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IMMUNOCHEMICAL EVIDENCE FOR THE ENZYMATIC DIFFERENCE OF Δ^6-DESATURASE FROM Δ^9- AND Δ^5-DESATURASE IN RAT LIVER MICROSOMES

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SUMMARY: The enzymatic properties of the three types of microsomal acyl-CoA desaturases, Δ^6-, Δ^9- and Δ^5-desaturases, were immunologically compared using a monospecific antibody raised against the purified linoleoyl-CoA desaturase (Δ^6-desaturase). By the double immunodiffusion technique, the anti-Δ^6-desaturase antibody showed a single precipitin line to the purified Δ^6-desaturase and microsomes treated with Triton X-100, but no line was observed with the partially purified Δ^9-desaturase. The antibody even inhibited definitely Δ^6-desaturase activity in microsomes, but neither stearoyl-CoA (Δ^9-) nor eicosatrienoic acid (Δ^5-) desaturations were inhibited. By these immunological investigations it was confirmed that terminal Δ^6-desaturase is different enzyme from desaturases Δ^9- and Δ^5.

Rat liver microsomes catalyze at least three types of acyl-CoA desaturation such as (18:2, $\Delta^{9,12}$ → 18:3, $\Delta^{6,9,12}$)-, Δ^9(18:0 → 18:1, Δ^9)- and Δ^5(20:3, $\Delta^{8,11,14}$ → $\Delta^{5,8,11,14}$)-desaturation depending upon the position of the newly introduced double bond (1-3). These desaturations have been considered to be catalyzed by different enzymes judging from their various responses to dietary induction and circadian rhythm (4), and their different behaviour to detergents (5). Moreover, recent reports based on the purification and reconstitution of the desaturase system (6,7) support the above prediction. The present communication provides further evidence for the existence of different acyl-CoA desaturases by immunological investigation.

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MATERIALS AND METHODS

Chemicals - The following chemicals were obtained from commercial sources. [1-^{14}C]Linoleic acid (56 Ci/mol), [1-^{14}C] stearoyl-CoA(59.8 Ci/mol) and [1-^{14}C]eicosatrienoic acid ($\Delta^{8,11,14}$)(54.9 Ci/mol) from New England Nuclear Co.; CoA, ATP, NAD(P)H from Kyowa Hakko Co.; Bio-Beads SM_2 from Bio-Rad Lab. All other chemicals were of reagent grade.

Preparation of microsomes - Acyl-CoA desaturase was induced by refeeding fasted rats as follows (5,8,9). Male Wister strain rats weighing 100-120 g were fed ad libitum a fat-free diet for 21 days, starved for 24 hours and then refed the same diet for 24 hours prior to being killed by decapitation. Liver microsomes were prepared as described previously (10) and suspended in 0.1 M Tris-HCl buffer (pH 7.2) at a protein concentration of 15-25 mg/ml. They were stored at -70°C until use.

Preparation of antibody to the purified Δ^6-desaturase - The terminal Δ^6-desaturase was purified from rat liver microsomes as described previously (7) and dialyzed against 10 mM potassium phosphate buffer (pH 7.5) to remove KCl and glycerol. The concentration of Triton X-100 in the final enzyme preparation was lowered to about 0.01% by the procedure of Holloway using Bio-Beads SM_2 (11). The Δ^6-desaturase was concentrated to a protein concentration of 0.5 mg/ml, emulsified with an equal volume of complete Freunds adjuvant and injected into three rabbits as described by Prasad et al. (12). The γ-globulin fraction of the antiserum was obtained by ammonium sulfate fractionation followed by DEAE-cellulose column chromatography (13).

Enzyme assays - Microsomal Δ^6-desaturase activity was determined as described previously (14), except that 50 µM [1-^{14}C]linoleic acid was used with the addition of 100,000 x g supernatant (0.28 mg of protein). Δ^9-Desaturase activity was measured by the method of Jones et al. (15) except that 50 µM [1-^{14}C]stearoyl-CoA was used. Δ^5-Desaturation of eicosatrienoic acid (20:3, $\Delta^{8,11,14}$) to arachidonic acid (20:4, $\Delta^{5,8,11,14}$) was assayed as described by Pollard et al. (16) with the following modification. The incubation mixture contained 30 µM [1-^{14}C] eicosatrienoic acid, 3 mg of microsomal protein, 5 mM ATP, 0.2 mM CoASH, 0.75 mM NADH, 5 mM $MgCl_2$ and 0.1 M potassium phosphate buffer (pH 7.2) in a final volume of 0.2 ml.

One unit of desaturase activity was defined as 1 nmol of desaturated product/min.

NADH-cytochrome c reductase activity was measured as described previously (17).

Immunological inhibition experiments were carried out by preincubating anti-Δ^6-desaturase antibody with microsomes for 10 min at 25°C and then measuring the enzymic activities.

Other methods - Δ^9-Desaturase was partially purified up to the "Fraction 5" according to the method of Strittmatter et al. (6). Protein was determined by the method of Lowry et al. (18) using bovine serum albumin as a standard. Double immunodiffusion was carried out by the method of Ouchterlony (19).

RESULTS AND DISCUSSION

Double immunodiffusion test - As shown in Fig. 1, a single precipitin line formed without spurring between anti-Δ^6-desaturase antibody and the purified terminal Δ^6-desaturase as well as the

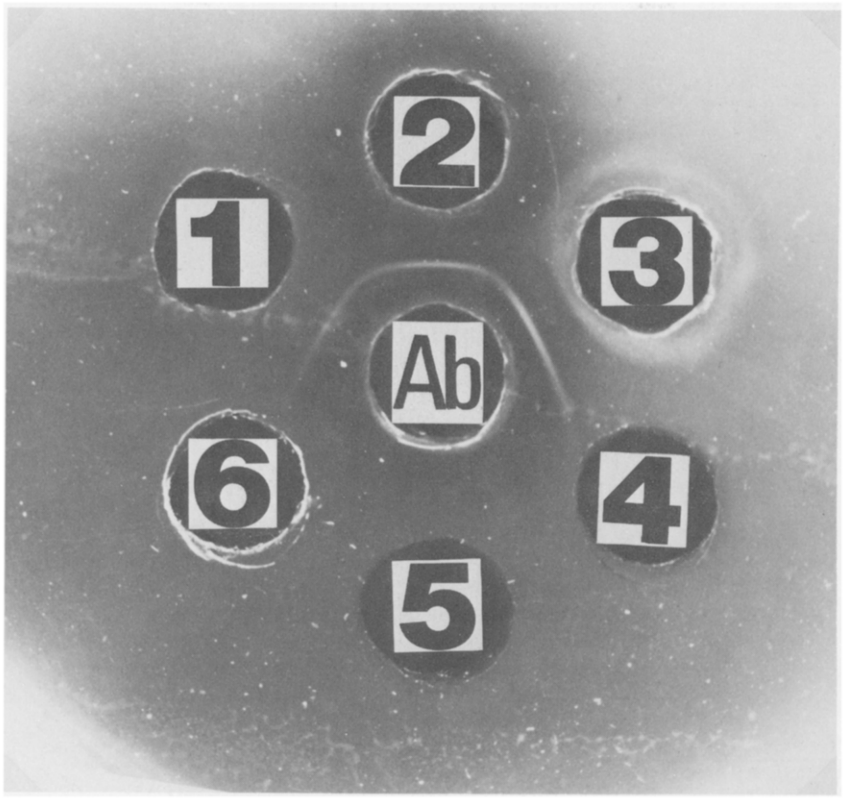

Fig. 1. Double immunodiffusion test. The center well contained anti-Δ^6-desaturase antibody; the contents of the outer one were as follows.
1. purified Δ^6-desaturase (0.70 unit as an enzymic activity)
2. partially purified Δ^6-desaturase (0.07 unit)
3. microsomes treated with 1% Triton X-100 (0.07 unit as a Δ^6-desaturase activity)
4. partially purified Δ^9-desaturase (0.70 unit)
5. partially purified Δ^9-desaturase (0.07 unit)
6. control γ-globulin

microsomes treated with 1% Triton X-100. On the other hand, the antibody did not make the precipitin line with the partially purified Δ^9-desaturase, indicating that the antibody recognizes only Δ^6-desaturase with antigenic specificity.

Effect of anti-Δ^6-desaturase antibody on microsomal Δ^6-, Δ^9- and Δ^5-desaturase activity - Following preincubation of anti-Δ^6-desaturase antibody with the microsomes, a significant decrease of Δ^6-desaturase activity was observed depending upon the amounts of antibody added (Fig. 2A). On the other hand, the antibody did not inhibit at all the microsomal NADH-cytochrome

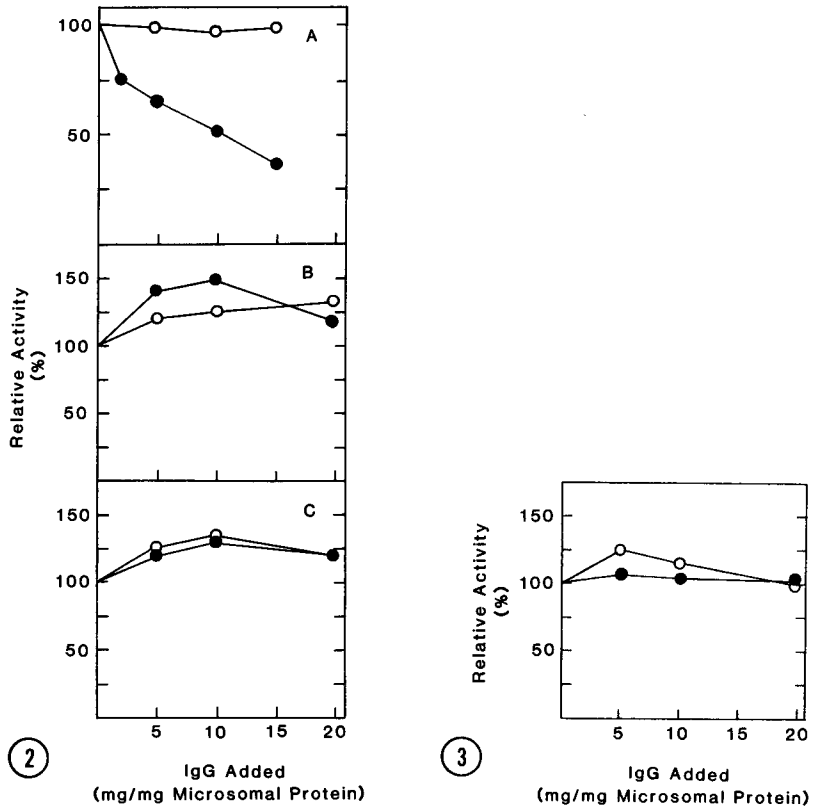

Fig. 2. Effect of anti-Δ^6-desaturase antibody on Δ^6-, Δ^9- and Δ^5-desaturase activity in microsomes. Enzymic activity was measured as described under Materials and Methods except that indicated amounts of anti-Δ^6-desaturase were added. The enzymic activity in the absence of the antibody was 0.35 (A , Δ^6), 1.54 (B,Δ^9) and 1.1 (C,Δ^5) unit/mg of microsomal protein respectively. (●): anti-Δ^6-desaturase antibody added; (O): non-immune antibody added.

Fig. 3. Effect of anti-Δ^6-desaturase antibody on microsomal NADH-cytochrome c reductase activity. NADH-cytochrome c reductase activity was measured as described in Materials and Methods except that indicated amounts of anti-Δ^6-desaturase antibody were added. The enzymic activity in the absence of the antibody was 0.20 μmol/min/mg of microsomal protein. (●): anti-Δ^6-desaturase antibody added; (O): non-immune antibody added.

c reductase (Fig. 3). These results suggest that anti-Δ^6-desaturase antibody recognizes antigenically only terminal desaturase, since NADH-cytochrome c reductase is known to catalyze an electron flow from NADH to cytochrome b_5 via cytochrome b_5 reductase in the microsomal fatty acid desaturase system (6,7).

The inhibition of enzymic activity of microsomal linoleoyl-CoA desaturase by a monospecific antibody supports the concepts that terminal Δ^6-desaturase is an extrinsic protein of the endoplasmic reticulum in which the immunogenic and catalytic sites are positioned possibly toward the cytosolic surface of the membrane.

Anti-Δ^6-desaturase antibody inhibited neither Δ^9- nor Δ^5-desaturation (Figs. 2 B, C). The antibody was rather stimulatory, to a degree of about 50%, in either immune or control γ-globulin, which might be due to a non-specific effect on the metabolic fate of fatty acids (20).

Thus the immunological differences among Δ^6-, Δ^9- and Δ^5-desaturases were demonstrated in either the double immunodiffusion or enzymatic inhibition test.

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