The Effect of Avidin on the Biosynthesis of Fatty Acids in *Aspergillus niger* and *Aspergillus flavus*

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Abstract

Submerged cultures of *Aspergillus flavus* and *Aspergillus niger* were grown in a medium containing avidin, a substance which inhibits the conversion of acetate to malonate. Control cultures were grown without avidin.

Mycelium samples were taken at time points over a 70-hour incubation period, harvested by centrifugation, washed with distilled water and re-suspended in absolute methanol. After saponification, the fatty acids were extracted with hexane, methylated, isolated by thin layer chromatography, and separated and identified by gas liquid chromatography.

An increase in C_{16} fatty acids and a decrease in C_{18} fatty acids by cultures grown with avidin suggest that malonate plays an important role in the elongation of long-chain fatty acids in these organisms during the time interval from 5 to 15 hours.

In control cultures, the initially high percentage of stearic acid decreased while the percentage of oleic acid and linoleic acid increased, further indicating the conversion of stearate to oleate.

Several monoenoic acids are present in these organisms, but the only dienoic acid found is linoleic acid. This suggests that the conversion of oleate to linoleate involves a highly specific desaturase.

The first step in the biosynthesis of long chain fatty acids by way of the malonate pathway involves the enzyme, acetyl-CoA-carboxylase. This enzyme is one of the biotin-containing carboxylases and catalyzes the overall reaction.

$$\text{Acetyl-CoA} + HCO_3^- + \text{ATP} \rightleftharpoons \text{Malonyl-CoA} + \text{ADP} + P_i$$

This reaction is followed by the successive addition of 2-carbon units in the form of malonyl-CoA or malonyl-ACP to acetyl-CoA (4). The end product of this sequence of reactions is dependent upon the type of organism and is either palmitate (9) or stearate (3).

Although the malonate pathway is thought to be the predominant pathway for the biosynthesis of fatty acids, evidence for the existence of alternate pathways has been presented (4). Mattoo *et al.* (5) reported that the biosynthesis of fatty acids in intact mycelium of *Aspergillus flavus* was only partially inhibited after increasing amounts of avidin, a biotin inhibitor, had been added to the medium. Their results suggested that the formation of malonate was not essential for the synthesis of fatty acids in this organism. The fatty acid distribution of the products recovered from the inhibited cultures was not determined.

In the present experiments avidin was used to study the effect of the inhibition of malonate formation on the types of fatty acids synthesized by *A. niger* and *A. flavus*.

Materials and Methods

Aspergillus niger and *Aspergillus flavus*, obtained from Dr. K. B. Raper, University of Wisconsin, were maintained on slants of Czapek

medium and stored at 4° C. Spores were lightly scraped and washed from the slants with sterile medium. Five ml of spore suspension (A = 0.1 at 525 mμ) was added to 25 ml of sterile (autoclaved; 15 psi, 15 min) culture medium (glucose, 40 g; NH_4NO_3, 1 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; KH_2PO_4, 0.3 g; H_2O, 1 liter) in 250 ml Erlenmeyer flasks. The inoculated cultures were incubated for 24 hours at 28°C on a reciprocating shaker. At the end of this period, stir cultures were prepared by adding the contents of the flask to 420 ml of culture medium in a 2-liter Erlenmeyer flask, aerating (500 ml/min) and stirring for 60 hours. Forty-five units of avidin (1 U=amount capable of inactivating 1 λ biotin) had been added to the experimental culture medium; no avidin was added to control cultures.

Aliquots of the mycelial suspension were removed from the stir culture at various time points. After centrifugation, the mycelial mat was washed with distilled water, placed in 15 ml absolute methanol, and sonified for 3 min (J-17A Sonifier, Branson Sonic Co. Power). The sonified material was diluted with an equal volume of 15% KOH in methanol, refluxed for 2 hours, acidified with concentrated HCl, and extracted with hexane. After washing the extract with distilled water, drying over anhydrous sodium sulfate and removing the solvent on a rotary evaporator, the resulting fatty acids were methylated with diazomethane (7). The methyl esters were isolated on silica gel G thin layer plates and separated and identified by gas liquid chromatography using a 10-foot glass column packed with 5% poly(diethylene-glycol adipate) on 60/80 mesh Chrom GA/W; column temperature, 190° C; gas flow, 70 ml/min; Varian Aerograph 90-P instrument. For further identification of unsaturated fatty acids, methyl esters were separated by thin layer chromatography on 10% $AgNO_3$-impregnated silica gel G with chloroform:acetone (99.5:0.5) as the developing solvent prior to GLC analysis.

Results and Discussion

Members of the class Ascomycetes have a fatty acid composition similar to that of plant seeds (2) and like the growing leaves and seeds of plants produce the stearate to linolenate series of unsaturated fatty acids. Shaw (8) reported that the only C_{18} fatty acids produced by the ascomycetes are the Δ9-unsaturated fatty acids. Analysis of the products in the present experiments revealed that linoleic acid was the only dienoic acid synthesized by *A. niger* and *A. flavus*, although the monoenoic form of C_9 through C_{18} acids were identified, suggesting that the desaturase which is responsible for the conversion of oleate to linoleate is highly specific. This finding makes members of the genus Aspergillus particularly well-suited as experimental organisms for studying the biosynthesis of unsaturated fatty acids in plant-like organisms.

Cultures of *Aspergillus niger* grown in a medium containing inhibitory amounts of avidin did not differ from those grown in the absence of avidin as to the relative rate of palmitate synthesis (Fig. 1).

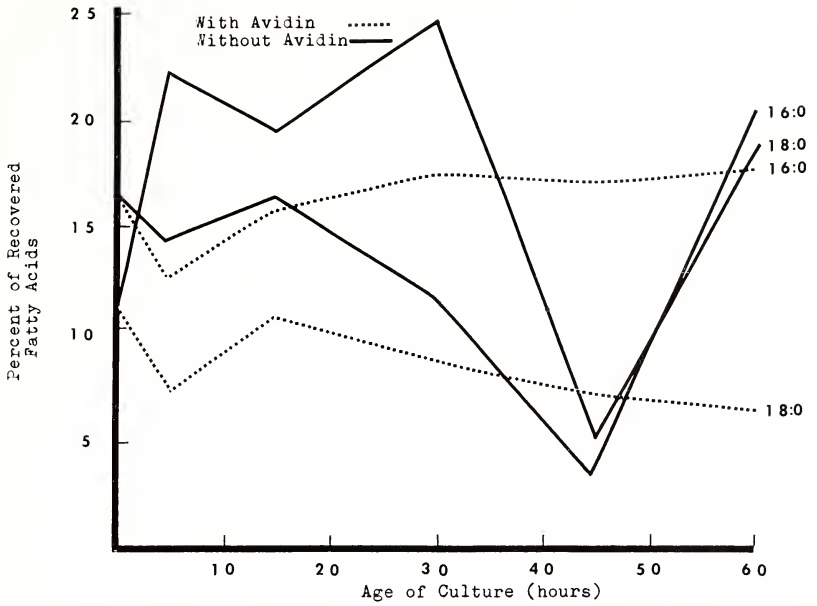

FIGURE 1. *Percent distribution of palmitic and stearic acids in mycelium of* Aspergillus niger *grown in media with and without avidin.*

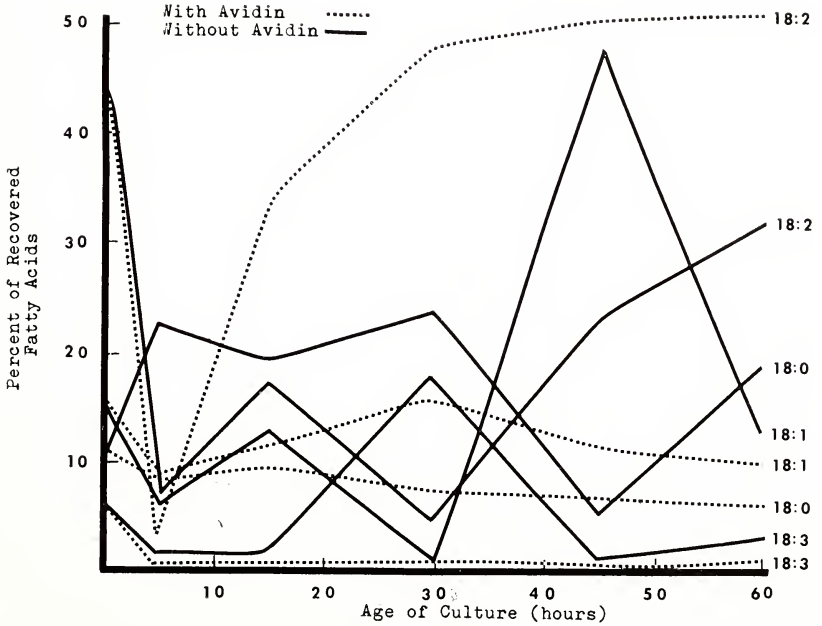

FIGURE 2. *Percent distribution of fatty acids in mycelium of* Aspergillus niger *grown in media with and without avidin.*

However, the relative amount of stearic acid produced was substantially lower in the mycelium grown in the avidin containing medium. In the control, the relative amount of stearic acid recovered increased from 12% in the inoculum to 22% after a 5-hour incubation period; with avidin, stearic acid decreased from 12% to 7%. This difference in stearate concentration is evident during the first 30 hours of the incubation period.

At the 5-hour time point only 23% of the fatty acids recovered from the mycelium grown in the avidin-containing medium was of the C_{18} series, compared to 33% in the control (Fig. 2).

These data suggest that malonate plays an important role in the elongation of palmitate to stearate. Although Mattoo (5) reported a partial overall inhibition of fatty acid synthesis upon the addition of avidin to the medium, our data show that all steps in the biosynthetic pathway were not affected to the same degree. In both *A. niger* and *A. flavus*, the addition of avidin to the culture medium inhibited the formation of long chain fatty acids to a greater degree than short chain. The importance of malonate in the elongation of palmitate to stearate in *Aspergillus* is in agreement with the report of Nagai and Bloch (6) who found that elongation to stearate in bacterial and plant extracts was dependent upon the presence of malonate. Barron (1) found that malonate was used for the elongation of preformed fatty acids by the soluble fraction of rat liver homogenates, while acetate was used by the mitochondrial fraction.

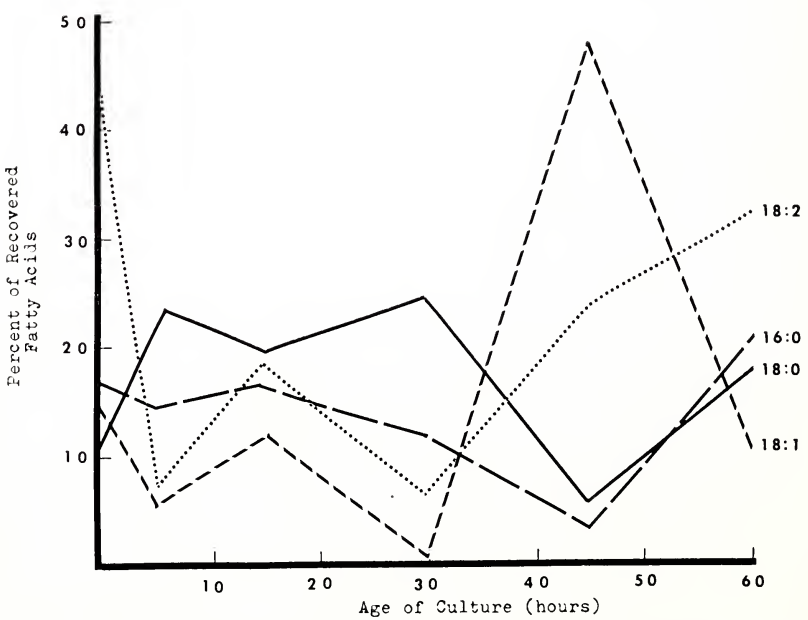

FIGURE 3. *Percent distribution of fatty acids in the mycelium of* Aspergillus niger *at various times in the incubation period.*

Bennett and Quackenbush (2) previously reported studies on the biosynthesis of fatty acids in *Penicillium chrysogenum* which indicated that endogenous palmitate was rapidly elongated to stearate and desaturated by the sequential pattern of oleate to linoleate to linolenate. In the present experiments, relatively large amounts of palmitate and stearate were synthesized at early time points in the growth period (22% and 12%, respectively, at 5 hours), followed by increases in oleate and linoleate concentration at 45 hours (Fig. 3), to give additional support for this pattern of elongation, then desaturation.

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