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Review

Omega-3 polyunsaturated lipophenols, how and why?

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ABSTRACT

Polyphenols and n-3 polyunsaturated fatty acids (PUFAs) are two classes of natural compounds, which have been highlighted in epidemiological studies for their health benefits. The biological activities of those two families of metabolites on oxidation, inflammation, cancer, cardiovascular and degenerative diseases have been reported *in vitro* and *in vivo*. On the other hand, chemical bonding between the two structures leading to n-3 lipophenol derivatives (or phenolipids) has been studied in numerous works over the last decade, and some examples could also be found from natural sources. Interest in lipophilization of phenolic structures is various and depends on the domain of interest: in food industry, the development of lipidic antioxidants could be performed to protect lipidic food matrix from oxidation. Whereas, on pharmaceutical purpose, increasing the lipophilicity of polar phenolic drugs could be performed to improve their pharmacological profile. Moreover, combining both therapeutic aspects of n-3 PUFAs and of polyphenols in a single lipophenolic molecule could also be envisaged. An overview of the synthesis and of the natural sources of n-3 lipophenols is presented here, in addition to their biological activities which point out in several cases the benefit of the conjugated derivatives.

1. Introduction

Health benefits of polyunsaturated fatty acids of the omega-3 family have been largely reported in the last decades [1,2]. The most common ones are α-linolenic acid (C18:3, ALA), eicosapentaenoic acid (C20:5, EPA) and docosahexaenoic acid (C22:6, DHA). They are reported as “essential fatty acids”, seem to be involved in the reduction of inflammation and may help lower risk of chronic diseases such as heart disease, cancer, and arthritis. As they are highly concentrated in the brain, they are also implicated in cognitive and behavioral functions [3]. Several omega-3-polyunsaturated fatty acid (n-3 PUFA) conjugates have been developed in recent literature to improve the efficiency of active molecules. Among them, this review will focus especially on PUFA-Phenol conjugates, including *inter alia*, flavonoid, catechol, phloroglucinol derivatives, already known for their therapeutic applications (anti-oxidative, anti-inflammatory, anticancer activities …) [4], as well as natural omega-3 lipophenols.

The rational to design lipophenol derivatives depends either on the initial phenolic drug or on the targeted pathology: linkage of highly hydrophilic drugs to n-3 PUFA may help to increase lipophilicity, cell penetration and bioavailability of specific polar phenolic drugs. On the contrary, conjugation with PUFA would be interesting to reach appropriate solubility of hydrophobic drugs, by facilitating its binding to human serum albumin (HSA) [5,6]. In some cases, PUFA conjugates have been investigated to target specific tissues, either ones rich in n-3 PUFA such as retina or brain (high content in DHA and EPA) [3,7], or tumor tissues in which PUFA uptake is particularly high. On one side, since PUFAs are prime targets for oxidation (due to numerous bis-allylic positions) [8], linkage with antioxidant such as phenolic compounds would also help to limit auto-oxidation, to prevent the resulting harmful effects of lipid oxidation and to preserve health benefits of PUFA. On the other side, esterification of phenolic drugs by PUFA is a good way to mask their hydroxyl polar functions and thus, to reduce their biotransformation or the pace of oxidative degradation. In addition, conjugation with a PUFA part may contribute to increase antioxidant properties of phenolic compounds in lipophilic media. Depending on the pathology studied, synergism effect between the phenolic and the PUFA parts could be expected. In those cases PUFA would be able to enhance the efficacy of the phenolic drug, not only by the release of the drug but by the combined functionality of both

Abbreviation: ALA, α-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AR, 5-alkenylresorcinol; ACP, acylphloroglucinol; n-3 PUFA, omega-3-polyunsaturated fatty acid; PC, phosphatidylcholine.

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moieties of the conjugate (including their metabolites).

For all those reasons several research teams worked on the synthesis and the evaluation of a wide range of lipophenols, however, natural sources offer also the possibility to access lipophenol structures. As an example, several lipophenols bearing a n-3 PUFA part and a phloroglucinol moiety have been identified, mainly in vegetable sources [9]. Interest in natural n-3 lipophenols resides in their diverse biological activities.

As an illustration, we describe herein the main synthetic strategies to access n-3 lipophenols, the source and/or the total synthesis of natural n-3 lipophenolic derivatives, and we will discuss the current and potential therapeutic applications of the resulting compounds presented in the literature. Even though other n-3 PUFA conjugates bearing phenolic residues have been described, this review will focus specifically on bioactive compounds in which the phenolic or polyphenolic moiety plays a major role in the biological properties of the molecule.

2. The chemical or enzymatic pathway to access n-3 lipophenols

Introduction of PUFA on phenolic cores can be performed chemically, enzymatically, or chemo enzymatically, most often through esterification/acylation of the phenolic-OH with fatty acids. Fig. 1 presents phenolic molecules that have been transformed into lipophilic derivatives using n-3 PUFAs, with position of the reported acylation in red/pink color.

2.1. Enzymatic synthesis

The synthesis of n-3 PUFA-phenols depends on the structure of the phenolic part. Indeed, different types of bioactive phenols have been linked to ALA, EPA or DHA. Among them, are reported polyphenolic compounds belonging to the flavonoids family such as rutin **5**, phloridzin **6**, isoquercitrin **7**, naringin **4**, quercetin **3** or epigallocatechins **1**–**2** (Fig. 1). The enzymatic way, involving lipases, most usually novozyme 435 (commercially immobilized lipase from *Candica antarctica* – CALB), is always preferred to the chemical one to introduce PUFA on heterosidic flavonoids. Its high regioselectivity allows introduction of only one fatty acid (FA) residue on the sugar part, and mild reaction conditions avoid substrate alteration. The most studied flavonoid for this purpose was rutin (**5**).

Enzymatic esterification using CALB is usually performed in acetone [10–12], 2-methyl-2-butanol [13,14] or in a mixture of both solvents [15], which were selected for their abilities to solubilize both the reactant and the final lipophenol, providing excellent activity and stability of the lipase. The lipase allows the introduction of ALA, EPA or DHA specifically at the 4‴-hydroxyl group of the rhamnosyl moiety. Mbatia et *al.* [10] performed the reaction using a mixture of PUFAs enriched in ALA, EPA and DHA, in proportion phenol/FA 1/4 at 50 °C during 96 h. They reported 30% yield of the 3 lipophenols (**5**) without purification. Using similar protocol with pure ALA (phenol/FA 1/5, 50 °C, 96 h), Mellou et *al.* [11] observed up to 68% of esterification (measured by HPLC) while Viscupicova et *al.* [13,14] obtained around 30% of conversion using the same PUFA (phenol/FA:1/5, 60 °C, 168 h). Those works pointed out that the yields of enzymatic rutin esterification, inversely correlates the chain length of fatty acid (with C4–C12 fatty acids, yield >50%), probably due to steric hindrance/constraints in the active site of the CALB. It has also been reported that the presence of double bonds could negatively influence the lipase specificity to a large extent. However this was not clearly demonstrated with rutin.

More recently Zheng et *al.* [15], introduced ultrasound activation to link ALA to rutin (**5**) and naringin (**4**) in the presence of CALB (wrong structure of naringin is reported by the authors). Microtip probe ultrasonic pretreatment to esterification of flavonoids (frequency 25 KHz, power 150–200 W), allowed to use lower PUFA's equivalents and reduced reaction time (48–72 h instead of 72–96 h), compared to stirring experiments to reach up to 80% of conversion without damaging the lipase. Ziaullah et *al.* [12] were the only ones to perform phloridzin acylation at the 6″ position of the glucose, with each of the three PUFAs ALA, EPA and DHA, using the stirring process (acetone, 45°–50 °C, 12–24 h) and obtained respectively 94, 85 and 82% yields after purification of phloridzin-ALA, -EPA and -DHA **6**. The same work on isoquercitrin (**7**) led to the acylation of the 6″-OH position of the sugar moiety with a complete regioselectivity and 91, 81 and 81% yield, respectively. In addition to the presence of flame dried molecular sieves to remove any *in situ* generated water in the reaction mixture, they focused on the need to dry the enzyme over P_2O_5 for 20 h before the reaction and to maintain extra dry condition so as to limit hydrolysis and drag the reaction forward.

Lipase-catalyzed esterification and transesterification were used to produce lipophenol structures having a catechol part like vanillyl alcohol, dihydrocaffeic acid or hydroxytyrosol. Using the specificity of CALB to acylate primary hydroxyl groups compared to secondary hydroxyl ones, MBatia et *al.* [10] reported, as for rutin but with an increased yield (60%), the synthesis of a mixture of ALA, EPA and DHA vanillyl esters **10**, using CALB in acetone. Additionally, EPA and DHA were introduced in the olive oil hydroxytyrosol (**13**) and in analogue structures through transesterification, catalyzed by CALB under stirring and vacuum (5–10 mmHg, 37 °C, 4–16 h, 29–97%) [16]. In this work, important decreased yields were observed, going from EPA and DHA ethyl esters to saturated ethyl palmitate and stearate esters. Weak conversions were explained by the partial hydrolysis of the PUFA ethyl esters during the reaction. Finally, CALB was used to investigate the lipase transesterification reaction of dihydrocaffeic acid (DHCA) with flaxseed oil (rich in ALA and other C18 fatty acids glycerides), in order to obtain phenolic mono or diacyl glycerol enriched in ALA (**14**). The high specificity and stereoselectivity of the enzyme towards C18:1 n-9 and C18:2 n-6 for transesterification reaction, led to an increased proportion of ALA in the phenolic diacyl glycerol structure [17].

2.2. Chemical synthesis

The introduction of a PUFA moiety onto chemical structures having a single free phenolic function (juglone **21**, propofol **19**, podophyllotoxin derivatives **23**), or one amino or aliphatic-alcohol group, with higher nucleophilicity than the phenolic one (dopamine and analogues **12**, trimethoxy anilide **18**, farinosone C **20**, shikonin **22**), was performed either by the well-established esterification procedure using either the proper preformed mixed anhydrides [18,19] or acyl chlorides [20–22] of the PUFA, or a classical coupling reagent, such as, DCC/DMAP [23–27], pentafluorophenol/TEA [28], TBTU/TEA [29] or TCTU/TEA [30]. Whatever the used procedure and reagents, moderate to good yields were obtained.

For more complex polyphenolic structures like epigallocatechine-3-*O*-gallate (EGCG) **1** [31–33], epigallocatechin (EGC) **2** [34,35], quercetin **3** [36], phloroglucinol **15** or resveratrol **17** [37], two different strategies are used: with or without phenolic protection. On the one hand, uncontrolled acylation leading to the introduction of the FAs on several phenolic functions have been reported for quercetin and EGCG. Penta, tetra and tri-esters of quercetin (**3**) were obtained using acyl chloride of ALA [36]. Unsaturated acyl chlorides/phenols molar ratio modulation allowed Mainini et *al.* to obtain preferentially pentaesters (74%) when using phenolic/FA in a 1 to 10 ratio or a mixture of tetra and triesters (45 and 15%, respectively) at 1/4 ratio. Zhong et *al.* [31–33] synthesized preferentially EGCG-3′, 5′, 3″,5″-*O*- tetraesters of DHA and EPA **1**, using acyl chloride reagents.

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