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INHIBITION BY CERULENIN OF LIPID SYNTHESIS IN *CRAMBE ABYSSINICA* TISSUES

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Key Word Index—*Crambe abyssinica*, Brassicaceae, leaf, seed, inhibition, cerulenin; fatty acid synthetases, lipid.

Abstract—Cerulenin is a potent inhibitor of fatty acid synthesis from ^{14}C-labelled acetate in leaves and developing seeds of *Crambe abyssinica*. The antibiotic is equally inhibitory on the elongation of [1-^{14}C]-oleic acid to erucic acid which is the major fatty acid of the seed. There is no significant inhibition of fatty acid desaturation in either tissue. Acylation of lipids is not a primary target of cerulenin's action.

INTRODUCTION

THE ANTIBIOTIC cerulenin, (2S)(3R) 2,3-epoxy-4-oxo-7,10-dodecadienoyl amide, is a potent inhibitor of fatty acid biosynthesis in yeasts,[1,2,4] bacteria[3,4] and rat liver.[4] It is reported to inhibit β keto acyl thioester synthetase in all the systems studied so far with the one exception of the Type II, palmitoyl CoA elongating, system of *Mycobacterium phlei*.[4] Evidence for this inhibition has been obtained from studies on homogeneous preparations of β-ketoacyl carrier protein synthetase isolated from *E. coli*.[5] The antibiotic has been shown to have no effect on either the biosynthesis of nucleic acids, proteins or cell walls, or the exogenous respiration of yeasts.[1] It has little effect on nucleic acid or protein synthesis in *E. coli*[3] except under conditions where cell growth has stopped. The data presented in this paper are concerned with the action of cerulenin on fatty acid and lipid metabolism of leaves and developing seeds of the crucifer *Crambe abyssinica*. The seeds are of particular interest since they contain an oleyl-thioester primed fatty acid synthetase whose product is erucic acid [docosa-cis-13-enoic acid].[6] The effect of the antibiotic on this system will make an interesting comparison with the effect on the sub-group of the Type II fatty acid synthetases found in *E. gracilis* and *M. phlei*.[4]

RESULTS

Cerulenin inhibits the synthesis of fatty acids in both *Crambe* leaves and maturing seeds. The effects on [2-^{14}C]-acetate uptake into lipids are similar in both tissues. Cerulenin (1 or 2 μmol) inhibits ^{14}C uptake into leaf lipids by 77 and 82%, and into seed lipids by 80

[1] NOMURA, S., HORIUCHI, T., OMURA, S. and HATA, T. (1972) *J. Biochem* **71**, 783
[2] NOMURA, S., HORIUCHI, T., HATA, T. and OMURA, S. (1972) *J. Antibiot* **15**, 365
[3] GOLDBERG, I., WALKER, J. R. and BLOCH, K. (1973) *Antimicrob. Aq. Chemother* **3**, 549
[4] VANCE, D., GOLDBERG, I., BLOCH, K. OMURA, S. and NOMURA, S. (1972) *Biochem. Biophys. Res. Commun.* **48**, 649
[5] D'AGNOLO, G., ROSENFELD, I. S., AWAYA, J., OMURA, S. and VAGELOS, P. R. (1973) *Biochim. Biophys. Acta*, **326**, 155
[6] APPLEBY, R. S., GURR, M. I. and NICHOLS, B. W. In preparation

and 83% respectively Examination of the patterns of incorporation of radioactive fatty acids into individual lipid classes demonstrates that cerulenin does not affect the acylation of polar lipids, typical of leaf systems. (Table 1) or the neutral lipids typical of seed systems (Table 2). The uptake pattern of [1-^{14}C]-oleate into acyl lipids is essentially the same with or without cerulenin, in both plant tissues The activities of all the lipids in both leaf and seed incubations, with [2-^{14}C]-acetate as substrate, are reduced considerably in the presence of cerulenin. The antibiotic causes the greatest reduction in those lipids which normally have high activity, such as phosphatidyl choline which shows rapid turnover of fatty acids [7] Thus inhibition of fatty acid formation will cause a more pronounced reduction in the activity of such lipids and also in rapidly accumulating lipids such as seed triglyceride.

TABLE 1 DISTRIBUTION OF ^{14}C-ACTIVITY IN *Crambe* LEAF LIPIDS

[2-^{14}C]-Na acetate (μmol cerulenin/g tissue)							[1-^{14}C]-Oleic acid (μmol cerulenin/g tissue)					
0		2		4			0		2		4	
nCi	Total lipids (%)	nCi	Total lipids (%)	nCi	Total lipids (%)	Lipid class	nCi	Total lipids (%)	nCi	Total lipids (%)	nCi	Total lipids (%)
726		165		134		Total lipids	1750		1701		1766	
290	40	99	60	78	58	Neutral lipids	990	56	1021	60	954	54
102	14	21	13	21	16	MGDG	53	3	51	3	35	2
94	13	23	14	21	16	PE	106	6	85	5	106	6
22	3	5	3	4	3	DGDG + PG	18	1	17	1	35	2
167	23	10	6	5	4	PC	530	30	476	28	583	33
51	7	7	4	5	4	SQDG + PI	53	3	51	3	53	3

Chopped leaf (0 5 g) was incubated in 5 ml of 0 05 M Hepes buffer at pH 7 4 with either [2-^{14}C]-acetate at 20 μCi (3 34 μmol)/g of tissue or [1-^{14}C]-oleate at 4 μCi (0 64 μmol)/g of tissue

Key monogalactosyl diglyceride (MGDG), phosphatidyl ethanolamine (PE), digalactosyl diglyceride (DGDG), phosphatidyl glycerol (PG), phosphatidyl choline (PC), sulphoquinovosyl diglyceride (SQDG), phosphatidyl inositol (PI)

TABLE 2 DISTRIBUTION OF ^{14}C-ACTIVITY IN *Crambe* SEED LIPIDS

[2-^{14}C]-Na acetate (μmol cerulenin/g tissue)							[1-^{14}C]-Oleic acid (μmol cerulenin/g tissue)					
0		2		4			0		2		4	
nCi	Total lipids (%)	nCi	Total lipids (%)	nCi	Total lipids (%)	Lipid class	nCi	Total lipids (%)	nCi	Total lipids (%)	nCi	Total lipids (%)
753		152		129		Total lipids	1277		1423		1467	
255	34	18	12	8	6	TG	240	19	168	12	159	11
56	7	17	11	16	12	FFA	592	46	727	51	806	55
101	13	35	23	33	26	DG	87	7	94	7	96	7
33	4	21	14	21	16	MG + PA	22	2	26	2	27	2
306	41	60	40	50	39	Polar lipids	336	26	410	29	380	26

Halved seeds (0 5 g) were incubated in 5 ml of 0 05 M Hepes buffer at pH 7 4 with either [2-^{14}C]-acetate at 20 μCi (3 34 μmol)/g of tissue, or [1-^{14}C]-oleate at 4 μCi (0 64 μmol)/g of tissue

Key triglyceride (TG); free fatty acids (FFA), diglyceride (DG) monoglyceride (MG), phosphatidic acid (PA)

There is very little effect on the distribution of ^{14}C-activity in fatty acids when cerulenin is present in [2-^{14}C]-acetate incubations of either leaf systems (Table 3) or seed systems

[7] NICHOLS, B W, JAMES A T and BRUCE J (1967) *Biochem J* **104**, 486

(Table 4) tissue, which suggests that the major site of action of the antiobiotic is on the fatty acid synthetase systems. Oleate metabolism in leaf is mainly directed towards desaturation to linoleic and linolenic acids (Table 3). Desaturation of [1-^{14}C]-oleic acid in the presence of 1 μmol of cerulenin is inhibited by 8% which is only 1/10 of the inhibitory effect on fatty acid synthesis. This indicates that cerulenin is exerting a minor effect on desaturation.

TABLE 3 PER CENT DISTRIBUTION OF ^{14}C-ACTIVITY IN FATTY ACID METHYL ESTER OF LEAF LIPIDS

Substrate	μmol Cerulenin/ g tissue	14's	16 0	16 1^{9}	16 1^{3}t	16 3	18 0	18 1	18 2	18 3
[2-^{14}C]-Acetate	0	2 0	28 6	4 8	5 4	2 7	3 4	23 8	21 1	8 2
	2	1 0	25 0	5 6	6 9	<0 1	3 1	30 3	18 7	9 4
	4*	—	—	—	—	—	—	—	—	—
[1-^{14}C]-Oleate	0							78 8	16 1	5 1
	2							80 6	15 0	4 4
	4							88 6	8 4	3 0

* Not determined due to very low ^{14}C-activity Incubation conditions as Table 1

Crambe seed fatty acid metabolism is orientated towards erucic acid synthesis (Table 4). Oleate desaturation is not so active in the seed and its inhibition by 1 μmol of cerulenin is only 4%. Exogenous [1-^{14}C]-oleate elongation to erucic acid is normally rather poor compared to erucate synthesis from acetate, probably due to poor permeability of the substrate. Despite this, Table 4 shows an 81% inhibition of the elongation in the presence of 1 μmol cerulenin which compares with 80% inhibition of total fatty acid synthesis at the same antibiotic level

TABLE 4 PER CENT DISTRIBUTION OF ^{14}C-ACTIVITY IN FATTY ACID METHYL ESTERS OF SEED LIPIDS

Substrate	μmol Cerulenin/ g tissue	14's	16's	18 0	18 1	18 2	18 3	20's	22's
[2-^{14}C]-Acetate	0	0 9	13 1	3 8	18 3	11 4	2 3	9 4	40 8
	2	6 6	13 0	3 5	17 7	7 3	2 0	9 4	40 6
	4*								
[1-^{14}C]-Oleate	0				75 2	13 1	4 0	3 7	3 7
	2				82 1	13 8	2 6	0 7	0 7
	4				88 2	8 1	2 9	0 8	<0 1

* Not determined due to very low ^{14}C-activity Incubation condition as Table 2

DISCUSSION

It has now been demonstrated that cerulenin inhibits fatty acid synthetase systems in bacteria,[4] yeasts,[2] rat liver,[4] and leaves and seeds of higher plants. The antibiotic is a specific inhibitor of β-keto-acyl-thio-ester synthetase in both the Type I, ACP independent synthetases, of yeasts, mammals and certain bacteria, and the Type II, ACP dependent synthetases, of bacteria, algae and plants. This inhibition of fatty acid synthesis has occurred in all acetyl–CoA primed systems that have been examined.

The position is not so clear with synthetases utilizing long chain acyl–CoA primers. Two palmityl–CoA primed ACP dependent synthetases (sub groups of Type II) have been studied by Vance *et al* ;[4] that from *Euglena gracilis* is inhibited by cerulenin whilst that from *Mycobacterium phlei* is not affected, even by high levels of the antibiotic

There are several plant and algal synthetases which are primed by unsaturated fatty acid–CoA esters. These include erucyl synthetase in *Brassica* seeds[6,8] primed by oleyl-CoA, dihomolinoleyl-CoA synthetase[9] primed by linoleyl CoA in *Euglena gracilis* and arachidonyl synthetase[9] primed by γ-linolenyl-CoA in *Porphyridium cruentum* and *Ochromonas danica*. Erucyl synthetase is the only one of these mono-, di- or tri-enoic acid primed synthetases to have been tested against cerulenin, and erucate formation was inhibited to the same extent as it was in other susceptible systems.

Cerulenin has been shown to be a potent inhibitor of a wide range of saturated fatty acid synthetases from a variety of sources, including the plant examples reported in this paper. The antibiotic also inhibits the elongation of a monoenoic substrate which poses the question of its action on elongation of polyenoic fatty acids. Finally desaturation of plant fatty acids are not affected by this antibiotic.

EXPERIMENTAL

Crambe abyssinica plants were grown in greenhouses on a daily minimum of 12 hr light. Seeds were removed for incubations between 12 and 16 days post anthesis. Young open leaves were detached from *Crambe* plants before flowering occurred. [2-^{14}C]-acetate and [1-^{14}C]-oleic acid were obtained from the Radiochemical Centre, Amersham. Incubations of tissue with 10 μCi [2-^{14}C]-acetate or 2 μCi [1-^{14}C]-oleate were done using 0·5 g of either chopped leaf or halved seeds in 4 ml of 0·05 M Hepes buffer at pH 7·4 at room temperature in light for 4 hr. Cerulenin, supplied by Dr. S. Omura of the Kitasato Institute, Japan, was tested at 1 μmol (44·5 μg/ml) and 2 μmol (89 μg/ml) per incubation. Lipids were extracted by macerating the leaf or seed tissue in *iso*-propanol (10 vol.) using an Ultra-Turrax; the probe was washed on a further 10 vol. The extracts combined and 2 vol. $CHCl_3$ added. Extraction was allowed to take place overnight at +2°, when the preparations were filtered, the residues washed with $CHCl_3$–MeOH (2:1) and the filtrates combined. The filtrates were concentrated to near dryness, taken up in $CHCl_3$–MeOH (2:1), and 1·5 vol. 0·7% saline added. The sample was shaken, the $CHCl_3$ phase containing the lipids recovered, concentrated and stored at −25° under N_2. Separation of lipids was achieved on layers of silica gel G developed in either petrol–Et_2O–HCOOH (70:30:1) for neutral classes or $CHCl_3$–MeOH–HOAc–H_2O (85:15:10:3·5) for glycolipids and phospholipids. Quantitative recovery of radioactivity in individual components was determined after location with iodine vapour by scraping the appropriate areas of silica gel from the plates and assaying the radioactivity by scintillation spectrometry.[10] Radioactivity in fatty acid classes was determined by interesterification of the lipid extracts by refluxing in MeOH–C_6H_6–H_2SO_4 (20:10:1). The methyl esters of the component fatty acids were extracted with petrol (bp 40–60°), dried and analysed on a radio-chemical gas chromatograph with 10% EGAP as stationary phase.

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