In vitro Conversion of Saturated to Monounsaturated Fatty Acid by Ehrlich Ascites Cells

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ABSTRACT

In this paper, evidence is presented on the capacity of Ehrlich ascites cells to synthesize in vitro monounsaturated fatty acids from radioactive palmitate. Localization of the double bond was determined by ozonolysis and subsequent reduction of the ozonides to aldesters followed by gas liquid chromatography. These results proved that Ehrlich ascites cells have a Δ9 desaturase that catalyzes the biosynthesis of palmitoleic acid from palmitic acid and oleic and vaccenic acid via elongation-desaturation and desaturation-elongation, respectively, using palmitic acid as substrate. Furthermore, it is shown that, as in the hepatic cells, Δ9 desaturase enzyme activity of the tumoral cells is associated with the endoplasmic reticulum. The electron transport components involved in the desaturase system, i.e., NADH-cytochrome b_5 reductase and NADH-cytochrome c reductase, were also measured. The activities of these enzymes do not appear to be rate-limiting in the desaturase activity of these tumoral cells.

INTRODUCTION

It is well established that there are significant alterations in the fatty acid composition of tumor membranes. The replacement of polyunsaturated fatty acids in cellular phospholipids from neoplastic tissues by monounsaturated fatty acids has been demonstrated (1-3).

Wood and Healy (4) had claimed that Ehrlich ascites cells are capable of elongating palmitoyl-CoA but are incapable of desaturating the stearoyl-CoA produced in vivo by these cells. Apparently, there is no discrepancy between lack of desaturation and high levels of octadecenoic fatty acids in Ehrlich ascites cells, because the fatty acids accumulated are derived by these cells from the ascites plasma (5).

Nevertheless, it is important to take into account that cultured cells preferentially use preformed fatty acids from the medium, but when they grow in a lipid-free medium, are capable of synthesizing unsaturated fatty acids from simple precursors (6). These experiments were performed in order to prove the presence of a Δ9 desaturase system in the endoplasmic reticulum of Ehrlich ascites cells, including 2 enzymes from the microsomal electron transport chain associated with the desaturase activity: NADH-cytochrome b_5 reductase and NADH-cytochrome c reductase.

MATERIALS AND METHODS

[1-^{14}C] Palmitic acid (58 mci/mmol) was purchased from the Radiochemical Centre, Amersham, England. Pentex bovine albumin, fatty acid poor, was purchased from Miles Laboratories, Kankakee, IL.

Animals and Diet

Weanling Swiss mice were fed a Purina chow diet and water ad libitum before being used. Experiments were done with groups of 5 male Swiss mice, 12 g in weight. Animals were fed ad libitum a control diet as previously described (7) for 5 weeks prior to intraperitoneal (ip) implantation of Ehrlich ascites cells. The experimental diet was continued during tumor growth and the cells were harvested 8 days after implantation.

Cells

We used one hyperdiploid strain of the Ehrlich ascites cells carcinoma which shows the following characteristics: mean survival time 15 days and mean generation time 38 hr. Transplantation was achieved by ip injection of 0.2 ml of a sterile suspension in isotonic NaCl containing ca. 2.0×10^7 cells.

The mice were sacrificed by cervical fracture and the tumor cells were drained after injection of 1 ml of heparinized NaCl solution into the peritoneal cavity. The cells were separated by sedimentation at 4 C for 20 min at 400 × g. After washing and resedimentation 2 or 3 times, the cells were resuspended in a modified Hank solution (8). Cell counting was done with a hemocytometer. Cell integrity was estimated by 10% trypan blue exclusion. Viability was usually greater than 96%. Livers from host animals were separated, homogenized and the microsomes isolated by differential centrifuga-

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tion as previously described (9). The microsomal protein was estimated by the biuret method (10).

Assay for in vitro Desaturation in Host Liver Microsomes

[1-^{14}C] Palmitic acid was diluted to a specific activity (sp act) of about 1.7 mCi/mmol with the corresponding unlabeled pure fatty acid. The assay conditions were: 5 mg of microsomal protein was incubated in an open tube with 100 nmol of the diluted, labeled fatty acid in a Dubnoff shaker at 37 C for 30 min in a total vol of 1.5 ml of 0.15 M KCl; 0.25 M sucrose containing, in μmol: ATP, 2; CoA, 0.1; NADH, 1.2; $MgCl_2$, 7.5; glutathione, 2.2; NaF, 62; nicotinamide, 0.5 and phosphate buffer (pH 7.0) 62.

After incubation, the mixture was saponified and the extracted fatty acids esterified (11). The conversion of saturated to monounsaturated fatty acid was measured by thin layer chromatography (TLC) of the fatty acid methyl esters on $AgNO_3$-impregnated silica gel plates as described (12). The areas containing labeled methyl esters were scraped off and counted directly in vials in a Packard Scintillation Spectrometer. Other monounsaturated fractions were analyzed by gas liquid chromatography (GLC), collecting the $^{14}CO_2$ at the exit chimney of the detector.

Assay for in vitro Desaturation in Intact Ehrlich Ascites Cells

The incubation of intact Ehrlich ascites cells was performed in 25-ml siliconized Erlenmeyer flasks in a total vol of 4 ml of modified Hank solution (8), containing 1 μCi of ^{14}C palmitic acid/albumin solution (13) and ca. 5×10^7 cells. The Erlenmeyer flasks were incubated in a Dubnoff shaker at 37 C at ca. 100 oscillations/min for 1 hr. All incubations were in duplicate. At the end of the incubation period, the flasks were placed in ice and their contents transferred to centrifuge tubes and centrifuged for 5 min at 3,000 x g. The cellular pellets were washed 3 times and extracted by the method of Folch-Pi et al. (14). The nonreacted free fatty acids were eliminated from the lipid extract by Elovson's method (15). The conversion of saturated to monounsaturated fatty acids was measured as already described.

Preparation of Smooth Membranes

The microsomal fraction from Ehrlich ascites cells obtained from groups of 20 mice was purified from free ribosomes by gradient centrifugation according to Holtzman et al. (16). Smooth microsomes were incubated in vitro as described for liver microsomes.

Determination of Structure of Monounsaturated Fatty Acids via Reductive Ozonolysis

The ozonization was done on the monoethylenic fatty acid fraction separated by Ag-TLC with a microozonizator, Supelco, Inc., according to Beroza and Bierl (17). The radioactivity was measured in the $^{14}CO_2$ effluent from the gas chromatograph according to Blank et al. (18).

Assay for the Microsomal Transport Chain Enzymes

NADH-cytochrome b_5 reductase was assayed at 25 C by measuring the NADH-ferricyanide reductase activity of the enzyme according to Strittmatter (19). NADH-cytochrome c reductase was determined at 550 nm as described by Rogers and Strittmatter (20).

RESULTS AND DISCUSSION

Results from in vitro experiments have shown that intact Ehrlich ascites cells are capable of desaturating saturated fatty acids. A conversion was found of 4.2% ± 1.4 [^{14}C]-palmitic acid to radioactive monoethylenic fatty acids. The analyses by GLC of the monoene fatty acid fraction separated by Ag-TLC shows that only hexadecenoic acid, 16:1, and octadecenoic acid, 18:1, were radioactive.

Further analyses of the saturated fatty acid fraction show that 91.7% of the total radioactivity remains in the palmitic acid, whereas only 4.1% of the label was found in the stearic acid (Table 1), indicating that this acid was produced by chain elongation of its radioactive predecessor as described previously by Wood and Healy (4).

The position of the double bond in the labeled products of the monoene fraction, determined by ozonolysis and reduction of

TABLE 1

Radioactivity from [1-^{14}C] Palmitic Acid Incorporated into Saturated and Monounsaturated Fatty Acid from Total Lipids of Ehrlich Ascites Cells (%)

Fatty acid	Distribution of radioactivity (%)
16:0 (Palmitic acid)	91.7[a]
18:0 (Stearic acid)	4.1
16:1Δ9 (Palmitoleic acid)	1.9
18:1Δ9 (Oleic acid)	0.8
18:1Δ11 (Vaccenic acid)	1.5

[a]Values are means of 2 pooled samples of total lipids from Ehrlich ascites cells.

the ozonide, shows that the aldesters of C_9 and C_{11} were produced during the molecular cleavage (Fig. 1). According to the radioactivity incorporated into 16:1 and 18:1, we can deduce that 2 isomers, oleic acid (18:1Δ9) and vaccenic acid (18:1Δ11) were present in the 18:1 fraction, as shown in Table 1.

Apparently, under the conditions of these experiments, desaturation of 16:0 → 16:1Δ9 preceded chain elongation 16:1Δ9 → 18:1Δ11, and chain elongation 16:0 → 18:0 preceded chain desaturation 18:0 → 18:1Δ9. The route of vaccenic acid biosynthesis in these cells appears to be the same as that reported in rat liver by Holloway and Wakil (21) and in HTC cells (22) by Wiegand and Wood.

The presence of the enzyme responsible for the Δ9 desaturase activity found in intact Ehrlich ascites cells was also analyzed in the microsomal fraction of the tumoral cells.

The electron microscope observations of microsome preparations from Ehrlich ascites cells showed that this subcellular fraction was rich in free ribosomes and lacking in membrane structures. Because desaturase enzyme activity is associated in mammalian cells with these membrane structures (23), it was necessary to isolate them in order to assay the Δ9 desaturating system.

The presence of a Δ9 desaturase was confirmed in 2 preparations of smooth endoplasmic reticulum from Ehrlich ascites cells. These results indicated that the sp act of the enzyme was 0.04 nmol/min/mg protein in this subcellular fraction (Table 1), contrasting with the value of 0.12 ± 0.02 nmol/min/mg protein corresponding to the microsomal fraction from host liver.

It is well known that the microsomal desaturase activity is associated with the electron transport chain (21-25). It has been reported that microsomal electron transport may be impaired in some cancer cells (2,26). The values obtained from the assay of the enzymes involved in the electron flux show an activity of 0.063 μmol/min/mg protein for NADH-cytochrome c reductase and 0.44 μmol/min/mg protein for NADH-cytochrome b_5 reductase as shown in Table 2. Electron transport does not appear to be rate-limiting in the Δ9 desaturase reaction in Ehrlich ascites cells.

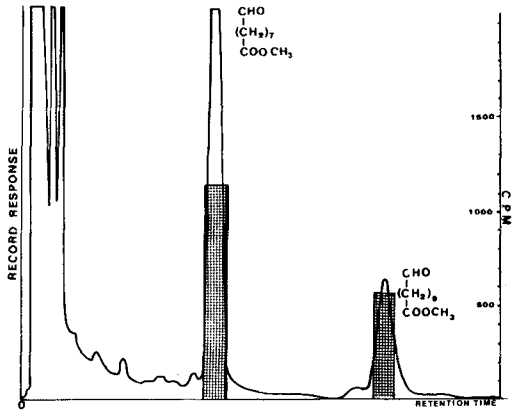

FIG. 1. Gas liquid chromatographic analysis and distribution of ^{14}C activity of the methyl esters of ozonolysis products of the methyl esters of monounsaturated fatty acids. Analyses were performed on glass column packed with 15% DEGS at 170 C.

In early experiments, Wood and Healy (4) studied the distribution of radioactivity in saturated and monoethylenic fatty acids incorporated into lipid fractions of Ehrlich ascites cells incubated in the peritoneal cavity of Swiss mice with labeled palmitoyl-CoA. We assume that 16:0-CoA is hydrolyzed to 16:0 before being taken up by the cells.

Despite these authors' conclusion that saturated fatty acids are not desaturated to the corresponding monoenoic acids, their results indicate that cells did exhibit a small amount of desaturase activity. Probably they arrived at this conclusion because, under the conditions of their in vivo experiments, it is not possible

TABLE 2

In vitro Activity of Fatty Acid Desaturase, NADH-Cytochrome b_5 Reductase and NADH-Cytochrome c Reductase from Smooth Endoplasmic Reticulum of Ehrlich Ascites Cells

Enzyme	Fatty acid desaturase	NADH-cytochrome b_5 reductase	NADH-cytochrome c reductase
	16:0 → 16:1 nmol/min/mg protein	μmol/min/mg protein	μmol/min/mg protein
	0.04[a]	0.44	0.06

[a]Values are means of 2 preparations of smooth endoplasmic reticulum from Ehrlich ascites cells.

to deduce to what extent the labeled palmitic acid is taken up by the host liver, elongated and desaturated, incorporated into lipoproteins and recirculated to the peritoneal cavity.

From the results of the present experiments, we can conclude that Ehrlich ascites cells have a Δ9 desaturase enzyme activity associated with the endoplasmic reticulum. The low capacity of these cells to desaturate palmitic acid to monounsaturated fatty acids could be the consequence of at least 2 contributing factors, i.e., the low content of endoplasmic reticulum in Ehrlich ascites cells associated with a poor Δ9 desaturase enzyme activity in this subcellular fraction.

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