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CHAPTER 100

Reaction of General Acyl-CoA Dehydrogenase with 3,4-Pentadienoyl-CoA

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Recently several thioester inhibitors of acyl-CoA dehydrogenases have been described whose mode of action probably involves an initial removal of a C-2 proton followed by isomerization of the thioester to the active species. Thus general acyl-CoA dehydrogenase is inhibited via irreversible covalent modification of the protein using 3-alkynoyl-CoA derivatives (1), and a similar inhibition has been reported for butyryl-CoA dehydrogenase from *Megasphaera elsdenii* and glutaryl-CoA dehydrogenase from *Pseudomonas fluorescens* using 3-alkynoyl-pantetheine thioesters (2). Methylenecyclopropylacetyl-CoA, a metabolite of hypoglycin, effects irreversible flavin modification on incubation with *M. elsdenii* butyryl-CoA dehydrogenase and pig kidney general acyl-CoA dehydrogenase (3). This paper deals with 3,4-pentadienoyl-CoA, a novel inhibitor of general acyl-CoA dehydrogenase. The mode of action of this allenic thioester is also consistent with an initial proton abstraction.

A titration of pig kidney general acyl-CoA dehydrogenase with 3,4-pentadienoyl-CoA is shown in Figure 1. The reduced spectrum obtained at one equivalent of thioester is strikingly different from that obtained on reduction with octanoyl-CoA (4); it exhibits neither a 350 nm transition nor detectable long wavelength absorbance, but shows a very high extinction at 316 nm (Figure 1). The spectrum resembles N-(5)-acetyl-1,5-dihydroflavins (5,6). Reduction with equimolar inhibitor is rapid and biphasic, with a rate constant of $\geqslant 200\ sec^{-1}$ at 25°C for the fast phase, accounting for >50% of the bleaching at 450 nm and a 4-fold slower second phase.

A slow regeneration of up to ~80% oxidized enzyme occurs on incubation of the reduced enzyme species. This process is independent of O_2 or the effective mediator phenazine methosulfate ($t_{1/2}$ 30 min, 25°; activation energy 19 kcal/mole). Increased concentrations of 3,4-pentadienoyl-CoA decrease the extent of A_{450} nm recovery and lengthen the lag phase preceding this apparent reoxidation, Figure 2. 3,4-pentadienoyl-CoA is not a substrate for general acyl-CoA dehydrogenase in the standard assay system (4), but is a potent inhibitor of the enzyme. Assays performed in the presence of 6 μM compound show progressive inhibition (exhibiting 60% and 11 % of control rates after 15 and 120 sec respectively). In contrast, preincubation of enzyme with inhibitor yields an initially inactive reduced species (see Figure 1), which regains up to 75% activity after 2 minutes exposure to octanoyl-CoA in the assay mixture.

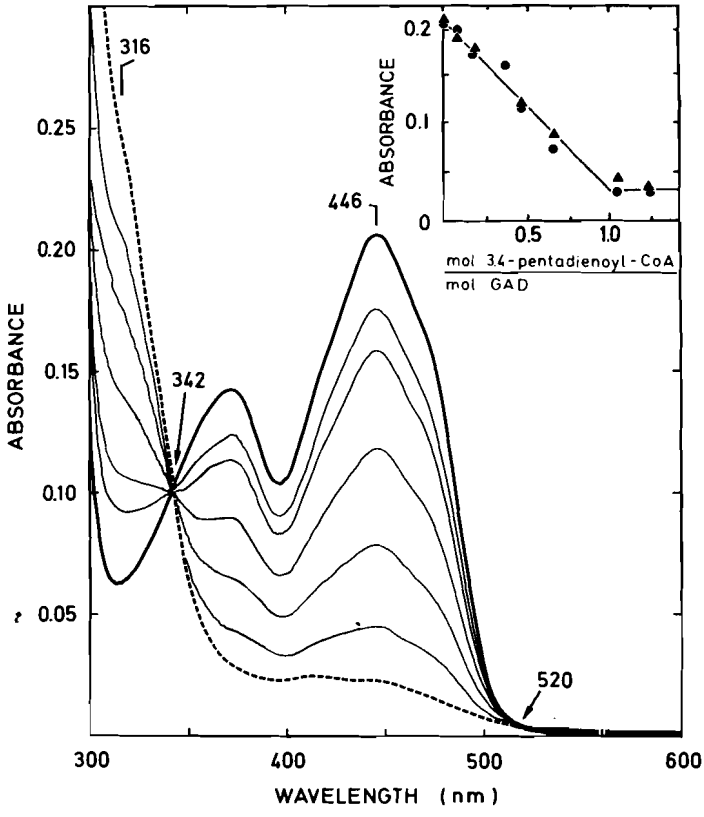


Figure 1. Spectral course of the reaction of general acyl-CoA dehydrogenase with 3,4-pentadienoyl-CoA. The oxidized enzyme, 1.3×10^{-5} M in 0.1 M phosphate buffer, pH 7.6 (curve: —), was titrated at 25° with the inhibitor concentrations shown in the inset, and the intermediate spectra were recorded within 5 min. The arrows denote the isobestic points of the conversion. Curve (---) represents the spectrum of the inactive enzyme after addition of 1.5 equivalents of inhibitor and before noticeable oxidation had occurred. The inset shows the absorbance changes at 450 nm measured under aerobic (●–●), and anaerobic (▲–▲–) conditions.

This work demonstrates that 3,4-pentadienoyl-CoA is a potent, substantially reversible inhibitor of general acyl-CoA dehydrogenase. The spectrum of the reduced enzyme and its resistance to reoxidation suggest that it may represent a covalent adduct. A complex of reduced enzyme and the corresponding cumulene seems much less likely, and would be expected to react with phenazine methosulfate and exhibit a long wavelength band. Adduct formation could reasonably involve attack of a C(2)- or C(4)- carbanion on oxidized flavin (Scheme 1).

Scheme 1 provides for the ready reversal of adduct formation (lower left) by octanoyl-CoA, since this substrate binds tightly to the oxidized enzyme. The apparent reoxidation of the reduced species in Figure 1 and the lag phase in Figure 2 suggest that GAD catalyzes turnover of the inhibitor. Indeed prolonged preincubation of 3,4-pentadienoyl-CoA with GAD yields 2,4-

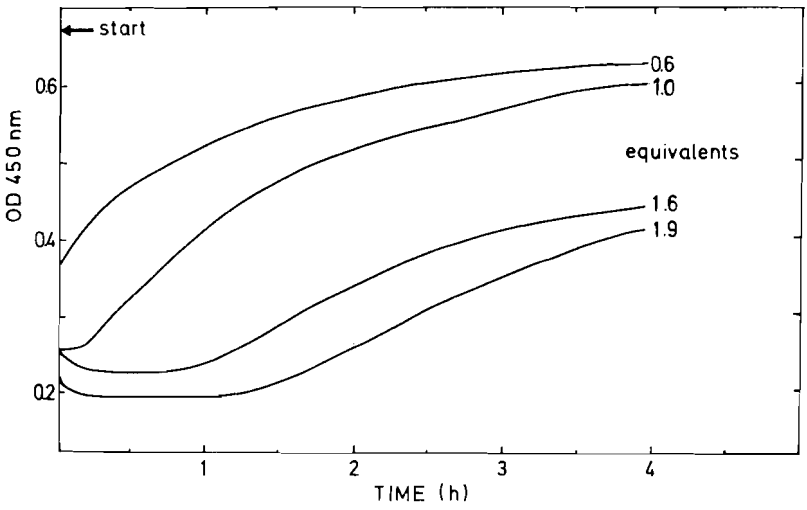
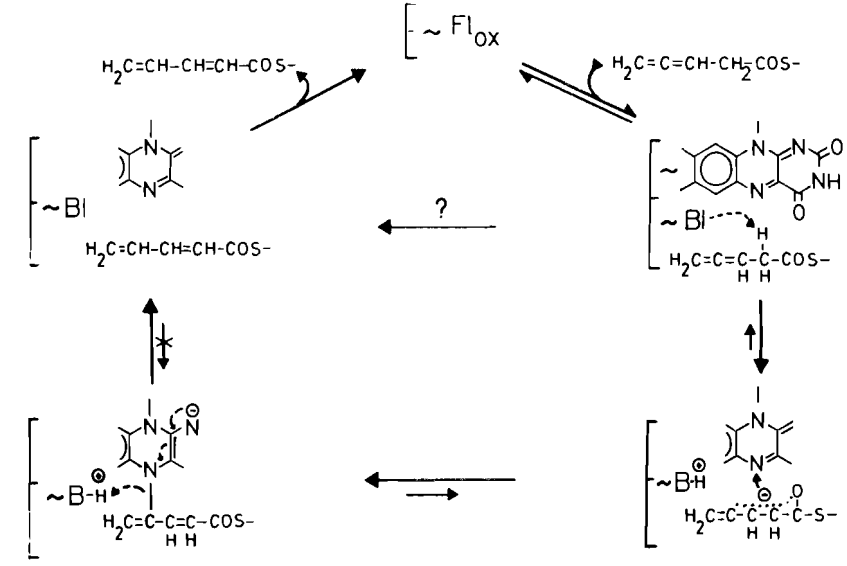


Figure 2. Time dependence of the oxidation state of general acyl-CoA dehydrogenase upon incubation with varying concentrations of 3,4-pentadienoyl-CoA. The enzyme, 4.4×10^{-6} M (0.8 ml) was incubated at 25° with the equivalents of inhibitor indicated. After a very rapid decrease of 450 nm absorption, a slow reoxidation occurs, which leads to a maximal 80% reappearance of the original 450 nm absorbance. This process is unaffected by the presence of oxygen or PMS. The recovery of catalytic activity parallels the spectral changes.

Scheme 1. Proposed mechanism for the reaction between oxidized general acyl-CoA dehydrogenase and 3,4-pentadienoyl-CoA. (B=enzyme active site base, $E \sim Fl_{ox}$ = oxidized GAD). In the lower left hand structure, the covalent bond could alternatively be to the α-position of the inhibitor.



pentadienoyl-CoA as evident by the immediate formation of the characteristic spectrum of the reduced enzyme 2,4-pentadienoyl-CoA complex (7) on the addition of dithionite.

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