DIETARY CONTROL OF STEARYL CoA AND ALKYLACYLGLYCEROPHOSPHORYLETHANOLAMINE DESATURASES IN TUMOR

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SUMMARY

The responses of stearyl CoA desaturase and alkylacylglycerophosphoryl-ethanolamine desaturase were compared in Fischer R-3259 tumors of rats maintained on normal and fat-free diets. The stearyl CoA desaturase activity significantly increased in control livers, host livers, and tumors in rats maintained on the fat-free diet over that in the same tissues from animals maintained on laboratory chow; however, no increase of alkylacylglycerophos-phorylethanolamine desaturase was observed in tumors of rats maintained on the fat-free diet. These findings suggest that the two desaturase systems are not identical and that they are controlled by different components of the microsomal electron transport system. Stearyl CoA desaturase activity was lower in the livers of tumor-bearing animals than in those of control animals. This finding supports the concept of others that indicates host livers tend to acquire the enzymic composition of immature livers.

INTRODUCTION

Recent work on the biosynthesis of plasmalogens reported by our laboratory (1) and by others (2) indicates that the properties of alkylacyl-glycerophosphorylethanolamine desaturase (alkylacyl-GPE desaturase)* resembles those of stearyl CoA desaturase. Both reactions require molecular oxygen and a reduced pyridine nucleotide and are inhibited by cyanide, but not by carbon monoxide. The stearyl CoA desaturation system has been shown to consist of at least four protein components, i.e., NADH-cytochrome b_5 reductase, NADPH-specific flavoprotein, cytochrome b_5, and a cyanide-sensitive factor (3,4). In normal liver stearyl CoA desaturase activity can be significantly induced when fasted rats are refed (5) or when rats are maintained on a fat-free diet

* This is a key enzyme in the biosynthesis of plasmalogens that catalyzes the conversion of the *O*-alkyl moiety of alkylacyl-GPE to an *O*-alk-1-enyl moiety on the intact glycerolipid.

(6). This response is thought to be regulated by the amount of the terminal enzyme(s) of the desaturation system, the cyanide-sensitive factor(s) (5).

The present study was carried out to determine whether the activity of alkylacyl-GPE desaturase is controlled by dietary conditions in a manner similar to that for stearyl CoA desaturase. We compared the response of stearyl CoA desaturase and alkylacyl-GPE desaturase in tumors of rats maintained on normal and fat-free diets; the stearyl CoA desaturase activities in control and host livers were also compared. The Fischer R-3259 sarcoma was selected for these studies because it contains relatively high levels of alkylacyl-GPE desaturase (1).

MATERIALS AND METHODS

Male weanling Charles River (CDF strain) rats were fed either a commercial laboratory chow (Dietrich and Gambrill Laboratory Diet) or a fat-free diet (Nutritional Biochemical Corporation). After 2 months on the diets, Fischer R-3259 sarcomas were implanted subcutaneously by the trocar method; after about 3 months on their respective diets, the animals were sacrificed.

Microsomes from control livers, host livers, and tumors of rats fed the normal or fat-free diets were prepared according to the method described by Oshino and Sato (5). A portion of the freshly prepared suspension was used to determine alkylacyl-GPE desaturase activity and the remainder was stored at -23°C. There was no significant decrease in stearyl CoA desaturase activity during storage at this temperature.

Alkylacyl-GPE desaturase was assayed in a final volume of 3 ml containing microsomes (0.5 to 3.0 mg protein), Tris-HCl (0.1 M, pH 7.2), ATP (10 mM), CoA (0.1 mM), $MgCl_2$ (5 mM), reduced glutathione (4 mM), NADH (2 mM), and 20 nmoles 1-[1-^{14}C]alkyl-*sn*-glycerophosphorylethanolamine (2.0 μCi/μmole) added in 20 μl of diethyl ether-ethanol (2:1, v/v). The preparation of substrate and the analysis of the products were carried out as described earlier (1). Stearyl CoA desaturase was assayed (5) in a reaction mixture

of 0.5 ml containing microsomes (0.5 to 3.0 mg protein), Tris-HCl (0.1 M, pH 7.2), [1-^{14}C]stearyl CoA (53 μM) (New England Nuclear Corporation), and NADH (0.4 mM) (P. L. Biochemicals, Inc.). The isolation of stearate and oleate and calculation of the desaturase activities were carried out according to the procedures of Oshino et al. (7). All enzyme assays were carried out at three or more concentrations of protein to establish zero-order kinetics. Protein was determined by the method of Lowry et al. (8), using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Table 1 summarizes the activities of stearyl CoA and alkylacyl-GPE desaturases found in normal livers, host livers, and tumors of rats fed the laboratory chow or the fat-free diet. In our system we found the value for stearyl CoA desaturase in normal rat liver microsomes to be 0.72 nmoles/min/mg protein, which is considerably higher than the 0.3 to 0.4 nmoles/min/mg protein reported by others (5,6). This difference is probably due to the different strains of rats used in various laboratories because we found that the activity was 0.24 nmoles/min/mg protein in livers from rats of the Buffalo strain.* The presence of neoplastic tissue lowered the stearyl CoA desaturase activity in the host livers. Desaturation of stearyl CoA has been found to be minimal in postnatal rat livers and to increase to a maximal level in the adult rats (9). These results support the observations that host livers tend to acquire the enzymic composition of immature livers (10).

The maintenance of rats on a fat-free diet resulted in a significant increase in stearyl CoA desaturase activities of control livers, host livers, and Fischer R-3259 sarcomas over that in the same tissue from animals maintained on laboratory chow. In a separate series of experiments we also found that stearyl CoA desaturase was induced (about 3-fold)* in microsomes prepared from 5123C hepatomas grown in rats fed a fat-free diet. In contrast,

* Lee, T-c., Stephens, N., and Snyder, F. -- unpublished observation.

TABLE 1

EFFECT OF ESSENTIAL FATTY ACID DEFICIENCY ON

STEARYL CoA AND ALKYLACYL-GPE DESATURASE ACTIVITIES*

	Stearyl CoA Desaturase		Alkylacyl-GPE Desaturase	
Tissue	Chow	Fat-free	Chow	Fat-free
	nmoles/min/mg protein			
Normal liver	0.72 ± 0.07 (4)	4.80 ± 0.43 (4)	†	n.d.
Host liver	0.12 ± 0.05 (7)	2.60 ± 0.43 (6)	n.d.	n.d.
Tumor	0.06 ± 0.01‡ (5)	0.12 ± 0.02‡ (5)	0.029 ± 0.004§ (3)	0.025 ± 0.006§ (3)

* The means ± S.E.; the numbers in parentheses refer to the number of experiments used to obtain mean values.

† Alkylacyl-GPE desaturase was not detectable in normal liver microsomes; n.d.=not determined.

‡ $P < 0.05$.

§ $P = 0.5$ to 0.6 (no significant difference in values).

feeding the host animals bearing Fischer sarcomas the fat-free diet did not change the alkylacyl-GPE desaturase activity.

Our results for the fatty acid desaturase are different from those reported by others (11-13) who found that the dietary regimen did not influence fatty acid biosynthesis in several transplantable tumors. These differences may be explained by the fact that our rats were maintained on the fat-free diet for about 3 months whereas in other studies, they were fed a fat-free diet for only 2 to 3 days, which was probably not long enough for the tumors to become deficient in essential fatty acids.

Oshino and co-workers (3,5) reported that the capacity of the microsomal system to supply reducing equivalents to b_5 is much greater than that needed for the desaturation of saturated fatty acyl CoA's and that the level of cyanide-sensitive factor(s) limits the overall desaturation. Furthermore, the cyanide-sensitive factor(s) has a rather short half-life (5) in comparison with NADH-cytochrome C reductase, cytochrome b_5, and total microsomal protein (14). It is proposed that the electron transport system containing cytochrome b_5 is transferring electrons to various kinds of inducible terminal components that may function in different metabolic reactions (5,15). Since we found that stearyl CoA desaturase and alkylacyl-GPE desaturase respond differently toward induction by dietary conditions, we conclude that a portion of the electron transport system [probably the cyanide-sensitive factor(s)] associated with these two types of desaturase activities is not identical.

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