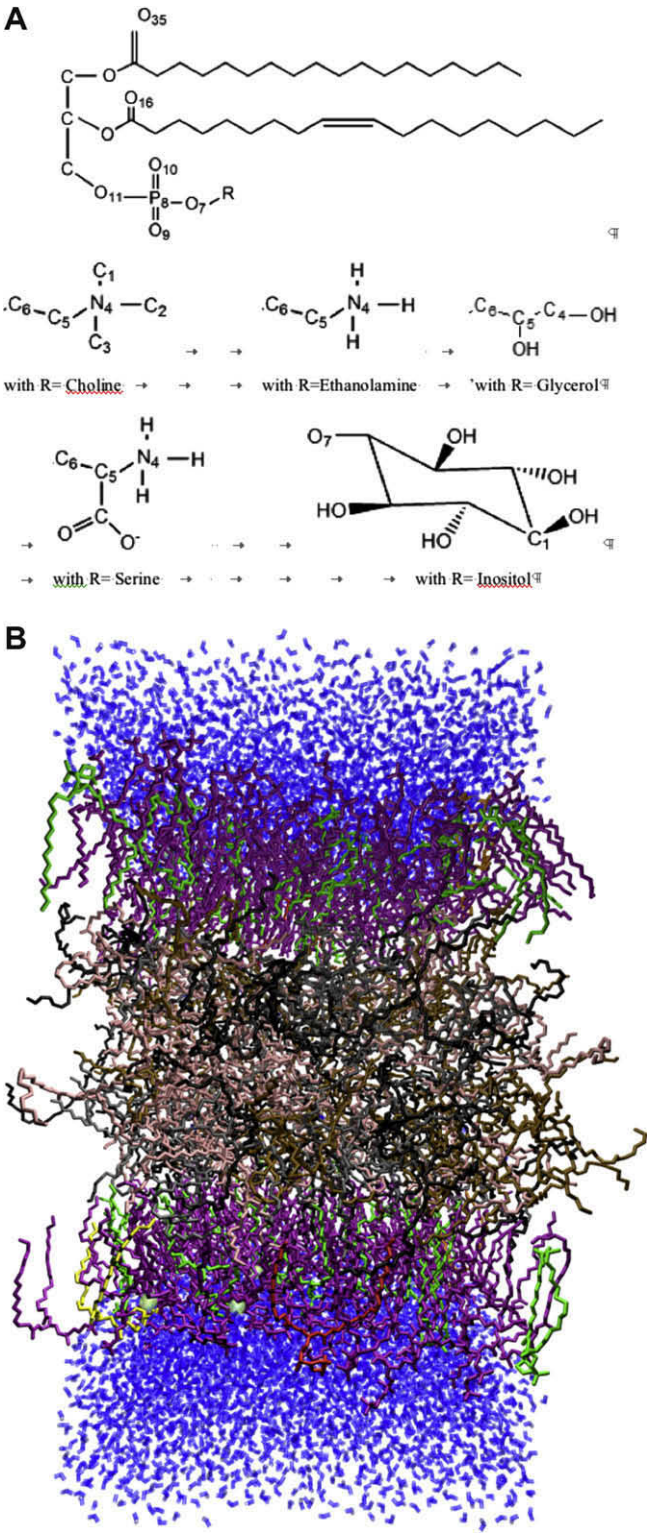

Fig. 1. Snapshot of the mixed emulsion model. Water (blue), Na^+ (lime spheres), POPC (purple), POPE (green), POPG (orange), POPS (red) and POPI (yellow), LLLn (black), LLL (pink), LLO (gray) and PLL (ochre).

structural and energetic properties for which well defined experimental values exist, are well predicted by all force fields, while uncertainties exist for ill determined properties. The main exception was the formation of ionic clusters which can be problematic at high ionic concentrations [29,30]. The absence of ion aggregation in our system, expected because of the very low ionic concentration, has