Lipid Biosynthesis in Sebaceous Glands: Regulation of the Synthesis of *n*- and Branched Fatty Acids by Malonyl-Coenzyme A Decarboxylase†

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ABSTRACT: Crude cell-free extracts isolated from the uropygial glands of goose catalyzed the carboxylation of propionyl-CoA but not acetyl-CoA. However, a partially purified preparation catalyzed the carboxylation of both substrates and the characteristics of this carboxylase were similar to those reported for chicken liver carboxylase. The K_m and V_{max} for the carboxylation of either acetyl-CoA or propionyl-CoA were 1.5×10^{-5} *M* and 0.8 μmol per min per mg, respectively. In the crude extracts an inhibitor of the acetyl-CoA carboxylase activity was detected. The inhibitor was partially purified and identified as a protein that catalyzed the rapid decarboxylation of malonyl-CoA. This enzyme was avidin-insensitive and highly specific for malonyl-CoA with very low rates of decarboxylation for methylmalonyl-CoA and malonic acid. V_{max} and K_m for malonyl-CoA decarboxylation, at the pH optimum of 9.5, were 12.5 μmol per min per mg and 8×10^{-4} *M*, respectively. The relative activities of the acetyl-CoA carboxylase and malonyl-CoA decarboxylase were about 4 μmol per min per gland and 70 μmoles per min per gland, respectively. Therefore acetyl-CoA and methylmalonyl-CoA should be the major primer and elongating agent, respectively, present in the gland. The major fatty acid formed from these precursors by the fatty acid synthetase of the gland would be 2,4,6,8-tetramethyldecanoic acid which is known to be the major fatty acid of the gland (Buckner, J. S. and Kolattukudy, P. E. (1975), *Biochemistry*, following paper). Therefore it is concluded that the malonyl-CoA decarboxylase controls fatty acid synthesis in this gland.

Multi-branched fatty acids occur in a variety of biological systems including humans (Murray, 1962; Asselineau and Bennet, 1964; Nicolaides, 1972). Yet very little is known about their biosynthesis. In animals these acids are usually found in sebacious glands. The uropygial gland, the sole sebacious gland of goose, duck, and a variety of other water birds, synthesizes large quantities of multi-branched fatty acids (Murray, 1962; Odham, 1966, 1967a,b). In our attempts to determine the mechanism and regulation of synthesis of multi-branched fatty acids we discovered that a partially purified fatty acid synthetase from the uropygial glands of goose synthesized multi-branched fatty acids from methylmalonyl-CoA and *n*-fatty acids from malonyl-CoA (Buckner and Kolattukudy, 1975). The substrate specificity of this synthetase preparation was in favor of *n*-fatty acid formation while the bulk of the naturally occurring fatty acids of this gland are multi-branched acids. Therefore, the regulation of synthesis of *n*- and multi-branched acids was suspected to be at the carboxylase level and hence we examined the gland extract for acetyl-CoA and propionyl-CoA carboxylase. In this paper we describe the isolation, purification, and some of the properties of an acetyl-CoA and propionyl-CoA carboxylase. Discovery of the presence of a very active malonyl-CoA decarboxylase in the uropygial gland is also reported. This enzyme specifically decarboxylated malonyl-CoA at rates much higher than that of the carboxylation of acetyl-CoA to malonyl-CoA. Properties of the partially purified decarboxylase are also presented. From the experimental evidence obtained for both enzymes the malonyl-CoA decarboxylase appears to play a regulatory role in the synthesis of multi-branched fatty acids.

Experimental Section

Materials. Domestic white geese were purchased from the Richards Goose Hatchery, Outlook, Wash., and maintained on a low poultry breeder ration. Bovine serum albumin, adenosine triphosphate, coenzyme A, avidin, biotin, dithioerythritol, acetyl-CoA, propionyl-CoA, and malonyl-CoA were obtained from Sigma Chemical Company, St. Louis, Mo. $NaH^{14}CO_3$ and hyamine hydroxide were obtained from Amersham-Searle, Arlington Heights, Ill. [1-^{14}C]Acetyl-CoA, [1,3-^{14}C]malonyl-CoA, and Omnifluor were obtained from New England Nuclear, Boston, Mass.

Methyl-[3-^{14}C]malonyl-CoA was prepared enzymatically from $H^{14}CO_3^-$ and propionyl-CoA in the presence of partially purified goose uropygial gland carboxylase and the other components of the HCO_3^- fixation assay. The enzymatically synthesized methyl-[3-^{14}C]malonyl-CoA was purified by DEAE-cellulose column chromatography utilizing a linear LiCl gradient (Gregolin et al., 1968), and subsequent desalting by gel filtration. The authenticity of the methyl-[3-^{14}C]malonyl-CoA was determined by reduction of the CoA ester with $NaBH_4$ and analysis of the reduction product, 2-methyl-3-hydroxy[1-^{14}C]propionic acid, by radio gas-liquid chromatography. The reduced methylmalonyl-CoA derivative cochromatographed with $NaBH_4$ reduction product of [methyl-^{3}H]methylmalonyl-CoA.

Isolation and Partial Purification of Enzymes. Geese were killed by exsanguination. The preen glands were excised and the adhering muscle tissue and fat were removed. The two lobes of each gland (4–8 g) were cut into thin slices and homogenized in a Ten-Broeck tissue grinder with a

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buffer consisting of 10 m*M* phosphate (pH 7.6), 250 m*M* sucrose, 1 m*M* $MgCl_2$, 20 m*M* sodium citrate, and 0.5 m*M* dithioerythritol.

The resulting homogenate was centrifuged at 15,000*g* for 30 min to sediment mitochondria and the supernatant was centrifuged again at 15,000*g* for 30 min to remove remaining mitochondria. Microsomes were collected by centrifugation of the 15,000*g* supernatant at 105,000*g* for 90 min. The supernatant was spun again at 105,000*g* for 60 min and filtered through cheesecloth to remove any floating fatty material. Mitochondrial and microsomal pellets were washed by resuspension in buffer and centrifugation at the appropriate rotor speeds. Fresh liver equal to the weight of the preen gland also was homogenized and centrifuged as described above for the glandular tissue.

Four milliliters of the 105,000*g* supernatant (150–200 mg of protein) were applied to a Sepharose 4B column (2 × 90 cm) previously equilibrated with 100 m*M* phosphate buffer (pH 7.6) containing 20 m*M* sodium citrate and 0.5 m*M* dithioerythritol. The same buffer was passed through the column for protein fractionation at a rate of 0.2 ml/min. Fractions of 2.5 ml were collected and assayed for carboxylase and decarboxylase activities. Active fractions were pooled and stored at 0–4° until use. All isolation and purification procedures were done at 0–4°.

Enzyme Assays. Acetyl-CoA and propionyl-CoA carboxylase activities were determined with the HCO_3^- fixation assay described by Gregolin et al. (1968). The standard reaction mixture consisted of 10 μmol of phosphate (pH 7.6), 0.05 μmol of dithioerythritol, 0.33 μmol of ATP, 1 μmol of $MgCl_2$, 2 μmol of sodium citrate, 3.8 μmol of $NaH^{14}CO_3$ (0.26 Ci/mol), 0.12 μmol of acetyl-CoA or propionyl-CoA, 75 μg of bovine serum albumin, and enzyme in a total volume of 0.1 ml. In control reaction mixtures acetyl-CoA or propionyl-CoA were omitted. After incubation for 20 min at 30°, the reaction was terminated with 0.1 ml of 6 *N* HCl and the acidified reaction mixture was evaporated to dryness. The acid-stable reaction products were dissolved in 0.2 ml of H_2O and transferred to a counting vial with 15 ml of a mixture of 30% ethanol in toluene containing 4 g of Omnifluor/l. The radioactivity was determined with a Packard Model 3003 TriCarb liquid scintillation spectrometer. An internal standard of [^{14}C]toluene was used to determine a counting efficiency of 74%.

Malonyl-CoA decarboxylase was assayed in stoppered 25-ml erlenmeyer flasks with center wells. The standard reaction mixture consisting of 10 μmol of Tris-Cl (pH 9.0), 30 μmol of [1,3-^{14}C]malonyl-CoA (0.63 Ci/mol), and enzyme in a total volume of 0.1 ml was placed in the center well of each flask. The main compartment of each flask contained 0.5 ml of 1 *N* hyamine hydroxide in methanol. After incubation for 10 min at 30° the reaction was terminated by the addition of 0.05 ml of 1 *M* H_3PO_4 into the center well. After gentle shaking for 30 min the hyamine hydroxide solution was transferred to counting vials with scintillation fluid and the $^{14}CO_2$ trapped was determined by liquid scintillation spectrometry. Control reaction mixtures contained heat treated enzyme solutions (100°, 10 min).

Malonyl-CoA decarboxylase was also occasionally assayed by terminating the reaction by the addition of 0.1 ml of 6 *N* HCl evaporating the acidified solution to dryness and counting the acid-stable residue by liquid scintillation spectrometry. The acid-stable reaction products were identified by paper chromatography as described in the following section.

Protein was determined by the method of Lowry et al. (1951) after precipitation of the protein with 10% trichloroacetic acid. Bovine serum albumin served as standard.

Analysis of Products. Labeled acetyl-CoA, malonyl-CoA, and methylmalonyl-CoA were identified as reaction products from acidified incubation mixtures by descending paper chromatography with isobutyric acid–1 *N* ammonium acetate–water (57:35:8) as the developing solvent. These coenzyme A esters were also identified by preparation of their hydroxamic acid derivatives and analysis by paper chromatography with isoamyl alcohol–pyridine–water (1:1:1) as the descending solvent. The hydroxamic acid derivatives were prepared by mixing the incubation mixtures with an equal volume of 14% hydroxylamine hydrochloride in 7% sodium hydroxide. Radioactive regions of paper chromatograms were located with a Berthold radioactivity scanner and the labeled compounds were eluted from the paper with H_2O. Other experimental details are described under the appropriate figures and tables.

Results and Discussion

Isolation and Partial Purification of Goose Uropygial Gland Carboxylase. The bulk of the fatty acids of the uropygial gland oil of goose is multi-branched (Murray, 1962; Odham, 1963; Buckner and Kolattukudy, unpublished results). However, a partially purified fatty acid synthetase from this gland catalyzed the synthesis of *n*-fatty acids from malonyl-CoA much faster than it did multi-branched fatty acid synthesis from methylmalonyl-CoA (Buckner and Kolattukudy, 1975). Therefore, availability of malonyl-CoA and methylmalonyl-CoA was suspected to regulate the synthesis of *n*- and multi-branched fatty acids in the uropygial glands and the availability of these coenzyme A derivatives should be controlled by acetyl-CoA and/or propionyl-CoA carboxylase. Therefore, several subcellular fractions from uropygial glands were examined for carboxylase activity and only the soluble supernatant contained substantial levels of activity (Table I). The soluble supernatant catalyzed carboxylation of propionyl-CoA but acetyl-CoA carboxylase activity was very low and often not detectable. This propionyl-CoA carboxylase activity required ATP and Mg^{2+} and was activated by citrate in a manner similar to that known for acetyl-CoA carboxylase from other animal sources (Greenspan and Lowenstein, 1968; Gregolin et al., 1968). As expected, it was inhibited by avidin and this inhibition could be overcome with the addition of biotin (data not presented).

These results indicated a possible mechanism by which preferential synthesis of multi-branched acids could occur. However, the specific carboxylation of propionyl-CoA (not acetyl-CoA) catalyzed by the crude cell-free preparations suggests either that the carboxylase itself is highly specific for propionyl-CoA or that some other component present in the system prevented carboxylation of acetyl-CoA. In order to distinguish between these possibilities, the 105,000*g* supernatant was fractionated. Removal of small molecular weight materials from the extract by gel filtration through Sephadex G-25 did not result in any increase in the acetyl-CoA carboxylase activity of the preparation (Table I). Fractionation of the proteins by gel filtration with Sepharose 4B gave an elution profile shown in Figure 1. The propionyl-CoA carboxylase activity was located in fractions eluted from the column just after the void volume. This procedure resulted in a 25–40-fold increase in the specific activity of the carboxylase. With this partially purified prepara-

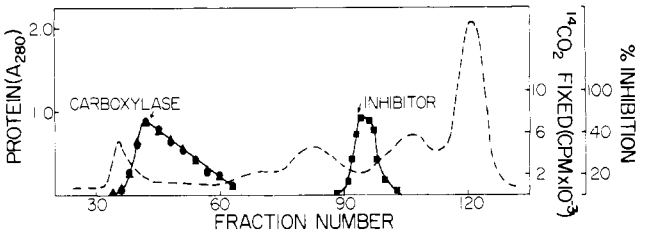

FIGURE 1: Chromatogram of 105,000*g* supernatant filtered through a Sepharose 4B column (2.3 × 90 cm). Absorbance at 280 nm (- - -); acetyl-CoA (●) and propionyl-CoA (▲) carboxylase activities and carboxylase inhibitor activity (■) are plotted. Carboxylase activity was determined with 20 μl of each fraction added to the reaction mixtures and assayed as described in the Experimental Section. Fractions assayed for inhibition of acetyl-CoA carboxylase activity were diluted tenfold and 5 μl was added to each assay.

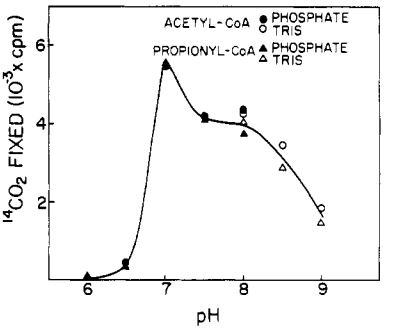

FIGURE 2: Effect of pH on the carboxylation of acetyl-CoA and propionyl-CoA by the partially purified carboxylase. The reaction mixtures contained 1.5 μg of enzyme and assay conditions were as described in the Experimental Section.

Table I: Subcellular Localization of Carboxylase Activity from the Uropygial Gland of Goose and the Effects of Sephadex G-25 Filtration on the Activity of the 105,000*g* Supernatant.[a]

Fraction	Carboxylase Activity (nmoles of $^{14}CO_2$ Fixed)	
	Acetyl-CoA	Propionyl-CoA
Experiment 1		
Crude homogenate	1.8	11.1
Mitochondria	<0.1	0.2
Microsomes	0.1	0.9
105,000*g* supernatant	0.7	9.9
Experiment 2		
105,000*g* supernatant	0.5	18.3
Sephadex G-25	0.2	14.1

[a] In Experiment 1, the reaction mixtures were incubated at 30° for 30 min. The protein added to each assay represented 0.07% of the crude homogenate from one uropygial gland. Amounts of protein added per assay were as follows: crude homogenate, 0.49 mg; mitochondria, 0.04 mg; microsomes, 0.02 mg; 105,000*g* supernatant, 0.35 mg. Collection of the subcellular fractions and other assay conditions are as described in the Experimental Section. In experiment 2, reaction mixtures contained 50 μg of protein. The Sephadex G-25 protein was obtained by filtering the 105,000*g* supernatant through a 2 × 30 cm column Sephadex G-25 equilibrated with 100 m*M* phosphate (pH 7.6) containing 20 m*M* citrate, and 0.5 m*M* dithioerythritol. Assay conditions are as described in the Experimental Section.

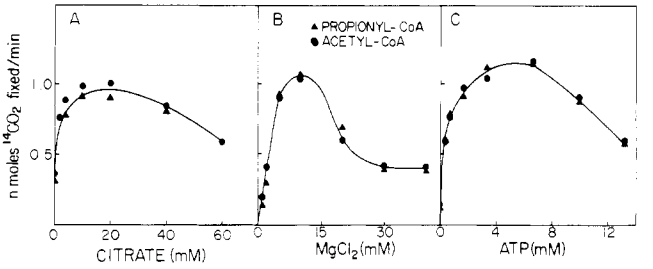

FIGURE 3: Effects of the concentrations of sodium citrate (A), $MgCl_2$ (B), and ATP (C) on acetyl-CoA and propionyl-CoA carboxylase activities. The reaction mixtures contained 3 μg of partially purified carboxylase and the assay conditions were as described in the Experimental Section except where indicated.

tion, carboxylation of propionyl-CoA was linear up to at least 20 min and 0.2 mg/ml of protein and all assays were done within the linear range for time and protein.

The Sepharose 4B column effluent was also examined for carboxylase activity with acetyl-CoA as substrate and the results showed that the same fractions which contained the propionyl-CoA carboxylase activity (Figure 1) also carboxylated acetyl-CoA at the same rate. Carboxylation rates for acetyl-CoA were also linear at least up to 20 min and 0.2 mg/ml of protein.

Properties of the Partially Purified Acetyl-CoA and Propionyl-CoA Carboxylase. The comparable rates of carboxylation for acetyl-CoA and propionyl-CoA and the fact that in the gel filtration step the two activities cochromatographed (Figure 1) suggest that the two substrates are carboxylated by the same enzyme. This possibility was examined by further characterization of the partially purified carboxylase using acetyl-CoA and propionyl-CoA as substrates. The identical pH dependence of the carboxylation of acetyl-CoA and propionyl-CoA is shown in Figure 2. Optimum rates for both substrates was at pH 7.0 with a shoulder extending to pH 8.0. The carboxylation rate of both acetyl-CoA and propionyl-CoA by the partially purified enzyme increased upon the addition of citrate and maximal stimulation of activity was obtained at concentrations between 10 and 20 m*M* citrate for both substrates (Figure 3A). The enzyme was also fully activated by 20 m*M* isocitrate but little, if any, activation could be demonstrated with 20 m*M* malate, succinate, or malonate. This carboxylase activity is similar to chicken liver carboxylase (Gregolin et al., 1968) in that the carboxylase was immediately activated upon addition to solutions containing citrate or isocitrate and did not require preincubation with the tricarboxylic acid. This property of immediate activation with citrate is in contrast to the acetyl-CoA carboxylases from rat liver and adipose tissue which require a 15–20-min incubation period with citrate and Mg^{2+} at 25 or 37° for full activation whereas the purified enzymes from *Escherichia coli* and Brewer's yeast are not activated by tricarboxylic acids (Numa and Ringelmann, 1965; Greenspan and Lowenstein, 1967; Matsuhashi et al., 1964; Alberts and Vagelos, 1968).

The effects of magnesium ion and ATP concentrations on the rates of carboxylation of acetyl-CoA and propionyl-CoA are illustrated in Figure 3B and C. The carboxylation rates of both substrates increased in an identical manner with increasing concentrations of Mg^{2+} and maximal rates were obtained with both substrates at 10 m*M* Mg^{2+}. The activity with 10 m*M* manganese ion was only 30% of that obtained with 10 m*M* Mg^{2+}. The acetyl-CoA and propionyl-CoA carboxylation rates with increasing concentrations of ATP were identical for both acetyl-CoA and propionyl-CoA and the optimum concentration of ATP was between 3.5 and 7.0 m*M* for the carboxylation of either substrate, Figure 3C.

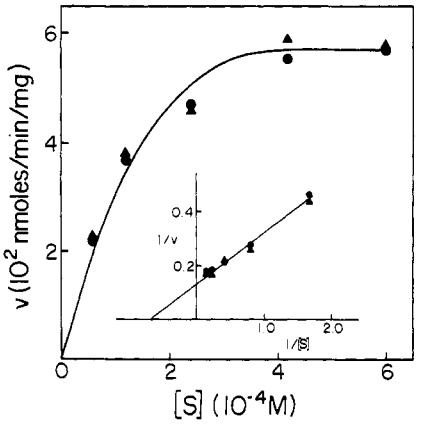

FIGURE 4: Effects of acetyl-CoA (●) and propionyl-CoA (▲) concentrations on the rate of $^{14}CO_2$ fixation by the partially purified carboxylase from goose uropygial glands. Each reaction mixture contained 0.8 μg of protein and assay conditions were as described in the Experimental Section.

The partially purified enzyme was incubated with increasing concentrations of acetyl-CoA and propionyl-CoA and the rates of carboxylation resulted in typical saturation curves and double reciprocal plots were linear (Figure 4). Calculation from these plots yielded an apparent K_m for both acetyl-CoA and propionyl-CoA of 1.5×10^{-4} *M*. The V_{max} for acetyl-CoA and propionyl-CoA was 770 nmol per min per mg. From these data as well as the precedent that chicken liver acetyl-CoA carboxylase also carboxylates propionyl-CoA (Gregolin et al., 1968), we tentatively conclude that both acetyl-CoA and propionyl-CoA are carboxylated by the same enzyme in the uropygial glands of goose.

Isolation of an Inhibitor of Acetyl-CoA Carboxylation. Since the crude preparations (105,000*g* supernatant) catalyzed carboxylation of only propionyl-CoA and not acetyl-CoA such preparations must have contained an agent which prevented specifically the carboxylation of acetyl-CoA. Since gel filtration with Sephadex G-25 did not remove this agent from the preparation (Table I), it must be a relatively large molecule.

In order to investigate the nature of this agent, the various fractions obtained by the Sepharose 4B gel filtration step were examined for their ability to prevent carboxylation of acetyl-CoA catalyzed by the partially purified carboxylase preparation. Such an acetyl-CoA carboxylase inhibitory activity was found in fractions which were eluted much later than the carboxylase (Figure 1). The addition of 9 μg of this inhibitor fraction to a carboxylase preparation which had a specific activity of over 400 nmol per min per mg decreased the rate of acetyl-CoA carboxylation by over 90% while the carboxylation of propionyl-CoA was hardly affected (Table II). Heat treatment of the inhibitor fraction at 90–100° for 10 min resulted in a total loss of inhibitory activity suggesting that the factor is a heat-labile protein. If the factor is truly an inhibitor of the carboxylation of acetyl-CoA, its effect might also be shown on acetyl-CoA carboxylase of other tissues. In fact the inhibitor was effective in decreasing the rates of carboxylation of acetyl-CoA catalyzed by liver extracts of the goose and chicken with little effect on the propionyl-CoA carboxylation by the goose liver extract (Table II).

Identity of the Inhibitor as Malonyl-CoA Decarboxylase. Since carboxylation of acetyl-CoA and propionyl-CoA involve the same reaction components, the specific effect of the inhibitory factor on acetyl-CoA carboxylase activity could be due to removal of either the substrate or the product of the reaction. For example, acetyl-CoA might undergo hydrolysis or condensation with some other components such as oxaloacetate. This possibility was ruled out by the fact that after incubation of [1-^{14}C]acetyl-CoA and the inhibitory factor with or without carboxylase, the labeled material was recovered virtually quantitatively and all the ^{14}C was shown to be in acetyl-CoA. Identity of acetyl-CoA was confirmed by paper chromatography of both the coenzyme-A ester and the hydroxamic acid derivative of the ester. Under the same assay conditions, incubation of labeled acetyl-CoA and the carboxylase without the factor gave the same amount of labeled malonyl-CoA as predicted from the carboxylase assays with labeled HCO_3^-. Therefore, the possibility that the inhibitory effect is due to destruction of the substrate can be ruled out.

Table II: Carboxylation of Acetyl-CoA and Propionyl-CoA by Cell-Free Preparations from Uropygial Glands and Liver of Goose and from Chicken Liver.[a]

Experimental Conditions	Carboxylase Activity (nmoles $^{14}CO_2$ fixed/min/mg)	
	Acetyl-CoA	Propionyl-CoA
Goose gland		
Sepharose 4B	443.6	418.8
Sepharose 4B + inhibitor	30.5	370.8
Sepharose 4B + boiled inhibitor	444.5	399.2
Goose liver		
Sephadex G-25	2.8	5.5
Sephadex G-25 + inhibitor	0.3	3.5
Chicken liver		
Sephadex G-25	2.9	
Sephadex G-25 + inhibitor	0.8	

[a] In experiments with the gland extracts and liver extracts 3 and 50 μg of protein, respectively, were used. Where indicated, 9 μg of the inhibitor fraction from Sepharose 4B was added.

In order to test whether the inhibitory effect is due to the removal of the product, [1,3-^{14}C]malonyl-CoA was incubated, under the carboxylation assay condition, with the inhibitor fraction obtained from the Sepharose 4B gel filtration. A paper chromatographic examination of the acid-stable products revealed two labeled materials one of which had an R_f (0.53) identical with that of authentic acetyl-CoA while the other was malonyl-CoA (R_f 0.32). Acetyl-CoA contained 38% of the total ^{14}C. Furthermore, the amount of $^{14}CO_2$ released from the malonyl-CoA by the inhibitor preparation was identical with the amount of labeled acetyl-CoA which was recovered from the reaction mixture. This stoichiometry shows that the inhibitor preparation catalyzed the decarboxylation of malonyl-CoA to produce acetyl-CoA and CO_2. Therefore, it appears probable that inhibition of carboxylation caused by this fraction is due to its malonyl-CoA decarboxylase activity.

Properties of the Partially Purified Decarboxylase. The partially purified decarboxylase from Sepharose 4B gel filtration catalyzed decarboxylation of malonyl-CoA at linear rates at least up to 20 min and 10 μg/ml of protein. As a result of these findings, standard enzyme assays were done with a 10-min incubation period and 5–10 μg/ml of protein. Specific activities and substrate specificity for the decarboxylase are given in Table III. Malonyl-CoA was decarboxylated at a rate of about 1 μmol per min per mg of protein. This specific activity for the decarboxylation of mal-

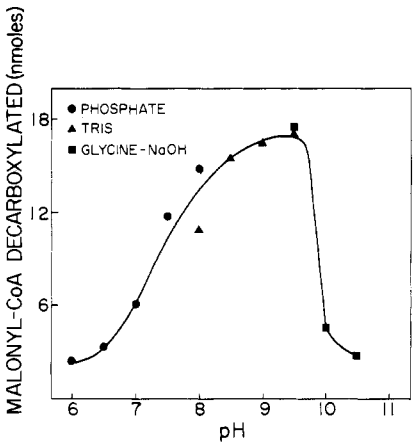

FIGURE 5: Effect of pH on the rate of decarboxylation of malonyl-CoA. Reaction mixtures contained 1 μg of partially purified decarboxylase in a total volume of 0.1 ml and assay conditions were as described in the Experimental Section.

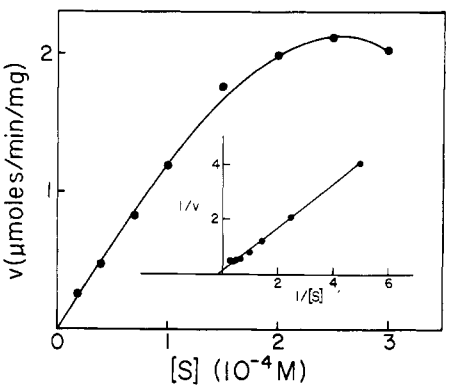

FIGURE 6: Effects of malonyl-CoA concentration on the rate of decarboxylation of malonyl-CoA by the partially purified decarboxylase. Assays were run at pH 9.5 in 100 m*M* Tris-Cl buffer containing 0.5 m*M* dithioerythritol with 0.4 μg of partially purified decarboxylase. Other assay conditions were as described in the Experimental Section.

Table III: Decarboxylase Activity of Cell-Free Preparations from Goose Uropygial Glands.[a]

Experimental Conditions	Decarboxylase Activity (nmoles of $^{14}CO_2$ per min per mg)
Sepharose 4B effluent	
Malonyl-CoA	1080
Malonyl-CoA + avidin	1120
Methylmalonyl-CoA	12
Malonic acid	20
Sephadex G-25 effluent	
Malonyl-CoA	366
Methylmalonyl-CoA	14

[a] Reaction mixtures contained, where indicated, 30 nmol of [1,3-^{14}C]malonyl-CoA (0.63 Ci/mol), [3-^{14}C]methylmalonyl-CoA (59.5 Ci/mol), or [1,3-^{14}C]malonic acid (0.63 Ci/mol), and 100 μg of avidin. Assays with the Sepharose 4B effluent contained 1 μg of protein and those with Sephadex G-25 effluent 2.3 μg of protein. Assay conditions were as described in the Experimental Section.

onyl-CoA is about threefold higher than the specific activity for the carboxylation of acetyl-CoA (Table II). In contrast to the high specific activity of the decarboxylase observed in the goose gland, the specific activity of malonyl-CoA decarboxylase preparations from other sources, such as plants (Hatch and Stumpf, 1962), yeast (Lynen et al., 1962), rat liver (Scholte, 1969), and rat brain (Koeppen et al., 1974), was relatively low and was often expressed as a contaminating activity in the purification procedures for fatty acid synthetase (Bressler and Wakil, 1961; Martin et al., 1961; Lynen, 1962).

The malonyl-CoA decarboxylase was not inhibited by avidin (Table III). This finding suggests that the decarboxylation reaction is not the reverse reaction of an avidin-sensitive acetyl-CoA carboxylase such as that reported for chicken liver carboxylase (Gregolin et al., 1968). If this decarboxylase activity is responsible for the inhibitory effect of this fraction on the acetyl-CoA carboxylase, the decarboxylase should preferentially decarboxylate malonyl-CoA and not methylmalonyl-CoA. In fact the rate of decarboxylation of methylmalonyl-CoA was less than 2% of that observed with malonyl-CoA. The substrate specificity of this enzyme is further illustrated by the observation that malonic acid was not decarboxylated at significant rates.

pH Dependence. The effect of pH on the activity of malonyl-CoA decarboxylase was determined and the results are shown in Figure 5. The rate of decarboxylation was highest between pH 8 and 9.5 and the activity dropped sharply above pH 9.5. This pH optimum is higher than the optimal pH range of 6–7.5 reported for the partially purified malonyl-CoA decarboxylase from yeast (Lynen et al., 1962) and slightly higher than the pH optimum for the mitochondria-bound malonyl-CoA decarboxylase from rat brain (Koeppen et al., 1974). The possibility of hydrolysis of the thioester substrate at higher pH to yield malonic acid and coenzyme-A was ruled out by paper chromatographic analysis of the reaction products showing that no malonic acid was present. The specific activity for the decarboxylase at pH 9.5 was threefold higher than the activity at pH 7.0, which was the pH of the prior decarboxylase assays (Table III).

Effect of Substrate Concentration on Decarboxylase Activity. Increasing concentrations of malonyl-CoA increased the rate of decarboxylation and further increases in substrate concentration resulted in a typical substrate saturation pattern (Figure 6). The double reciprocal plot was linear and from it a K_m and V_{max} of 8×10^{-4} *M* and 12,500 nmol per min per mg were calculated. At pH 7.0 the K_m decreased to 3×10^{-4} *M* and V_{max} decreased to 2400 nmol per min per mg (data not presented). Although the rate of decarboxylation of methylmalonyl-CoA was very low, the effect of its concentration on the decarboxylation rate was also determined at pH 7.0. From linear double reciprocal plots K_m and V_{max} for this substrate was calculated to be 2×10^{-3} *M* and 80 nmol per min per mg, respectively. These values clearly demonstrate that the decarboxylase is highly specific for malonyl-CoA.

Malonyl-CoA Decarboxylase as a Possible Regulator of Multi-Branched Fatty Acid Synthesis. The summary of the kinetic parameters for acetyl-CoA carboxylation, propionyl-CoA carboxylation, malonyl-CoA decarboxylation, and methylmalonyl-CoA decarboxylation is shown in Table IV. Although these values cannot be applied directly to in vivo conditions, they indicate that the rate of malonyl-CoA decarboxylation could prevent availability of malonyl-CoA for *n*-fatty acid synthesis. On the other hand, methylmalonyl-CoA should be readily available for the synthesis of multi-branched fatty acids. Thus if these properties of the isolated

Table IV: Summary of the Kinetics of the Partially Purified Carboxylase and Decarboxylase Activities from Goose Uropygial Glands.[a]

Experimental Conditions	K_m (M)	V_{max} (nmoles per min per mg)
Decarboxylase, pH 7.0		
Malonyl-CoA	3×10^{-4}	2,400
Methylmalonyl-CoA	2×10^{-3}	80
Decarboxylase, pH 9.5		
Malonyl-CoA	8×10^{-4}	12,500
Carboxylase, pH 7.6		
Acetyl-CoA	1.5×10^{-4}	770
Propionyl-CoA	1.5×10^{-4}	770

[a] Apparent K_m and V_{max} values for the partially purified decarboxylase were calculated from data presented in Figure 6. Assay conditions for decarboxylase activity are as described in the Experimental Section. K_m and V_{max} values for carboxylase were calculated from data presented in Figure 4.

enzymes are relevant to what actually occurs in the gland, in vivo, acetyl-CoA should be the most available primer and methylmalonyl-CoA the available elongating agent for fatty acid synthesis. Therefore, one should expect the major fatty acids to be those derived from acetyl-CoA and methylmalonyl-CoA. In fact, analysis of the naturally occurring fatty acids of the gland shows clearly that the major fatty acid of the gland is 2,4,6,8-tetramethydecanoic acid which is synthesized from one molecule of acetyl-CoA and four molecules of methylmalonyl-CoA (Buckner and Kolattukudy, 1975). Thus, these in vitro studies do reflect the in vivo situation and the major control for the synthesis of multibranched fatty acids appears to be at the carboxylase level in that the availability of the carboxylated product is most probably controlled by the malonyl-CoA decarboxylase.

Since malonyl-CoA decarboxylase occurs in relatively large quantities in the glandular tissue, it might prevent malonyl-CoA synthesis in vivo resulting in the preferential formation of methylmalonyl-CoA and thus allow preferential synthesis of multibranched fatty acids. If this explanation is valid, other tissues such as liver which do not synthesize multi-branched fatty acids, may not contain high levels of malonyl-CoA decarboxylase. To test this possibility, the 105,000g supernatant, prepared from fresh liver homogenates, was filtered through Sephadex G-25. The resulting filtrate did not contain measurable levels of decarboxylase activity. This apparent specific localization of the decarboxylase in the gland, and not in the liver, supports the hypothesis that the enzyme plays a key role in the production of multi-branched fatty acids.

If the regulatory role proposed for malonyl-CoA decarboxylase is valid, one might expect to find high malonyl-CoA decarboxylase activity in other systems which synthesize large quantities of multi-branched fatty acids. Multibranched fatty acids are known to be a major constituent of the uropygial gland lipids of the muscovy duck, a species of bird from a different subfamily than the goose (Odham, 1967a,b). The glandular tissue from this bird was examined for malonyl-CoA decarboxylase in a manner similar to that described for the goose. A very active malonyl-CoA decarboxylase was isolated and purified from this tissue. The activity level and the properties of the enzyme were very similar to those of the decarboxylase from the goose gland (data not presented).

It might appear that decarboxylation of malonyl-CoA is a wasteful method of channeling carbon into multibranched fatty acids. However, this route might be advantageous from an evolutionary point of view as it allows conservation of the complex regulatory carboxylase enzyme. Furthermore, such a regulation could allow a single fatty acid synthetase to catalyze the formation of n- and multibranched fatty acids. However, it is not known whether n- and multi-branched fatty acids are synthesized by the same enzyme complex, although experimental results obtained thus far in this laboratory have indicated the involvement of a single enzyme complex.

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