INHIBITION OF FATTY ACID SYNTHETASES BY THE ANTIBIOTIC CERULENIN

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Summary: The antibiotic cerulenin is a potent and apparently non-competitive inhibitor of fatty acid synthetase systems isolated from various microorganisms and from rat liver. Cerulenin specifically blocks the activity of β-keto acyl thioester synthetase (condensing enzyme). This effect may account for the inhibition of overall fatty acid synthesis by the antibiotic.

Introduction: Cerulenin, an antibiotic of the structure (2S)(3R)2,3-epoxy-4-oxo-6,10-dodecadienoylamide (1,2) inhibits the growth of a variety of yeasts, fungi and bacteria (3). Since this inhibition can be overcome by the addition of certain lipid molecules to the growth medium, it was proposed that cerulenin interferes with the utilization of a precursor common to the biosynthesis of several lipid structures. In support of this proposal, cerulenin was found to reduce markedly the incorporation of ^{14}C-acetate into fatty acids and sterols of yeast cells in vivo (4). These effects can also be demonstrated in vitro (5). Addition of the antibiotic (2-3 μM) to yeast extracts reduced the incorporation of 1-^{14}C-acetate, 1-^{14}C-acetyl-CoA or 1,3-^{14}C-malonyl-CoA into the fatty acid or non-saponifiable fractions by 60-90% (5). On the basis of these findings, it was suggested that cerulenin may exert its inhibitory effects on some step beyond the formation of acetyl-CoA and malonyl-CoA interfering most likely with their condensation to acetoacetyl-thioesters (5).

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Table I

Percent Inhibition of Type I Fatty Acid Synthetases (Multienzyme Complexes) by Cerulenin

Cerulenin, µg/ml \ Enzyme Source	Rat Liver	Yeast	Euglena gracilis Type I	Corynebacterium diphtheriae	Mycobacterium phlei, Type I
4	13	90	100	82	-
8	14	94	100	88	56
20	51	97	-	93	79
40	-	97	-	99	87
100	-	-	-	-	95

We now report the effects of cerulenin on the activities of several crude or partially purified fatty acid synthetases from diverse sources. In all but one instance the incorporation of ^{14}C-malonyl-CoA into long chain fatty acids was strongly inhibited by low concentrations of the antibiotic (Tables I and II). Testing the effect of cerulenin on several of the partial reactions catalyzed by the high molecular weight synthetase of M. phlei, we have obtained evidence that the antibiotic specifically inhibits the formation of β-ketoacyl thioesters, the reaction catalyzed by condensing enzyme.

Experimental:

Fatty Acid Synthetases - Fatty acid synthetase I from M. phlei ATCC-356 was isolated as described (6), except that the calcium phosphate step and sucrose gradient centrifugation were omitted and substituted by Bio-Gel A-5 chromatography. M. phlei synthetase II (palmityl CoA elongating system) was prepared and assayed as described (7).

Corynebacterium diphtheriae was kindly provided by Dr. A. Pappenheimer. This synthetase was isolated and purified by H. Knoche (8).

Euglena gracilis strain Z was the source of three fatty acid synthetases.

Table II

Percent Inhibition of ACP-Dependent (Type II) Fatty Acid Synthetases by Cerulenin

Cerulenin, µg/ml \ Enzyme Source	E. coli	Mycobacterium phlei Type II*	Euglena gracilis II	Euglena gracilis III*
4	-	0	99	77
8	95	-	100	87
20	99	0	-	93
40	-	-	-	-
100	-	10	-	-
200	-	10	-	100

*palmityl-CoA elongation

Enzyme I is the multienzyme complex and Enzyme II the ACP-requiring synthetase isolated from green Euglena cells (9). Enzyme III is an activity that catalyzes an ACP-dependent elongation of palmityl CoA to stearate and arachidate and is present both in etiolated and green Euglena cells (14).

E. coli - The enzyme was fraction A as described by Vagelos et al. (10).

Yeast - The synthetase was partially purified as described by Lynen (11).

Rat Liver - This enzyme was prepared according to Brady et al. (12).

Fatty acid synthesis activity was assayed in the various systems under the conditions shown in Table III. In all cases 2-^{14}C-malonyl-CoA was the labelled substrate.

Condensing enzyme activity of M. phlei synthetase I was assayed as described by Kumar et al. (13). The assay system (total volume of 0.2 ml)

Table III

Composition of Assay Mixtures For Fatty Acid Synthetases

Substrates \ Enzyme Source	Rat Liver	Yeast	Corynebacterium diphtheriae	Euglena gracilis I	Euglena gracilis II	Euglena gracilis III	Mycobacterium phlei I	Mycobacterium phlei II
Acetyl CoA, μM	5	100	100	10	10	10*	300	100*
Malonyl CoA, μM	20	25	25	30	10	15	20	30
TPNH, μM	125	19	19	15	15	15	30	200
DPNH, μM	19	-	19	15	15	15	30	200
FMN, μM	1	2	2	0.5	0.5	0.5	1	-
Dithiothreitol, mM	5	6	6	2	2	2	5	5
KPO_4 buffer, pH 7.2, M	0.1	0.2	0.5	0.05	0.05	0.05	0.1	0.2
Protein, μg	1-2.5	2-8	0.4-0.8	24-60	12-48	28-112	2	74
E. coli ACP, μM	-	-	-	-	10	10	-	2

*Palmityl-CoA

All incubations (total vol. 0.5 ml) were carried out for 20 min. at 37°

contained 100 mM potassium phosphate buffer (pH 7.0), 5 mM dithiothreitol, 1 mM EDTA, 300 μM acetyl-CoA, 50 μM malonyl-CoA, and 100 mM ^{14}C-sodium bicarbonate (1 μC/μmole). The reaction was initiated by addition of 100 μg of enzyme.

Results: Cerulenin inhibits the synthesis of long-chain fatty acids as judged by ^{14}C-malonyl-CoA incorporation *in vitro* as effectively as it does *in vivo*. For testing the antibiotic, we have chosen representatives of the two major types of synthetases that catalyze the *de novo* formation of long-chain fatty acids from acetyl-CoA and malonyl-CoA. Type I synthetases are represented by the multienzyme complexes from *M. phlei* (6), *Corynebacterium diphtheriae* (8), *Saccharomyces cerevisiae* (11), *Euglena gracilis* (9) and rat liver (12). These enzyme systems are characterized by high molecular weights and by the lack of a requirement for external acyl carrier protein (ACP). Cerulenin inhibits the activities of these systems to varying degrees. In some instances inhibition is complete with 4 μg/ml (Table I).

Fatty acid synthetases of the second class (type II) consist of non-aggregated individual enzymes and show a requirement for added ACP. In this category we have tested the synthetase systems from *E. coli* (10) and photoauxotrophic *Euglena gracilis* (9). They are as sensitive to cerulenin as the synthetases of class I (Table II). In a subgroup of type II synthetases are palmityl-CoA elongating systems which are also non-aggregated ACP-dependent systems. The palmityl-CoA elongating activity from *Euglena gracilis* (14) is strongly inhibited by cerulenin, but the corresponding ACP-dependent synthetase from *M. phlei* (7) retains full activity even at 200 μg/ml of antibiotic. The exceptional cerulenin resistance of *M. phlei* synthetase II is unexplained.

Apart from catalyzing the *de novo* synthesis of fatty acids, *M. phlei* synthetase I is also active with palmityl-CoA as the primer converting it to longer chain (C_{24}) acids (6). This process is likewise sensitive to the antibiotic.

Partial reactions of fatty acid synthesis were tested with model substrates in the presence and absence of cerulenin in order to explore the mode of action of the antibiotic. The enzyme source was *M. phlei* synthetase I and the assay procedures were those described by Lynen (11) for the fatty acid synthetase of yeast. The following enzyme activities were not affected by antibiotic in concentrations of 100 μg per ml: acyl-CoA-transacylase, malonyl-CoA-transacylase and β-keto acyl reductase. On the other hand

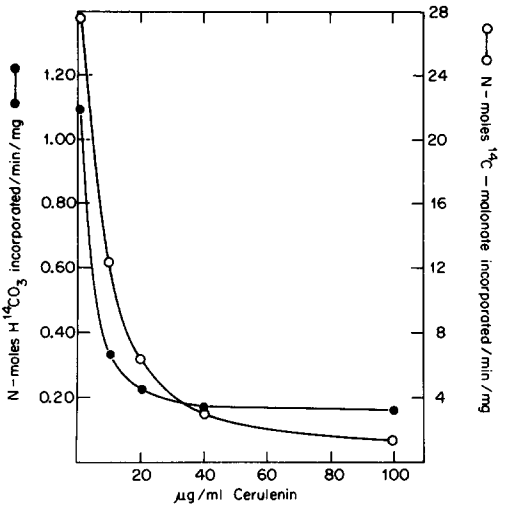

Figure 1. Effect of Cerulenin on Type I Fatty Acid Synthetase From M. phlei. (o-o) fatty acid synthesis; (●-●) condensing enzyme activity.

condensing enzyme (β-keto acyl thioester synthetase) was highly sensitive to cerulenin (Fig. 1). This activity was assayed by measuring $^{14}CO_2$ fixation into malonyl-CoA in the presence of acetyl-CoA (13).

The cerulenin concentrations required for 50% inhibition of condensing enzyme activity were 7 µg/ml as compared to 12 µg/ml for 50% inhibition of overall fatty acid synthetase activity (Fig. 1). In one experiment, enzyme was preincubated for 10 min. with 10 µg/ml of cerulenin. Fatty acid synthesis was 98% inhibited, whereas simultaneous incubation of inhibitor with the complete system gave only 57% inhibition. Therefore, inhibition appears to be non-competitive.

Discussion: Cerulenin is a uniquely potent and apparently specific inhibitor of fatty acid synthetases from all sources so far tested with the single exception noted. Condensing enzyme appears to be the specific and possibly the only target for the antibiotic. Inhibition of this activity is non-competitive suggesting that cerulenin may combine non-covalently with an active enzyme site. Cerulenin affects fatty acid synthetases of all known types, whether they are multienzyme complexes or non-aggregated systems. Moreover, cerulenin interferes both with de novo fatty acid synthesis from acetyl-CoA and with chain elongation starting with palmityl-CoA. Chain-length specificity, therefore, does not appear

to be a factor in the mode of cerulenin action. Cerulenin should be a useful tool for controlling the fatty acid content and composition of cells in studies on the function of lipids in membranes. The one agent used for this purpose so far, 3-decynoyl-N-acetyl cysteamine (15) interferes only with the biosynthesis of unsaturated fatty acids in certain bacteria (16).

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