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Biotransformation of S-13 thiaoleic acid to the corresponding sulfoxide by *Chlorella vulgaris* 211/8k

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Abstract

S-13 thiaoleic acid was transformed to the corresponding sulfoxide by *Chlorella vulgaris* (211/8k) and the likely intervention of the cytoplasmic oleoyl desaturase in this biotransformation was studied by the time-course incorporation and the transformation of the thiafatty acid into the complex lipids of the algae.

Keywords: Biotransformation; Algae; *Chlorella*; Thiafatty acid; Sulfoxide; Oxygenase

1. Introduction

Desaturases are enzymatic complexes playing an important part in animal and plant lipids metabolisms [1–4]. Particularly, the transformation of oleic acid to linoleic acid in plants is ensured by a membranous enzymatic system. The active enzyme named oleoyl desaturase is still very enigmatic, especially concerning the structure of its active site and its specificity towards the substrate [5,6].

In the course of our first studies [7,8], we explored the characteristics of this enzyme–substrate interaction using whole cells of *Chlorella vulgaris*, by exogenous supplying of modified acyl chains and by evaluating the effects of these synthetic analogues on the desaturation of exogenous labelled oleic acid. For instance, methyl 11 to 17-methyl-octadec-9-enoates [7], iso-vernolic and iso-linoleic acids [8] have been synthesized in order to study the influence of steric and/or electronic effects of the substituents on desaturation. We then chose to test thiaoleic acids, which could significantly interfere with the desaturation process by affinity of the sulfur atom with the non-heme iron of the active site. A very significant effect was observed when the substrate contains a sulfur atom instead of the methylene 13 [9]. Moreover, in this case, we isolated from the bulk, the corresponding sulfoxide with an excellent yield. In this paper, we report a study on the transformation of S-13-thiaoleic acid in the complex lipids

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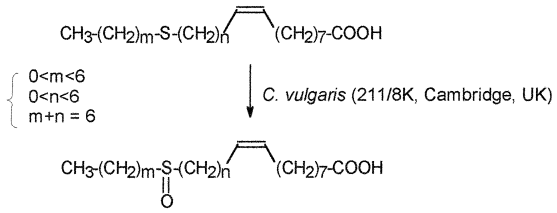

Fig. 1. Biotransformation of thiaoleic acids by *C. vulgaris*.

of *C. vulgaris* in order to investigate its likely interaction with the oleoyl desaturase.

2. Results and discussion

The 13-thiaoleic S-oxide acid from the bioconversion of S-13-thiaoleic acid by *C. vulgaris* was detected in the cellular fraction from GC/MS analysis and isolated from the bulk by semi-preparative HPLC. It was then fully characterized on the basis of ^{1}H NMR and MS data and compared with a synthetic standard. Moreover, it appeared that others, S-12, S-14, S-15 or S-16 thiaoleic acids were also converted to the corresponding sulfoxide in moderate to good yield by whole cells *C. vulgaris* 211/8k (Fig. 1). However, S-13-thiaoleic acid was the only one among the whole of thia analogues that dramatically decreased the oleic acid desaturase activity. We were then wondering about a likely specific interaction between S-13 oleic acid and the oleoyl desaturase. Actually, it was previously shown that the Δ9-desaturase of *Saccharomyces cerevisiae* could behave as a regio- and enantio-selective oxygenase towards S-9 and S-10 thiastearic acids [10,11]. It was then demonstrated by Buist et al. that the high enantioselectivity of the reaction conducted to the R stereoisomer sulfoxide.

Distinctly from these experiments on *S. cerevisiae*, our sulfoxides were trapped into the cells. Therefore, we followed the incorporation of S-13 oleic and SO-13 oleic acids into complex lipids of *C. vulgaris* (Fig. 2). According to the excellent percentages of sulfoxide recovery obtained among the cellular fraction or the soluble one, we could assess that sulfoxidation was the only kind of biotransformation in these assays. Moreover, the chemical process of lipids extraction and separation was alone responsible for the less effective (75–80%) percentage of sulfoxide recovery obtained after the compartmentation experiment (Fig. 2). The lipids separation on TLC indicated that S-13-thiaoleic acid readily incorporated into the cells, was mainly transformed into its CoA ester whereas 25% was oxidized and accumulated as free fatty acid after a 3-min incubation period. However, it should be pointed out that about 10% of S-13 oleic acid was detected into phospholipids (PL),

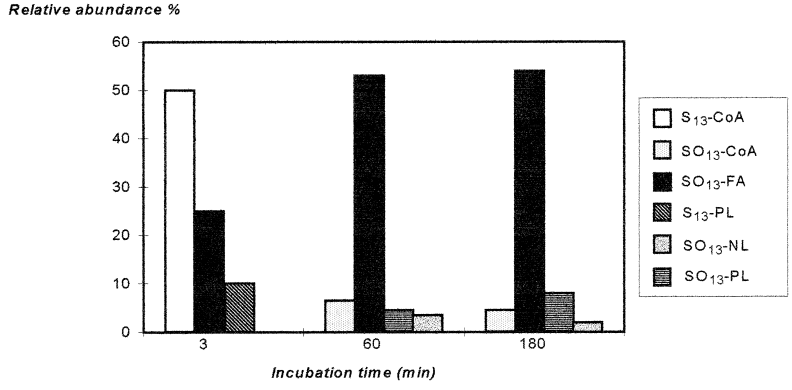

Fig. 2. Incorporation of the S13-oleic acid into complex lipids of *C. vulgaris* and distribution of the SO-13 oleic acid. FA, Fatty acid; NL, Neutral lipid; PL, Phospholipid.

especially phosphatidylcholine (PC), which is the endoplasmic C18:1 desaturation site. After 1 h incubation, the entirely oxidized thiafatty acid was mainly recovered as free fatty acid into the cells (53%) and to a less extent esterified as CoA ester (6.5%), as phospholipid (4.5%) or as neutral lipid (NL) (diacyl- and triacylglycerides) (3.5%). The S-13 and SO-13 oleic acids incorporation pattern was very similar after a 3-h incubation period.

It is still not clear whether the sulfoxide encountered into these different lipid classes was mainly transferred from CoA ester as S-13 or as SO-13 oleic acid. However, some of the thiafatty acid encountered in PL after a few minutes incubation period should have been transformed into the PL as sulfoxide and partly transferred elsewhere (10% S-13-PL at 3 min and 8% SO-13-PL at 3 h). Moreover, we previously showed an accumulation of endogenous C18:1 as well as a decrease of C18:2 in PL when S-13 oleic acid was supplied to *C. vulgaris* in standard conditions of incubation [12]. Both these experiments strongly suggested that the oleoyl desaturase could behave as an oxygenase in this oxidative pathway and to a small extent could be responsible for the transformation of S-13-oleic acid into the corresponding sulfoxide. Careful examination of the stereochemistry of this sulfoxide by the use of efficient NMR shift reagents [13] is still in progress and would assert or not our present statement.

3. Experimental

Cultivation of *C. vulgaris*, Δ12-desaturase activity assays as well as thiaoleic acids biotransformation assays were fully described in previous papers [9,12].

3.1. Lipids separation on TLC

After standard incubation and lipids extraction [12], the residue was resuspended in 300 μl of $CHCl_3$. The lipid solution was deposited on silica gel plates (Merck G60) which were previously activated at 60°C for an hour. Polar and neutral lipids were separated with a first development (two-thirds of the plate) in $CHCl_3$–Me_2CO–MeOH–CH_3COOH–H_2O (50:20:10:10:5) and a second development in petroleum ether–Et_2O–CH_3COOH (70:30:1). After detection with I_2 vapor, the lipids were cut off and transesterified by addition of 3 ml of the mixture MeOH–H_2SO_4 (2.5%). The reaction mixture was stirred for 2 h at 70°C. Fatty acid methyl esters were extracted and analyzed by capillary GC as previously described [12].

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