A PROPOSED MECHANISM FOR THE CONVERSION OF o-COUMARIC ACID TO 4-HYDROXY-COUMARIN IN Aspergillus fumigatus Fresenius

A. Bye, W.M. Ashton and H.K. King
Department of Biochemistry and Agricultural Biochemistry,
University College of Wales, Aberystwyth

Received June 10, 1968

Summary

Current investigations suggest that the conversion of o-coumaric acid to 4-OH coumarin is by a route analagous to the β-oxidation of fatty acids.

Introduction

Melilotic acid (A) and o-coumaric acid (B) both found in sweet vernal grass (Anthoxanthum odoratum) are converted to 4-OH coumarin (D) [a precursor of dicoumarol (E)] by Aspergillus fumigatus isolated from decomposing Anthoxanthum. This conversion has been previously reported in a variety of fungi [Bocks 1967; Bellis, Spring and Stoker, 1967; Shieh and Blackwood, 1967]. It seemed possible that a sequence similar to the β-oxidation of fatty acids might be involved, and a search was made for intermediates.

Methods

The fungus was grown in Czapek Dox liquid medium with 1% glucose as carbon source. Inoculation of the liquid medium was by spore transfer from agar slopes under sterile conditions. When the metabolism of a 'coumarin' was being studied two methods were employed. In the first method the 'coumarin' (5×10^{-3}M) was supplied together with glucose (1%) at the time of spore inoculation. In this case a lag of 36 to 48 hr., associated with a pH rise from 4.8 to 7.0, occurred before the 'coumarin' was metabolised. Alternatively, cells were grown, harvested, washed free of 'spent' medium, and resuspended in fresh Czapek Dox medium (pH 6.5-7.0) containing the 'coumarin' (5×10^{-3}M) being investigated.

In this case the medium used for resuspension was not enriched by added glucose.

In both methods metabolic products were isolated from the medium and cells following acidification of the medium and extraction with diethyl ether. The resulting ether extract was subjected to thin layer chromatography on Kieselgel-G and metabolites characterised by a variety of solvent systems.

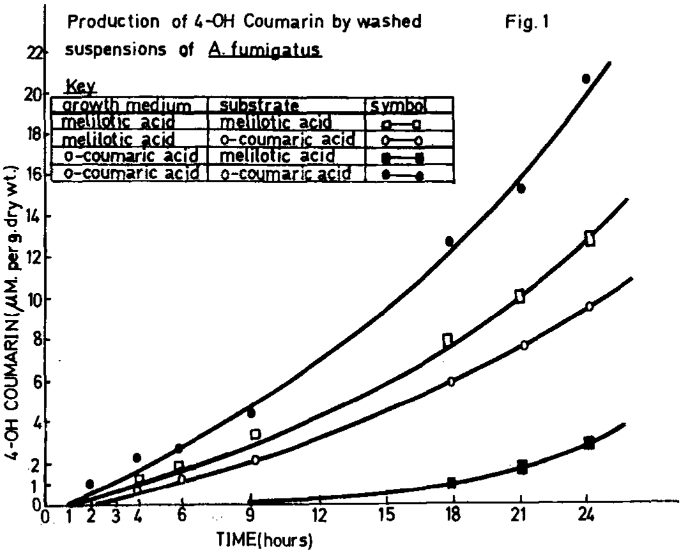
Results and Discussion

It was established that o-coumaric acid followed melilotic acid in the sequence by a "sequential induction" experiment. Cells grown in the presence of melilotic acid (and glucose) were separated from the medium and washed aseptically, then resuspended in fresh media containing only melilotic acid or o-coumaric acid respectively. In both cases 4-OH coumarin formation proceeded 'with only a short lag'. In a similar experiment with cells grown initially on o-coumaric acid however, melilotic acid was converted to 4-OH coumarin only after a lag of several hours; no comparable delay occurred with o-coumaric acid itself (Fig. 1.).

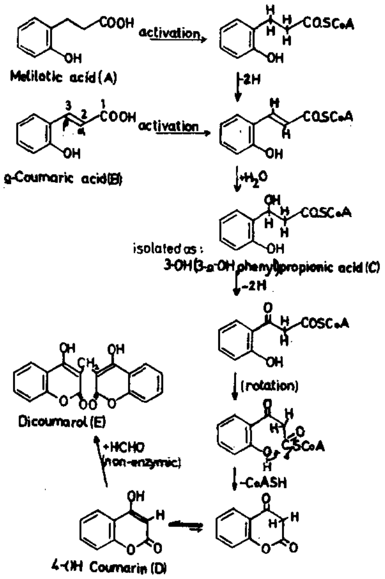
On incubation of washed suspensions of the organism with o-coumaric acid or melilotic acid in presence of ATP and hydroxylamine, the corresponding xanthates were produced, indicating that the organism was converting these substrates into their coenzyme A esters.

When the organism was grown in presence of glucose and ^{3}H- generally labelled o-coumaric acid, a radioactive product was obtained which was tentatively identified as 3-OH(3-o-OH phenyl)propionic acid (C) by co-chromatography with the authentic compound and from its ultra violet absorption spectrum. When incubated with the fungus in presence of glucose, it was removed with concurrent formation of 4-OH coumarin.

It was thought that a keto intermediate might be formed at some stage and accordingly washed suspensions were incubated with o-coumaric acid and semicarbazide. A semicarbazone was obtained and converted to the 2,4-dinitrophenylhydrazone for identification, and the carbonyl compound regenerated from the latter by treatment with benzaldehyde. Mass-spectroscopy and co-chromatography



SUGGESTED SCHEME FOR THE CONVERSION OF MELILOTIC AND o-COUMARIC ACIDS TO 4-OH COUMARIN AND DICOUMAROL.



with the authentic compound, also reduction to the corresponding alcohol and identification of the latter, indicated that the carbonyl compound was o-coumaraldehyde.

The role of 3-OH(3-o-OH phenyl)propionic acid as an intermediate in 4-OH coumaric formation is obvious but the formation of o-coumaraldehyde is difficult to fit into any plausible sequence. It is possible that it might be an 'unnatural' product arising from metabolism of the organism in the presence of semicarbazide, but its role is under further investigation.

The studies so far have suggested that the conversion of melilotic acid to 4-OH coumarin involves α,β unsaturation followed by a specific α,β hydration. However there is no subsequent severing of the side-chain such as occurs in the well-known oxidation of ω-phenyl fatty acids in animals.

References

Bellis, D.M., Spring, M.S. and Stoker, J.R., Biochem. J. 103, 202 (1967).
Bocks, S.M., Phytochemistry 6, 127 (1967).
Shieh, H.S. and Blackwood, A.C., Can. J. Biochem. 45, 2045 (1967).

Acknowledgements

We wish to thank M.S. Spring for his generous gift of authentic 3-OH (3-o-OH phenyl)propionic acid.