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Substrate Specificity of Microsomal 1-Acyl-*sn*-glycero-3-phosphocholine Acyltransferase in Rat Salivary Glands for Polyunsaturated Long-Chain Acyl-CoAs

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The substrate specificities of microsomal 1-acyl-sn-glycero-3-phosphocholine acyltransferase were observed in the rat submandibular and parotid glands. The most preferable substrate in the submandibular gland was 5,8,11,14,17-eicosapentaenoyl-CoA followed by 11,14,17-eicosatrienoyl-, arachidonoyl- and oleoyl-CoAs. However, the specificity for saturated acyl-CoAs was lower than those for the polyunsaturated acyl-CoAs. In the parotid gland, the highest enzyme activity was also for 5,8,11,14,17-eicosapentaenoyl-CoA followed by 11,14,17-eicosatrienoyl-, γ-linolenoyl- and arachidonoyl-CoAs. These specificities also closely resemble those of the liver. When 1-acyl-sn-glycero-3-phosphocholine acyltransferase activity is compared among the salivary glands and liver, activity in the submandibular gland was 2-5 times and about two times higher than those in the parotid gland and liver, respectively. 1-Acyl-sn-glycero-3-phosphocholine acyltransferase also utilized acyl-CoAs having trans-unsaturated such as elaidoyl- and linoelaidoyl-CoAs or branched chain acyl-CoA like 13-methyltetradecanoyl-CoA as substrates. Furthermore, the activities of trans-unsaturated acyl-CoAs were very similar to those of cis-unsaturated acyl-CoAs. However, it is still not clear whether the reacylation enzyme protein recognizes the cis and trans configurations. From the analysis of relative activities, 1-acyl-sn-glycero-3-phosphocholine acyltransferase from both salivary glands preferred a substrate with an acyl chain length more than 18 carbon atoms with more than 3-5 double bonds in the acyl chain. These findings suggest that eicosanoid-related acyl-CoAs are good substrate for 1-acyl-sn-glycero-3-phosphocholine acyltransferase in salivary glands. Thus,the microsomal reacylation enzyme may play a role in the storage of eicosanoid precursors for metabolism into phosphatidylcholine.

Key words: 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase activity, acyl-CoA specificity, phosphatidylcholine, salivary glands

INTRODUCTION

Phosphatidylcholine, a major class of glycerophospholipids present in all mammalian and some prokaryotic cells, plays critical roles in membrane structure and cellular signaling[1]. An extensive network of acyltransferases, phospholipases and other metabolizing enzymes makes phosphatidylcholine[2]. The known signaling molecules generated from phosphatidylcholine are phosphatidic acid, diacylglycerol, lysophosphatidylcholine, and arachidonic acid[2]. Among this unique group of signaling mediators, arachidonic acid, which is on behalf of polyunsaturated fatty acids and released from the *sn*-2 position of phosphatidylcholine by the action of phospholipase A_2, is metabolized to eicosanoids, which have a variety of biological functions[3]. Therefore, phosphatidylcholine is thought to comprise a major pool in the biological membrane for eicosanoid-related fatty acyl-moieties[4]. In general, the polyunsaturated fatty acids at the *sn*-2 position of phospholipid is transferred through lysophospholipid acyltransferase acting on the deacylation-reacylation system, but not de novo synthesis[5]. It is reported that the submandibular and parotid glands have high reacylation enzyme activities to synthesize phosphatidylcholine[6]. Furthermore, 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase was specifically enhanced in microsomes from rat submandibular gland[7]. The properties of the reacylation enzyme to form phosphatidylinositol was observed in detail[5], but little is known about the transfer activity, especially for polyunsaturated fatty acids into phosphatidylcholine in the submandibular gland.

In this paper, we examined the substrate specificity of microsomal 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase in rat salivary glands, submandibular and parotid, for various acyl-CoAs having different chain-lengths, numbers of double bonds and double bond positions including eicosanoid-related acyl-CoAs.

MATERIALS AND METHODS

1. Animals

Male Wistar rats (9-11 weeks old) were maintained ad libitum on Oriental MF solid chow (Oriental Yeast Co., Tokyo) and water. The present experimental protocol was approved by the Animal Ethics Committee of Asahi University (No. 05-003)[8].

After fasting overnight, all rats were killed by bleeding under light diethyl ether anesthesia. The parotid and submandibular glands, and liver were immediately removed and carefully trimmed of connective tissue, blood vessels and capsule. Liver was perfused by saline solution. The obtained gland and liver tissues were stored at −60℃ until use.

2. Preparation of microsomes

All procedures were carried out at 0-4℃. Frozen tissue was thawed, cut into small pieces with McIlwain Tissue Chopper (Mickel Laboratory Engineering Co., Gomshall, Surrey, UK), then homogenized with a Potter-Elvehjem Teflon pestle homogenizer in 0.1M Tris-HCl buffer (pH7.2) containing 0.25M su-

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crose. Microsomes were obtained as a precipitate of centrifugation (105,000×*g*, 60min) as described previously[9]. The resulting pellet was suspended to approximately 10mg microsomal protein per ml in 0.1M Tris-HCl buffer (pH7.2) without sucrose, using a glass pestle homogenizer, and stored at-85℃ until use.

3. Preparation of substrates

1-Acyl-*sn*-glycero-3-phosphocholine was prepared from egg yolk phosphatidylcholine by hydrolysis with snake venom phospholipase A_2[10]. The following fatty acids were purchased from Serdary Research Laboratories (London, Canada); palmitoleic [16:1 (*n*-7)], elaidic [*trans*18:1 (*n*-9)], linoelaidic [*trans*18:2 (*n*-6)], α-linolenic [18:3 (*n*-3)], γ-linolenic [18:3 (*n*-6)], bishomo-γ-linolenic [20:3 (*n*-6)], arachidonic [20:4 (*n*-6)], 4,7,10,13,16,19-docosahexaenoic acids [22:6 (*n*-3)]. Linoleic acid [18:2 (*n*-6)] was from Nacalai Tesque (Kyoto, Japan). 5,8,11,14,17-Eicosapentaenoic [20:5(*n*-3)] and 13-methyltetradecanoic acids [*iso*15:0] were from Funakoshi (Tokyo, Japan) and Larodan Fine Chemicals (Malmö, Sweden), respectively. Coenzyme A (sodium salt) was obtained from Kyowa Hakko Kogyo (Tokyo, Japan). Oleoyl [18:1 (*n*-9)]-CoA and stearoyl [18:0]-CoA were purchased from GE Healthcare Bio-Sciences KK (Tokyo, Japan). Acyl-CoAs from fatty acids were synthesized by coupling reaction of acylchloride derived from fatty acid and coenzyme A[11,12]. Other acyl-CoAs used here were of the highest grade available from commercial sources as described[9].

4. Assay of acyltransferase activity

Acyl-CoA: 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase activities were measured spectrophotometrically using 5,5'-dithiobis (2-nitrobenzoic acid)[10]. The reaction mixture contained 20μM acyl-CoA, 150μM 1-acyl-glycerophosphocholine, 1mM DTNB and 100μg/ml of microsomal protein in 90mM Tris-HCl buffer (pH7.2) and incubation was carried out at25℃ in duplicate.

5. Other methods

The protein concentration was determined according to the method of Lowry *et al.*[13] or with the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) according to Bradford[14], using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Phosphatidylcholine in submandibular and parotid glands is the most abundant membrane glycerophospholipid[6] and occupied by polyunsaturated fatty acids at the *sn*-2 position[15]. Acyl-CoA: 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase is thought to be one of the key enzymes for the determination of fatty acids composition of the *sn*-2 position in phosphatidylcholine. Table 1 shows the substrate specificity of microsomal 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase for various acyl-CoAs in the salivary glands and liver. Under optimal conditions[10], the most preferable substrate for enzyme from submandibular gland was 5,8,11,14,17-eicosapentaenoyl [20:5 (*n*-3)]-CoA followed by 11,14,17-eicosatrienoyl [20:3 (*n*-3)]-, arachidonoyl [20:4 (*n*-6)]- and oleoyl [18:1 (*n*-9)]-CoAs. However, the specificity for saturated acyl-CoAs such as myristoyl [14:0]-, palmitoyl [16:0]- and stearoyl [18:0]-CoAs was lower than those for the polyunsaturated acyl-CoAs. In parotid gland, the highest enzyme activity was also for 5,8,11,14,17-eicosapentaenoyl [20:5 (*n*-3)]-CoA followed by 11,14,17-eicosatrienoyl [20:3 (*n*-3)]-, γ-linolenoyl [18:3 (*n*-6)]- and arachidonoyl [20:4 (*n*-

Table 1 Substrate specificities of microsomal 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase for various acyl-CoAs in the rat submandibular and parotid glands, and liver.

Acyl-CoA	Specific activity (nmol/min per mg of protein)			Ratio of specific activity		
	Submandibular gland (A)	Parotid gland (B)	Liver (C)	A/B	A/C	B/C
Myristoyl[14:0]-CoA	10.5	2.3	6.9	4.6	1.5	0.3
13-Methyltetradecanoyl[*iso*15:0]-CoA	25.3	4.8	14.5	5.3	1.7	0.3
Palmitoyl[16:0]-CoA	14.8	4.4	6.5	3.4	2.3	0.7
Palmitoleoyl[16:1(*n*-7)]-CoA	12.9	3.9	10.8	3.3	1.2	0.4
Stearoyl[18:0]-CoA	9.7	-*	-	-	-	-
Oleoyl[18:1(*n*-9)]-CoA	50.1	11.6	37.1	4.3	1.4	0.3
Elaidoyl[*trans*18:1(*n*-9)]-CoA	46.9	12.0	22.8	3.9	2.1	0.5
Linoleoyl[18:2(*n*-6)]-CoA	32.5	10.9	25.3	3.0	1.3	0.4
Linoelaidoyl[*trans*18:2(n-6)]-CoA	32.9	9.5	23.8	3.5	1.4	0.4
α-Linolenoyl[18:3(*n*-3)]-CoA	10.5	4.0	6.6	2.6	1.6	0.6
γ-Linolenoyl[18:3(*n*-6)]-CoA	40.0	14.0	33.9	2.9	1.2	0.4
11,14,17-Eicosatrienoyl[20:3(*n*-3)]-CoA	63.9	19.4	40.6	3.3	1.6	0.5
Bishomo-γ-linolenoyl[20:3(*n*-6)]-CoA	37.2	8.1	-	4.6	-	-
Arachidonoyl[20:4(*n*-6)]-CoA	55.6	13.8	33.1	4.0	1.7	0.4
5,8,11,14,17-Eicosapentaenoyl[20:5(*n*-3)]-CoA	99.4	33.6	54.7	3.0	1.8	0.6
4,7,10,13,16,19-Docosahexaenoyl[22:6(*n*-3)]-CoA	20.1	5.0	15.7	4.0	1.3	0.3

Specific activities represent the averages of duplicate assays. Acyl chains are desighnated as the number of carbon atoms:the number of double bonds followed by the position of double bonds.

* : not determined

6)]-CoAs. Although the order of specificity for various polyunsaturated fatty acyl-CoAs was slightly different between submandibular and parotid glands, tendencies, such as the preference for polyunsaturated and long-chain acyl-CoAs and less activity for saturated acyl-CoAs, were very similar. These specificities also closely resembled those from the liver. However, analysis of the positional distribution of fatty acid in phosphatidylcholine shows that the abundant fatty acid in the *sn*-2 position were linoleic [18:2 (*n*-6)] acid (33%) and arachidonic [20:4 (*n*-6)] acid (17%), not 5,8,11,14,17-eicosapentaenoic [20:5 (*n*-3)] acid[15]. The supplement and availability of acyl-CoA are limited by the metabolic pathways, such as fatty acid synthase, acyl-CoA synthase[16] and acyl-CoA hydrolase[17]. Using similar types of acyl-CoAs, the specificity of 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase for acyl-CoAs is different from that of 1-acyl-*sn*-glycero-3-phosphoinositol acyltransferase in submandibular gland[5]. These observations support that substrate specificity plays an important role in determining the fatty acid composition in the sn- 2 position of phospholipids. When the microsomal 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase activity is compared among the salivary glands and liver, the activity in submandibular gland was 2-5 times and about two times higher than those in the parotid gland and liver, respectively. Usually, common metabolic enzyme activity is very high in the liver. These findings suggest that the high reacylation activity might play a specific and functional role in the submandibular gland but not the parotid gland. Table 1 shows very interesting findings that 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase also utilized acyl-CoAs having *trans*-unsaturated such as elaidoyl [*trans*18:1 (*n*-9)]- and linoelaidoyl [*trans*18:2 (*n*-6)]-CoAs or branched chain acyl-CoA like 13-methyltetradecanoyl [*iso*15:0]-CoA as substrates. Furthermore, the activities for *trans*-unsaturated acyl-CoAs were very similar to those for *cis*-unsaturated acyl-CoAs. The affinity of acyl-CoA for the enzyme protein may be related to the physical and structural properties of the acyl chain. However, it is still not clear whether the reacylation enzyme protein recognizes the *cis* and *trans* configurations.

Table 2 shows relative activities expressed as ratios of the specific activity to the specific activity with palmitoyl [16:0]-CoA in microsomal 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase from the submandibular gland. The enzyme preferred as a substrate has an acyl chain length of more than 18 carbon atoms and more than 3-5 double bonds in the acyl chain. The position of the double bond dose not seem to be important. These properties of 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase was also very similar in those from parotid gland (Table 3). These findings suggest that eicosanoid-related acyl-CoAs are a suitable substrate for 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase in the salivary glands, but the concentration or availability of acyl-CoAs must be considered. Thus, microsomal reacylation enzyme may play a role in the storage of eicosanoid precursors for metabolism into phosphatidylcholine.

To synthesize the membrane phosphatidylcholine, there are not only de novo formation by CDP-choline citidyltransferase, but also by the methylation of phosphatidylethanolamine or the base-exchange reaction of phospholipids[1]. The characteristics of other reacylation enzymes such as phosphatidylethanolamine and other phospholipids are also considered to determine the acyl chain composition in phosphatidylcholine. Recently, another substrates for acyltransferase, 1-acyl-*sn*-glycero-3-phosphocholine, has been the focus of attention as one of lysophospholipids with properties resembling extracellular growth factors or signaling molecules[18,19].

Phosphatidylcholine containing arachidonic acid in the *sn*-2 position becomes the substrate for specific phospholipase A_2, which produces 2-arachidonoyl-*sn*-3-glycerophosphocholine[20]. This unique lysophospholipid is hydrolyzed by phospholipase C, producing 2-arachidonoyl-*sn*-glycerol, which plays a biological role as an endocannabinoid signaling molecule. Further studies are necessary to determine the role of 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase in the establishment of arachidonic acid-enriched phosphatidylcholine in the salivary glands.

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Table 2 Relative activities of microsomal 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase for various acyl-CoAs in the rat submandibular gland

Number of carbon atom	Position of double bond	Relative activity: Number of double bond 0	1	2	3	4	5
16	*n*-7	1.0	0.9				
18	*n*-9	0.7	3.4				
18	*n*-6			2.2	0.7		
18	*n*-3				2.7		
20	*n*-6				4.3	3.8	
20	*n*-3				2.5		6.7

Acyl chains are desighnated as the number of carbon atoms, the number of double bonds and the position of double bonds.

Relative activities are expressed as ratios of the specific activity to the specific activity with palmitoyl-CoA which are from the Data of Table 1.

Table 3 Relative activities of microsomal 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase for various acyl-CoAs in the rat parotid gland

Number of carbon atom	Position of double bond	Relative activity: Number of double bond 0	1	2	3	4	5
16	*n*-7	1.0	0.9				
18	*n*-9		2.6				
18	*n*-6			2.5	0.9		
18	*n*-3				3.2		
20	*n*-6				4.4	3.1	
20	*n*-3				1.8		7.6

Acyl chains are desighnated as the number of carbon atoms, the number of double bonds and the position of double bonds.

Relative activities are expressed as ratios of the specific activity to the specific activity with palmitoyl-CoA which are from the Data of Table 1.

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ラット顎下腺および耳下腺ミクロソームの 1-アシル-*sn*-グリセロ-3-ホスホコリンアシルトランスフェラーゼの 多価不飽和脂肪酸アシル-CoA に着目した基質特異性

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ラット顎下腺および耳下腺ミクロソームの1-アシル-*sn*-グリセロ-3-ホスホコリンアシルトランスフェラーゼの基質特異性を検討した。顎下腺の酵素は，5,8,11,14,17-エイコサペンタエノイル-CoA に対して一番活性が高く，次いで11,14,17-エイコサトリエノイル-，アラキドノイル-，オレオイル-CoA の順であった。一方，飽和アシル-CoA に対する基質特異性は不飽和アシル-CoA のそれより低かった。耳下腺では，5,8,11,14,17-エイコサペンタエノイル-CoA に対して一番活性が高く，次いで11,14,17-エイコサトリエノイル-，γ-リノレノイル-，アラキドノイル-CoA の順であった。顎下腺ミクロソームの1-アシル-*sn*-グリセロ-3-ホスホコリンアシルトランスフェラーゼ活性は耳下腺のそれより2-5倍高かった。この酵素は，トランス型および分枝鎖型アシル-CoA に対しても高い活性を示した。顎下腺，耳下腺における酵素の比活性の解析から，本酵素は炭化水素鎖長：18以上，二重結合の数：3-5個のアシル-CoA に対して高活性を示した。これらの結果は，唾液腺の1-アシル-*sn*-グリセロ-3-ホスホコリンアシルトランスフェラーゼがエイコサノイドに関連したアシル-CoA を良い基質にすることが示唆された。このことは，ミクロソームの再アシル化系酵素がホスファチジルコリン内にエイコサノイド前駆物質を蓄積するのに重要であると考えられる。

キーワード：1-アシル-*sn*-グリセロ-3-ホスホコリンアシルトランスフェラーゼ，アシル-CoA 基質特異性，ホスファチジルコリン，唾液腺

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