SC 2288

Studies of inhibitors of fatty acid biosynthesis.

III. Mechanism of action of tetrolyl-coenzyme A

We have recently shown that tetrolyl-CoA, the acetylenic analogue of butyryl-CoA, is a potent inhibitor of the enzymic synthesis of long-chain fatty acids[1,2]. This compound appeared to inhibit the condensation of acetyl-CoA with malonyl-CoA and the reduction of α,β-unsaturated acyl-CoA derivatives such as crotonyl-CoA. These reactions are known to require the participation of enzyme sulfhydryl groups[3]. The inhibition appears to be of the noncompetitive type and is illustrated by the effect of tetrolyl-CoA on the reduction of crotonyl-CoA catalyzed by a partially purified rat-brain fatty acid-synthesizing enzyme preparation (Fig. 1).

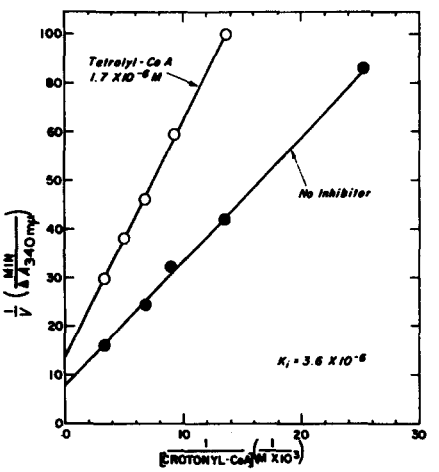


Fig. 1. Noncompetitive inhibition of the reduction f crotonyl-CoA by tetrolyl-CoA. Each cuvette contained 30 μmoles of potassium phosphate buffer (pH 7.0), 30 mμmoles of TPNH, rat-brain fatty acid-synthesizing enzyme Fraction II (ref. 3) (0.8 mg of protein), and substrate and inhibitor as indicated in a final volume of 0.3 ml.

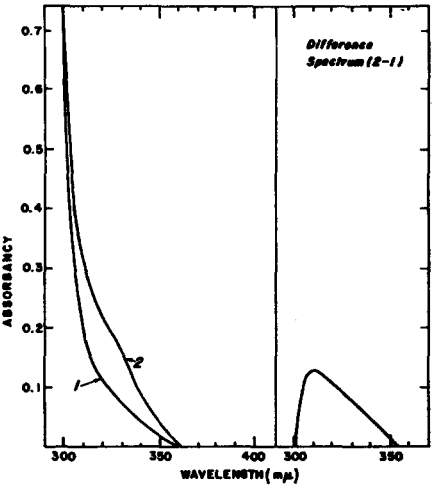


Fig. 2. Spectrophotometric demonstration of the reaction between rat-brain fatty acid-synthesizing enzyme Fraction III (ref. 3) and tetrolyl-CoA. 1, Absorption spectrum of fatty acid-synthesizing enzyme (5.3 mg of protein) in 0.01 M phosphate buffer (pH 6.7). 2, Spectrum of enzyme plus $1.8 \cdot 10^{-5}$ M tetrolyl-CoA *vs.* $1.8 \cdot 10^{-5}$ M tetrolyl-CoA in 0.01 M phosphate buffer (pH 6.7).

A reaction between mercaptans and tetrolyl-CoA has been observed under mildly alkaline conditions and is attended by a change in the absorption spectrum of tetrolyl-CoA and the appearance of a new peak at 308 mμ (see ref. 1). Such new compounds probably occur by the formation of a thioester mercaptide since the facile addition of sulfhydryl-containing materials to triply-bonded compounds has been amply demonstrated[4]. The extinction coefficients of the compounds resulting from the reaction of 2-mercaptoethanol, cysteine, or glutathione with tetrolyl-CoA have been calculated to be $7 \cdot 10^3$ at 308 mμ. Free tetrolic acid does not give rise to such compounds in the presence of mercaptans. In the present experiments using relatively large amounts of fatty acid-synthesizing enzyme from rat-brain tissue, the addition of tetrolyl-CoA to a solution of the enzyme caused a shift in the absorption spectrum

TABLE I

REACTION OF TETROLYL-CoA WITH FATTY ACID-SYNTHESIZING ENZYME

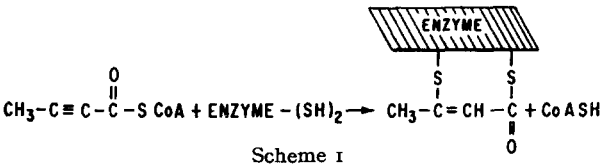
Each tube contained 10 μmoles of potassium phosphate buffer (pH 6.7), rat-brain enzyme Fraction II (0.6 mg of protein), and 132 mμmoles of tetrolyl-CoA in a final volume of 0.20 ml at 37°. The reaction was stopped by the addition of neutralized hydroxylamine and residual CoA esters were determined.

Time of incubation (min)	*Change in tetrolyl-CoA over control without enzyme Tetrolyl-CoA (mμmoles)*	*Change in absorbancy at 308 mμ*	*Tetrolic thioester mercaptide formation (mμmoles*)*
0	−5.8	+0.143	+4.1
15	−8.0	+0.213	+6.1
30	−8.5	+0.234	+6.7

* Based on $\varepsilon_{308} = 7.0 \cdot 10^3$.

and the difference spectrum showed the appearance of a new peak in the region of 310 mμ (Fig. 2).

It has been previously demonstrated that fatty acid-synthesizing preparations catalyze the deacylation of butyryl- and palmityl-CoA and these reactions appear to follow a first-order kinetic course[1]. An investigation of the kinetics of the deacylation of tetrolyl-CoA by such enzyme preparations indicated that there was very little progression of the deacylation of tetrolyl-CoA with time (Table I). Under similar conditions, 80 and 109 mμmoles of butyryl-CoA were found to be deacylated after 15 and 30 min, respectively. Furthermore, using the value $\varepsilon_{308} = 7 \cdot 10^3$, the amount of tetrolic thioester mercaptide formed agreed fairly well with the disappearance of tetrolyl-CoA. It has been shown previously that vicinal sulfhydryl groups on the



Scheme 1

enzyme participate in fatty acid synthesis[5] and that acyl–S–enzyme complexes can be formed from acyl-CoA derivatives and sulfhydryl groups of fatty acid-synthesizing enzymes[6–8]. These observations together with the present data suggest that the inhibition of fatty acid synthesis by tetrolyl-CoA occurs because of the formation of a thioester mercaptide from tetrolyl-CoA and vicinal sulfhydryl groups on the enzyme according to Scheme 1.

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