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Molecular interactions governing the incorporation of cholecalciferol and retinyl-palmitate in mixed taurocholate-lipid micelles

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ABSTRACT

Cholecalciferol (D_3) and retinyl palmitate (RP) are the two main fat-soluble vitamins found in foods from animal origin. It is assumed that they are solubilized in mixed micelles prior to their uptake by intestinal cells, but only scarce data are available on the relative efficiency of this process and the molecular interactions that govern it. The extent of solubilization of D_3 and RP in micelles composed of lipids and sodium taurocholate (NaTC) was determined. Then, the molecular interactions between components were analyzed by surface tension and surface pressure measurements. The mixture of lipids and NaTC allowed formation of micelles with higher molecular order, and at lower concentrations than pure NaTC molecules. D_3 solubilization in the aqueous phase rich in mixed micelles was several times higher than that of RP. This was explained by interactions between NaTC or lipids and D_3 thermodynamically more favorable than with RP, and by D_3 self-association.

1. Introduction

Retinyl esters and cholecalciferol (D_3) (Fig. 1) are the two main fat-soluble vitamins found in foods of animal origin. There is a renewed interest in deciphering their absorption mechanisms because vitamin A and D deficiency is a public health concern in numerous countries, and it is thus of relevance to identify factors limiting their absorption to tackle this global issue. The fate of these vitamins in the human upper gastrointestinal tract during digestion is assumed to follow that of dietary lipids (Borel, Caillaud, & Cano, 2015). This includes emulsification, solubilization in mixed micelles, diffusion across the unstirred water layer and uptake by the enterocyte via passive diffusion or apical membrane proteins (Reboul & Borel, 2011). Briefly, following consumption of vitamin-rich food sources, the food matrix starts to undergo degradation in the acidic environment of the stomach, which contains several enzymes, leading to a partial release of these lipophilic molecules and to their transfer to the lipid phase of the meal. Upon reaching the duodenum, the food matrix is further degraded by pancreatic secretions, promoting additional release from the food matrix, and both vitamins then transfer from oil-in-water emulsions to mixed micelles (and possibly other structures, such as vesicles, although not demonstrated yet). As it is assumed that only free retinol can be taken up by enterocytes, retinyl esters are hydrolyzed by pancreatic enzymes, namely pancreatic lipase, pancreatic lipase-related protein 2 and cholesterol ester hydrolase (Desmarchelier et al., 2013). Bioaccessible vitamins are then taken up by enterocytes via simple passive diffusion or facilitated diffusion mediated by apical membrane proteins (Desmarchelier et al., 2017). The apical membrane protein(s) involved in retinol uptake by enterocytes is(are) yet to be identified but in the case of D_3, three proteins have been shown to facilitate its uptake: NPC1L1 (NPC1 like intracellular cholesterol transporter 1), SR-BI (scavenger receptor class B member 1) and CD36 (Cluster of differentiation 36) (Reboul & Borel, 2011). Both vitamins then transfer across the enterocyte towards the basolateral side. The transfer of vitamin A is mediated, at least partly, by the cellular retinol-binding protein, type II (CRBPII), while that of vitamin D is carried out by unknown mechanisms. Additionally, a fraction of retinol is re-esterified by several enzymes (Borel & Desmarchelier, 2017). Vitamin A and D are then incorporated in chylomicrons in the Golgi apparatus before secretion in the lymph.

The solubilization of vitamins A and D in mixed micelles, also called micellarization or micellization, is considered as a key step for their

Abbreviations: NaTC, sodium taurocholate; RP, retinyl palmitate; D_3, cholecalciferol; cmc, critical micelle concentration; cac, critical aggregation concentration; LDP, lipid digestion products; PC, phosphatidylcholine; Lyso-PC, L-α-lysophosphatidylcholine palmitoyl; POPC, 2-oleoyl-1-palmitoyl-*sn-glycero*-3-phosphocholine

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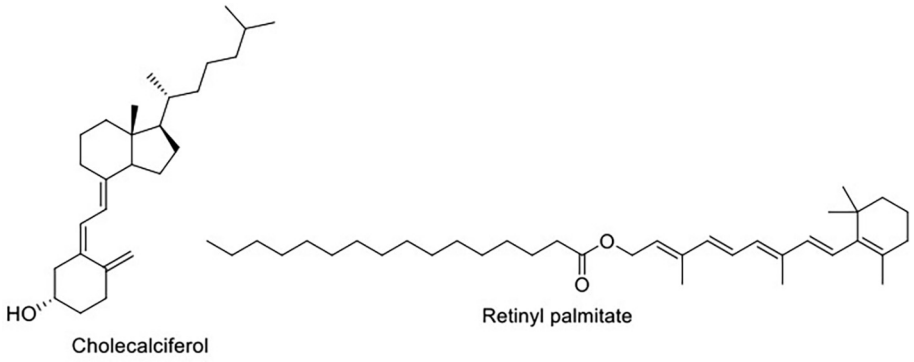

Fig. 1. Chemical structures for D_3 and RP.

bioavailability because it is assumed that the non-negligible fraction of fat-soluble vitamin that is not micellarized is not absorbed (Desmarchelier et al., 2013). Mixed micelles are mainly made of a mixture of bile salts, phospholipids and lysophospholipids, cholesterol, fatty acids and monoglycerides (Hernell, Staggers, & Carey, 1990). These compounds may form various self-assembled structures, e.g., spherical, cylindrical or disk-shaped micelles (Leng, Egelhaaf, & Cates, 2003; Walter, Vinson, Kaplun, & Talmon, 1991) or vesicles, depending on their concentration, the bile salt/phospholipid ratio (Walter et al., 1991), the phospholipid concentration, but also the ionic strength, pH and temperature of the aqueous medium (Cheng, Oh, Wang, Ragavan, & Tung, 2014; Madenci & Egelhaaf, 2010; Salentinig, Sagalowicz, & Glatter, 2010). Fat-soluble micronutrients display large variations with regards to their solubility in mixed micelles (Gleize, Nowicki, Daval, Koutnikova, & Borel, 2016; Sy et al., 2012) and several factors are assumed to account for these differences (Desmarchelier & Borel, 2017, for review).

The mixed micelle lipid composition has been shown to significantly affect vitamin absorption. For example, the substitution of lysophospholipids by phospholipids diminished the lymphatic absorption of vitamin E in rats (Koo & Noh, 2001). In rat perfused intestine, the addition of fatty acids of varying chain length and saturation degree, i.e. butyric, octanoic, oleic and linoleic acid, resulted in a decrease in the rate of D_3 absorption (Hollander, Muralidhara, & Zimmerman, 1978). The effect was more pronounced in the ileal part of the small intestine following the addition of oleic and linoleic acid. It was suggested that unlike short- and medium-chain fatty acids, which are not incorporated into micelles, long-chain fatty acids hinder vitamin D absorption by causing enlargement of micelle size, thereby slowing their diffusion towards the enterocyte. Moreover, the possibility that D_3 could form self-aggregates in water (Meredith, Bolt, & Rosenberg, 1984), although not clearly demonstrated, has led to question the need of mixed micelles for its solubilization in the aqueous environment of the intestinal tract lumen (Maislos & Shany, 1987; Rautureau & Rambaud, 1981).

This study was designed to compare the relative solubility of D_3 and RP in the aqueous phase rich in mixed micelles that exists in the upper intestinal lumen during digestion, and to dissect, by surface tension and surface pressure measurements, the molecular interactions existing between these vitamins and the mixed micelle components that explain the different solubility of D_3 and RP in mixed micelles.

2. Materials and methods

2.1. Chemicals

2-oleoyl-1-palmitoyl-*sn-glycero*-3-phosphocholine (POPC) (phosphatidylcholine, ≥99%; Mw 760.08 g/mol), 1-palmitoyl-*sn-glycero*-3-phosphocholine (Lyso-PC) (lysophosphatidylcholine, ≥99%; Mw 495.63 g/mol), free cholesterol (≥99%; Mw 386.65 g/mol), oleic acid (reagent grade, ≥99%; Mw 282.46 g/mol), 1-monooleoyl-*rac*-glycerol (monoolein, C18:1,-*cis*-9, Mw 356.54 g/mol), taurocholic acid sodium salt hydrate (NaTC) (≥95%; Mw 537.68 g/mol)), cholecalciferol (> 98%; Mw 384.64 g/mol; melting point 84.5 °C; solubility in water: 10^{-4}–10^{-5} mg/mL; logP 7.5) and retinyl palmitate (> 93.5%; Mw 524.86 g/mol; melting point 28.5 °C; logP 13.6) were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Chloroform and methanol (99% pure) were analytical grade reagents from Merck (Germany). Ethanol (99.9%), *n*-hexane, chloroform, acetonitrile, dichloromethane and methanol were HPLC grade reagents from Carlo Erba Reagent (Peypin, France). Ultrapure water was produced by a Milli-Q® Direct 8 Water Purification System (Millipore, Molsheim, France). Prior to all surface tension, and surface pressure experiments, all glassware was soaked for an hour in a freshly prepared hot TFD4 (Franklab, Guyancourt, France) detergent solution (15% v/v), and then thoroughly rinsed with ultrapure water. Physico-chemical properties of D_3 and RP were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

2.2. Micelle formation

The micellar mixture contained 0.3 mM monoolein, 0.5 mM oleic acid, 0.04 mM POPC, 0.1 mM cholesterol, 0.16 mM Lyso-PC, and 5 mM NaTC (Reboul et al., 2005). Total component concentration was thus 6.1 mM, with NaTC amounting to 82 mol%. Two vitamins were studied: crystalline D_3 and RP.

Mixed micelles were formed according to the protocol described by Desmarchelier et al. (2013). Lipid digestion products (LDP) (monoolein, oleic acid, POPC, cholesterol and Lyso-PC, total concentration 1.1 mM) dissolved in chloroform/methanol (2:1, v/v), and D_3 or RP dissolved in ethanol were transferred to a glass tube and the solvent mixture was carefully evaporated under nitrogen. The dried residue was dispersed in Tris buffer (Tris-HCl 1 mM, $CaCl_2$ 5 mM, NaCl 100 mM, pH 6.0) containing 5 mM taurocholate, and incubated at 37 °C for 30 min. The solution was then vigorously mixed by sonication at 25 W (Branson 250 W sonifier; Danbury, CT, U.S.A.) for 2 min, and incubated at 37 °C for 1 h. To determine the amount of vitamin solubilized in structures allowing their subsequent absorption by enterocytes (bioaccessible fraction), i.e. micelles and possibly small lipid vesicles, whose size is smaller than that of mucus pores (Cone, 2009), the solutions were filtered through cellulose ester membranes (0.22 µm) (Millipore), according to Tyssandier et al., 2003. The resulting optically clear solution was stored at −20 °C until vitamin extraction and HPLC analysis. D_3 and RP concentrations were measured by HPLC before and after filtration. For surface tension measurements and cryoTEM experiments, the mixed micelle systems were not filtered.

2.3. Self-micellarization of D_3

Molecular assemblies of D_3 were prepared in Tris buffer using the same protocol as for mixed micelles. D_3 was dissolved into the solvent mixture and after evaporation, the dry film was hydrated for 30 min at 37 °C with taurocholate-free buffer. The suspension was then sonicated.