Vol. 80, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

January 13, 1978 Pages 133-139

THE TEMPERATURE-MEDIATED METABOLISM OF 1-ACYL-LYSOPHOSPHATIDYL GLYCEROL IN CERULENIN-TREATED CULTURES OF BACILLUS MEGATERIUM

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Received November 23, 1977

SUMMARY: When [U-^{14}C]palmitate was added to a culture of B. megaterium that had been grown at 35°, transferred to 20° and treated with cerulenin, label was initially incorporated into lysophosphatidyl glycerol. The labeled lyso derivative, in turn, was converted to phosphatidyl glycerol, apparently by esterification of the 2-position with endogenous acyl groups. Labeled lysophosphatidyl glycerol synthesis at 20° was observed only when a culture was treated with cerulenin prior to the addition of [U-^{14}C]palmitate. When [U-^{14}C] palmitate was added before cerulenin, labeled lysophosphatidyl glycerol formation was not detected. When chloramphenicol was added with cerulenin at the time of culture transfer from 35° to 20°, the synthesis of lysophosphatidyl glycerol was unaffected but the rate of its esterification to phosphatidyl glycerol was significantly retarded. Transfer of such a culture back to 35° resulted in a marked acceleration in the rate of conversion of lysophosphatidyl glycerol to phosphatidyl glycerol.

In the preceding paper, we described the isolation and identification of 1-palmitoyl-lysophosphatidyl glycerol which was formed when palmitate was added to cerulenin-treated cultures of B. megaterium. In the work reported here, we have investigated the synthesis and metabolism of lysophosphatidyl glycerol and shown how temperature changes affect these processes.

MATERIALS AND METHODS

The materials and methods used in this work are described in the preceding paper.

RESULTS

One possible explanation for the appearance of 1-acyl-lysophosphatidyl glycerol in cerulenin-treated cultures at 20° is that it was derived by partial deacylation of phosphatidyl glycerol. This hypothesis would require that labeled palmitate be initially incorporated into the 1-position of phosphatidyl

Abbreviation: ACP for acyl carrier protein.

0006-291X/78/0801-0133$01.00/0

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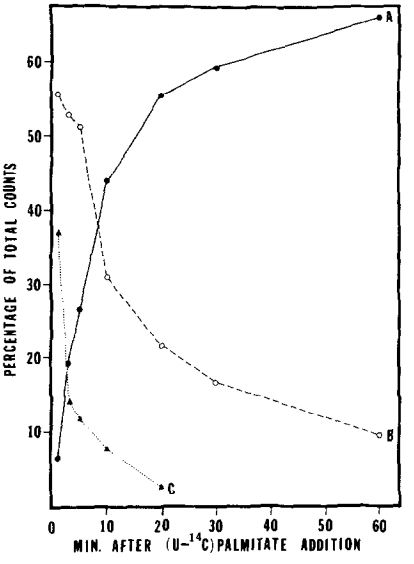

Figure 1. Incorporation of [U-^{14}C]palmitate into lysophosphatidyl glycerol and phosphatidyl glycerol in a cerulenin-treated culture at 20°. A culture growing at 35° (300 ml, 160 Klett units) was harvested by centrifugation and resuspended in 220 ml of fresh medium (7) at 20°. The resuspended cells were divided into 2, 110 ml portions and transferred to flasks in a 20°-water-bath shaker. One portion served as a control while cerulenin (20 μg/ml) was added to the second portion. After 28 min, [U-^{14}C]palmitate (0.025 nmoles/ml) was added to each flask. At intervals over the next 60 min, cells from 5 ml samples of the control and cerulenin-treated cultures were harvested by filtration and the cell lipids extracted and separated into chloroform-soluble and aqueous-methanol-soluble fractions. The distribution of ^{14}C in the lipids of each fraction was then determined by thin-layer silica gel radiochromatography. The total counts recovered in the extracted lipid from the samples of the cerulenin-treated culture remained constant (103,500 ± 3,500 cpm) over the time course of the experiment. The count distribution in phosphatidyl glycerol (curve A), lysophosphatidyl glycerol (curve B) and free fatty acid (curve C) are shown in the figure above. In the control culture (data not shown) phosphatidyl glycerol was the major radioactive lipid at all time intervals and no lysophosphatidyl glycerol was detected after 1 min. In the 1 min sample, however, a component in the aqueous-methanol phase, corresponding to lysophosphatidyl glycerol was found to contain about 3% of the total lipid counts. This component contained insufficient radioactivity (3,400 cpm) for further purification and analysis.

glycerol with subsequent deacylation at the 2-position. We found, however, that labeled lysophosphatidyl glycerol appeared only when cerulenin was added to a culture prior to the addition of [U-^{14}C]palmitate; when cerulenin was added after palmitate incorporation into phosphatidyl glycerol (i.e. 5-15 min after palmitate addition), lysophosphatidyl glycerol formation was never observed. These results indicated that phosphatidyl glycerol could not have

been the precursor of lysophosphatidyl glycerol. Indeed, data on the flow of label from [U-^{14}C]palmitate into these two lipids strongly suggest the opposite view. As Fig. 1 shows, 1 min after addition of labeled palmitate to a cerulenin-treated culture, 55% of the incorporated counts were found in lysophosphatidyl glycerol and less than 7% in phosphatidyl glycerol. The remaining counts were in free fatty acid. The relative changes in these 3 parameters with increasing incubation time indicate that exogenous free fatty acid was rapidly incorporated into lysophosphatidyl glycerol and that the lyso derivative, in turn, served as a major precursor of labeled phosphatidyl glycerol. When [U-^{14}C]palmitate-labeled phosphatidyl glycerol, isolated from control or cerulenin-treated cultures, was subjected to phospholipase A_2 action, almost all of the radioactivity was found in lysophosphatidyl glycerol and less than 5% was detected in free fatty acid. This result suggests that the lysophosphatide was converted to phosphatidyl glycerol by esterification of the 2-position with endogenous (i.e. unlabeled) acyl moieties.

As noted in the preceding paper, the effects of temperature on the appearance of lysophosphatidyl glycerol were similar to those observed on fatty acid desaturation in the same organism. In the latter case, fatty acid desaturase was not present in cultures growing at 35° but was rapidly induced when the temperature of the medium was shifted to 20° (1). If chloramphenicol, at a concentration sufficient to totally inhibit protein synthesis, was added before or at the time of temperature down-shift, desaturase induction was prevented and no unsaturated fatty acid synthesis took place. We thought it possible that a system requiring the temperature-induced synthesis of a new protein might also be responsible for lysophosphatidyl glycerol formation since this lipid was not synthesized at 35° but always appeared after labeled palmitate addition in cerulenin-treated cultures either growing at or transferred to 20°. As Fig. 2 shows, however, chloramphenicol added at the time of culture transfer from 35° to 20° did not inhibit lysophosphatidyl glycerol synthesis. To the contrary, in the culture treated with both cerulenin and

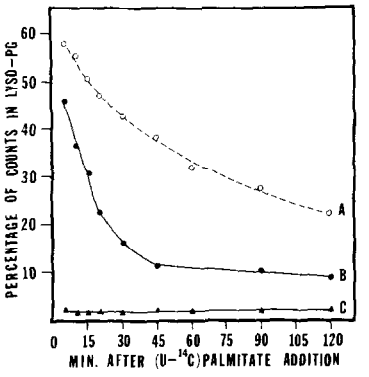

Figure 2. The effect of chloramphenicol on lysophosphatidyl glycerol metabolism. A culture growing at 35° (180 ml, 216 Klett units) was divided into 3, 60 ml portions. Cerulenin (20 μg/ml) and chloramphenicol (200 μg/ml) were added to the first portion (A), cerulenin (20 μg/ml) was added to the second portion (B) and chloramphenicol (200 μg/ml) was added to the third portion (C). The flasks containing the 3 portions were swirled in an ice bath to bring the medium temperature rapidly to 20° and transferred at once to a 20°-water-bath shaker and incubated for 15 min. [U-^{14}C]palmitate (0.025 nmoles/ml) was then added to each portion and cells from 5 ml samples were harvested at intervals and extracted in the usual manner to obtain the lipids. The counts in the aqueous-methanol phase, expressed as a percentage of the total counts extracted, were taken as a direct measure of lysophosphatidyl glycerol radioactivity. These values are shown above for portions treated with both chloramphenicol and cerulenin (curve A), with cerulenin alone (curve B) and with chloramphenicol alone (curve C). Thin-layer silica gel radiochromatography of selected aqueous-methanol phase samples confirmed that essentially all of the radioactivity shown by curves A and B was located in lysophosphatidyl glycerol. No labeled lysophosphatidyl glycerol could be detected, however, in the aqueous-methanol phases from portion C samples. The small levels of radioactivity shown in curve C arose from contamination by free fatty acid, phosphatidyl glycerol and traces of unidentified lipid components.

chloramphenicol (Fig. 2, curve A), the initial level of lysophosphatidyl glycerol was slightly higher and its rate of acylation to phosphatidyl glycerol significantly slower than in the culture treated with cerulenin alone (Fig. 2, curve B). The formation of lysophosphatidyl glycerol was not observed in the culture treated only with chloramphenicol.

The experiment described in Fig. 3 demonstrated that there is a marked acceleration in the rate of conversion of lysophosphatidyl glycerol to phosphatidyl glycerol when a culture is shifted from 20° to 35°. At the same time,

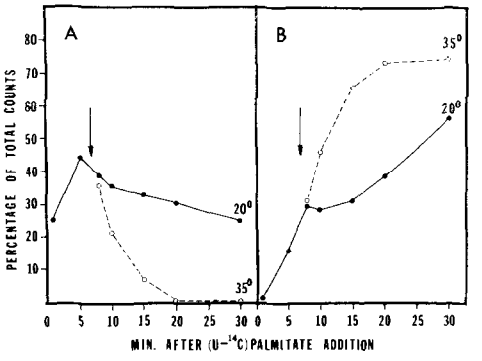

Figure 3. The effect of temperature on the rate of conversion of lysophosphatidyl glycerol to phosphatidyl glycerol. A culture growing at 35° (90 ml, 200 Klett units) was transferred to a 20°-water-bath shaker and cerulenin (20 μg/ml) and chloramphenicol (200 μg/ml) were added. After incubation at 20° for 15 min, [U-^{14}C]palmitate was added (zero-time) and samples were taken at 1 and 5 min. At 7 min (indicated by arrows in figure above) the culture was separated into 2, 45 ml portions. To one portion was added 45 ml of fresh medium at 50° to bring the culture temperature to 35°. The flask containing this portion was immediately transferred to a 35°-incubator shaker. To the second portion was added 45 ml of fresh medium at 20°, resulting in no temperature change. This portion was returned to the 20°-water-bath shaker. Samples were then taken from each portion as soon as possible (8 min) and at intervals thereafter up to 30 min. Analyses for labeled lipids were carried out as described in the legend for Fig. 1. The radioactivity in lysophosphatidyl glycerol (Fig. 3A) and in phosphatidyl glycerol (Fig. 3B) is shown above for the culture maintained at 20° (●) and for the portion transferred to 35° (○). At 1 min, essentially all of the remaining activity (73%) was found in free fatty acid. In samples taken after 1 min, radioactivity not accounted for by the two phospholipids was distributed between free fatty acid and diglyceride.

these results show that this acceleration cannot be explained by the rapid induction of a lysophosphatidyl glycerol acylating enzyme at 35° since chloramphenicol sufficient to totally inhibit protein synthesis was present in the culture at the time of temperature shift.

DISCUSSION

The results reported here indicate that in cerulenin-treated cultures of B. megaterium at 20°, the incorporation of added palmitate into phosphatidyl glycerol proceeds via the initial synthesis of 1-palmitoyl-lysophosphatidyl glycerol. It is also clear that the rate of esterification of lysophosphatidyl glycerol and its synthesis are mediated by temperature. Although a complete explanation for these observations must await the characterization of the

enzymes involved, the results do permit us to advance a working hypothesis.

Cerulenin, at 20 μg/ml of culture medium, blocks fatty acid synthesis in B. megaterium and we assume that the appearance of significant levels of ^{14}C-lysophosphatidyl glycerol in cerulenin-treated cultures at 20° is related to this effect. Presumably, acyl-ACP derivatives from fatty acid synthesis are normally utilized at 20° for esterification of the 2-position of either lysophosphatidyl glycerol or a precursor (possibly lysophosphatic acid). The initial accumulation of lysophosphatidyl glycerol in cerulenin-treated cultures after palmitate addition indicates that the substrate specificities for acylation of the 1 and 2 positions of the glycerol moiety are significantly different. This conclusion is supported by the results of phospholipase A_2 action on phosphatidyl glycerol from control or cerulenin-treated cultures which showed that, in both cases, added [U^{14}C]palmitate was incorporated only in the 1-position while unlabeled (endogenous) fatty acid was found at the 2-position. Since the transport of exogenous fatty acid into the cell probably involves the synthesis of the CoA ester (2), it seems likely that palmitoyl-CoA is utilized in the acylation of the 1-position. Assuming that acyl-ACP thioesters are the required or preferred substrates for acylation at the 2-position at 20° there are several possible explanations for the eventual conversion of lysophosphatidyl glycerol to phosphatidyl glycerol in cerulenin-treated cultures. E. coli contains a fatty acid synthetase-independent system that activates fatty acids to acyl-ACP thioesters (3); B. megaterium may contain a similar system. Alternatively, there could still be a very low level of fatty acid synthesis continuing in the presence of 20 μg/ml of cerulenin. However, we noted no decrease in the rate of lysophosphatidyl glycerol acylation when 80 μg/ml of cerulenin was used. It is also possible that, in the absence of the ACP thioesters, a less efficient substrate such as acyl-CoA could esterify the 2-position. In this regard, it should be noted that palmitoyl-CoA and palmitoyl-ACP serve equally well to esterify glycerol-3-phosphate to phosphatidic acid in cell-free systems from E. coli, but acylation

by the CoA thioester is inhibited by guanosine-5'-diphosphate-3'-diphosphate while acylation with the ACP derivative is not (4,5).

There are several possible explanations for the effects of temperature on lysophosphatidyl glycerol synthesis and acylation but we prefer to withhold speculation until more evidence is available. Nevertheless, the experiments employing chloramphenicol do demonstrate that temperature-mediated induction of specific enzymes is not directly involved in these processes. The significant decrease in the rate of lysophosphatidyl glycerol acylation, observed when chloramphenicol is added with cerulenin at the time of culture transfer from 35° to 20°, could be explained in terms of the inhibition by chloramphenicol of fatty acid desaturation in such cultures (6). Such an effect would be expected if an unsaturated acyl thioester was preferred over the saturated analog for the esterification of the 2-position of lysophosphatidyl glycerol. Other explanations for the chloramphenicol effect may be equally possible, however.

Finally, we may ask whether lysophosphatidyl glycerol is a normal intermediate in the synthesis of phosphatidyl glycerol in B. megaterium. It may be that the esterfication of [U-^{14}C]palmitoyl-lysophosphatidyl glycerol in cerulenin-free cultures is so rapid that detection of this intermediate, in vivo, is unlikely. We are presently attempting to study this proposed pathway in cell-free preparations with the hope of obtaining unequivocal answers to this and other questions raised in the course of the work reported here.

ACKNOWLEDGMENTS: We thank Sue Tsuda for excellent technical assistance in this work and Ellin James for preparation of the figures for publication. These studies were supported in part by Contract EY-76-C-03-0012 between the Environmental Research and Development Administration and the University of California and by Research Grant AI-09829 from the National Institute of Allergy and Infectious Diseases, NIH, USPHS.

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