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Functional characterization of rat glutaryl-CoA dehydrogenase and its comparison with straight-chain acyl-CoA dehydrogenase

Long Wu [a], Yuqin Qiao [a], Jinbo Gao [a], Guisheng Deng [a], Wenhua Yu [a], Gong Chen [a], Ding Li [b,*]

[a] Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, PR China
[b] School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou University City, Waihuan East Road 132, Guangzhou 510006, PR China

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ABSTRACT

Glutaryl-CoA dehydrogenase catalyzes the oxidative decarboxylation of the γ-carboxylate of the substrate, glutaryl-CoA, to yield crotonyl-CoA and CO_2. The enzyme is a member of the acyl-CoA dehydrogenase (ACD) family of flavoproteins. In the present study, the catalytic properties of this enzyme, including its substrate specificity, isomerase activity, and interactions with inhibitors, were systematically studied. Our results indicated that the enzyme has its catalytic properties very similar to those of short-chain and medium-chain acyl-CoA dehydrogenase except its additional decarboxylation reaction. Therefore, the inhibitors of fatty acid oxidation targeting straight chain acyl-CoA dehydrogenase could also function as inhibitors for amino acid metabolism of lysine, hydroxylysine, and tryptophan.

Glutaryl-CoA dehydrogenase (GCD, EC 1.3.99.7) is a member of the acyl-CoA dehydrogenase (ACD) family of flavoproteins that catalyzes the oxidation of acyl-CoA thioesters to the corresponding 2-enoyl-CoA products. There are nine known members in the ACD family, five of which are involved in fatty acid oxidation and four, including GCD, involved in amino acid oxidation.[1,2] The catalytic mechanisms of these enzymes have common features.[3] The position of the catalytic base, a glutamate residue that initiates the catalytic cycle, is located in the same relative position in seven of the nine mammalian ACDs.[4–6] In the remaining two dehydrogenases, long-chain acyl-CoA dehydrogenase (LCAD) and isovaleryl-CoA dehydrogenase (IVD), the glutamate catalytic base is located on a different helix, topologically conserved, but situated over 100 residues away from the conserved glutamate of other seven ACDs in the primary sequence.[7,8] GCD catalyzes the oxidative decarboxylation of the γ-carboxylate of the substrate, glutaryl-CoA, to yield crotonyl-CoA and CO_2 as shown in Figure 1. This enzyme is involved in the catabolism of lysine, hydroxylysine, and tryptophan, and the deficiency of this enzyme can cause neurological problems[9] due to an accumulation of the marker metabolites 3-hydroxyglutaric acid, glutaric acid, and glutarylcarnitine. The enzyme contains one noncovalently bound FAD per subunit. Interestingly, a structurally related but nondecarboxylating GCD has been recently characterized in the obligately anaerobic bacteria Desulfococcus multivorans.[10] The comparison of their three-dimensional structures has indicated decarboxylating and nondecarboxylating capabilities are provided by structural changes around the glutaconyl carboxylate group.[11]

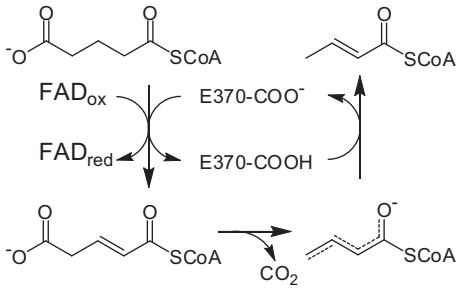

Figure 1. GCD catalyzes the oxidative decarboxylation of the γ-carboxylate of the substrate, glutaryl-CoA, to yield crotonyl-CoA and CO_2.

It has been reported that GCD has some unique features among the ACD family members, besides its additional catalytic function of decarboxylation.[12] Although the rate-limiting step for other ACDs involves the abstraction of a proton at alpha carbon, the major rate-determining step for GCD has been found to be the release of crotonyl-CoA product.[13] In addition, inhibitor 2-pentynoyl-CoA has been found to inactivate GCD at a rate that considerably exceeds the rates of inactivation of short and medium chain ACDs.[14] Although the overall fold of the GCD has been found to be similar to those of other known ACDs,[15] it should be noted that three-dimensional structures of the enzymes give static pictures, while the enzymes are rather flexible in nature. Therefore, in order to gain further understanding of GCD as a possibly unique enzyme

* Corresponding author. Tel./fax: +86 20 3994 3058.
E-mail address: liding@mail.sysu.edu.cn (D. Li).

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Table 1
Kinetic parameters for the dehydrogenase activity of rat GCD wild-type and variant enzymes with glutaryl-CoA as substrate

	V_{max} (μmol mg^{-1}min^{-1})	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (s^{-1} μM^{-1})
GCD WT	5.2 ± 0.4	3.8 ± 0.3	4.0 ± 1.6	0.95
GCD E370A	ND	ND	ND	ND
GCD E370Q	0.048 ± 0.005	0.035 ± 0.004	12 ± 2	0.0029

ND, not detectable.

Table 2
The straight chain substrate specificity of rat GCD

	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M (s^{-1} μM^{-1})
Butyryl-CoA	12 ± 1	2.7 ± 0.3	0.23
Hexanoyl-CoA	20 ± 3	2.1 ± 0.2	0.11
Octanoyl-CoA	28 ± 3	1.1 ± 0.1	3.9×10^{-2}
Decanoyl-CoA	54 ± 6	0.18 ± 0.02	3.3×10^{-3}
Dodecanoyl-CoA	ND		
Tetradecanoyl-CoA	ND		

ND, not detectable.

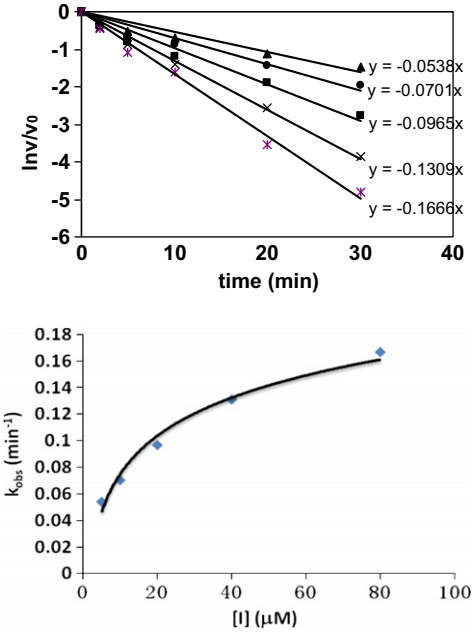

Figure 2. Kinetic studies for time- and concentration-dependent inactivation of the GCD by 2-octynoyl-CoA (▲, 5 μM 2-octynoyl-CoA; ●, 10 μM; ■, 20 μM; ×, 40 μM; ∗, 80 μM).

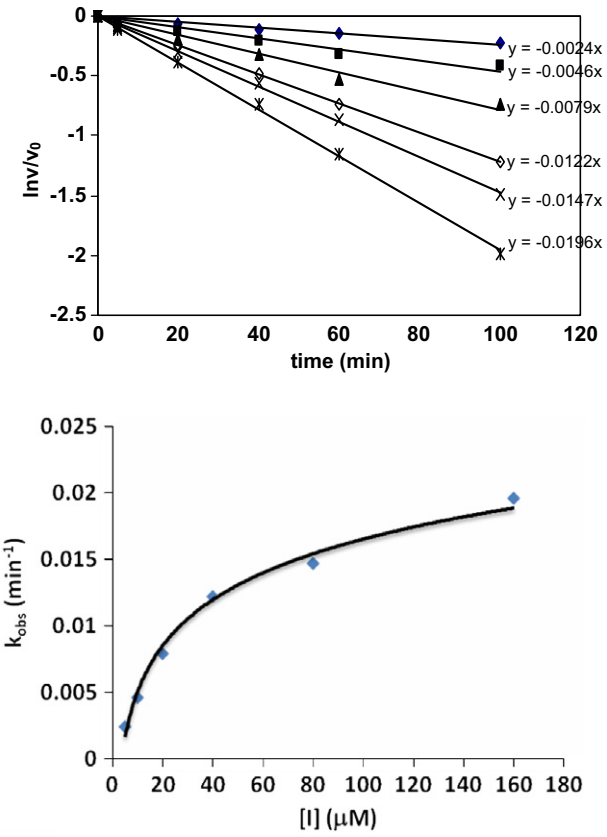

Figure 3. Kinetic studies for time- and concentration-dependent inactivation of the GCD by oct-2-yn-4-enoyl-CoA (♦, 5 μM oct-2-yn-4-enoyl-CoA; ■, 10 μM; ▲, 20 μM; ◇, 40 μM; ×, 80 μM; ∗, 160 μM).

in ACD family, in the present study, we carried out extensive experiments to investigate its catalytic properties systematically, including substrate specificity, isomerase activity, and interactions with inhibitors. These results were compared with those obtained for straight-chain acyl-CoA dehydrogenase. This study increased our understanding of GCD as a special enzyme in ACD family.

GCD gene cloning, protein expression, purification and characterization: Rat liver GCD was cloned, and its mutants, E370A and E370Q, were constructed using molecular biology techniques. All rat GCD wild type and variant enzymes were purified to be more than 95% pure. The procedures for above experiments were shown in the supporting information. The assay mixture for determining the activity of GCD consisted of 100 mM potassium phosphate buffer (pH 7.2), 75 μM DCIP (2,6-dichlorophenol indophenol), 0.6 mM PMS (phenazine methosulfate), 50 μM glutaryl-CoA, and 0.2 μg of GCD in a final volume of 1 mL. The reaction mixtures were pre-incubated for 1 min at 25 °C, and were initiated by the addition of the enzyme. The decrease in the optical density at 600 nm was monitored. Rates were calculated using $\varepsilon_{600} = 21.0\ mM^{-1}cm^{-1}$ for the reduction of the dye. The reaction progress curves were recorded for 2 min on a Hitachi U-2800 UV–visible spectrophotometer. Determination of the K_M and the V_{max} was performed using this assay buffer with varying substrate concentrations ranging from 1 to 75 μM. The averages of two assays were used for each point, and the result was obtained as shown in Table 1. The k_{cat}/K_M value of the mutant GCD E370Q was found to be 330 times lower than that of the wild-type enzyme, while the mutant GCD E370A had complete loss of its dehydrogenase activity, which confirmed that Glu370 is the catalytic residue for rat GCD. The straight chain substrate specificity of GCD was determined as shown in Table 2. When extending acyl chain of substrate, the K_M values increased, while its k_{cat} values decreased. The shorter acyl chain substrates were more suitable for rat GCD. Rat GCD could catalyze the conversion of substrates with up to 10 carbons in the acyl chain. The natural substrate of GCD is glutaryl-CoA with five carbon atoms in its acyl chain and a hydrophilic γ-carboxylate group in its terminal chain. Based on the crystal structure of GCD,[15] the γ-carboxylate group is tightly hydrogen-bonded with several hydrophilic residues, including Tyr369, Glu87, Arg94, Ser95, and Thr170.