SOLUBILIZATION OF LINOLEOYL-COENZYME A DESATURASE OF RAT LIVER MICROSOMES

Evidence of different responses of Δ^6- and Δ^9-desaturase to detergents

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1. Introduction

The biosynthesis of unsaturated fatty acids in the rat takes place in the endoplasmic reticulum via an aerobic mechanism. The conversion of linoleoyl-CoA to γ-linolenoyl-CoA takes place when a double bond is formed between carbon 6 and 7. The formation of this bond has been termed Δ^6-desaturation and is a principal regulatory step in the biosynthesis of polyunsaturated fatty acids. On the other hand, the conversion of stearoyl-CoA to oleoyl-CoA, also occurs when a new double bond is formed between carbon 9 and 10, and has been called Δ^9-desaturation. Either type of desaturation has been shown to be catalyzed by multienzyme components, which consisted of cytochrome b_5 reductase, cytochrome b_5 and desaturase, which are bound to microsomal membranes [1–3].

Several reports provide evidence that the formation of Δ^6-desaturase is different from that of Δ^9-desaturase. This has been shown in experiments of enzyme kinetics, dietary induction, circadian rhythm, hormonal stimulation [4] and by examination of hepatomas with different growth rates [5]. Stearoyl-CoA desaturase has been purified by Strittmatter et al. [1] and characterized by Enoch et al. [6], but few data have been available on the purification of linoleoyl-CoA desaturase. This report describes the solubilization of linoleoyl-CoA desaturase from rat liver microsomes and presents data to show that Δ^6- and Δ^9-desaturase respond differently to detergents.

2. Materials and methods

2.1. *Substrate and chemicals*

[1-^{14}C]stearoyl-CoA and [1-^{14}C]linoleic acid were provided by the Radio Chemical Center, England. Coenzyme A and NADH were purchased from Kyowa Hakko Co., Japan. [1-^{14}C]linoleyl-CoA (1 mCi/mmol) was synthesized from the free acid via the *N*-hydroxysuccinmide esters [7] by the method of Al-Arif and Blecher [8]. Its radiochemical purity was estimated to be greater than 95% using either paper chromatography with 1-butanol, acetic acid, water (5/2/3, v/v) as the solvent system, or gas–liquid radiochromatography. The chemical purity as CoA ester was determined from its absorption at 232 and 260 nm as previously described [9]. Sodium cholate was obtained from Sigma Chemical Co., USA. Sodium deoxycholate was obtained from Difco Laboratories, USA. Triton X-100 from Hokkaido Wako Co., Japan and Emalgen 913 was donated by the Kao-Atlas Co., Japan.

2.2. *Animals and preparation of microsomes*

Male Wistar strain rats which weighed 100 to 120 g were fed ad libitum a fat-free diet which consisted of 44% cornstarch, 25% milk-casein, 10% α-starch, 8% cellulose powder, 6% minerals, 5% sucrose and 2% vitamin mix (from Oriental Co., Japan) for 21 days, starved for 24 h, and then re-fed the same diet for 17 h prior to death by decapitation. Liver microsomes were prepared as described previously [10]. The

microsomal pellets were suspended in 0.1 M Tris–HCl buffer (pH 7.5) which contained 1 mM EDTA at a protein concentration of 20–30 mg/ml. They were stored at −70°C until use.

2.3. *Assay of desaturases*

The activity of stearoyl-CoA or linoleoyl-CoA desaturase was determined by measuring the formation of labeled oleic acid or γ-linolenic acid, respectively, as previously described [3,11]. The reaction mixture (0.5 ml) contained 25 nmol of [1-^{14}C]-stearoyl-CoA or linoleoyl-CoA, 0.5 μmol of NADH and 0.1–1.0 mg of protein of microsomes or solubilized preparations in 0.1 M Tris–HCl buffer (pH 7.2). When detergents were added, an appropriate volume of 5% (w/v) detergents was mixed with the reaction mixture to bring the final concentration of detergents to 0.01–1.0%. The reaction was run for 10 min at 30°C.

2.4. *Solubilization of desaturases*

To a suspension of rat liver microsomes (20 mg of protein/ml) in 0.1 M Tris–HCl buffer (pH 7.5) which contained 1 mM EDTA, the detergent (Triton X-100, Emalgen 913, sodium cholate or sodium deoxycholate) was added so that 20 mg of detergent was added to 20 mg of protein/ml. The mixture was stirred at 4°C for 20 min, and then was diluted with 20 mM Tris–HCl buffer (pH 7.5) so as to bring the concentration of the detergent to 0.5%. The mixture was then centrifuged at 105 000 × *g* for 60 min and then the activities of desaturases were measured in whole mixture or in the supernatant. The final concentrations of each detergent in the assay system were 0.01% or 0.05%, and that of protein were 0.1–0.2 mg/ml or 0.34 mg/ml, respectively.

3. Results

The effect of increasing concentration of detergents on linoleoyl-CoA desaturase activity is shown in fig.1. The specific activity in native microsomes was 0.18 nmol/min/mg of microsomal protein and was stimulated significantly in the presence of the ionic detergents. This was especially true at the lower detergent concentrations. Non-ionic detergents were less effective than the ionic detergents.

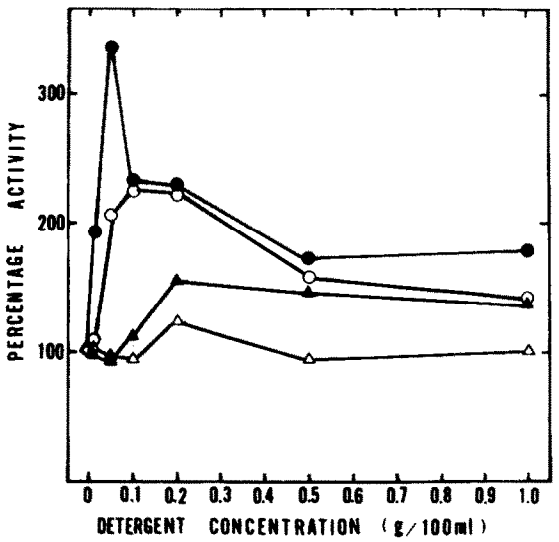

Fig.1. The effect of increasing concentrations of sodium deoxycholate (●), sodium cholate (○), Triton X-100 (▲), and Emalgen 913 (△) on the activity of linoleoyl-CoA desaturase. Incubations of microsomes were carried out as described in Materials and methods.

Table 1 shows the effect of detergents on the solubilization of Δ^6- and Δ^9-desaturase from microsomes. Treatment with Triton X-100 or sodium deoxycholate caused stimulation of the Δ^6-desaturase in the whole microsomal mixture and the activity was recovered in the supernatant. The specific activity in supernatant was 0.23 or 0.24 nmol/min/mg. Emalgen 913 seemed to be effective in the whole mixture but Δ^6-desaturase was scarcely solubilized. In contrast to Δ^6-desaturase, Δ^9-desaturase was poorly solubilized by any detergents.

4. Discussion

We have succeeded in the solubilization of Δ^6-desaturase from rat liver microsomes using Triton X-100 or sodium deoxycholate. Δ^9-desaturase was inactivated by the same solubilization procedures. Safford et al. [11] have reported that Δ^9-desaturase activity was 70–90% inhibited by the addition of detergents at low concentrations and that it could not be restored by either diluting out the detergent or by its removal with Sephadex G-25. In our study

Table 1
Solubilization and recoveries of Δ^9- and Δ^6-desaturase activity on treatment of rat liver microsomes with various detergents

Detergent	Microsomal protein solubilized (%)	Total desaturase activity			
		Δ^6		Δ^9	
		Whole mixture (%)	Recovery in supernatant (%)	Whole mixture (%)	Recovery in supernatant (%)
Triton X-100	83	169	101	0	0
Emalgen 913	84	92	12	0	0
Na cholate	85	92	79	7	16
Na deoxycholate	94	138	133	1	11

Mean values from 3 experiments

the detergents stimulated Δ^6-desaturase. Recently Pugh et al. partially purified eicosatrienoyl-CoA desaturase (Δ^5-desaturase) using procedures similar to those of Δ^9-desaturase [12]. The different responses of Δ^6- and Δ^9-desaturase to detergents has led us to postulate that the localization of Δ^6-desaturase in the microsomal membrane is quite different from that of Δ^9-desaturase. Δ^9-desaturase has 62% non-polar amino acids [1] and is believed to be rather deeply buried in the microsomal membranes [13]. The relative ease with which we solubilized Δ^6-desaturase suggest that the enzyme may have a location similar to cytochrome b_5. Cytochrome b_5 has been shown to consist of a hydrophilic part containing the active site and hydrophobic part which serves to anchor the enzyme to the surface of the endoplasmic reticulum.

The studies presented in this paper demonstrated that the different molecular localization in the microsomal membrane may be one possible reason to explain differences seen between Δ^6- and Δ^9-desaturases with variations of dietary and hormonal stimuli. Future experiments will attempt to purify Δ^6-desaturase.

References

[1] Strittmatter, P., Spatz, L., Corcoran, D., Rogers, M. J., Setlow, B. and Redline, R. (1974) Proc. Natl. Acad. Sci. USA 71, 4656–4669.
[2] Shimakata, T., Mihara, K. and Sato, R. (1972) J. Biochem. (Tokyo) 72, 1163–1174.
[3] Okayasu, T., Ono, T., Shinojima, K. and Imai, Y. (1977) Lipids 12, 267–271.
[4] Brenner, R. R. (1974) Mol. Cell. Biochem. 3, 41–52.
[5] Chiappe, L. E., De Tomas, M. E. and Mercuri, O. (1974) Lipids 9, 489–490.
[6] Enoch, H. G., Catala, A. and Strittmatter, P. (1976) J. Biol. Chem. 251, 5095–5103.
[7] Lapidot, Y., Rappoport, S. and Wolman, Y. (1967) J. Lipid Res. 8, 142–145.
[8] Al-Arif, A. and Blecher, M. (1969) J. Lipid Res. 10, 344–345.
[9] Seubert, W. and Pappajohn, D. (1960) Biochem. Prep. 7, 80–83.
[10] Okayasu, T., Kameda, K., Ono, T. and Imai, Y. (1977) Biochim. Biophys. Acta 489, 397–402.
[11] Safford, R., Jeffcoat, R. and James, A. T. (1975) Biochim. Biophys. Acta 409, 86–96.
[12] Pugh, E. L. and Kates, M. (1979) Lipids 14, 159–165.
[13] Jeffcoat, R., Brown, P. R., Safford, R. and James, A. T. (1977) Biochem. J. 161, 431–437.