Incorporation of Positional Isomers of *cis*- and *trans*-Octadecenoic Acids into the Acyl Lipids of Cultured Soya Cells

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Cell suspension cultures of soya were incubated with *cis*-[1-^{14}C]octadecenoic acids and *trans*-[1-^{14}C]octadecenoic acids, each of known composition with regard to positional isomers. Each of the positional isomers ranging from Δ8- to Δ15-*cis*-octadecenoic acids and Δ7- to Δ16-*trans*-octadecenoic acids was readily incorporated into the acyl lipids of the cells, yet, the Δ9-*cis*- and the Δ9-*trans*-isomers were the preferred substrates. *cis-trans* Isomerization did not occur during the incorporation of the fatty acids into the acyl lipids.

Introduction

Oleic acid (*cis*-9-octadecenoic acid) as well as octadecenoic acids of unusual structure are known to be metabolized by animal organisms and cell organelles of animal tissues [1–3]. It is not known so far, whether unusual octadecenoic acids can also be metabolized by whole plants or isolated plant cells. The use of intact plants or parts thereof in the study of lipid metabolism is severely limited by their relative inability to accept added precursors, such as long-chain fatty acids [4]. Plant cell cultures, however, are known to incorporate exogenous substrates quite readily and, therefore, they appear to be more suitable for such biochemical studies [5, 6]. Recently, it has been shown that acetate as well as saturated and unsaturated fatty acids, that are synthesized by most plants, are rapidly incorporated into the acyl moieties of glycerolipids in cell suspension cultures of soya [7, 8]. Thus, oleic acid, added to the culture medium, is almost quantitatively incorporated into the glycerolipids of the cells [8]. We have studied the incorporation of oleic acid and its various positional and geometrical isomers by soya cells in culture, grown heterotrophically. It was our aim to elucidate the substrate specificity of enzymes involved in the acyl transfer of exogenous fatty acids, especially of those that are neither synthesized by the intact soya plant nor by its cell cultures.

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Material and Methods

cis-[1-^{14}C]Octadecenoic acids and *trans*-[1-^{14}C]-octadecenoic acids, each of known composition with regard to positional isomers, were prepared from carboxyl-labelled methyl linolenate by partial hydrogenation and subsequent fractionation by argentation thin-layer chromatography and preparative gas chromatography [9]. Suspension cultures of soya cells were grown heterotrophically [8]. The *cis*- and *trans*-[1-^{14}C]octadecenoic acids, 4 μCi (0.15 MBq)/4 μmol, each, dissolved in 200 μl ethyleneglycolmonomethylether, were added to 10 g portions of freshly harvested soya cells, suspended in 60 ml 0.1 M phosphate buffer, pH 7.3, and incubated by shaking for 3 h, as described elsewhere [8]. Thereafter, the cells were separated by centrifugation and the lipids extracted with isopropanol and chloroform according to an established procedure [10]. After evaporation of the solvents, the total lipids extracted were dissolved in chloroform and the unesterified fatty acids were neutralized with aqueous sodium carbonate. The aqueous phase containing sodium salts of the fatty acids was separated from the chloroform phase containing the total acyl lipids, which were found to contain ca. 47% and 41%, respectively, of the radioactivity applied in the form of *cis*- and *trans*-octadecenoic acids. The acyl lipids were converted to methyl esters by transesterification [11], and, subsequently, methyl *cis*- and *trans*-octadecenoates were recovered by argentation thin-layer chromatography [12]. The distribution of positional isomers in the methyl *cis*- and *trans*-octadecenoates derived from the two substrates and from the total methyl esters obtained from the corresponding acyl lipids, was determined by reductive ozonolysis [13] and radio gas chromatography of the resulting [1-^{14}C]aldesters [9]. The distribution of isomers of octadecenoic acids in the endogenous acyl lipids of cultured soya cells was determined similarly.

Results and Discussion

Table I gives the composition of the *cis*- and *trans*-octadecenoic acids, which had been used as

Table I. Percentage composition of *cis*- and *trans*-[1-^{14}C] octadecenoic acids added to cell suspension cultures of soya and of the corresponding octadecenoic acids incorporated into acyl lipids of the cells *.

Positional isomer (Δ)	*cis*-[1-^{14}C] octadecenoic acids added	*cis*-[1-^{14}C] octadecenoic acids incorporated into acyl lipids	*trans*-[1-^{14}C] octadecenoic acids added	*trans*-[1-^{14}C] octadecenoic acids incorporated into acyl lipids
7			2	<1
8	3	3	5	4
9	21	28	13	18
10	9	9	12	11
11	9	9	12	12
12	33	30	19	18
13	7	7	11	13
14	5	5	11	10
15	13	9	10	9
16			5	4

* Composition of octadecenoic acids in the endogenous acyl lipids of cultured soya cells: *cis:* 3% Δ8, 77% Δ9, <1% Δ10, 17% Δ11, <1% Δ12, <1% Δ13; *trans:* not detected.

substrates and the distribution of the positional isomers that were incorporated into the octadecenoyl moieties of the acyl lipids in the soya cells. The composition of the isomeric octadecenoic acids in the endogenous acyl lipids of the cells are also included in Table I. These results show clearly that each of the positional isomers ranging from Δ8- to Δ15-*cis*-octadecenoic acids and Δ7- to Δ16-*trans*-octadecenoic acids is readily incorporated into the acyl lipids of the cultured soya cells, yet, the naturally predominant Δ9-*cis*-isomer, *i. e.* oleic acid, and the Δ9-*trans*-isomer, *i. e.* elaidic acid, are the preferred substrates.

It should be noted that in the acyl lipids of the soya cells incubated with labelled *cis*-octadecenoic acids, only the positional isomers of labelled *cis*-octadecenoic acids were detected. Similarly, the cells incubated with radioactive *trans*-octadecenoic acids produced acyl lipids containing only positional isomers of radioactive *trans*-octadecenoic acids. It appears, therefore, that geometrical isomerization of these substrates does not occur during their incorporation into the acyl lipids of the cells.

Our results show that the heterotrophic soya cell culture is, with regard to its metabolic behavior towards natural and unusual exogenous fatty acids, similar to the animal cell systems studied so far. In this context, it should be of interest to know, whether photoautotrophic plant cell cultures [14] are different with regard to fatty acid metabolism from the heterotrophic culture we have studied.

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