Affinity purification of alkylglycerol monooxygenase from rat liver microsomes by chimyl alcohol-Sepharose 4B column chromatography

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Summary Alkylglycerol monooxygenase of rat liver microsomes was purified approximately to 97-fold with a 30% yield by procedures including affinity chromatography on chimyl alcohol-Sepharose 4B. Chimyl alcohol (1-O-hexadecylglycerol) was converted to the *p*-aminobenzylidene derivative and then coupled to 6-carboxyhexyl-Sepharose. The final enzyme preparation was in nearly a homogeneous state, judging from the results of sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis, and it migrated to a position corresponding to an apparent molecular weight of 45,000. The results revealed that the native form of the enzyme (estimated to have a molecular weight of 400,000 as judged by Sepharose 6B column chromatography in a previous report, Ishibashi, T., and Y. Imai. 1983. *Eur. J. Biochem.* **132:** 23–27) will polymerize to large aggregates. **Ishibashi, T., and Y. Imai.** Affinity purification of alkylglycerol monooxygenase from rat liver microsomes by chimyl alcohol-Sepharose 4B column chromatography. *J. Lipid Res.* 1985. **26:** 393–395.

Supplementary key words liver microsomes • 1-O-hexadecylglycerol

In 1964 Tietz, Lindberg and Kennedy (1) demonstrated that rat liver microsomes contained a tetrahydropteridine-dependent hydroxylase that could split the ether bond present in alkylglycerols; they proposed a mechanism whereby the α-carbon of the O-alkyl moiety was hydroxylated to yield an unstable hemiacetal. The hemiacetal spontaneously breaks down to produce the first detectable products: glycerol and long-chain fatty aldehydes.

Neoplasm and brain cells, which are rich in ether-linked lipids, exhibit low or no ether cleavage activity (2). In contrast, rat liver, which is almost devoid of ether lipids, is a rich source of this enzyme. Soodsma, Piantadosi, and Snyder (3, 4) revealed that ammonium ions and reduced glutathione are required for maximum activity of the alkylglycerol monooxygenase. Recently we succeeded in solubilizing and partially characterizing the enzyme from rat liver microsomes (5). However, the final enzyme preparation was still not homogeneous. In this communication we report that affinity chromatography using Sepharose-immobilized substrate, prepared through the *p*-nitobenzylidene derivative of 1-O-hexadecylglycerol, rendered the enzyme into a homogeneous state.

METHODS AND RESULTS

The following chemicals were obtained from commercial sources: 1-O-hexadecylglycerol (chimyl alcohol) from

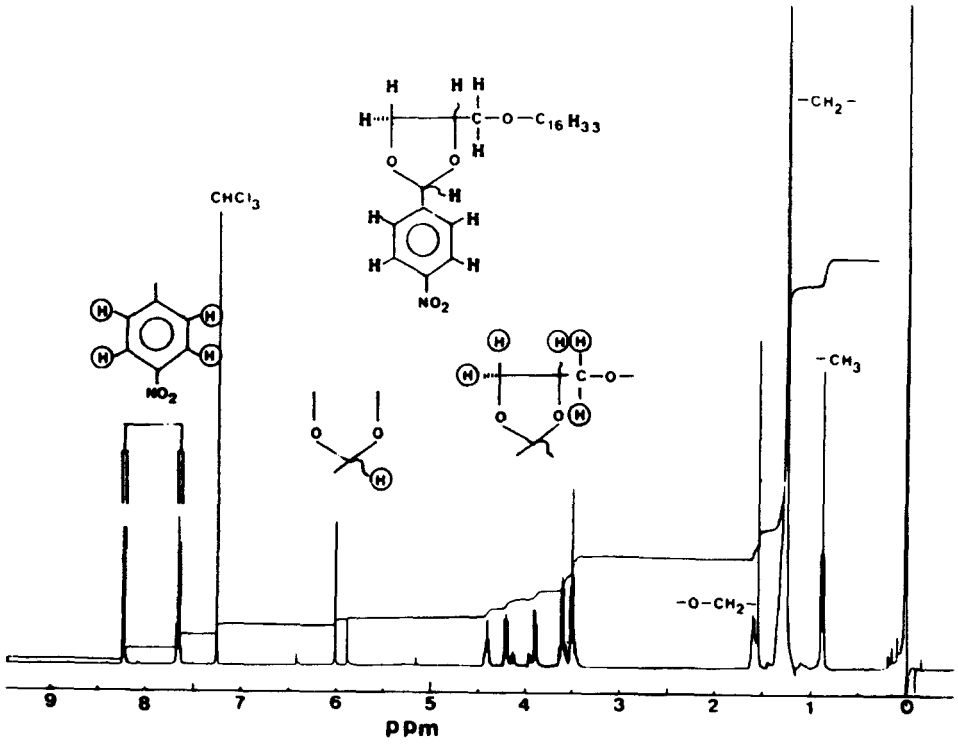

Fig. 1. Proton magnetic resonance spectroscopy of the *p*-nitrobenzylidene derivative of 1-O-hexadecylglycerol in $CDCl_3$ at 400 MHZ and 25°C.

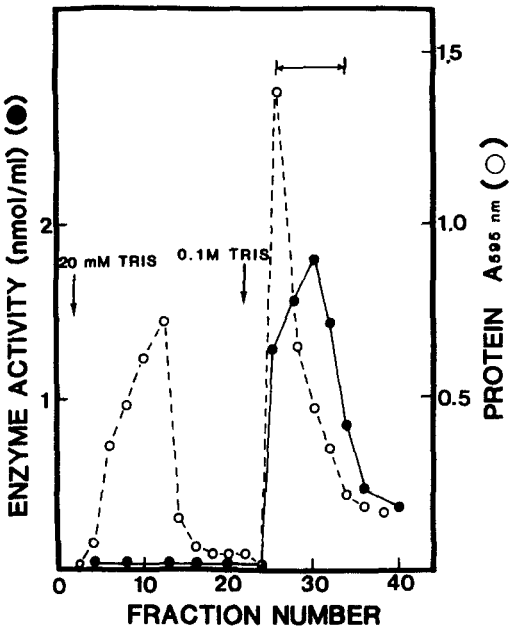

Fig. 2. Affinity chromatography on chimyl alcohol-Sepharose 4B column (1 × 2.5 cm). The partially purified alkylglycerol monooxygenase (fraction IV, 10.5 mg of protein) was applied on the column, and 1 ml of the fractions was collected at a flow rate of 10 ml/hr. Protein was measured in 10 μl of an aliquot using a Bio-Rad protein assay kit.

Sigma; 2-amino-6,7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropteridine hydrochloride (Pte. H_4) from Aldrich; reduced glutathione from Yamanouchi; Triton X-100; *p*-nitrobenzaldehyde, and Florisil (60–100 mesh) from Wako; paladium carbon and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride from Nakarai; DEAE-cellulose (DE52) from Whatman; 6-carboxyhexyl (CH)-Sepharose 4B from Pharmacia; pre-coated silica gel F_{254} plate from E. Merck; dye reagent for protein determination and molecular weight protein standards from Bio-Rad. 1-O-[1-^{14}C]Hexadecylglycerol (1.1 Ci/mol) and asolectin were kindly donated by Drs. H. Mangold and T. Takenawa, respectively. All of the other chemicals were of analytical grade.

The alkylglycerol monooxygenase activity was determined by Method I using 1-O-[1-^{14}C]hexadecylglycerol as a substrate, which was described in our previous report (5). Protein was measured by the method of Lowry et al. (6) or the Bio-Rad protein assay kit, using bovine serum albumin as a standard. Polyacrylamide disc gel (10%) electrophoresis in the presence of 0.1% sodium dodecyl sulfate was carried out according to the method of Weber and Osborn (7). Phospholipid vesicles were prepared as described previously (8), and phospholipid phosphorus was determined by the use of the Wako test kit (9).

1-O-Hexadecylglycerol was immobilized to CH-Sepharose 4B as follows. A mixture consisting of 1-O-hexadecylglycerol (700 mg), *p*-nitrobenzaldehyde (700 mg), benzene (500 ml), and $HClO_4$ (20 drops) was stirred at room temperature for 1 hr. The mixture was evaporated, dissolved in a minimum volume of $CHCl_3$, and then applied to a silicic acid column (2.6 × 55 cm), which was developed with $CHCl_3$. The *p*-nitrobenzylidene fractions were pooled, and reduced with H_2 gas in the presence of 5% paladium carbon. The reaction product, the *p*-aminobenzylidene derivative of 1-O-hexadecylglycerol, was purified by silicic acid column (1.8 × 50 cm) chromatography developed with chloroform–ethyl acetate 9:1 (v/v). The recovery was approximately 40–50%. The *p*-nitrobenzylidene derivative was identified as various stereoisomers by proton magnetic resonance spectroscopy (**Fig. 1**). The *p*-aminobenzylidene derivative was coupled to CH-Sepharose 4B by a carbodiimide method as described by Porath (10).

Rat liver microsomes and the partially purified alkylglycerol monooxygenase were prepared exactly according to the procedure outlined in our previous report (5). In the present study, the fraction IV noted in the preceding paper was used as the starting source for the enzyme purification. The partially purified alkylglycerol monooxygenase was applied to a chimyl alcohol-Sepharose column (**Fig. 2**). The enzyme effectively adsorbed on this affinity column and could be eluted by increasing the ionic strength. In typical purification procedures, the enzyme was purified approximately 97-fold with a 31% yield compared with microsomes (**Table 1**).

The final enzyme preparation was nearly homogeneous, judging from sodium dodecyl sulfate polyacrylamide disc gel electrophoresis (**Fig. 3**), and consisted of a single 45,000 dalton polypeptide chain (**Fig. 4**). In our previous

TABLE 1. Purification of alkylglycerol monooxygenase from rat liver microsomes

Fraction[a]	Volume	Protein	Specific Activity	Total Activity	Yield	Purification
	ml	*mg*	*units/mg*	*units*	*%*	*-fold*
I. Microsomes	11.2	369.6	10.6	3917.8	100	1
II. Triton X-100	47	223.3	16.2	3617.5	94	1.5
III. 6-Aminohexyl-Sepharose	18.8	94.9	51.8	4915.8	125.5	4.9
IV. DEAE-cellulose	5.8	10.5	170.9	1794.5	45.8	16.1
V. Chimyl alcohol-Sepharose	2.9	1.2	1025.4	1230.5	31.4	96.7

[a]Details of the purification procedures from fractions I to IV are described elsewhere (5).

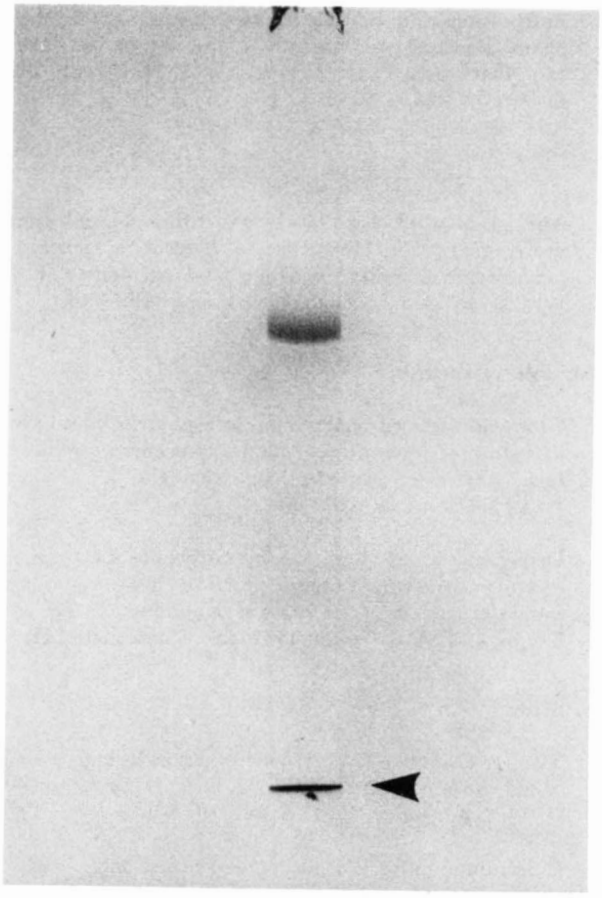

Fig. 3. Sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis of the purified enzyme. Electrophoresis of 30 μg of protein on 10% acrylamide gel was carried out as described in Methods. The arrow indicates the position of the tracking dye, bromphenol blue.

report, the native molecular weight of the enzyme was estimated to be 400,000 by Sepharose 6B column chromatography (5). The native form, therefore, was likely polymerized to a large aggregate due to the presence of Triton X-100 during column chromatography.

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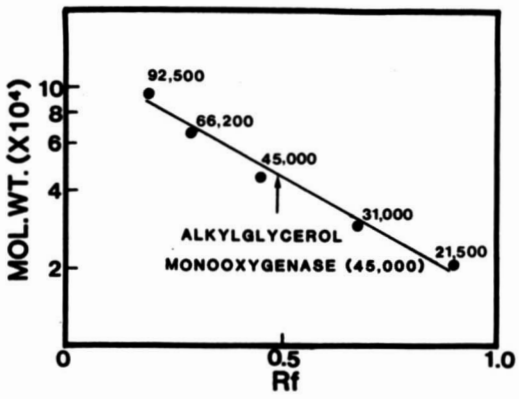

Fig. 4. Determination of the molecular weight of the purified alkylglycerol monooxygenase by sodium dodecyl sulfate disc gel electrophoresis. Electrophoretic conditions were the same as those for Fig. 2. Proteins of known subunit molecular weight used to calibrate in the gel system included lysozyme (21,500), carbonic anhydrase (31,000), ovalbumin (45,000), bovine serum albumin (66,200), and phosphorylase b (92,500).

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