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Cholesterol photo-oxidation: A chemical reaction network for kinetic modeling

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ABSTRACT

In this work we studied the effect of polyunsaturated fatty acids (PUFAs) methyl esters on cholesterol photo-induced oxidation. The oxidative routes were modeled with a chemical reaction network (CRN), which represents the first application of CRN to the oxidative degradation of a food-related lipid matrix. Docosahexaenoic acid (DHA, *T-I*), eicosapentaenoic acid (EPA, *T-II*) and a mixture of both (*T-III*) were added to cholesterol using hematoporphyrin as sensitizer, and were exposed to a fluorescent lamp for 48 h. High amounts of Type I cholesterol oxidation products (COPs) were recovered (epimers 7α- and 7β-OH, 7-keto and 25-OH), as well as 5β,6β-epoxy. Fitting the experimental data with the CRN allowed characterizing the associated kinetics. DHA and EPA exerted different effects on the oxidative process. DHA showed a protective effect toward 7-hydroxy derivatives, whereas EPA enhanced side-chain oxidation and 7β-OH kinetic rates. The mixture of PUFAs increased the kinetic rates several fold, particularly for 25-OH. With respect to the control, the formation of β-epoxy was reduced, suggesting potential inhibition in the presence of PUFAs.

1. Introduction

Cholesterol is an essential building block of lipids constituting mammalian cell membranes, forming a two-dimensional fluid matrix, in which membrane-associated proteins are able to carry out their various functions [1]. The chemical structure of cholesterol, specifically the presence of a double bond between C5–C6 carbons, makes it susceptible to oxidation processes [2,3], resulting in mono- or poly-oxygenated products called oxysterols or cholesterol oxidation products (COPs).

Oxygen concentration [4], light exposure [5–8], and high temperatures [3,9] are the main factors contributing to cholesterol oxidation. There are three basic mechanisms of sterol oxidation: auto-oxidation [10], photo-oxidation [5,7] and enzymatic oxidation [11,12]. Visible light (400–700 nm), together with an appropriate sensitizer and molecular oxygen, produce oxidative degradation of cholesterol through photodynamic reactions. Girotti [13] classified cholesterol photo-oxidation as either Type I or Type II reaction. Briefly, in the presence of oxygen, singlet oxygen ($^1O_2$) is generated (Type II photo-reactions). When a small amount of oxygen is present, the energy from light directly decomposes the sensitizers, and free radicals are formed (Type I photoreactions) [14] (Fig. 1).

The most common products of cholesterol oxidation are the epimeric 7-hydroxycholesterol (7α-OH and 7β-OH stereoisomers), epimeric 5,6-epoxycholesterol (5α,6α and 5β,6β stereoisomers), 7-ketocholesterol (7-keto), 25-hydroxycholesterol (25-OH) and the 5α-cholestane-3β,5α,6β-triol (3β,5α,6β-triol) [2,15]. The formation and occurrence of COPs have been studied in depth, because of their negative and controversial biological effects on human health [16]. Apoptosis, neurodegenerative diseases, decreased cholesterol efflux and the formation of crystalline domains in aortic muscle cells are among the pathologies related to high content of COPs in plasma [17].

Triacylglycerols have been suggested to induce the oxidation of free cholesterol at 25–180 °C [4,18–20]. Free fatty acids, on the other hand, not only increased the oxidation of cholesterol, but also changed the oxidative profile [19,21,22]. Eicosapentaenoic acid (20:5 ω-3, EPA) and docosahexaenoic acid (22:6 ω-3, DHA) are two polyunsaturated fatty acids (PUFAs) and essential nutrients for mammals; both have been associated with prevention of cardiovascular disease due to their ability to lower triacylglycerols

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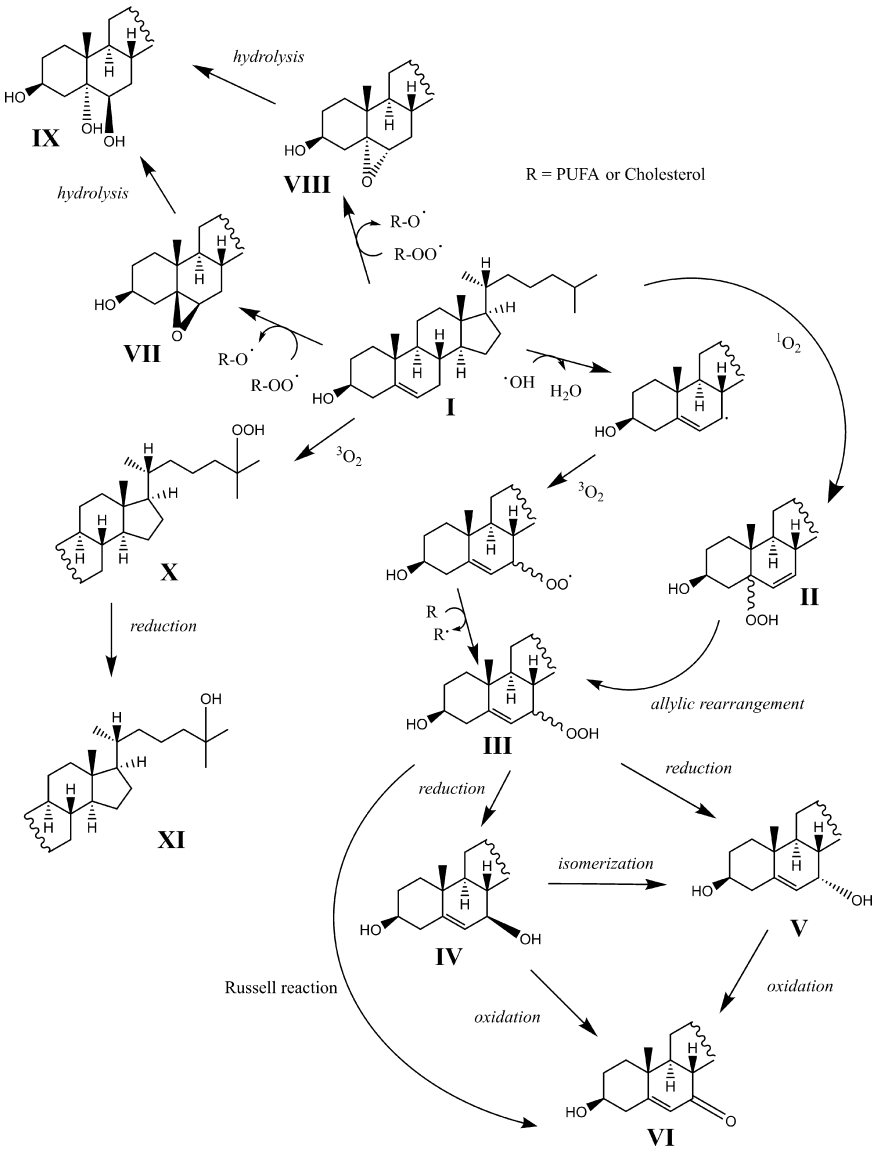

Fig. 1. Cholesterol free-radical mediated oxidative mechanism. I, Cholesterol; II, 5-hydroperoxycholesterol (α and β isomers); III, 7-hydroperoxycholesterol (α and β isomers); IV, 7α-hydroxycholesterol; V, 7β-hydroxycholesterol; VI, 7-ketocholesterol; VII, 5β,6β-epoxycholesterol; VIII, 5α,6α-epoxycholesterol; IX, 3β,5α,6β-cholestanetriol; X, 25-hydroperoxycholesterol; XI, 25-hydroxycholesterol.

and cholesterol [23,24]. However, lipid peroxy radicals generated from the oxidation of PUFA may react with cholesterol to form cholesterol peroxy radicals, which are precursors of several COPs [25] (Fig. 1).

Given the complexity of the oxidative process, there is a need to understand and predict the formation of oxidation compounds. As a matter of fact, attempts have been made in the past to model complex reactions in food-related systems, such as the Maillard reaction, as in the pioneering work of van Boekel's group [26], who extensively used the chemical reaction network (CRN) approach. For this multiresponse methodology, precursor, intermediates and final compounds are identified; then, a chemically consistent reaction network is built and described by a set of ordinary differential equations (ODEs). ODEs systems are numerically solved and experimental data are fitted, using either software packages or computational programming.

In the case of cholesterol oxidation, the kinetic analysis has been scarce or mathematically poor, as recently reviewed by the authors [2]. To the best of our knowledge, there are no reports of kinetic modeling of cholesterol oxidation using CNR; and this is one of the first applications of this methodology on food-related lipid systems. The present study aims to provide an understanding of the pro- and/or anti-oxidative effects of PUFAs methyl esters on the photo-induced oxidation of cholesterol. For this reason, we used three model systems containing cholesterol and EPA/DHA: peroxides content (POV), residual cholesterol and oxysterols formation were monitored for 48 h. A reaction network coupled with a numerical method was used to fit the time-dependent data and obtain kinetic rates.

2. Materials and methods

2.1. Reagents, solvents and standards

Chemicals of analytical grade or better were purchased from Sigma-Aldrich (St Louis, MO, USA) and J.T. Baker (Deventer, The Netherlands). Commercial standards of COPs supplied by Sigma-Aldrich were: cholesta-5-en-3β-ol (cholesterol), cholesta-5-en-3β-ol-7-one (7-ketocholesterol, 7-keto), 5α,6α-epoxycholestan-