COMMUNICATIONS

Desaturation of Isomeric *cis* 18:1 Acids

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ABSTRACT

The desaturation of positional *cis* 18:1 isomers (Δ4 through Δ11) was studied, using essential fatty acid deficient rat liver microsomes. The *cis* Δ4, Δ5, Δ6 and Δ7 isomers were not desaturated. The *cis* Δ10 and Δ11 isomers were desaturated at a very low rate. The maximum desaturation was obtained for Δ8 and Δ9 isomers. The *cis* Δ8 and Δ11 isomers were desaturated by Δ5 desaturase; the *cis* Δ9 isomer was desaturated by Δ6 desaturase; and the *cis* Δ10 isomer was desaturated to Δ7,10 and 5,10-18:2 acids.

INTRODUCTION

In the study of the effect of double bond position in fatty acids upon biochemical phenomena, each biochemical reaction or biological response has shown a unique pattern of response relative to the position of the double bonds (1-7). Each biological system was unique in discriminating between locations of *cis* double bonds, and in many cases the displacement of the double bond system one carbon atom along the chain caused drastic difference in acceptability of the fatty acid as substrate.

The hydrolysis by pancreatic lipase of a series of triglycerides each containing one positional isomer of *cis* 18:1 revealed that when the double bond was near the carboxyl group, pancreatic lipase was inhibited (7). The *cis* 18:1 isomers were incorporated at different rates in the cholesteryl ester (CE), triglyceride (TG) and phospholipid (PL) fractions by rat liver mitochondria, and at different positions of these TG and PL fractions according to their double bond position (8). The *cis* 6-18:1 acid was more extensively metabolized than the *cis* 9-18:1 acid in Novikoff hepatoma cells (9). We investigated the desaturation of the isomeric *cis* 18:1 acids by liver microsomes from rats deficient in essential fatty acids (EFA) to see how the position of the double bond affects the rate as well as the site of desaturation of these isomers.

MATERIALS AND METHODS

The labeled positional isomers of *cis* 18:1 acids were prepared in this laboratory. Some of the unlabeled isomers were obtained from Dr. F.D. Gunstone. The purity of the labeled and unlabeled isomers was checked by thin layer chromatography (TLC) and gas chromatography (GC). The purity of all the labeled and unlabeled acids was more than 90%. The double bond position was determined by ozonolysis of the methyl esters and reduction of the ozozonides as described by Privett and Nickell (10). The chain length of the labeled aldehyde ester, which indicates the position of the double bond, was determined by preparative GC using unlabeled carriers of aldehyde esters with chain lengths from C3 to C17.

The optimal concentrations of the substrate, microsomal protein and the time of incubation used for the desaturation of oleic acid were used as reported in a previous study (11).

Each substrate acid was incubated in a concentration of 120 nmoles per ml as Na salt-complex of bovine serum albumin. The substrate contained an amount of 1-^{14}C labeled acid equivalent to 0.1 μCi. Each incubation in 1 ml of a 0.15 M KCl-0.25 M sucrose solution contained in μmoles: ATP, 5; CoA, 0.25; NADH, 1.0; magnesium chloride, 5; glutathione, 1.5; NaF, 45: nicotinamide, 0.5; phosphate buffer (pH 7.0), 100; and 2 mg protein of a microsomal suspension. The microsomes were separated according to the procedure of Marcel et al. (12) by centrifugation at 105,000 x g for 2 hr. Microsomes from EFA deficient rats were used because desaturase activity has been shown to increase in EFA deficiency (12). After 20 min incubation in O_2 at 37 C, the reaction was stopped by adding 1 ml of 5% HCl in methanol, and the lipids were extracted with chloroform/methanol (2:1). The lipids of the extract were dried under nitrogen and transesterified with 10% HCl in methanol at 80 C for 2 hr. The extracts were evaporated to dryness under nitrogen, petroleum ether and unlabeled carriers of 18:0, 18:1 and 18:2 methyl esters were added, and the esters were

separated on 10% $AgNO_3$ TLC plates with petroleum ether (40-60 C)/diethyl ether (85:15, v/v). The quantitative distribution of the radioactivity between the substrate and product as well as the percent conversion and nmoles of product/mg protein/min were calculated as described before (13). The recovery of the radioactivity was more than 90%.

The structure of the dienoic acid esters isolated by TLC was identified by using preparative radio gas chromatography. All the labeled diene esters were found in the fraction corresponding to the 18:2 methyl ester peak. The double bond position was determined as described by Privett and Nickell (10), by partial reduction of the dienoic acids with hydrazine hydrate in methanol to give a mixture of monoenoic and saturated fatty acids. The extent of the reaction was monitored using gas chromatography. When the dienoic esters had just disappeared, the mixture was separated into saturated and monoenoic esters by $AgNO_3$ TLC, using the solvent system of light petroleum ether/diethyl ether (95:5, v/v). The isolated monoenes were ozonized, and the ozonides were reduced to aldehyde and aldehyde esters by Lindlar's catalyst (10). The chain length of the labeled aldehyde esters was identified by preparative GC using unlabeled carriers of aldehyde esters with chain lengths from C3 to C17.

RESULTS AND DISCUSSION

Table I shows that the *cis* 18:1 isomers with the double bond at carbons 4 through 7 were not desaturated at measurable rates by EFA deficient rat liver microsomes. Their percent conversion to 18:2 ranged from 0.48% for Δ4 to 0.76% for Δ6 18:1 acids. The maximum conversion to 18:2 was obtained for *cis* Δ8 and Δ9 isomers which gave 3.4 and 3.9%, respectively. The rate of desaturation of oleic acid to 6,9-octadecadienoate was 0.118 nmoles/min/mg protein, which is comparable to that obtained by Castuma et al. (14) (0.128 ± 0.002 nmoles/mg protein/min). The *cis* Δ10 and Δ11 isomers gave ca. 1.5% conversion to the 18:2 acid.

These results show that the *cis* 18:1 acids with double bond position between carbons 4 and 11 from the carboxyl group are poor substrates for the desaturation, except the *cis* Δ8 and Δ9 isomers were desaturated at relatively higher rates to the 18:2 acid. The *cis* Δ12 18:1 isomer was not desaturated by rat liver microsomes as reported by Gurr et al. (15).

The data in the present study show that the position of the double bond in a *cis* 18:1 acid is a determinant factor for the desaturase which acts on it. The *cis* Δ9 18:1 acid was desaturated by Δ6 desaturase, in agreement with the previous reports (16-18). The site of desaturation was similar for eicosa-9,12,15-trienoic, eicosa-9,12-dienoic (19), heptadeca-9,12-dienoic (20), and hexadeca-9-enoic (18) acids which have different chain lengths and number of double bonds. All of them have the first double bond at the 9-position from the carboxyl group. The *cis* Δ8 and Δ11-18:1 isomers were desaturated by Δ5 desaturase as are eicosa-8,11-dienoic (14), eicosa-8,11,14-trienoic (18), eicosa-11-enoic and eicosa-11,14-dienoic (21) acids, which also have the first double bond at the 8- or 11-positions from the carboxyl group. The desaturation of the *cis* Δ11 18:1 isomers to 5,11-18:2 acid (not to 8,11) confirms the absence of Δ8 desaturase from the rat liver microsomes as reported by Ullman and Sprecher (21).

The *cis* Δ10 18:1 isomers gave 1.57% conversion to the 18:2 acid with ca. 70% (1.1% conversion) of the double bonds at Δ7,10 and

TABLE I

Desaturation of *cis*-18:1 Isomers to 18:2 by EFA Deficient Rat Liver Microsomes Expressed as Percent Conversion and nmoles of 18:2 Produced/mg Protein/min. Values are Means ± SD, n=24

cis-Isomers	% Conversion	nmoles of 18:2/ min/mg protein	Position of double bond of 18:2	Desaturase
cis-Δ4-18:1	0.48 ± 0.47	0.014 ± 0.014		
Δ5	0.47 ± 0.9	0.014 ± 0.027		
Δ6	0.76 ± 0.72	0.023 ± 0.022		
Δ7	0.58 ± 0.32	0.017 ± 0.009		
Δ8	3.44 ± 0.69	0.103 ± 0.021	Δ5,Δ8	Δ5
Δ9	3.92 ± 0.32	0.118 ± 0.009	Δ6,Δ9	Δ6
Δ10	1.57 ± 0.34	0.047 ± 0.010	Δ5,Δ10 + Δ7,Δ10	Δ5 + Δ7
Δ11	1.50 ± 0.25	0.045 ± 0.007	Δ5,Δ11	Δ5

30% (0.47% conversion) as the Δ5,10 18:2 acid. Bernett and Sprecher (18) found that the eicosa-10,13-dienoic acid produced through the palmitoleate sequence was converted in vitro by rat liver microsomes to 7,10,13-20:3 at a very slow rate of only 0.8 nmoles in the 3 min incubation period. The rate of desaturation of *cis* Δ10 18:1 isomer to 7,10-18:2 was only ca. 0.05 nmole/min/mg protein which is lower than that reported for 10,13-20:2 acid (18).

The most abundant *cis* 18:1 isomers in the hydrogenated soybean oil were reported to be the *cis* Δ9 18:1 (76%), and *cis* Δ11 18:1 (10%) (22). The *cis* 18:1 isomers which were found in the present study not to be desaturated by rat liver microsomes (Δ4 → Δ7) were present in less than 1% of the total *cis* 18:1 acids (22). The major *cis*-18:1 acids present in hydrogenated oils are thus potentially metabolizable to *cis,cis*-18:2 acids which, in turn, may be converted to higher polyunsaturated acids and prostaglandin-like compounds.

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REFERENCES

1. Holman, R.T., P.O. Egwin, and W.W. Christie, J. Biol. Chem. 244:1149 (1969).
2. Reitz, R.C., W.E.M. Lands, W.W. Christie, and R.T. Holman, J. Biol. Chem. 243:2241 (1968).
3. Jenkin, H.M., L.E. Anderson, R.T. Holman, I.A. Ismail, and F.D. Gunstone, J. Bacteriol. 98:1026 (1969).
4. Jenkin, H.M., L.E. Anderson, R.T. Holman, I.A. Ismail, and F.D. Gunstone, Exp. Cell Res. 59:1 (1970).
5. Jensen, R.G., D.T. Gordon, W. Heimermann, and R.T. Holman, Lipids 7:738 (1972).
6. Chang, H.C., J. Janke, F. Pusch, and R.T. Holman, Biochim. Biophys. Acta 306:21 (1973).
7. Heimermann, W.H., R.T. Holman, D.T. Gordon, D.E. Kowalyshyn, and R.G. Jensen, Lipids 8:45 (1973).
8. Sgoutas, D., R. Jones, P. Pefanis, and F. Szlam, Biochim. Biophys. Acta 441:14 (1976).
9. Wennerstrom, D.E., and H.M. Jenkin, Biochim. Biophys. Acta 431:469 (1976).
10. Privett, O.S., and E.C. Nickell, Lipids 1:98 (1966).
11. Mahfouz, M., A.J. Valicenti, and R.T. Holman, Biochim. Biophys. Acta (In press).
12. Marcell Y.L., K. Christiansen, and R.T. Holman, Biochim. Biophys. Acta 164:25 (1968).
13. Hill, E.G., S. Johnson, and R.T. Holman, J. Nutr. 109:1759 (1979).
14. Castuma, J.C., A. Catala, and R.R. Brenner, J. Lipid Res. 13:783 (1972).
15. Gurr, M.I., M.P. Robinson, A.T. James, L.J. Morris, and D. Howling, Biochim. Biophys. Acta 280:415 (1972).
16. Mead, J.F., and W.H. Slaton, J. Biol. Chem. 219:705 (1956).
17. Mead, J.F., and D.R. Howton, J. Biol. Chem. 229:575 (1957).
18. Bernett, J.T., and H. Sprecher, Biochim. Biophys. Acta 398:354 (1975).
19. Castuma, J.C., R.R. Brenner, and W. Kunau, Adv. Exp. Med. Biol. 83:124 (1977).
20. Schlenk, H., D.M. Sand, and N. Sen, Biochim. Biophys. Acta 84:361 (1964).
21. Ullman, D., and H. Sprecher, Biochim. Biophys. Acta 248:186 (1971).
22. Reichwald-Hacker, I., K. Ilsemann, and K. Mukherjee, J. Nutr. 109:1051 (1979).

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