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THE INHIBITORY EFFECTS OF ACYL-COENZYME A ESTERS ON THE PYRUVATE AND α-OXOGLUTARATE DEHYDROGENASE COMPLEXES

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SUMMARY

1. Acyl-CoA inhibition of the pyruvate and α-oxoglutarate dehydrogenase complexes was studied in order to gain some insight into possible factors which might regulate these two enzymes.

2. Short chain acyl-CoA esters inhibit the overall pyruvate and α-oxoglutarate dehydrogenase complex by competing for the CoASH site. The efficiency with which CoASH overcomes this inhibition in the two complexes is a function of the K_m for CoASH of the two enzymes.

3. Long chain acyl-CoA inhibition of α-oxoglutarate dehydrogenase was studied in detail. Inhibition results from irreversible binding of acyl-CoA to the CoASH site by virtue of the interaction between the acyl-CoA side chain with hydrophobic regions in the enzyme. The potency of this inhibition is related to the properties of the acyl side chain.

4. The interaction between hydrocarbon side chain of acyl-CoA esters and the intact α-oxoglutarate dehydrogenase ultimately results in the dissociation of lipoamide dehydrogenase from the complex.

5. Control of α-oxo acid oxidation is considered to be related to short chain acyl-CoA and free CoASH levels rather than to inhibition by long chain acyl-CoA esters.

INTRODUCTION

The two enzyme complexes which catalyze the oxidative decarboxylation of pyruvate and α-oxoglutarate have many properties in common. Both can be regarded as being situated at a branch point of carbon flow; each is essentially irreversible and subject to product inhibition[1,2], and both are a complex of three separate enzymes held together by noncovalent bonds[3]. The sequence of reactions catalyzed by the three enzymes which constitute the α-oxoglutarate dehydrogenase complex are completely analogous with the reactions catalyzed by the pyruvate dehydrogenase complex[4]. The

Abbreviations: DTNB, 5,5′-dithio-bis-(2-nitrobenzoic acid); TPP, thiamine pyrophosphate; $lip(S)_2$ and $lip(SH)_2$, oxidized and reduced lipoate (or lipoamide); FAD, flavin adenine dinucleotide

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three individual enzymes, α-oxoglutarate or pyruvate decarboxylase (E_1), dihydrolipoyl transsuccinylase or transacetylase (E_2) and lipoamide dehydrogenase (E_3) (EC 1.6.4.3) are held together in each case as a macromolecular complex. Molecular weights for the mammalian pyruvate and α-oxoglutarate

$$R^*COCOOH + [TPP]\text{-}E_1 \rightarrow [RCH(OH)\text{-}TPP]\text{-}E_1 + CO_2 \quad (1)$$

$$[RCH(OH)\text{-}TPP]\text{-}E_1 + [LipS_2]\text{-}E_2 \rightarrow [R\text{-}CO\text{-}S\text{-}LipSH]\text{-}E_2 + [TPP]\text{-}E_1 \quad (2)$$

$$[R\text{-}CO\text{-}S\text{-}LipSH]\text{-}E_2 + CoASH \leftrightarrows R\text{-}CO\text{-}S\text{-}CoA + [Lip(SH)_2]\text{-}E_2 \quad (3)$$

$$[Lip(SH)_2]\text{-}E_2 + E_3\text{-}FAD \leftrightarrows [LipS_2]\text{-}E_2 + \text{reduced } E_3\text{-}FAD \quad (4)$$

$$\text{Reduced } E_3\text{-}FAD + NAD^+ \leftrightarrows E_3\text{-}FAD + NADH + H^+ \quad (5)$$

$$\text{Sum: } RCOCOOH + CoASH + NAD^+ \rightarrow RCO\text{-}S\text{-}CoA + CO_2 + NADH + H^+ \quad (6)$$

dehydrogenase complexes have been reported as $9 \cdot 10^6$ (ref. 5) and $2.7 \cdot 10^6$ (ref. 6), respectively.

The overall reaction (Reaction 6) is inhibited by NADH and this has been equated to an increased reduction of part of the enzyme complex (*i.e.*, reduction by reversal of Reactions 5 and 4). GARLAND[1] also proposed that succinyl-CoA inhibition (which only occurred in the presence of NADH) resulted from transsuccinylation of reduced enzyme-bound lipoic acid by reversal of Reaction 3. The overall reaction is also inhibited by other CoA esters, notably palmitoyl-CoA[7].

In this report the mechanism of inhibition by short and long chain acyl-CoA esters is compared in the overall reaction catalyzed by α-oxoglutarate dehydrogenase. The discovery of a model reaction in which DTNB is cleaved by the α-oxoglutarate dehydrogenase complex (and as well by the pyruvate dehydrogenase complex) in an NADH-dependent reaction provided a means by which the inhibition by acyl-CoA esters could be studied directly[8]. The mechanism of this model reaction is visualized as a lipoamide dehydrogenase-NADH-dependent reversal of Reactions 5 and 4. The resultant reduced lipoic acid reacts nonenzymatically with DTNB to form a disulfide bond with the active sulfhydryl of lipoic acid. This disulfide now undergoes catalytic reduction by NADH and lipoamide dehydrogenase in a fashion analogous to reversal of Reactions 5 and 4. Acylation of the active lipoic acid sulfhydryl blocks DTNB cleavage by the complex, hence this inhibition of the reaction can be used as a measure of the specificity of acyl-CoA esters to transacylate to dihydrolipoic acid. In addition, competition for the CoASH site on the enzyme results in a protection against succinyl-CoA inhibition of the DTNB cleavage reaction.

Product inhibition of pyruvate and α-oxoglutarate dehydrogenase was studied and compared, and the significance of this inhibition is discussed.

METHODS AND MATERIALS

Isolation of the α-oxoglutarate and pyruvate dehydrogenase complexes

The α-oxoglutarate dehydrogenase complex was isolated from beef heart by the method of HIRASHIMA *et al.*[6]. The following modifications were introduced into the method. In place of calcium phosphate gel-cellulose column chromatography the complex was passed through a 2.5 cm × 40 cm column of Sephadex G-200. The enzyme was eluted with 0.05 M potassium phosphate buffer containing 2 mM EDTA (pH 7.0).

* R is $COOH\text{-}CH_2\text{-}CH_2$ in α-oxoglutarate or CH_3 in pyruvate.

The final preparation obtained by this method had specific activities of 0.5–1.0 units/mg. In some later experiments, preparations were further purified by centrifugation in 20–5% sucrose density gradient according to ISHIKAWA *et al.*[3]. In this case the specific activity was 2–4 units/mg. Pyruvate dehydrogenase was isolated by the method of HAYAKAWA *et al.*[5].

Enzyme assays

The overall reaction (Reaction 6) catalyzed by the α-oxoglutarate dehydrogenase complex was determined according to the method described by GARLAND[1]. The reaction was followed at 340 nm in a 1-cm cuvette which contained 50 μmoles potassium phosphate (pH 6.5), 1 μmole $MgCl_2$, 3 μmoles cysteine, 1 μmole α-oxoglutarate, 1 μmole NAD^+, 0.1 mg TPP, 0.45 μmole EDTA, and 0.15 μmole CoASH in a final volume of 1 ml. Antimycin A (1 μg) was added when crude enzyme fractions were assayed. The reaction was initiated by the addition of enzyme or by α-oxoglutarate in the case of the inhibition studies.

The pyruvate dehydrogenase complex was assayed as described by BACHMANN *et al.*[9]. The reaction was followed at 340 nm in a 1-cm cuvette which contained 50 μmoles Tris–HCl (pH 7.0), 5 μmoles $MgCl_2$, 3 μmoles cysteine, 0.1 mg TPP, 10 μmoles pyruvate, 1 μmole NAD^+, 0.1 μmole CoASH and pyruvate dehydrogenase (specific activity, 3.8 units/mg protein), in a final vol. of 1 ml.

Lipoamide dehydrogenase was assayed according to MASSEY[10]. The oxidation of NADH was measured at 340 nm in a 1-cm cuvette which contained 50 μmoles potassium phosphate buffer (pH 6.5), 2 μmoles lipoamide, 0.2 μmole NADH and 0.6 mg bovine serum albumin in a final volume of 1.0 ml. The reaction was initiated with the addition of enzyme.

For the assay of dihydrolipoyl transsuccinylase in the palmitoyl-CoA inhibition studies, a radioactive assay was developed with [2,3-$^{14}C_2$]succinyl-CoA as substrate. Incubations contained in a volume of 1 ml 100 μmoles Tris–HCl buffer (pH 7.2), 5 μmoles EDTA, 2.5 μmoles dihydrolipoic acid, 0.1–0.25 μmole [2,3-$^{14}C_2$]succinyl-CoA (specific activity, 0.2 μC/μmole) and α-oxoglutarate dehydrogenase (30 μg; specific activity, 4.25 μmoles/min per mg in the overall reaction). After preincubation for 1 min at 30° the reaction was started with the addition of [2,3-$^{14}C_2$]succinyl-CoA. The reaction was stopped after 0.5–5.0-min incubation by the addition of 0.1 ml of 5 M HCl. Succinylhydrolipoic and dihydrolipoic acids were extracted from the incubation with 2–5-ml aliquots of toluene containing 5 g 2,5-diphenyloxazole and 50 mg 1,4-bis-(5-phenyloxazolyl-2)benzene per l. The extracts were combined with an additional 5 ml of scintillation fluid and counted. The counting efficiency (55–65%) was determined by the channels ratio method. A time and succinyl-CoA concentration dependent nonenzymatic transsuccinylation necessitated the inclusion of appropriate controls when incubation time or substrate concentration was varied. The validity of the assay was based on the following criteria. The toluene-extractable radioactive compound formed a hydroxamate, and the quantity of succinate extracted with toluene was equal to the CoASH liberated in the incubation.

The activity of α-oxoglutarate decarboxylase was determined by the method of MASSEY[11]. The incubation was adapted to a 1 ml final volume and the decrease in absorbancy at 420 mμ measured after initiation of the reaction by the addition of enzyme.

The DTNB cleavage reaction catalyzed by α-oxoglutarate dehydrogenase was measured as previously described[8]. Details of the incubations are given in the legends to corresponding tables and figures.

Protein was determined by the biuret method of GORNALL *et al.*[12] or by ultraviolet absorption.

Synthesis of acyl thioesters

Long chain acyl-CoA and pantetheine esters (C_{10} to C_{22}) were synthesized according to the method of GOLDMAN AND VAGELOS[13] and were assayed by the alkaline hydroxylamine method[14] and by ultraviolet absorption.

[2,3-$^{14}C_2$]Succinyl-CoA was prepared from the anhydride by the method of SIMON[15]. [2,3-$^{14}C_2$]Succinic anhydride was synthesized according to BLATT[16]. Succinyl-CoA was purified by Ecteola-cellulose chromatography as previously described[17].

Dihydrolipoic acid was prepared by sodium borohydride reduction of lipoic acid according to GUNSALUS *et al.*[18].

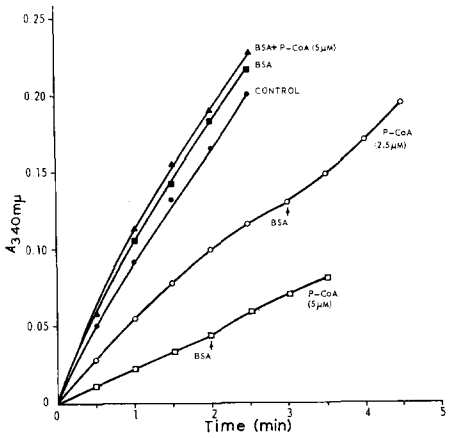
CoASH was obtained from P-L Biochemicals, lipoic acid, lipoamide, erucic acid, palmitoleic acid, and linoleic acid were products of Sigma Chemical Co. Elaidic acid was obtained from Applied Sciences Laboratory and [2,3-$^{14}C_2$]succinic acid from New England Nuclear Corp. DTNB was a product of Aldrich Chem. Co.

RESULTS

The effects of acyl-CoA esters on the overall reaction

Palmitoyl-CoA was a potent inhibitor of the overall reaction (Reaction 6) catalyzed by the α-oxoglutarate dehydrogenase complex (Fig. 1). The results with palmitoyl-CoA typify the inhibition by all long chain acyl-CoA esters. Inhibition, which occurs at very low levels, was prevented but not reversed by addition of bovine serum albumin. As was found for a number of other enzymes[19], this inhibition was a function of the ratio of enzyme to acyl-CoA ester rather than CoA ester concentration *per se*. Inhibition by palmitoyl-CoA was not altered by increased levels of α-oxoglutarate (1–10 mM), CoASH (0.027–0.27 mM) or NAD^+ (1–10 mM). The lack of inhibition by palmitoylpantetheine, neutralized palmitic acid and strong surfactants such as Tween-80 and Triton X-100 indicated that the detergent properties alone of long chain acyl-CoA esters were not the primary cause of inhibition. However, there can be no doubt that the potency of inhibition of these CoA esters is related to the properties of the acyl side chain. Fig. 2 illustrates the inhibitory potency of long chain acyl-CoA esters as a function of chain length. Inhibition was negligible for acyl-CoA esters below C_{12}. Further support for the correlation between the properties of acyl side chain and inhibitory potency is presented by the data in Table I. Inhibition decreased with increased unsaturation.

Product inhibition of the overall reaction catalyzed by pyruvate and α-oxoglutarate dehydrogenases is compared in Table II. This inhibition was unlike that by palmitoyl-CoA in that it occurred at higher acyl-CoA concentrations and was reversed by increased CoASH levels. Both enzymes were subject to inhibition by acetyl-CoA as well as succinyl-CoA. Of particular interest was the fact that inhibition of α-oxoglutarate dehydrogenase occurred at a lower CoASH level (*i.e.*, approx. 20 μM) than



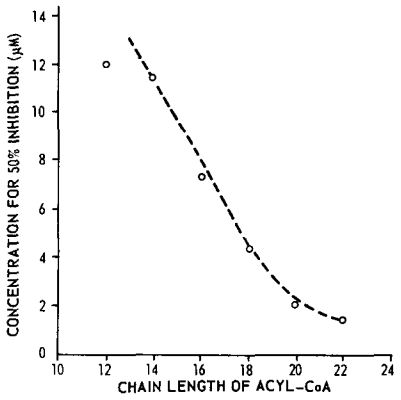


Fig. 1. Inhibition of the overall α-oxoglutarate dehydrogenase reaction by palmitoyl-CoA (P-CoA). The assay procedure is outlined under METHODS. The enzyme used had a specific activity of 0.5 unit/mg. The contents of the cuvette were incubated 1 min at 30° prior to starting the reaction with α-oxoglutarate. Five mg bovine serum albumin (BSA) was added as indicated or added to the cuvette prior to preincubation.

Fig. 2. Acyl-CoA inhibition as a function of the acyl chain length. The conditions for assay were similar to those described in Fig. 1. Each incubation contained 28 μg of enzyme (specific activity, 0.5 unit/mg). The concentration of acyl-CoA ester for 50% inhibition was determined by measurement of activity over a range of inhibitor concentrations and interpolation to 50% inhibition.

did the inhibition of pyruvate dehydrogenase (*i.e.*, approx. 100 μM CoASH). This effect probably resulted from the difference in K_m for CoASH of the two enzymes[2,11].

Effect of palmitoyl-CoA on the physical integrity of the α-oxoglutarate dehydrogenase complex

The three species of enzyme molecules which constitute the intact complex are

TABLE I

THE EFFECT OF ACYL SIDE CHAIN ON ACYL-CoA INHIBITION OF α-OXOGLUTARATE DEHYDROGENASE

The inhibition was determined with the incubation system described in Fig. 1. The 50% inhibition value was determined by interpolation. For purposes of comparison the inhibition by the saturated analogue was taken as unity.

Acyl group		*Relative concentration for 50% inhibition*
Palmitic	C_{16}	1.00
Palmitoleic	$C_{16}\Delta^9$ *(cis)*	1.33
Stearic	C_{18}	1.00
Oleic	$C_{18}\Delta^9$ *(cis)*	3.34
Elaidic	$C_{18}\Delta^9$ *(trans)*	5.00
Linoleic	$C_{18}\Delta^{9,12}$ *(cis, cis)*	7.50
Behenic	C_{22}	1.0
Erucic	$C_{22}\Delta^{13}$ *(cis)*	2.0

TABLE II

SUCCINYL-CoA AND ACETYL-CoA INHIBITION OF THE REACTIONS CATALYZED BY PYRUVATE AND α-OXOGLUTARATE DEHYDROGENASE

The details of assay procedures for the overall reaction are outlined in the text. Reactions were initiated by the addition of CoASH.

Acyl-CoA	*Concn. (mM)*	*CoASH (mM)*	*α-Oxoglutarate dehydrogenase activity (%)*	*Pyruvate dehydrogenase activity (%)*
None	—	0.133	—	100
	—	0.026	100	—
Acetyl-CoA	0.092	0.133	—	63
	0.184	0.133	—	47
	0.092	0.013	43	—
	0.184	0.013	39	—
	0.184	0.027	72	—
Succinyl-CoA	0.068	0.133	—	53
	0.102	0.133	—	43
	0.096	0.013	38	—
	0.096	0.027	69	—

held together by noncovalent bonds[3]. In the case of the complex from mammalian tissue these bonds can be disrupted with 2.5 M urea[11]. This treatment completely dissociates lipoamide dehydrogenase from the other two enzyme molecules which can still be sedimented by high speed centrifugation. The effect of palmitoyl-CoA on the dissociation of lipoamide dehydrogenase from the complex is shown in Table III.

TABLE III

DISSOCIATION OF LIPOAMIDE DEHYDROGENASE FROM THE α-OXOGLUTARATE DEHYDROGENASE COMPLEX BY INCUBATION WITH PALMITOYL-CoA

Palmitoyl-CoA and α-oxoglutarate dehydrogenase (1.3 mg) were incubated for 10 min at 30° in 0.3 ml of 0.033 M potassium phosphate buffer (pH 7.0) containing 1.3 mM EDTA. After incubation the enzyme was assayed for activity in the overall reaction and for lipoamide dehydrogenase. The incubations were then centrifuged at 50 000 rev./min (Spinco Model L-2) for 2.5 h. After centrifugation the supernatant fluid was assayed for lipoamide dehydrogenase activity. The control incubation was not centrifuged but was assayed after 2.5 h storage on ice.

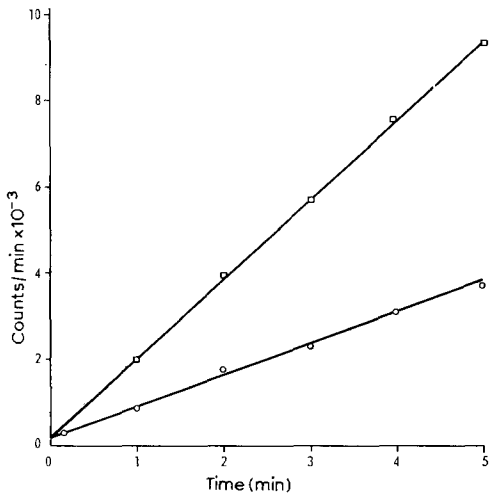
Palmitoyl-CoA (mM)	*Enzyme activity (units)**		
	Before centrifugation		*Supernatant Lipoamide dehydrogenase*
	α-Oxoglutarate dehydrogenase	*Lipoamide dehydrogenase*	
0.00	1.26	14.52	3.10
0.24	0.78	14.03	5.23
0.48	0.14	13.74	3.82
0.96	0.03	13.31	4.94
1.91	0.00	15.00	10.23
3.81	0.00	16.60	17.61
Control	1.31	15.14	—

* Units of activity are μmoles/min.

Inhibition of the overall reaction determined prior to centrifugation is complete at 1 mM palmitoyl-CoA. There was little or no inhibition of lipoamide dehydrogenase at any level of palmitoyl-CoA. The entire lipoamide dehydrogenase from the inhibited complex was found in the supernatant fluid after centrifugation at 50 000 rev./min for 2.5 h. The dissociation of the lipoamide dehydrogenase took place after the inhibition of the overall reaction was complete. Hence the inhibition was not primarily caused by dissociation and loss of lipoamide dehydrogenase from the complex. Dissociation into subunits caused by long chain acyl-CoA esters has been reported for glucose-6-phosphate dehydrogenase[20] and the fatty acid synthetase complex[21]. These reports do not indicate if inhibition precedes or parallels this dissociation.

The site of long chain acyl-CoA inhibition in the overall α-oxoglutarate dehydrogenase complex

The intact α-oxoglutarate dehydrogenase complex consists of 3 separate enzymes with a concerted enzymatic activity which is inhibited by acyl-CoA esters. The specific



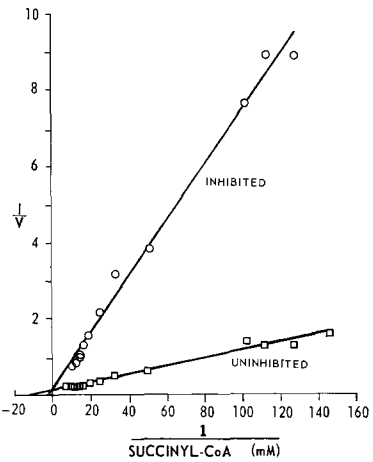


Fig. 3. Progress curve of the dihydrolipoyl transsuccinylase reaction. The enzyme assay is described in METHODS. ○—○, nonenzymatic transsuccinylation to dihydrolipoic acid: □—□, reaction catalyzed by 0.17 mg α-oxoglutarate dehydrogenase (specific activity, 0.96 unit/mg in the overall reaction).

Fig. 4. Double reciprocal plots of initial velocities of succinyl-CoA transesterification to dihydrolipoic acid as a function of succinyl-CoA concentration. The assay is described under METHODS. Initial velocities for the inhibited reaction were determined when palmitoyl-CoA (5 μM) was included in the incubation during a 1-min period of preincubation.

enzyme or enzymes which are inhibited by acyl-CoA esters can only be determined by a separate study of each enzyme. The lack of inhibition of palmitoyl-CoA on lipoamide dehydrogenase is demonstrated in Table III. The effect of palmitoyl-CoA on dihydrolipoyl transsuccinylase was studied with an assay to measure the reverse of Reaction 3. In this model reaction dihydrolipoate acts as an acceptor of succinyl units from succinyl-CoA[22]. The sensitivity of the assay was increased by the substitution of [2,3-$^{14}C_2$]

succinyl-CoA for a succinyl-CoA generating system. Succinyl-hydrolipoic acid was extractable from acidified incubation media and counted directly. The time-dependent enzymatic and nonenzymatic transsuccinylation from CoASH to dihydrolipoate is shown in Fig. 3. Palmitoyl-CoA showed a competitive-type inhibition with respect to succinyl-CoA (Fig. 4). K_m for succinyl-CoA is $30 \cdot 10^{-6}$ M while K_i for palmitoyl-CoA is 10^{-6} M. These results suggest that at least one site of palmitoyl-CoA binding was the succinyl-CoA site. This conclusion was further substantiated by an experiment in which enzyme was preincubated with succinyl-CoA prior to incubation with palmitoyl-CoA. The enzyme was inhibited 58, 40 and 24% by 0.41 mM palmitoyl-CoA in the presence of 0, 0.8 and 3.2 mM succinyl-CoA, respectively. Hence succinyl-CoA did afford some protection of the enzyme to palmitoyl-CoA inhibition.

The α-oxoglutarate decarboxylase activity of the complex was determined in another model reaction in which potassium ferricyanide acts as an electron acceptor[11]. Palmitoyl-CoA was inhibitory in this reaction and this inhibition was not affected by increased α-oxoglutarate concentration or by the addition of thiamine pyrophosphate.

The results thus far give no indication if long chain acyl-CoA esters inhibit irreversibly beceause of transacylation to functional sulfhydryl groups as was shown to be the case with yeast fatty acid synthetase[23]. Transacylation from CoA esters to lipoic acid in the enzyme by reversal of Reaction 3 has not been ruled out as part of the mechanism of action for long chain acyl ester inhibition. The possibility of transacylation to lipoic acid was studied with the NADH-dependent DTNB cleavage reaction catalyzed by the intact α-oxoglutarate dehydrogenase complex[8]. The mechanism of this reaction is a reduction of the disulfide formed between thionitrobenzoate and the sulfhydryl at C-6 of lipoic acid by lipoamide dehydrogenase and NADH. Acylation of this sulfhydryl by succinyl-CoA or α-oxoglutarate inhibits the DTNB cleavage. Other acyl-CoA esters with the capacity to transacylate should cause a similar inhibition. The ability of CoA esters of monocarboxylic acids from C_2 to C_{12} to inhibit DTNB cleavage is compared in Table IV with that of succinyl-CoA. Acetyl-CoA, butyryl-CoA and hexanol-CoA demonstrate a marked capacity to transacylate

TABLE IV

THE INHIBITION OF THE DTNB CLEAVAGE REACTION BY ACYL-CoA ESTERS

The assay for DTNB cleavage by the α-oxoglutarate dehydrogenase was to follow the increase in absorbance at 412 mμ in a 1-cm cuvette. Incubations contained potassium phosphate (pH 7.0), 50 μmoles; NADH, 0.15 μmole; EDTA, 1.0 μmole; DTNB, 1.0 μmole; α-oxoglutarate dehydrogenase, 50–100 μg (specific activity, 1.4–2.7 units/mg) and acyl-CoA esters in a final vol. of 1 ml. The contents of the incubation were preincubated 5 min at 30° and the reaction started by addition of DTNB.

Acyl-CoA ester	*Concentration causing 50% inhibition (μM)*
Succinyl-CoA	0.5
Acetyl-CoA	1.0
Butyryl-CoA	7.0
Hexanoyl-CoA	7.0
Octanoyl-CoA	200.0
Decanoyl-CoA	325.0
Myristyl-CoA	750.0

to the lipoic acid sulfhydryl, however very limited transacylation took place with octanoyl, decanoyl and myristyl-CoA esters. Longer chain esters were not potent inhibitors of the DTNB cleavage reaction and therefore did not transacylate to enzyme-bound lipoic acid.

It now becomes possible to show direct evidence for the competition between long chain acyl-CoA and succinyl-CoA for the succinyl-CoA site by means of the DTNB cleavage reaction. Preincubation of palmitoyl-CoA and enzyme prior to incubation with succinyl-CoA gave a concentration dependent protection against succinyl-CoA inhibition and hence prevented succinyl transfer to enzyme-bound lipoic acid by binding to the CoA site (Fig. 5). Similar protection against succinyl-CoA inhibition was afforded by lauryl-CoA and myristyl-CoA at higher concentrations. Decanoyl-CoA and octanoyl-CoA gave no protection, while the effect of hexanoyl-CoA and butyryl-CoA was one of additive inhibition. The same effect of acyl chain length on inhibition of the overall reaction (*i.e.*, decreasing inhibition with decreasing chain length) is seen

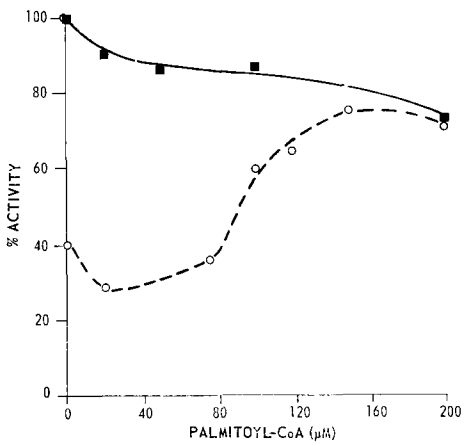


Fig. 5. The protective effect of palmitoyl-CoA against succinyl-CoA inhibition of the DTNB cleavage reaction catalyzed by α-oxoglutarate dehydrogenase. The DTNB cleavage reaction was measured as described in Table IV. Palmitoyl-CoA was preincubated with the enzyme 5 min before addition of succinyl-CoA and an additional 2 min preincubation. The reaction was initiated by addition of DTNB. ■—■, palmitoyl-CoA alone; ○— — ○, palmitoyl-CoA *plus* succinyl-CoA (0.5 m μmole/ml).

here with regards to prevention of succinyl-CoA transfer to lipoic acid in the enzyme. Taken together these results indicate that the long chain acyl-CoA inhibition of the overall reaction is caused by a tight binding to the CoASH site, but does not involve transacylation to lipoic acid. Short chain acyl-CoA inhibition also involves binding to the CoASH site with the additional possibility of transacylation.

DISCUSSION

The acyl-CoA esters which inhibit the overall α-oxoglutarate dehydrogenase reaction can be divided in two groups on the basis of their mechanism of inhibition. The short chain acyl-CoA esters (acetyl, butyryl, hexanoyl and succinyl) inhibit by

virtue of their ability to compete for the CoASH site as well as transacylate to enzyme-bound lipoate. The results which demonstrate the dissociation of the complex (Table III) indicate an interaction between the acyl chain of the CoA ester and the enzyme molecule. The evidence for interactions between a long chain hydrocarbon and a hydrophobic region on a protein has been discussed by CHASE[24]. The mechanism of long chain acyl-CoA inhibition of the α-oxoglutarate dehydrogenase complex can be regarded as a blocked CoASH site. This blockage probably results when the acyl chain becomes irreversibly associated with hydrophobic regions in the enzyme. The results in Fig. 5 would suggest that the nonpolar lipoic acid moieties in the enzyme are not strongly involved in the acyl chain–protein interaction. The interaction of acyl-CoA esters with the enzyme ultimately causes dissociation of the lipoamide dehydrogenase, however, this dissociation is not the primary cause of inhibition of the overall reaction. Similar dissociation into subunits has been reported for glucose-6-phosphate dehydrogenase[20] and the fatty acid synthetase complex[21], but these reports do not attempt to correlate inhibition with dissociation.

The first suggestion of a regulatory role for long chain acyl-CoA esters was the result of their inhibition of fatty acid synthesis in what appeared to be a feedback type of mechanism[25,26]. An extensive number of reports of enzymes inhibited by long chain acyl-CoA esters have since appeared[20,21,27–29]. The diversity of the catalytic activity of the enzymes inhibited and the almost complete irreversibility of the inhibition has resulted in a general consensus that this inhibition is of no physiological significance[19,27]. Under these circumstances, it is interesting to speculate how the cell prevents the indiscriminate binding of acyl-CoA esters to enzymes. One possible mechanism is provided by the long chain acylcarnitine reversal of this inhibition which FRITZ has demonstrated with purified[7,30], as well as crude enzyme systems[31]. It is also possible that certain proteins (*e.g.*, albumin) specifically bind and transport long chain acyl-CoA esters in the cell, however no evidence to prove this point is available.

The inhibitory nature of the short chain acyl-CoA esters on the overall reaction catalyzed by the α-oxoglutarate and pyruvate dehydrogenase complexes has been shown to result from competition for the CoASH site (Table II), since this inhibition is overcome by increased CoASH concentrations. The reversal of Reaction 3 is not implicit in this inhibition, since presumably in the presence of NAD^+, enzyme-bound lipoic acid would be predominantly in the oxidized state. The ratio of acyl-CoA to free CoASH could thus provide a means for the regulation of both pyruvate and α-oxoglutarate dehydrogenase. The results presented in Table II indicate that succinyl-CoA inhibition of α-oxoglutarate dehydrogenase in the absence of NADH occurred when the succinyl-CoA to CoASH ratio was greater than 1.5. On the other hand, the results of GARLAND[1] demonstrated that inhibition of the α-oxoglutarate dehydrogenase complex occurred at a succinyl-CoA to CoASH ratio of 0.5 when the NAD^+ to NADH ratio was 4.5. The redox state of the system thus appears to determine the ratio of acyl-CoA to CoASH necessary to obtain inhibition. If indeed these dehydrogenases are subject to metabolic regulation both the redox state of the system as well as the level of acylated and free CoA may be implicated.

From the view point of metabolic control of α-oxo acid oxidation, it is of interest to compare the properties of pyruvate and α-oxoglutarate dehydrogenase. From the results in Table II, it is evident that certain striking differences exist with regard to level of free CoASH necessary for the prevention of succinyl or acetyl-CoA inhibition.

This difference probably relates to the K_m of the two enzymes for CoASH. MASSEY[11] found the K_m of CoASH for α-oxoglutarate dehydrogenase too low to determine accurately but set the value at less than 10^{-7} M. The K_m for CoASH of the pyruvate dehydrogenase complex was found to be $6.7 \cdot 10^{-6}$ M (ref. 2). Therefore, at any given concentration of free CoASH, the inhibition of pyruvate dehydrogenase by acetyl or succinyl-CoA would be greater than the corresponding inhibition of α-oxoglutarate dehydrogenase. The preferential oxidation of α-oxoglutarate over pyruvate by isolated mitochondria supports this conclusion[32]. The conditions which favor decreased pyruvate oxidation (*i.e.*, increased acetyl-CoA levels) are known to stimulate pyruvate carboxylation[33] and hence may be considered to control the route by which pyruvate enters the tricarboxylic acid cycle.

Of the two enzyme complexes studied, pyruvate dehydrogenase would appear to be the one upon which control is exerted. Inhibition by long chain acyl-CoA esters is not favored in either case as a mechanism of control, while short chain acyl-CoA esters and free CoA levels appear to be prime factors in regulation of both enzyme complexes.

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