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Succinylation and inactivation of 3-hydroxy-3-methylglutaryl-CoA synthase by succinyl-CoA and its possible relevance to the control of ketogenesis

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Succinyl-CoA (3-carboxypropionyl-CoA) inactivates ox liver mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (EC 4.1.3.5) in a time-dependent manner, which is partially prevented by the presence of substrates of the enzyme. The inactivation is due to the enzyme catalysing its own succinylation. Complete inactivation corresponds to about 0.5 mol of succinyl group bound/mol of enzyme dimer. The succinyl–enzyme linkage appears to be a thioester bond and is probably formed with the active-site cysteine residue that is normally acetylated by acetyl-CoA. Succinyl-CoA binds to 3-hydroxy-3-methylglutaryl-CoA synthase with a binding constant of 340 μM and succinylation occurs with a rate constant of 0.57 min^{-1}. Succinyl-enzyme breaks down with a half-life of about 40 min ($k = 0.017\ min^{-1}$) at 30 °C and pH 7 and is destabilized by the presence of acetyl-CoA and succinyl-CoA. A control mechanism is postulated in which flux through the 3-hydroxy-3-methylglutaryl-CoA cycle of ketogenesis is regulated according to the extent of succinylation of 3-hydroxy-3-methylglutaryl-CoA synthase.

INTRODUCTION

The existence of the HMG-CoA cycle of acetoacetate synthesis from acetyl-CoA in liver is well established, but its regulation remains poorly understood (McGarry & Foster, 1980). There has been much discussion of the importance of the mitochondrial [acetyl-CoA]/[CoA] ratio on the process (Fritz & Lee, 1974; Siess *et al.*, 1978), but no control mediated by changes in enzyme activities has been identified. It has been suggested that HMG-CoA synthase (EC 4.1.3.5) activity may be rate-limiting (Dashti & Ontko, 1979), but so far this enzyme has not been shown to be affected by influences other than the concentrations of its substrates and products.

In the present paper we report that succinyl-CoA (3-carboxypropionyl-CoA) inactivates ox liver mitochondrial HMG-CoA synthase, as a consequence of the enzyme catalysing its own succinylation. This is analogous to acetylation of HMG-CoA synthase by acetyl-CoA, which is the first step in the enzyme-catalysed synthesis of HMG-CoA (Scheme 1) (Middleton & Tubbs, 1974; Miziorko & Lane, 1977; Lowe & Tubbs, 1985*a*,*b*), but the succinyl-enzyme is incapable of reacting with acetoacetyl-CoA.

Siess *et al.* (1980) have shown that glucagon treatment of perfused livers and of isolated hepatocytes strikingly lowers the mitochondrial succinyl-CoA content. Similar treatment stimulates ketogenesis (McGarry *et al.*, 1975; Keller *et al.*, 1977; McGarry & Foster, 1980). We suggest a possible control mechanism in which glucagon (and perhaps other hormones) regulates the ketogenic flux through the HMG-CoA pathway by affecting the concentration of succinyl-CoA and, hence, the extent of succinylation of HMG-CoA synthase.

$$E + \text{Ac-CoA} \rightleftharpoons \text{E-Ac} + \text{CoA} \quad (1)$$

$$\text{E-Ac} + \text{Acac-CoA} + H_2O \rightleftharpoons E + \text{HMG-CoA} \quad (2)$$

Scheme 1. Enzymic formation of HMG-CoA

Abbreviations: E, enzyme; Ac, acetyl; Acac, acetoacetyl.

EXPERIMENTAL

Materials

CoA and acetoacetyl-CoA were obtained from P-L Biochemicals (Milwaukee, WI, U.S.A.). Acetyl-CoA was prepared by treating CoA with acetic anhydride. All other acyl-CoA thioesters and GTP (type VII, lithium salt) were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). Desulpho-CoA was prepared by the action of Raney nickel on CoA (Chase *et al.*, 1966), and *S*-methyl-CoA was prepared by treating CoA (25 mM) with methyl iodide (75 mM) for 1 h at 20 °C. CoA disulphide was prepared by incubating a solution of CoA in O_2-saturated 0.1 M-KH_2PO_4/KOH buffer, pH 8, at 22 °C for 16 h. [1-^{14}C]Acetyl-CoA (4 or 5.55 Ci/mol) and [2,3-^{14}C]succinic acid (100 Ci/mol) were obtained from Amersham International (Amersham, Bucks., U.K.). [2,3-^{14}C]Succinyl-CoA was prepared by exchange between [2,3-^{14}C]succinate (0.25 μmol; 25 μCi) and unlabelled succinyl-CoA (2.25 μmol), catalysed by succinyl-CoA:3-oxo-acid CoA-transferase (EC 2.8.3.5; from pig heart) (Hersh & Jencks, 1967). The reaction was performed at 30 °C for 3 min with 10 units of CoA transferase in a final volume of 0.3 ml. The [2,3-^{14}C]succinyl-CoA was purified by h.p.l.c. by the method of Corkey *et al.* (1981).

Abbreviation used: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA.

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Succinyl-CoA synthetase (EC 6.2.1.4; from pig heart) was obtained from Boehringer (Mannheim, W. Germany).

HMG-CoA synthase from ox liver mitochondria was purified and assayed as described by Lowe & Tubbs (1985*a*). The assay follows the breakdown of acetoacetyl-CoA (10 μM) at 30 °C in the presence of acetyl-CoA (100 μM) by monitoring the A_{303} of the enol form. One unit of enzyme activity is defined as the amount of enzyme that causes 1 μmol of acetoacetyl-CoA to be transformed/min. The final specific activity was between 0.5 and 1.0 unit/mg of protein. Unless otherwise stated, all experiments were performed with HMG-CoA synthase dissolved in 0.1 M-KH_2PO_4/KOH buffer, pH 7, containing 15% (v/v) glycerol, 0.1 mM-EDTA and 0.5 mM-dithiothreitol.

Protein determination

Protein was determined by using a modified Lowry technique as described by Peterson (1977), with bovine serum albumin as a standard. This assay gave protein concentrations for purified HMG-CoA synthase that agreed to within 10% of that determined by amino acid analysis.

Determination of covalently bound [^{14}C]succinyl-enzyme

Covalently bound [^{14}C]succinyl-enzyme was determined as trichloroacetic acid-precipitated protein, as described by Lowe & Tubbs (1985*b*). The amount of [^{14}C]succinyl-enzyme is expressed as mol of succinyl group bound/mol of synthase dimer (M_r 100000) throughout.

Centrifuge gel filtration

Protein was separated rapidly from unbound ligand by centrifuge gel filtration as described by Lowe & Tubbs (1985*b*).

RESULTS

Time-dependent inhibition by succinyl-CoA

Inclusion of succinyl-CoA (100 μM) in a standard ox liver HMG-CoA synthase assay resulted in a curved assay trace due to a time-dependent decrease in enzyme activity during the time of the assay (approx. 2 min). Preincubation of HMG-CoA synthase at 30 °C with succinyl-CoA (100 μM) and removal of samples for assay showed a rapid progressive inactivation of the enzyme followed by a slow recovery of activity (Fig. 1). For this experiment, the HMG-CoA synthase activity was assayed at intervals by diluting samples 100-fold into the standard assay mixture. This gave linear, inhibited, initial rates, demonstrating that the inhibition was irreversible over the time period of an assay. Preincubation in the presence of the substrates of HMG-CoA synthase gave some protection from the inhibition by succinyl-CoA, with acetoacetyl-CoA being more effective than acetyl-CoA (Fig. 1).

When the preincubation was performed at 0 °C, the extent of inhibition by succinyl-CoA was greatly diminished (Fig. 2). This behaviour is analogous to that seen for acetylation by acetyl-CoA and for inhibition by reagents that are thought to react with a cysteine residue at the active site of HMG-CoA synthase (Lowe & Tubbs, 1985*b*).

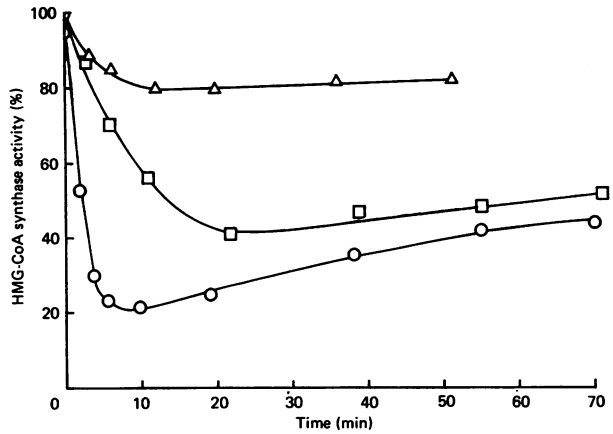


Fig. 1. Time course of inhibition of HMG-CoA synthase activity by succinyl-CoA

HMG-CoA synthase (1.1 mg of protein/ml; 11 μM) was incubated at 30 °C with 100 μM-succinyl-CoA, alone (○) or plus 100 μM-acetoacetyl-CoA (△), or plus 100 μM-acetyl-CoA (□). Samples (10 μl) were removed at the times indicated and assayed for HMG-CoA synthase activity. Results are expressed as percentages of controls, from which succinyl-CoA was omitted.

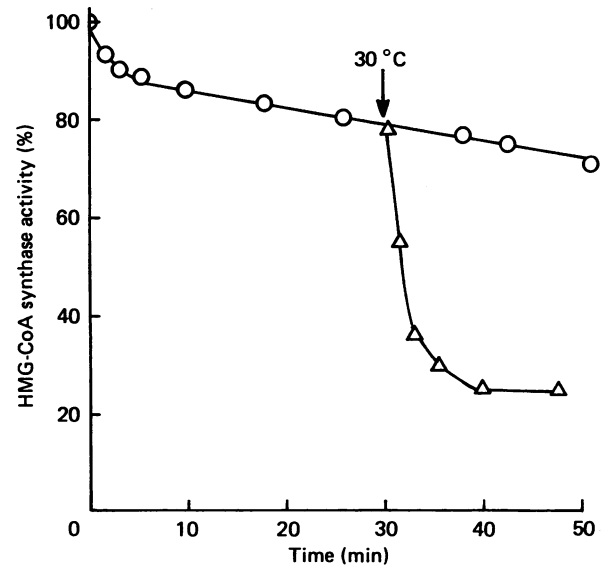


Fig. 2. Effect of temperature on the inhibition of HMG-CoA synthase activity by succinyl-CoA

HMG-CoA synthase (1.2 mg of protein/ml; 12 μM) was incubated with 200 μM-succinyl-CoA at 0 °C (○). After 30 min, the incubation was divided; half was moved to an incubation temperature of 30 °C (△), while the remainder was left at 0 °C. Samples (10 μl) were removed at the times indicated and assayed for HMG-CoA synthase activity. Results are expressed as percentages of controls, from which succinyl-CoA was omitted.

To test whether this type of inhibition occurs with any other CoA derivative, HMG-CoA synthase (10 μM) was incubated at 30 °C with between 0.2 and 1.5 mM of propionyl-CoA, *RS*-methylmalonyl-CoA, butyryl-CoA, malonyl-CoA, *S*-methyl-CoA, desulpho-CoA or CoA disulphide. None of these CoA derivatives caused any

time-dependent inactivation of the type seen with succinyl-CoA, although they all acted as poor reversible inhibitors when added directly to an assay system.

Formation of succinyl-*S*-enzyme

HMG-CoA synthase, that had been inhibited by about 80% by incubation with succinyl-CoA, was separated from excess reagent by rapid centrifuge gel filtration at 4 °C. The isolated enzyme remained inhibited during this procedure, but on incubation at 30 °C and pH 7 it slowly recovered about 90% of the original enzyme activity, with a half-life for the inhibited enzyme species of about 43 min. This isolated succinyl-CoA-modified enzyme (6 μM) was not acetylated when mixed immediately with 100 μM-[1-^{14}C]acetyl-CoA, although as enzyme activity returned so did the potential to be acetylated. This result suggests that succinyl-CoA might be succinylating the active-site thiol group to form an inactive succinyl-*S*-enzyme, which then breaks down.

To investigate this possibility we followed the time course of inhibition of the HMG-CoA synthase activity by [2,3-^{14}C]succinyl-CoA, simultaneously with taking samples to measure incorporation of covalently bound radioactivity (Fig. 3). Fig. 3 shows that there is a clear correlation between inactivation and incorporation of succinyl groups in a linkage that is acid-stable. The stoichiometry of about 0.5 mol of succinyl group bound/mol of synthase dimer is similar to that found for acetylation of HMG-CoA synthase by acetyl-CoA (Lowe & Tubbs, 1985*b*). Treatment of trichloroacetic acid-precipitated [^{14}C]succinyl-enzyme with performic acid for 1 h at 30 °C resulted in 95% release of [^{14}C]succinyl groups from the protein, compared with a control incubated with water. This is compatible with the bond being a thioester.

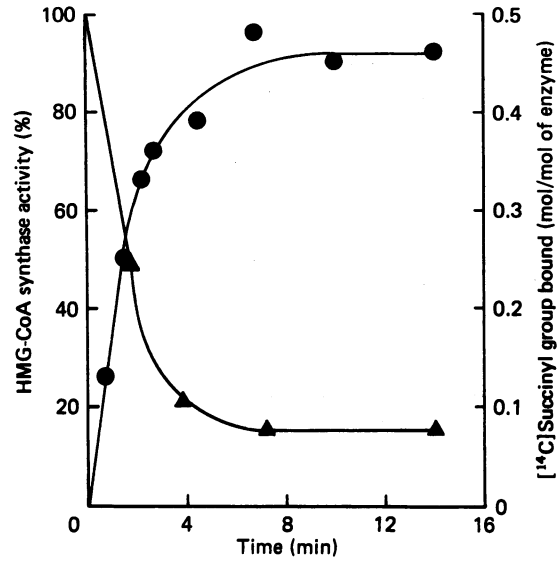


Fig. 3. Correlation between loss of enzyme activity and incorporation of radioactivity on reaction of HMG-CoA synthase with [^{14}C]succinyl-CoA

HMG-CoA synthase (1.6 mg of protein/ml; 16 μM) was incubated with 200 μM-[2,3-^{14}C]succinyl-CoA (9.2 Ci/mol) at 30 °C. At intervals, 10 μl samples were assayed for HMG-CoA synthase activity (▲), and 25 μl samples were precipitated with ice-cold 6% (w/v) trichloroacetic acid (1 ml) and used to determine the amount of protein-bound radioactivity (see the Experimental section) (●). The enzyme activities are expressed as percentages of a control, from which [2,3-^{14}C]succinyl-CoA was omitted. The enzyme activity of the control remained unchanged over the course of the experiment.

Breakdown of succinyl-enzyme

The stability of the thioester bond of succinyl-CoA was determined by monitoring the decrease in its absorbance band at 232 nm on incubation at 30 °C and pH 7. This gave a half-life for succinyl-CoA of 41 min. This half-life is very similar to that for succinyl-enzyme, measured either by monitoring the return of enzyme activity (see above) or by monitoring the loss of covalently bound radioactivity from isolated [^{14}C]succinyl-enzyme (Table 1). These two types of experiment were repeated in the presence of various derivatives of CoA to see what effect they had on the stability of succinyl-enzyme (Table 1). The two approaches give sufficiently similar results to confirm that the return of enzyme activity is due to breakdown of the covalent succinyl-enzyme. Acetyl-CoA

Table 1. Effect of CoA derivatives on the stability of succinyl-enzyme

[^{14}C]Succinyl-enzyme was prepared by incubating HMG-CoA synthase (1.8 mg of protein/ml; 18 μM) with 200 μM-[2,3-^{14}C]succinyl-CoA (9.2 Ci/mol) for 10 min at 30 °C and was isolated by centrifuge gel filtration. The isolated [^{14}C]succinyl-enzyme had 0.6 mol of [^{14}C]succinyl group bound/mol of synthase dimer and had only about 20% the original enzyme activity. The rate of breakdown of the [^{14}C]succinyl-enzyme was determined by incubating portions at 30 °C with and without various derivatives of CoA and taking samples at intervals for measurement of HMG-CoA synthase activity and the amount of protein-bound radioactivity (see the Experimental section). The half-lives given were obtained from semi-logarithmic plots of the data, which showed apparent first-order kinetics for about the first 10 min.

	Enzyme re-activation		Breakdown of [^{14}C]succinyl-enzyme	
Incubation conditions	$t_{\frac{1}{2}}$ (min)	k (min^{-1})	$t_{\frac{1}{2}}$ (min)	k (min^{-1})
No addition	43	0.016	38	0.018
+100 μM-CoA	—	—	35	0.020
+200 μM-CoA	24	0.029	—	—
+100 μM-acetoacetyl-CoA	35	0.020	28	0.025
+100 μM-acetyl-CoA	17	0.041	14	0.050
+100 μM-succinyl-CoA	—	—	20	0.035

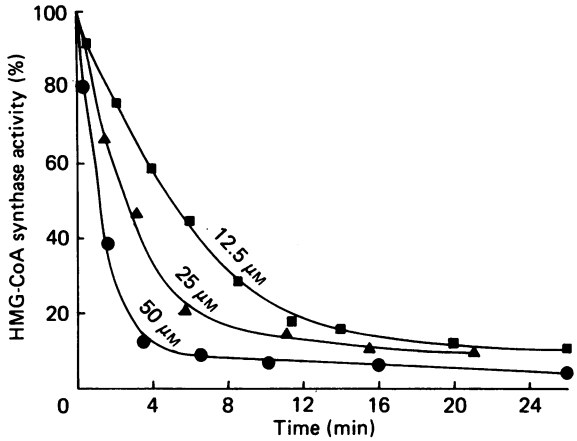


Fig. 4. Time course of inhibition of HMG-CoA synthase activity by various concentrations of succinyl-CoA in the presence of a succinyl-CoA-regenerating system

HMG-CoA synthase was gel-filtered into buffer containing 25 mM-succinate, adjusted to pH 7 with Tris (approx. 50 mM), 2 mM-$MgCl_2$, 0.2 mM-dithiothreitol, 15% (v/v) glycerol and 0.1 mM-EGTA. HMG-CoA synthase (0.2 ml of 1.6 mg of protein/ml; 16 μM) was warmed to 30 °C and mixed with GTP to a concentration of 0.5 mM and 2 μl (10 μg; 0.1 unit at 25 °C) of succinyl-CoA synthetase, before the inhibition reaction was initiated by the addition of succinyl-CoA (12.5, 25 or 50 μM). The concentrations of succinyl-CoA are indicated next to each line. Results are expressed as percentages of a control, from which succinyl-CoA was omitted.

was the most effective compound at stimulating de-succinylation, although succinyl-CoA was also effective, with CoA and acetoacetyl-CoA being less so (Table 1).

Kinetics of formation of succinyl-enzyme

The relative instability of succinyl-CoA and its stimulation of succinyl-enzyme hydrolysis result in deviation from pseudo-first-order kinetics and marked incomplete inhibition (cf. Figs 1, 2 and 3), which complicate kinetic studies on the inhibition of HMG-CoA synthase activity by succinyl-CoA. In an attempt to overcome this problem, incubations were carried out in the presence of a succinyl-CoA-regenerating system, with the enzyme succinyl-CoA synthetase (EC 6.2.1.4). The inclusion of this regenerating system in incubations of HMG-CoA synthase with succinyl-CoA had a dramatic effect on the potency of this inhibitor (Fig. 4). It was now possible to use equimolar amounts of succinyl-CoA and synthase and still get rapid inhibition of HMG-CoA synthase activity to extents close to 90%. As the concentration of succinyl-CoA was decreased, the extent of maximum inhibition was correspondingly diminished (Fig. 4). The rate of inhibition, however, was so rapid at high concentrations of succinyl-CoA as to preclude a sufficient number of consecutive spectrophotometric synthase activity assays being performed for the construction of accurate time courses. A different approach was therefore adopted, in which incorporation of radioactivity from [2,3-^{14}C]succinyl-CoA into the protein was measured (Fig. 5*a*). HMG-CoA synthase was found to have a lower activity after incubation in the buffer required for the regenerating system, which is

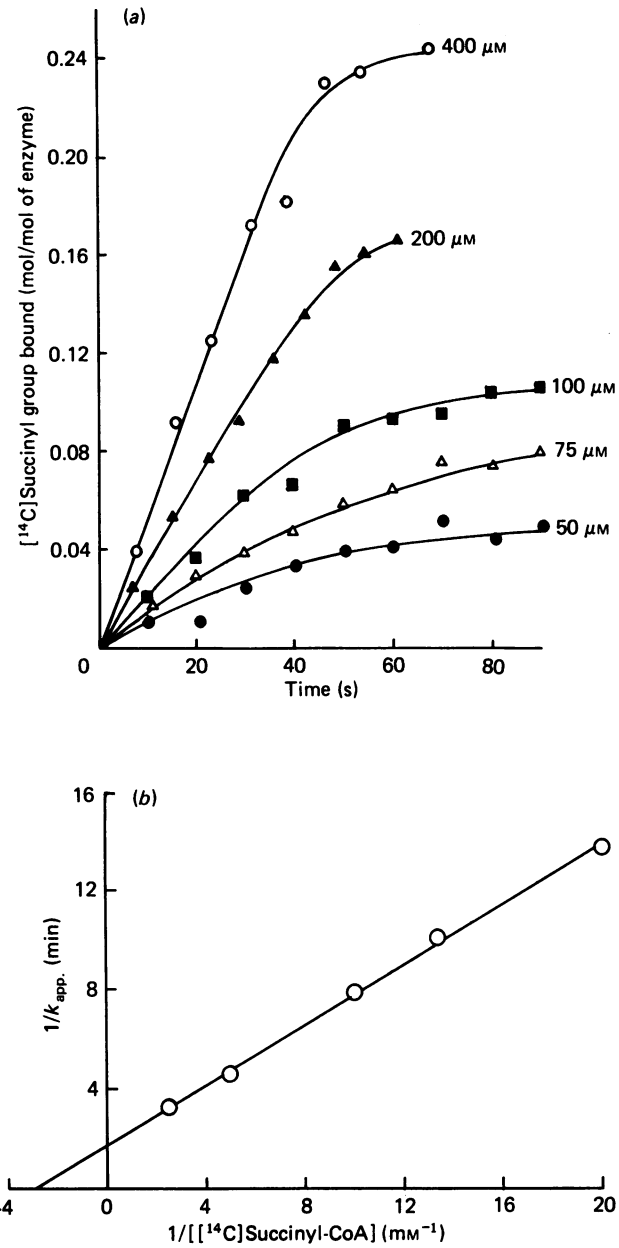


Fig. 5. Relationship between the concentration of [^{14}C]succinyl-CoA and the rate of formation of [^{14}C]succinyl-enzyme

(*a*) Incubations (0.1 ml) were performed at 30 °C with HMG-CoA synthase dissolved at 3 mg of protein/ml (30 μM) in medium composed of 25 mM-succinate/Tris, pH 7, 2 mM-$MgCl_2$, 0.2 mM-dithiothreitol, 15% (v/v) glycerol, 0.1 mM-EGTA and 0.5 mM-GTP and containing 1 μl (5 μg; 0.05 unit at 25 °C) of succinyl-CoA synthetase. The reaction was started by the addition of [2,3-^{14}C]succinyl-CoA (9.2 Ci/mol) (50, 75, 100, 200 or 400 μM). The reaction vessel was stirred with a magnetic 'flea' so that mixing occurred immediately. Samples (10 μl) were removed at intervals and precipitated into ice-cold trichloroacetic acid, before determination of the amount of protein-bound radioactivity (see the Experimental section). The concentrations of [2,3-^{14}C]succinyl-CoA are indicated next to each line. (*b*) Apparent first-order rate constants ($k_{app.}$) for the formation of [^{14}C]succinyl-enzyme were obtained from the initial rates of the data in (*a*) and are plotted against the concentration of [2,3-^{14}C]succinyl-CoA in double-reciprocal form.

thought to explain the low extents of [^{14}C]succinyl-group incorporation seen in this experiment. However, apparent first-order rate constants ($k_{app.}$) for the formation of [^{14}C]succinyl-enzyme were obtained from the initial rates of the data in Fig. 5(*a*) and are plotted against the concentration of [2,3-^{14}C]succinyl-CoA, in double-reciprocal form, in Fig. 5(*b*). For an inhibitor that binds to the enzyme in a reversible complex before undergoing a transition, with rate constant k_{+2}, to an irreversibly inhibited form, this double-reciprocal plot can be used to determine the values of k_{+2} and K_m^1, a binding constant (Kitz & Wilson, 1962; Chen & Engel, 1975). This gave values of 340 μM for K_m^1 and 0.57 min^{-1} for k_{+2} for the interaction of succinyl-CoA with HMG-CoA synthase.

Succinic anhydride (50 μM) was found to inhibit HMG-CoA synthase activity, with 70% inhibition being achieved in less than 1 min, and the inhibition slowly reversed with time. Acetic anhydride (50 μM), however, was without effect.

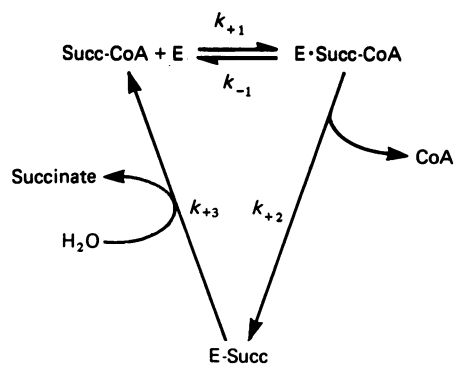
DISCUSSION

The inactivation of HMG-CoA synthase by succinyl-CoA can be represented by Scheme 2, in which succinyl-CoA binds reversibly at a specific site on the enzyme to form an E·Succ-CoA complex, which then reacts to form a thioester linkage with the enzyme, E–Succ, with the loss of CoA. This inactive succinyl-enzyme is then slowly hydrolysed to regenerate active enzyme (Scheme 2).

The site of succinylation is probably the same cysteine residue that is acetylated by acetyl-CoA during the normal enzyme reaction mechanism (Lowe & Tubbs, 1985*b*). Acetyl-CoA gives some protection against inactivation by succinyl-CoA (Fig. 1), although this protection is not complete, presumably owing to the turnover of the acetyl-enzyme (Lowe & Tubbs, 1985*b*). Acetoacetyl-CoA gives more effective protection, probably because it is bound with very high affinity by HMG-CoA synthase (Lowe & Tubbs, 1985*a*).

Scheme 2 accounts for the failure of succinyl-CoA to cause complete inactivation of HMG-CoA synthase (cf. Figs 1 and 4), since in the steady state at a saturating concentration of succinyl-CoA:

$$\frac{d[\text{E–Succ}]}{dt} = k_{+2}[\text{E·Succ-CoA}] - k_{+3}[\text{E–Succ}] = 0$$



Scheme 2. Proposed mechanism of reaction of succinyl-CoA with HMG-CoA synthase

Abbreviations: E, enzyme; Succ, succinyl.

Hence:

$$\frac{[\text{E–Succ}]}{[\text{E·Succ-CoA}]} = \frac{k_{+2}}{k_{+3}}$$

With values for k_{+2} of 0.57 min^{-1} (Fig. 5*b*) and for k_{+3} of 0.017 min^{-1} (Table 1), the ratio of k_{+2}/k_{+3} is 33.5, so that a steady-state maximum inhibition of about 97% should be achieved. This is very close to the maximum inhibition observed experimentally in the presence of the regeneration system for succinyl-CoA (Fig. 4). The observation that k_{+3} is considerably smaller than k_{+2} justifies using the initial rates of formation of succinyl-enzyme for the determination of k_{+2} (Fig. 5), since the breakdown rate would be initially insignificant relative to the rate of formation.

The situation described by Scheme 2 is closely analogous to that for the reaction of carbamates with cholinesterase (O'Brien *et al.*, 1966; O'Brien, 1968), and the kinetic characteristics are also identical with those for the interaction of various enzymes with pyridoxal 5′-phosphate (Chen & Engel, 1975).

Succinyl thioesters are, generally, less stable than the corresponding acetyl derivative, owing to intramolecular hydrolysis of the thioester bond (cf. Jencks, 1969). Succinyl-enzyme ($t_{\frac{1}{2}} \simeq 40$ min) (Table 1), however, is considerably more stable at 30 °C than is the acetyl-enzyme ($t_{\frac{1}{2}} \simeq 7$ min) (Lowe & Tubbs, 1985*b*), since, unlike acetyl-enzyme, it does not catalyse its own hydrolysis but breaks down at the same rate as the succinyl-CoA thioester bond. The stimulation of breakdown of succinyl-enzyme by acetyl-CoA, succinyl-CoA and, to a much lesser extent, acetoacetyl-CoA and CoA (Table 1) may be related to the acetyl-CoA-stimulated breakdown of acetyl-enzyme discussed by Lowe & Tubbs (1985*b*). The finding that acetoacetyl-CoA does not cause rapid desuccinylation is consistent with an observation that succinyl-CoA is not a substrate for HMG-CoA synthase.

Succinyl-CoA is the only CoA thioester that has been found to inhibit HMG-CoA synthase by covalent modification. An explanation for this unique property could be that, on binding to the CoA site of HMG-CoA synthase, succinyl-CoA is orientated so as to favour the intramolecular displacement of CoA. This would generate highly reactive succinic anhydride at the active site, which could attack the near-by reactive cysteine residue. Indeed, succinic anhydride, unlike acetic anhydride, was found to inhibit HMG-CoA synthase activity extremely rapidly.

Middleton & Tubbs (1974) reported that mixing succinyl-CoA with yeast HMG-CoA synthase resulted in a stoichiometric release of CoA, identical with that produced in the presence of acetyl-CoA. This is consistent with succinylation of the enzyme, as is described here for ox liver HMG-CoA synthase. Reed *et al.* (1975) also observed inhibition of chicken liver mitochondrial HMG-CoA synthase by succinyl-CoA, with 50% inhibition occurring in the presence of 0.2 mM-succinyl-CoA. The time-dependence and reversibility of this inhibition was not commented upon.

Although this has been a study performed *in vitro* with purified enzyme, it is interesting to speculate whether there is a metabolic significance of succinylation of HMG-CoA synthase and, in particular, its possible relevance to the control of ketogenesis. The mitochondrial concentration of succinyl-CoA of normal liver is about 0.2 mM (Barritt *et al.*, 1976; Siess *et al.*, 1980), and,

although the mitochondrial concentration of HMG-CoA synthase is very high (Lowe & Tubbs, 1985*a*), it is reasonable to assume, from the work reported in the present paper, that mitochondrial HMG-CoA synthase could be significantly inhibited by succinyl-CoA under normal conditions.

Succinate inhibits ketone-body formation when added to coupled rat liver mitochondria far more than, for example, malate does, and the inhibition is accompanied by a decrease in the concentration of free CoA (Bremer, 1968). Bremer (1968) concluded that this was due to formation of succinyl-CoA, and postulated that succinate was not merely acting as a precursor of oxaloacetate and, hence, as an acetyl-CoA sink, but might also be inhibiting the pathway of ketogenesis directly.

Glucagon treatment stimulates ketogenesis by a direct effect on the liver, in addition to increasing delivery of free fatty acids (McGarry *et al.*, 1975; Keller *et al.*, 1977; McGarry & Foster, 1980), and one effect that has been shown is a glucagon-stimulated decrease in the concentration of succinyl-CoA in liver cells (Siess *et al.*, 1980). We suggest that a fall in the concentration of succinyl-CoA after glucagon treatment might lead to de-inhibition of HMG-CoA synthase and, thus, an increase in the ketogenic flux. The inhibition of HMG-CoA synthase activity by succinyl-CoA is slowly reversible on a time-scale of minutes (Table 1), which is compatible with the 1 h time period for the activation of hepatic ketogenesis capacity after administration of glucagon (McGarry *et al.*, 1975).

Thus a control mechanism can be postulated in which flux through the HMG-CoA cycle is regulated according to the extent of succinylation of HMG-CoA synthase. This would be determined by the mitochondrial concentration of succinyl-CoA, relative to that of acetyl-CoA, and it is well established that the mitochondrial [acetyl-CoA]/[CoA] ratio rises during enhanced ketogenesis (Siess *et al.*, 1978).

Succinyl-CoA is thought to be a negative effector of the 2-oxoglutarate dehydrogenase complex in the citric acid cycle (Williamson *et al.*, 1972) and of the gluconeogenic enzyme pyruvate carboxylase (Barritt *et al.*, 1976; Siess *et al.*, 1980). Its inhibition of HMG-CoA synthase means it is now implicated in the regulation of three major pathways within the mitochondria.

It remains to be established whether an inverse correlation between the concentration of succinyl-CoA in liver and ketone-body production does exist, and whether this is related to the activity of HMG-CoA synthase *in vivo*.

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