Effect of Thiolactomycin on *de novo* Fatty Acid Biosynthesis in Plants

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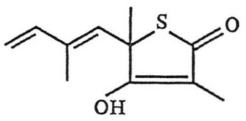
Thiolactomycin, Fatty Acid Biosynthesis, Acetyl-CoA : ACP Transacylase, Condensing Enzyme

Thiolactomycin was shown to be a potent inhibitor of *de novo* fatty acid biosynthesis in intact isolated chloroplasts (measured as [^{14}C]acetate incorporation into total fatty acids). In our attempt to further localize the inhibition site we confirmed the inhibition with a fatty acid synthetase preparation, measuring the incorporation of [^{14}C]malonyl-CoA into total fatty acids. From the two proposed enzymic targets of the fatty acid synthetase by thiolactomycin we could exclude the acetyl-CoA : ACP transacetylase. It appears that the inhibition by thiolactomycin occurs on the level of the condensing enzymes, *i.e.* the 3-oxoacyl-ACP synthases. We also demonstrated that the two starting enzymes of *de novo* fatty acid biosynthesis, the acetyl-CoA synthetase and the acetyl-CoA carboxylase, are not affected by thiolactomycin.

Introduction

Inhibition of *de novo* fatty acid biosynthesis can result in growth retardance and death of cells and organisms by shortening the supply of fatty acids, which are essential components of the glycerolipids of all types of biomembranes. Such inhibitors therefore are of dual interest: 1) because of there potential use in plant protection and 2) for scientists as a probe for a more detailed understanding of the fatty acid biosynthesis, metabolism and related metabolic pathways. Thiolactomycin (for chemical structure see Fig. 1), a natural occurring antibiotic from *Norcardia* sp. [1], is inhibiting specifically the prokaryotic-type fatty acid synthetases (FAS) of higher plants and most bacteria. This is in contrast to the antibiotic cerulenin, which inhibits prokaryotic and eukaryotic-type fatty acid synthetases [2, 3]. Prokaryotic fatty acid synthetases are composed of different discrete peptides, each catalyzing only one step in the *de novo* fatty acid biosynthesis, whereas eukaryotic FAS consists of multifunctional peptides with more than one enzymic activity on each protein. In *Escherichia coli* thiolactomycin was believed to inhibit two enzymic targets, the acetyl-CoA : ACP transacylase as well as the 3-oxoacyl-ACP synthase [4]. This was an interesting hypothesis with particular relevance to plants, because in the plant fatty acid biosynthesis these two enzymes were described as bottle-neck enzymes [5]. Hitherto it had not yet been investigated in plants, whether these two possible target enzymes are really affected by thiolactomycin. It had only been described that the incorporation of [^{14}C]acetate and [^{14}C]malonyl-CoA into fatty acids was blocked by thiolactomycin in isolated FAS preparations or plastids from spinach, castor bean, avocado mesocarp and oat [2, 6].



THIOLACTOMYCIN

Fig. 1. Chemical structure of the antibiotic thiolactomycin.

In this paper the inhibitory effect of thiolactomycin was reinvestigated with the aim to further localize the inhibition site. It was also tested whether the starting enzymes of *de novo* fatty acid biosynthesis, the acetyl-CoA synthetase and the acetyl-CoA carboxylase, are affected.

Materials and Methods

Chloroplasts were isolated as described in [7]. An enzymic, FAS-containing fraction, capable to incorporate different labelled precursors into fatty acids, was prepared from isolated barley chloroplasts as outlined in [8]. This enzyme preparation

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was also used to determine the activity of acetyl-ACP transacylase by measuring the transfer of [^{14}C]acetyl-CoA to ACP which then was precipitated by acids. Controls without acyl carries protein (ACP) were included. We followed the assay procedure described in [9]. Acetyl-CoA synthetase activity was measured *via* determination of the non-enzymic acylation of dithioerythritol with [^{14}C]acetyl-CoA [10]. The acetyl-CoA carboxylase, in turn, was determined by measuring the heat- and acid-stable radioactivity (incorporation of [^{14}C]hydrogencarbonate into malonyl-CoA).

Results and Discussion

Due to the fact that the *de novo* fatty acid biosynthesis in higher plants is exclusively localized in plastids [5], the effect of thiolactomycin was tested with a well established test-system monitoring the fatty acid biosynthesis in isolated chloroplasts [7, 8]. Thiolactomycin inhibited the incorporation of ^{14}C-labelled acetate into the total fatty acid fraction of isolated oat and spinach chloroplasts in a dose-dependent manner (Fig. 2). The I_{50}-value for thiolactomycin was in the range of 4 μM, which indicates a relatively high inhibitory potency for this natural occurring antibiotic. These results could be confirmed using a FAS enzyme preparation in which the incorporation of [^{14}C]malonyl-CoA into fatty acids was blocked by thiolactomycin (Fig. 3).

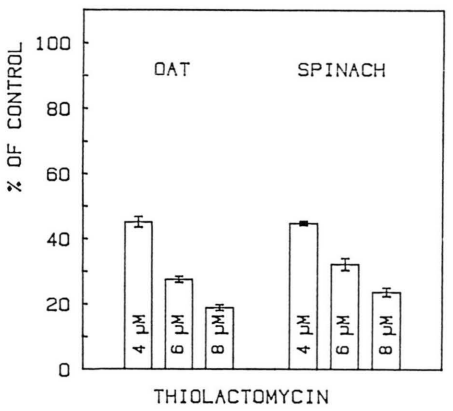

Fig. 2. The dose-dependent inhibition by thiolactomycin of *de novo* fatty acid biosynthesis from [^{14}C]acetate of isolated oat and spinach chloroplasts. Mean of 3 determinations with SD.

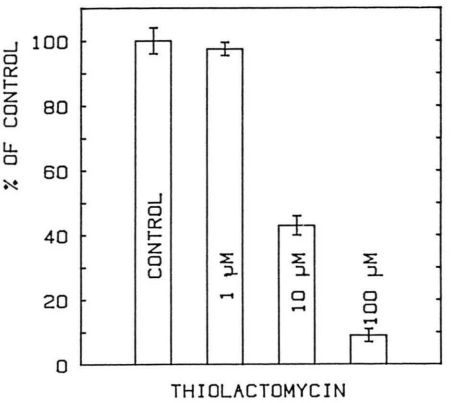

Fig. 3. Inhibition of the incorporation of [^{14}C]malonyl-CoA into total fatty acids by a fatty acid synthetase preparation of barley by thiolactomycin. Mean of 3 determinations with SD.

To further characterize the mode of action of thiolactomycin, we decided to measure the activity of acetyl-CoA:ACP transacylase, which was proposed to be one of the two target enzymes of thiolactomycin in *E. coli* [4]. Surprisingly no inhibition could be observed, even at concentrations of 10^{-4} M. This is in accordance with recent observations by other groups which reinvestigated the sensitivity of this enzyme in *E. coli* towards thiolactomycin and found no inhibition [9]. Based on the results in *E. coli* and on our results in barley only the 3-oxoacyl-ACP synthase remains as a possible target enzyme of the plant FAS.

At the present stage it is not possible to decide whether the target of thiolactomycin is solely the hitherto known condensing enzyme (3-oxoacyl-ACP synthase) and/or also the newly described short-chain 3-oxoacyl-ACP synthase [11]. In a preliminary notice the new short-chain synthase of *E. coli* was proposed to be one of the critical target enzymes for thiolactomycin [11]. The presence of such a short-chain 3-oxoacyl-ACP synthase could recently be demonstrated also in spinach, but the inhibitory activity of thiolactomycin was not tested [12]. To decide the question whether one or two of the condensing enzymes 3-oxoacyl-ACP synthases of the plant FAS are affected by thiolactomycin can only be decided after additional experiments.

The starting enzymes of the fatty acid biosynthesis sequence, the acetyl-CoA synthetase and the acetyl-CoA carboxylase are not affected by thiolactomycin, even at a concentration of 10^{-4} M thiolactomycin, indicating the specificity of this antibiotic.

Acknowledgement

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