Food Research International 88 (2016) 70–78

Contents lists available at ScienceDirect

Food Research International

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

Biopolymer nanovehicles for essential polyunsaturated fatty acids: Structure–functionality relationships

Maria G. Semenova *, Anna S. Antipova, Darya V. Zelikina, Elena I. Martirosova, Irina G. Plashchina, Nadezda P. Palmina, Vladimir I. Binyukov, Natalia G. Bogdanova, Valerii V. Kasparov, Elena A. Shumilina, Natalia S. Ozerova

N. M. Emanuel Institute of Biochemical Physics of Russian Academy of Sciences, Kosygin str. 4, 119334 Moscow, Russian Federation

ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 29 April 2016
Accepted 8 May 2016
Available online 10 May 2016

Keywords:
Covalent conjugate
Encapsulation
Soy phospholipids
Essential fatty acids
Protection against oxidation
Water solubility

ABSTRACT

Design of stimuli-sensitive (i.e., smart) nano-sized delivery systems for nutraceuticals, having both a nutritional and pharmaceutical value, is very important for the formulation of novel functional food. Omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) are among the most needed nutraceuticals for the maintenance of good health. It is medically proven that in order to get the best effect on the human health the weight ratio of ω−6/ω−3 PUFAs should be within the range between 1/1 and 5/1. Thus, our work was focused on the molecular design of the delivery systems based on the nano-sized complexes formed between covalent conjugate (sodium caseinate + maltodextrin (a dextrose equivalent = 2)) and the combinations of polyunsaturated lipids, which are mutually complementary in the ω−6 and ω−3 PUFAs content: α-linolenic (ALA) + linoleic (LA) acids; liposomes of soy phosphatidylcholine (PC) + ALA; and micelles of soy lysophosphatidylcholine (LPC) + ALA. For such complex particles the high extent (>95%) of encapsulation of these all combinations of lipids by the conjugate was found along with both the high protection of the lipids against oxidation and their high solubility in an aqueous medium. To gain a better insight into such functionality of the complex particles a number of their structural (the weight-averaged molar weight, M_w; the radius of gyration, R_G; the hydrodynamic radius, R_h; the architecture; the volume; the density; the ζ-potential; the microviscosity of both the bilayers of PC liposomes and LPC micelles), and thermodynamic (the osmotic second virial coefficient, A_2, reflecting the nature and intensity of both the complex-complex and complex-solvent pair interactions) parameters were measured by a combination of such basic physico-chemical methods as static and dynamic multiangle laser light scattering, particle electrophoresis, atomic-force microscopy and electron spin resonance spectroscopy.

1. Introduction

There is considerable current interest in the design of delivery vehicles providing both protection and controlled release for a variety of nutraceuticals. Such delivery vehicles, containing nutraceuticals, could be used as health-promoting food ingredients in functional food formulation in particular (Arroyo-Maya & McClements, 2015; Dickinson, 2014; McClements, 2014; Semenova & Dickinson, 2010). It is common knowledge that polyunsaturated fatty acids (PUFAs: ω−3 (α-linolenic (ALA, 18:3), eicosapentaenoic (EPA, 20:5), docosahexaenoic (DHA, 22:6)) and ω−6 (linoleic (LA, 18:2), arachidonic (AA, 20:4))) are the important nutraceuticals in maintaining well-being. In particular, EPA, DHA, and AA are very significant in the optimization of the biological functions of brain, retina, heart, liver, and kidneys (Brenna, Salem, Sinclair, & Cunnane, 2009; Candela Gómez, Bermejo López, & Loria Kohen, 2011; Dietary Guidelines for Americans, 2010; Harnack, Andersen & Somoza, 2009; McClements, 2014; Michalski et al., 2013).

ALA and LA are essential fatty acids in the sense that they cannot be synthesized from precursors in the body and must be provided in a diet. ALA and LA are synthesized in plants and can be converted in the body to EPA/DHA and AA, respectively, requiring the successive enzymatic reactions of the desaturation and elongation (Candela Gómez et al., 2011; Harnack et al., 2009). The biotransformation of ALA to DHA depends strongly on the weight balance of LA to ALA in their daily intake and decreases significantly in the presence of an excessive amount of LA (Candela Gómez et al., 2011; Harnack et al., 2009; Brenna et al., 2009). Moreover, a very high ω−6/ω−3 weight ratio (15/1–16.7/1), as it is found in today's Western diets, promotes the pathogenesis of many diseases, including cardiovascular disease, cancer, inflammatory and autoimmune diseases, whereas the increased levels of ω−3 PUFA (the lower ω−6/ω−3 weight ratio, that is in the range 1/1–5/1) exert suppressive effects (Simopoulos, 2008; Candela Gómez et al., 2011). In addition, EPA, DHA and AA are also available directly from the diet. EPA and DHA are found in the lipids from marine sources like

* Corresponding author.
E-mail address: mariagersem@mail.ru (M.G. Semenova).

http://dx.doi.org/10.1016/j.foodres.2016.05.008
0963-9969/

oil of cold-water fishes or algae, while AA is found in plants, eggs or the dietary fats from grain-fed animals. A recommended daily intake of the PUFAs (from 20 mg up to 1–2 g of ω − 3 PUFAs per day) (Walker, Decker, & McClements, 2015) should be taken into account under the design of delivery vehicles for PUFAs as ingredients for functional food formulation.

In spite of the proven importance of the PUFAs in maintaining well-being, their use as health-promoting food ingredients runs into problems, which are mainly attributable to both their high susceptibility to an oxidative degradation (during the food product preparation, transport, and storage (McClements, 2014)) and their low solubility in water that hinders their addition into low fat food in particular. To tackle these difficulties we have tried to encapsulate the essential PUFAs (ALA and LA) as well as phospholipids containing these PUFAs (soy phosphatidylcholine (PC) and soy lysophosphatidylcholine (LPC)) by the complex formation with the nanoparticles of covalent conjugate (sodium caseinate (SC) + maltodextrin (MD-SA2)), which have the high extent of both encapsulation ability relative to different in nature molecules and solubility in an aqueous medium (Grigorovich et al., 2012; Markman & Livney, 2012; Semenova et al., 2014b, 2016; Semenova & Dickinson, 2010). In addition, in order to get a new possibility in an attainment of the increased level of ω − 3 PUFAs in the formulation of functional foods we have used the equimass ratio of ω − 6/ω − 3 PUFAs for such complex formation. In so doing to gain a better insight into the impact of the structure of polyunsaturated lipids on the structure and functionality of their complexes with the conjugate we have chosen three different combinations of the lipids: (i) ALA + LA, (ii) liposomes of PC enriched in LA + ALA, and (iii) micelles of LPC enriched in LA + ALA. The studied phospholipids (PC and LPC) are of interest for the formulation of health-promoting food ingredients not only as natural sources of the essential PUFAs but also due to their own well known biological activity as anti-aging agents and as superior protectants against liver damage (Kidd, 1996, 2000). Moreover, they are well known and promising carriers for both hydrophobic and hydrophilic bioactive molecules and drugs.

In order to characterize both the overall and local structure of the complexes formed we have used a combination of such basic physicochemical methods as static and dynamic multiangle laser light scattering, particle electrophoresis, atomic-force microscopy, and electron spin resonance spectroscopy. As one of the main functional properties of the complex particles we have considered their protective abilities against oxidation of the encapsulated lipids and their solubility in an aqueous medium.

2. Materials and methods

2.1. Materials

The sample of SC was purchased from Sigma (C8654, New Zealand) and used as received. The sample of Paselli MD-SA2 was kindly supplied by AVEBE (the Netherlands) and used as received. The phospholipids (PC, Lipoid S 100 (phospholipids [g/100 g]: phosphatidylcholine (by anhydrous weight) = 94; *N*-acyl-phosphatidylethanolamine = 0.5; phosphatidylethanolamine = 0.1; phosphatidylinositol = 0.1; lysophosphatidylcholine = 3.0. Nonpolar lipids [g/100 g]: triglycerides = 2.0; free fatty acids = 0.5; DL-a-tocopherol = 0.15 ÷ 0.25. Typical fatty acid composition in % to total fatty acids: palmitic acid = 12 ÷ 17; stearic acid = 2 ÷ 5; oleic acid = 11 ÷ 15; linoleic acid = 59 ÷ 70; linolenic acid = 3 ÷ 7) and LPC, Lipoid LPC 80 (phospholipids [g/100 g]: lysophosphatidylcholine = 80; phosphatidylcholine = 20 (typical fatty acid composition in % of total fatty acids: palmitic acid = 20 ÷ 27; stearic acid = 5 ÷ 8; oleic acid = 7 ÷ 9; linoleic acid = 48 ÷ 56; linolenic acid = 4 ÷ 6; arachidonic acid <0.2)) were kindly supplied by Lipoid, Germany. ALA (L 2376) and LA (L 1376) (>99% of purity) were purchased from Sigma. 16-Doxylstearic acid radical (810604P, 16-DSA, >99% of purity) was purchased from Avanti Polar Lipids. All other reagents (HCl, NaOH, ethanol, sodium azide, trichloroacetic acid, 2-thiobarbituric acid (TBA), Na_2HPO_4, and NaH_2PO_4) were analytical grade (≥99% of purity) and purchased from (Laverna, Russia). Reagents of SENTINEL DIAGNOSTICS (Italy) were used for the direct enzymatic-colorimetric determinations of the phospholipids. All solutions were prepared using a double-distilled water. Sodium azide (0.02% w/v) was added into all solutions prepared as antimicrobial agent.

2.2. Methods

2.2.1. Preparation of the complex (conjugate + lipids) particles

The covalent conjugate ((SC + MD-SA2), having the weight ratio SC:MD-SA2 = 1:2), was prepared and characterized as described previously (Semenova et al., 2016). It was dissolved in a phosphate buffer at pH 7.0 and ionic strength = 0.001 M.

The lipid solutions were prepared by the following method: the required amount of the lipids, having the equimass ω − 6/ω − 3 PUFAs ratio, was dissolved in pure ethanol and mixed with the buffered aqueous solution (a phosphate buffer, pH 7.0, ionic strength = 0.001 M). Ultimately, these solutions contained 50% v/v of ethanol. Thereafter these solutions were successively exposed to the mechanical homogenization at 22,000 r/min (Heidolph, Germany) for 2 min and the ultrasound sonication (VCX-130, Sonics & Materials, USA) in an ice bath (three times for 5 min (30 s - operation/30 s - rest). In the case of PC liposomes mixed with ALA, the prepared solutions were passed 19 times through the membrane filter (a pore size of which was equal to 100 nm), using the (AVANTI Polar Lipid extruder, USA).

The prepared lipid solutions were mixed with the conjugate solution (at the conjugate (1% w/v) to the total lipids (0.1% w/v) weight ratio = 10:1) in a shaker (GFL 3032, Germany) at 40 °C for 1 h that was followed by additional shaking at a model pasteurization temperature (60 °C) for 1 h.

In order to remove ethanol from the prepared solutions of both pure lipids (PC + ALA; LPC + ALA) and the complexes (conjugate (SC + MD-SA2) + either (ALA + LA) or (PC + ALA), or (LPC + ALA)) we have used the equilibrium dialysis (Visking Dialysis Tubing (Type 36/32), Serva; the ratio of the volumes of an outer vessel to an inner one equalled to 20, the duration time of the dialysis was 24 h) against a pure phosphate buffer (pH 7.0, ionic strength = 0.001 M). For an atomic force microscopy the prepared solutions were dialyzed against water (pH 7.0). Preliminary estimation showed no transmission of ALA-loaded both PC liposomes and LPC micelles through the dialyzing tubes. The residual amount of ethanol in the prepared solutions was equal to 0.1% v/v.

2.2.2. Estimation of the extent of encapsulation of the lipids by the conjugate particles

In the cases of both (ALA + LA) and (PC + ALA) combinations, the method of estimation of the extent of encapsulation of the lipids by the conjugate particles was based on the separation of free lipids from aqueous solutions of their complexes with the conjugate by the extraction with diethyl ether: an aliquot of 5 ml of the tested sample solutions was placed into a glass vial and then diethyl ether (3 ml) was added. The two-layer mixture was shaken and aged 24 h in a cool place (~7 °C). Thereafter, the organic layer was separated and the concentration of lipids in this extract was determined using SF-2000 (UKB Spectrum, Russia) spectrophotometer by measuring the optical density of the extract against a pure diethyl ether, as a blank, at a wavelength of 215 nm. The value of the measured optical density was used in order to calculate the concentration of free and encapsulated lipids in the tested sample. Prior to these experiments, calibration curves were constructed by plotting the optical density values for the diethyl ether extracts of the lipids from the buffered aqueous solutions (without the conjugate) against known concentrations of the lipids in these solutions. The results presented in this work are the average of at least of