Lipase-catalyzed synthesis of designer lipids with improved nutritional properties

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Abstract

Lipid designing using plant and microbial *sn*-1,3-specific triacylglycerol lipases has been employed for the preparation of structured lipids for functional foods and nutraceuticals. Human milk fat replacer containing palmitic acid, esterified at the *sn*-2 position of triacylglycerols and unsaturated fatty acids at the *sn*-1,3-positions have been prepared by transesterification of tripalmitin with fatty acids of rapeseed oil using a *sn*-3-regiospecific lipase as a biocatalyst present in an inexpensive crude papaya (*Carica papaya*) latex preparation. Structured triglycerides containing medium-chain fatty acids or ω3 and ω6 polyunsaturated fatty acids esterified at definite positions can be prepared using such a lipase from papaya latex, which may find good acceptance as compared to lipases from transgenic microorganisms.

Introduction

Designing of lipids using lipases, such as *sn*-1,3-specific triacylglycerol acylhydrolase (EC 3.1.1.3) as biocatalysts (Figure 1) has been employed for the preparation of structured lipids for use in functional foods and nutraceuticals (Mukherjee, 1998). Structured triglycerides containing palmitic (C16:0) acid, esterified predominantly at the *sn*-2-position and C18-unsaturated fatty acids at the *sn*-1,3-positions of triacylglycerols (Figure 2) are produced for use as human milk fat replacer by transesterification of tripalmitin with oleic acid or polyunsaturated fatty acids using *sn*-1,3-specific microbial lipases as biocatalysts (Kavanagh, 1997).

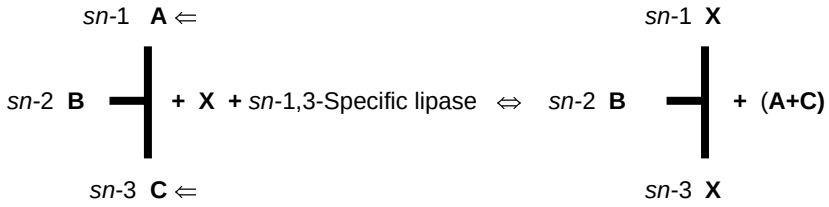

Figure 1: Interesterification of triacylglycerols using *sn*-1,3-specific triacylglycerol lipases: (A,B,C,X = Fatty acids / Acyl moieties).

Lipases from plants rather than those from transgenic microorganisms may find an easier acceptance as biocatalysts for the preparation of the above type of designer lipids for use in infant food formulations and nutraceuticals (Mukherjee, 1994). This communication reports the preparation of structured triacylglycerols resembling human milk fat by transesterification of tripalmitin with fatty acids of low-erucic rapeseed oil using lipase present in crude latex of papaya (*Carica papaya*) as biocatalyst; for comparison an immobilized microbial *sn*-1,3-specific lipase preparation (Lipozyme®) was studied.

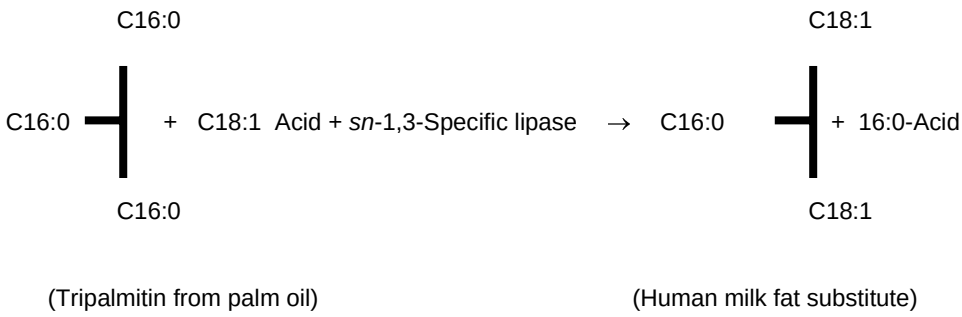

Figure 2: Interesterification of tripalmitin with oleic acid using *sn*-1,3-specific triacylglycerol lipase for the preparation of human milk fat substitute (Betapol).

Experimental

Tripalmitin (0.5 mmol) and rapeseed oil fatty acids (0.5 mmol) containing 10% palmitic (C16:0), 59% oleic (C18:1) and 20% linoleic (C18:2) together with 54 mg of either finely ground (<0.8 mm mesh) *Carica papaya* latex (Sigma) or immobilized lipase from *Rhizomucor miehei* (Lipozyme IM 20, Novo) were stirred under nitrogen at 60°C for various periods. The reaction products were centrifuged to separate the biocatalyst. The products were fractionated by thin-layer chromatography into fatty acids and triacylglycerols and subsequently analyzed by gas chromatography of their methyl esters (Mukherjee and Kiewitt, 1998). Aliquots of triacylglycerols were subjected to hydrolysis by porcine pancreatic lipase and the *sn*-2-acylglycerols formed were isolated by thin-layer chromatography, converted to methyl esters and analyzed by gas chromatography to determine the composition of the acyl moieties at the *sn*-2-position (Christie, 1980). Aliquots of triacylglycerols were also subjected to Grignard degradation and *sn*-1,3-diacylglycerols formed were isolated by thin-layer chromatography, converted to methyl esters and analyzed by gas chromatography to determine the composition of the acyl moieties at the *sn*-1,3-positions (Christie, 1980).

Results and discussion

Transesterification of tripalmitin with rapeseed oil fatty acids, catalyzed by papaya latex and Lipozyme, was found to result in incorporation of 10 to 25% C18:1 plus C18:2 acids into the triacylglycerols (Figure 3). Simultaneously, C16.0 moieties from tripalmitin were released as palmitic acid into the reaction products.

The proportion of C16:0 moieties at the *sn*-2-position of triacylglycerols was very slowly reduced during transesterification and little increase in the levels of C18:1 and C18:2 moieties occurred at this position. Thus, very little exchange of C18:1 and C18:2 acids against the C16:0 moieties at the *sn*-2-position of triacylglycerols occurred with both enzyme preparations which is in agreement with their known regioselectivity (Mukherjee, 1998; Foglia and Villeneuve, 1997; Villeneuve et al., 1995).

Transesterification led to reduction in the level of C16:0 and increase in the concentrations of C18:1 and C18:2 moieties at the *sn*-1,3-positions of the triacylglycerols at a much higher rate as compared to the changes in the concentration of these acyl moieties at the *sn*-2-position (Figure 4).

The data presented in Figures 3 and 4 show that transesterification of tripalmitin with fatty acids of low-erucic rapeseed oil using either papaya latex or Lipozyme as biocatalyst yielded triacylglycerols resembling those of human milk fat containing C16:0 moieties predominantly at the *sn*-2-position of the glycerol backbone and C18 unsaturated acyl moieties at the *sn*-1,3-positions (Figure 2).

Crude papaya latex containing papain is available commercially in bulk scale, it is inexpensive and is widely used in food and beverage industries. Papaya latex also exhibits lipolytic activity with a strong *sn*-3-selectivity (Villeneuve *et al*., 1995). The regiopreference of this enzyme has been utilized for the synthesis of structured triacylglycerols (Foglia and Villeneuve, 1997). Moreover, lipase in papaya latex exhibits a strong selectivity for specific unsaturated fatty acids, i.e. the ability to discriminate against fatty acids having a *cis* double bond at Δ^4 (*all-cis*-4,7,10,13,16,19-docosahexaenoic acid, DHA), Δ^6 (*all-cis*-6,9,12-octadecatrienoic acid, i.e. γ-linolenic acid, GLA and *all-cis*-6,9,12,15-octadecatetraenoic acid, i.e stearidonic acid) and Δ^8 (*all-cis*-8,11,14-eicosatrienoic acid, i.e. dihomo-γ-linolenic acid, DGLA) in esterification with *n*-butanol (Figure 5).

The data reported here show that crude papaya latex can be efficiently used as a biocatalyst for the preparation of designer lipids resembling human milk fat and possibly other structured triglycerides containing medium-chain or specific long-chain polyunsaturated fatty acyl moieties at definite positions of the glycerol backbone. For example fatty acids such as DHA and GLA with specific beneficial effects on health can be enriched from marine oils and borage oil, respectively, via kinetic resolution (selective hydrolysis or esterification) (Mukherjee, 1995) catalyzed by papaya latex. Subsequently, it should be possible to insert these fatty acids into the *sn*-1,3-positions of triacylglycerols by transesterification or esterification catalyzed by papaya latex to obtain designer lipids for use in functional foods.

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