BIOSYNTHESIS OF SIDERAMINES IN FUNGI. MEVALONATE AS A PRECURSOR OF *cis*- AND *trans*-5-HYDROXY-3-METHYL-2-PENTENOIC ACIDS*

H. ANKE and H. DIEKMANN

Institut für Biologie, Lehrbereich Mikrobiologie, Universität Tübingen, D-74 Tübingen, Auf der Morgenstelle, Germany

Received 12 July 1971

1. Introduction

The two isomeric 5-hydroxy-3-methyl-2-pentenoic acids I and II (Δ^2-anhydromevalonic acids) were found in the culture fluids and bound in some metabolic products of ascomycetes and deuteromycetes [2–5]. As nothing is known about the biosynthesis and the metabolic function of these acids, we have investigated the incorporation of labelled precursors. When we had evidence that the anhydromevalonic acids are synthesized from mevalonic acid, we also investigated the stereospecificity of dehydration.

2. Materials and methods

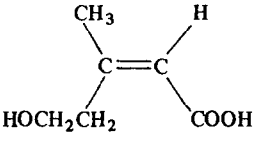
Fusarium cubense Smith (Tü 133) and *Fusarium sp.* (Tü 511) were cultured and the products isolated and purified as previously published [6–8]. For the labelling experiments the mycelia were harvested after 3–4 days by filtration, washed with saline and resuspended in the original volume of water with addition of 100 mg L-ornithine·HCl/150 ml. The flasks were shaken at 27° for 15 hr.

For the determination of radioactivity in the anhydromevalonic acid part, iron was removed from the sideramines and the hydroxamic acid bond split by hydrochloric or periodic acid [8, 9]. L-Ornithine · HCl and L-leucine were purchased from Merck, Darmstadt, DL-mevalonolactone from Fluka AG, Buchs, U-^{14}C-L-leucine (10 mCi/mmole), 1-^{14}C-L-leucine (58 mCi/mmole), 2-^{14}C-DL-mevalonolactone (5.8 mCi/mmole) and the mixtures of 2-^{3}H-mevalonic acids [10] from the Radiochemical Centre, Amersham.

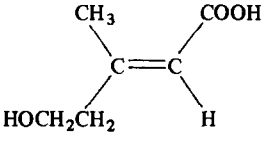
* Metabolic products of microorganisms, 93; for preceding publication see [1].

3. Results and discussion

Formally, the two Δ^2-anhydromevalonic acids can be synthesized



cis-Δ^2-anhydromevalonic acid (I)



trans-Δ^2-anhydromevalonic acid (II)

(a) by dehydration of mevalonic acid and (b) by reduction of β-methylglutaconic acid, which is – as the coenzyme A-derivative – an intermediate in the pathway from leucine to β-hydroxy-β-methylglutaryl-coenzyme A. Therefore, we incubated washed cells of *Fusarium cubense* with 2-^{14}C-mevalonate or U-^{14}C-leucine, and found incorporation of radioactivity into I from both precursors (table 1). Addition of unlabelled leucine to labelled mevalonate led to a decrease in the incorporation of mevalonate although the amount of sideramine synthesized increased. Estimation of the dilution factor for both

Table 1
Incorporation of labelled L-leucine (Leu) and DL-mevalonic acid (Mev) into sideramines with washed cells of *Fusarium cubense*.

Experiment No.	Labelled precursor	Unlabelled precursor	Sideramine produced	Incorporation (*calculated for D-isomer)	Specific activity of fusigen B	Dilution
	[μCi]	[mg]	[mg]	%	[dpm/mg]	
1	10 1-^{14}C-Leu	–	32	0.2	0.3×10^4	11,000
2	10 U-^{14}C-Leu	–	17.2	21.4	27.4×10^4	90
3	13 2-^{14}C-Mev	–	31.8	85 *	37.5×10^4	31
4a	10 U-^{14}C-Leu	–	9.5	7.3	17.0×10^4	140
4b	10 U-^{14}C-Leu	200 Mev	14	7.0	11.7×10^4	210
5a	20 2-^{14}C-Mev	–	9	35 *	70.1×10^4	20
5b	20 2-^{14}C-Mev	100 Leu	19.4	24 *	27.0×10^4	53

precursors in relation to the amount of unlabelled mevalonate or leucine (unpublished results) led to the conclusion that the internal pool of mevalonate is smaller than that of leucine. This could be the reason why leucine is incorporated with a higher dilution factor (90) than mevalonate (31). L-Leucine is incorporated directly and not via degradation to acetate. This was demonstrated by another experiment with 1-^{14}C-L-leucine, the labelling of which is lost during the decarboxylation of oxoleucine. Accordingly, almost no activity was found in the ornithine and anhydromevalonic acid part of the molecule. In all other cases more than 90% of the radioactivity was found in the anhydromevalonic acid part. From the facts mentioned it can be concluded that mevalonate is the precursor of I, and L-leucine can be the precursor of mevalonate.

An enzyme that dehydrates mevalonate has not been described so far. Looking for anhydromevalonic acids in the culture fluids of 22 fungi we had found that in most cases only one of the isomers could be detected [5]. Thus it seemed possible that there are two dehydratases in fungi with different stereospecificity.

To answer this question 2-tritiated mevalonic acids were fed to washed cells of *Fusarium cubense*, which produces fisigen (containing I), and *Fusarium sp.*, a producer of coprogen B (containing II). After incubation the sideramines were extracted, purified and split at the hydroxamate bonding. The radioactivity of the anhydromevalonic acid parts of the molecules were counted (table 2).

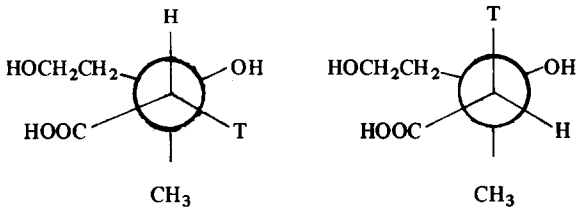
Table 2
Incorporation of 2-^{3}H-mevalonic acids in the anhydromevalonic acid part of sideramines.

Fungus	Sideramine produced	Specific activity of the anhydromevalonic acid part after incubation with 2-^{3}H-mevalonic acids	
		(2S, 3R) and (2R, 3S)	(2R, 3R) and (2S, 3S)
		[dpm/mg]	
Fusarium cubense	fusigen	54.0×10^4	5.6×10^4
Fusarium sp.	coprogen B	1.4×10^3	13.6×10^3

In interpreting the results it should be considered that *Fusarium cupense* incorporates more than 80%, *Fusarium sp.* less than 3% of 2-^{14}C-D-mevalonate. The amount of sideramines produced during the incubation time is nearly equal.

Most of the tritium label is lost when incubating (2R, 3R)-2-^{3}H-mevalonate with the producer of fusigen or when incubating the (2S, 3R)-isomer with *Fusarium sp.* The correct geometry of the product of elimination of water results when the substituents at C-2 and C-3 are eclipsed by rotation in clockwise (enzyme of *Fusarium cubense*) or anticlockwise (enzyme of *Fusarium sp.*) direction, as shown in fig. 1

In the growth test with *Lactobacillus homohiochi* ATCC 15434 [11] *cis*-Δ^2-anhydromevalonic acid



(2S, 3R) (2R, 3R)

Fig. 1. Newman projection for the (3R)-2-^{3}H-mevalonic acids.

could not replace mevalonate in a concentration 100-fold greater than mevalonate.

Acknowledgement

This investigation was supported by the Deutsche Forschungsgemeinschaft.

References

[1] M. Brufani et al., Experientia, in preparation.
[2] W. Keller, Schierlein, Helv. Chim. Acta 46 (1963) 1920.
[3] W. Keller-Schierlein, H. Zähner, V. Pünter-Streit and H. Bär, Biochem. Z. 341 (1965) 378.
[4] E. Fetz, B. Böhner and C. Tamm. Helv. Chim. Acta 48 (1965) 1669.
[5] H. Diekmann, Arch. Mikrobiol. 62 (1968) 322.
[6] H. Diekmann, Arch. Mikrobiol. 58 (1967) 1.
[7] H. Diekmann and H. Zähner, European J. Biochem. 3 (1967) 213.
[8] H. Diekmann, Arch. Mikrobiol. 73 (1970) 65.
[9] H. Diekmann, Arch. Mikrobiol. 74 (1970) 301.
[10] R.H. Cornforth and G. Popják, in: Methods in Enzymology, Vol. 15, ed. R.B. Clayton (Academic Press, New York and London, 1969) p. 359.
[11] G. Tamura, K. Ando, K. Kodama and K. Arima, Appl. Microbiol. 16 (1968) 965.