INHIBITORY EFFECTS OF LONG-CHAIN ACYL COENZYME A ANALOGUES ON RAT LIVER ACETYL COENZYME A CARBOXYLASE

Jun-ichi NIKAWA, Tadashi TANABE, Hideo OGIWARA*, Tetsuo SHIBA+ and Shosaku NUMA

Department of Medical Chemistry, Kyoto University Faculty of Medicine, Yoshida, Sakyo-ku, Kyoto 606 and *+Department of Chemistry, Osaka University Faculty of Science, Toyonaka, Osaka 560, Japan*

Received 17 April 1979

1. Introduction

Acetyl coenzyme A carboxylase (acetyl-CoA: carbon-dioxide ligase (ADP-forming), EC 6.4.1.2), which catalyzes the initial step in the biosynthesis of long-chain fatty acids, plays a critical role in the regulation of this synthetic process [1]. Acetyl-CoA carboxylase from animal tissues is activated by tricarboxylic acids such as citrate, while long-chain acyl-CoA thioesters inhibit this enzyme [2]. We have demonstrated [3] that 1 mol palmitoyl-CoA binds tightly and reversibly to 1 mol rat liver acetyl-CoA carboxylase to inhibit the enzyme, the inhibition constant (K_i) being as low as 5.5 nM. This finding supports the view that long-chain acyl-CoA represents a physiological inhibitor which binds to a specific regulatory site of the enzyme. The present investigation deals with the inhibitory effects of various structural analogues of palmitoyl-CoA on rat liver acetyl-CoA carboxylase. The results obtained indicate that the 3′-phosphate of the CoA moiety and the long-chain acyl residue are essential for the inhibition of the enzyme.

2. Materials and methods

2.1. *Chemicals*

For the abbreviations for the CoA analogues used, refer to [4,5]. CoA was obtained from Boehringer (Mannheim) and 1,N^6-etheno-CoA from P-L Biochemicals (Milwaukee). Synthetic CoA analogues, i.e., CoA(L) [6], keto-CoA [6], inosino-CoA [7] and 4′,4″-diphosphopantethine [8] were generous gifts of Dr M. Shimizu (Daiichi Seiyaku Co., Tokyo). Dephospho-CoA and adenosine 3′,5′-diphosphate as well as oleic acid and arachidonic acid were purchased from Sigma (St Louis) and arachidic acid from Merck (Darmstadt). All other fatty acids were products of Nakarai (Kyoto). Cetyl bromide was obtained from Wako Pure Chemical (Osaka). Other chemicals, including the reagents for the assay of acetyl-CoA carboxylase, were as in [3].

2.2. *Preparation and determinations*

Long-chain acyl thioesters of CoA and palmitoyl-1,N^6-etheno-CoA were prepared according to [9]. Palmitoyl thioesters of CoA(L), keto-CoA, inosino-CoA and dephospho-CoA were prepared by the method in [10]. The concentrations of the acyl-CoA derivatives and their analogues were determined spectrophotometrically with the use of the molar extinction coefficients for CoA [11], inosine [12] and 1,N^6-etheno-adenine [5]. Palmitoyl-4′-phosphopantetheine was prepared by the reduction of 4′,4″ diphosphopantetheine with sodium amalgam, followed by acylation [9] and was determined spectrophotometrically ($\epsilon_{233} = 4600\ M^{-1}cm^{-1}$) [11]. The concentrations of all these thioesters were also assayed by quantitating the free sulfhydryl group released by mild alkaline hydrolysis with the use of 5,5′-dithiobis(2-nitrobenzoic acid) [13]; this method and those mentioned above gave essentially identical values. *S*-cetyl-CoA was synthesized as follows: The

* Present address: Department of Biochemistry, Gunma University Faculty of Medicine, Maebashi, Gunma 371, Japan

mixture of 20 mg CoA, 7.9 mg cetyl iodide [14] and 20 mg sodium bicarbonate in 5 ml 70% aqueous tetrahydrofuran was stirred at room temperature for 16 h under a nitrogen atmosphere. The product formed was purified as in [9]. The possibility of *N*-alkylation on the adenine ring was excluded by the fact that acid hydrolysis (1 M HCl, 100°C, 10 min) of the product led to the liberation of adenosine 3′,5′-diphosphate, which was identified by thin-layer chromatography on a silica gel 60 F_{254} plate (Merck) with 1-propanol/28% ammonia/water (55/10/35, by vol.) as the developing solvent (R_F 0.46). The concentration of *S*-cetyl-CoA was determined by measuring the absorbance of CoA [11]. All the acyl-CoA derivatives and their analogues yielded a single spot when they were subjected to thin-layer chromatography on a silica gel 60 F_{254} plate with 1-butanol/acetic acid/water (5/2/3, by vol.) as the developing solvent and were visualized by ultraviolet irradiation and/or by staining as in [15].

2.3. *Purification and assay of acetyl-CoA carboxylase*

Pure acetyl-CoA carboxylase from rat liver was prepared by the method in [16] as modified in [17]. The activity of the enzyme was determined at 37°C in the absence of bovine serum albumin by the spectrophotometric method as in [3]. The enzyme used was 13–30 nM as calculated on the basis of the mol. wt 230 000 of the multifunctional monomeric subunit containing 1 molecule of biotin [18].

2.4. *Determination of inhibition constants*

The K_i values of acetyl-CoA carboxylase for long-chain acyl-CoA derivatives and their analogues were determined by the Dixon graphical method [19]. *K* values, which were determined with at least three different concentrations (2.5–10 mM) of citrate, were plotted against the citrate concentrations, and the K_i value was obtained by extrapolation to zero citrate concentration as in [3].

3. Results

The graphical method in [19], which was used in the present investigation to determine K_i values, is applicable to tight-binding inhibitors. Table 1 lists the K_i values of rat liver acetyl-CoA carboxylase for the palmitoyl thioesters of CoA and its analogues as well as for *S*-cetyl-CoA. Modification of the free hydroxy group in the pantoic acid moiety (palmitoyl-CoA(L) and palmitoyl-keto-CoA) or of the adenine ring (palmitoyl-1,N^6-etheno-CoA and palmitoyl-inosino-CoA) resulted in little increase in the K_i value. In contrast, palmitoyl-dephospho-CoA, which lacks the 3′-phosphate of the CoA moiety, exhibited a K_i value 40-fold higher than that for palmitoyl-CoA. Interestingly, the K_i value for palmitoyl-4′-phosphopantetheine was of the same order of magnitude as that for palmitoyl-dephospho-CoA. These results indicate that the 3′-phosphate of the CoA moiety is essential for the specific inhibition of the enzyme. It is noteworthy that *S*-cetyl-CoA, in which the thioester of palmitoyl-CoA is replaced by thioether, inhibited the enzyme almost as strongly as palmitoyl-CoA.

Table 1
Inhibition constants of acetyl-CoA carboxylase for palmitoyl-CoA and its analogues

Inhibitor	K_i (nM)
Palmitoyl-CoA	6.5
Palmitoyl-CoA(L)	22
Palmitoyl-keto-CoA	21
Palmitoyl-1,N^6-etheno-CoA	15
Palmitoyl-inosino-CoA	14
Palmitoyl-dephospho-CoA	260
Palmitoyl-4′-phosphopantetheine	650
S-Cetyl-CoA	10

The work in [20] has shown that the inhibition constant of rat liver acetyl-CoA carboxylase for fatty acyl-CoA varies with the chain length of its acyl residue. This study was carried out with the use of a partially purified enzyme preparation and of the reaction mixture containing serum albumin, which is known to bind long-chain acyl-CoA [21]. In the experiment represented in table 2, the CoA thioesters of various fatty acids were tested for their ability to inhibit pure rat liver acetyl-CoA carboxylase in the absence of serum albumin. Among the compounds examined, arachidoyl-CoA, stearoyl-CoA and palmitoyl-CoA exhibited the lowest K_i values. The CoA thioesters of saturated fatty acids of shorter or longer chain lengths were less inhibitory. The CoA

Table 2
Inhibition constants of acetyl-CoA carboxylase for CoA thioesters of various fatty acids

Inhibitor	K_i (nM)
Saturated acyl-CoA	
Lauroyl-CoA	–[a]
Myristoyl-CoA	680
Palmitoyl-CoA	6.5
Stearoyl-CoA	1.3
Arachidoyl-CoA	<1
Docosanoyl-CoA	40
Tetracosanoyl-CoA	150
Unsaturated acyl-CoA	
Palmitoleoyl-CoA	130
Oleoyl-CoA	44
Linolenoyl-CoA	27
Linolenoyl-CoA	66
Arachidonoyl-CoA	48

[a] Essentially no inhibition was observed at ⩽10 μM

thioesters of unsaturated fatty acids exhibited higher K_i values than those of saturated fatty acids of corresponding chain lengths.

4. Discussion

The present investigation clearly indicates that the 3′-phosphate of the CoA moiety is essential for the inhibition of acetyl-CoA carboxylase by palmitoyl-CoA. The importance of this phosphate group for the inhibitory effect of stearoyl-CoA on citrate synthase has been described [22]. Modification of the pantoic acid or adenine moiety of palmitoyl-CoA does not appreciably influence its inhibitory effect on acetyl-CoA carboxylase, although it was reported [5] that oleoyl-1,N^6-etheno-CoA is a less potent inhibitor of citrate synthase than oleoyl-CoA. The inhibitory effect of fatty acyl-CoA on various enzymes increases with increasing chain lengths of its acyl residue has been observed in [20,22–25]. The present study reveals that the CoA thioesters of saturated fatty acids with 16–20 carbon atoms inhibit acetyl-CoA carboxylase more effectively than those of saturated fatty acids of shorter or longer chain lengths. The fact that the CoA thioesters of unsaturated fatty acids are less inhibitory than those of saturated fatty acids of corresponding chain lengths may be accounted for by the different conformations of their acyl residues. The rather strict structural requirement for the inhibitory effect of long-chain acyl-CoA indicates that the inhibitor binds to a specific site on the acetyl-CoA carboxylase molecule. This, together with the reversible formation of the equimolar enzyme–inhibitor complex reported [3], supports the concept that long-chain acyl-CoA is a physiological regulator of acetyl-CoA carboxylase.

Acknowledgements

We are indebted to Dr M. Shimizu for generous gifts of some coenzyme A analogues and to Professor T. Hashimoto for helpful discussions. This investigation was supported in part by research grants from from the Ministry of Education, Science and Culture of Japan, the Mitsubishi Foundation, the Foundation for the Promotion of Research on Medicinal Resources and the Japanese Foundation of Metabolism and Diseases.

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