FEMS Microbiology Letters 49 (1988) 411–415
Published by Elsevier

FEM 03104

Metabolism of L-threonine and fatty acids and tylosin biosynthesis in *Streptomyces fradiae*

Aleš Vančura [a], Tomáš Řezanka [b], Jaroslav Maršlálek [c], Karel Melzoch [a], Gabriela Basařová [a] and Vladimir Křišťan [c]

[a] *Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology, 16628 Prague,* [b] *Department of Biogenesis of Natural Substances, Institute of Microbiology, Czechoslovak Academy of Sciences, 14220 Prague, and* [c] *Research Institute of Biofactors and Veterinary Drugs, 28161 Kouřim, Czechoslovakia*

Received 12 October 1987
Accepted 18 November 1987

Key words: *Streptomyces fradiae*; L-Threonine; Threonine aldolase; Threonine dehydratase; Fatty acids; Tylosin

1. SUMMARY

In *Streptomyces fradiae* L-threonine is catabolized by threonine dehydratase or threonine aldolase to 2-ketobutyrate or acetaldehyde and glycine, respectively. Threonine dehydratase synthesis is repressed and its activity is inhibited by NH_4^+ ions. Threonine aldolase is not repressed by NH_4^+ ions and its activity is slightly stimulated by these ions. The addition of threonine to the medium increased pronouncedly the fraction of non-branched fatty acids with an even carbon number under conditions when threonine dehydratase was repressed and inhibited. The results indicate that threonine serves as a source of propionyl-CoA and 2-methylbutyryl-CoA and also of acetyl-CoA required for tylosin and fatty acid biosynthesis.

Correspondence to: A. Vančura, Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology, 16628 Prague, Czechoslovakia.

2. INTRODUCTION

Streptomyces fradiae produces a macrolide antibiotic tylosin. Tylactone, the lactone ring of tylosin, is formed by condensation of 2 acetate units, 5 propionate units and 1 butyric acid unit [1]. In addition to malonyl-CoA, methylmalonyl-CoA and isobutyric acid produced from valine [2,3], some other amino acids can serve as precursors of these units [4]. Cell fatty acids (FA) are synthesized from the same subunits. The mutual regulation of the metabolism of amino acids, FA and the oligoketide antibiotic is a suitable model for studying the relationship between the primary and secondary metabolism and yields valuable information for a directed selection of high-production mutants. By influencing the synthesis or catabolism of certain amino acids it is possible to study the regulation of tylosin synthesis [5] and the flow of carbon atoms between the metabolism of amino acids and of oligoketides [6].

Omura found that the addition of L-threonine to a minimal medium led to an increased synthesis of the tylosin lactone from 18.0 μg/ml to 30.1

μg/ml. The author assumed that threonine was metabolized to propionyl-CoA [4,5].

Therefore, in the present work we studied the metabolism of threonine, its regulation and its relation with the tylosin and FA biosynthesis and found that *S. fradiae* metabolizes threonine to 2-ketobutyrate, occasionally to propionate and, in addition, to acetyl-CoA. This metabolic pathway apparently predominates under conditions of tylosin biosynthesis inhibition by NH_4^+ ions.

3. MATERIALS AND METHODS

3.1. Microorganisms and cultivation conditions

S. fradiae 30/1 was obtained from the collection of the Research Institute of Biofactors and Veterinary Drugs in Koufim, Czechoslovakia. Characteristics of the strain have already been published [7]. The first generation (inoculum) was cultivated in a synthetic medium containing (%, w/v): sucrose, 2.0; NaCl, 0.5; $CaCO_3$, 0.3; $MgSO_4 \cdot 7\ H_2O$, 0.05; K_2HPO_4, 0.1; $(NH_4)_2SO_4$, 0.33; *tris*-(hydroxymethyl)-aminomethane (Trizma base), 1.21. One ml of a solution of trace elements containing $FeSO_4 \cdot 7\ H_2O$, $MnCl_2 \cdot 4\ H_2O$, $ZnSO_4 \cdot 7\ H_2O$, $CuSO_4 \cdot 5\ H_2O$ and $CoCl_2 \cdot 2\ H_2O$ (each at 0.1%) was added to 1 litre of the medium.

The first generation was cultivated for 48 h, the second generation was inoculated with 5% of the first generation and was cultivated in medium A containing (%, w/v): sucrose, 2.5; $(NH_4)_2SO_4$, 0.17 (25 mM NH_4^+); K_2HPO_4, 0.05; concentrations of other compounds were identical with those in the medium for the first generation, or in medium B identical with medium A but containing sodium L-glutamate, 0.47% (25 mM) instead of $(NH_4)_2SO_4$; pH of all media was adjusted to 7.3 with 1 M HCl before heat sterilization. Solutions of L-threonine, $(NH_4)_2SO_4$ and L-glutamate were always sterilized separately and added before the inoculation. The cultivation was carried out in 500-ml flasks containing 70 ml of the medium on a reciprocal shaker (1.7 Hz). Biomass of 10–20 flasks was harvested by centrifugation (4°C). The presented values of the FA profile and of enzyme activities were obtained in a 72-h culture (end of the exponential phase of growth).

3.2. Analytical methods

Biomass was determined gravimetrically, cell-free extract was prepared from the mycelium disintegrated in a bacterial X-press (Biox) [8], and proteins were determined by means of the absorption method of Whitaker and Granum [9].

Threonine dehydratase (EC 4.2.1.16) was assayed by determining 2-ketobutyric acid produced with 2,4-dinitrophenylhydrazine [10] modified so that the reaction mixture contained 120 mM L-threonine and 1 mM AMP. Activity of threonine aldolase was assayed by means of alcohol dehydrogenase [11]. The activity values determined were also confirmed by determining the acetaldehyde produced by gas–liquid chromatography (GLC) [12]. The results obtained by means of GLC and alcohol dehydrogenase did not mutually differ by more than ±10%. Activity of transaminase branched-chain amino acids (EC 2.6.1.42) was assayed by determining 2-ketoisocaproate produced in a reaction with 2,4-dinitrophenylhydrazine [13]. Determinations of tylosin (biological titration using *Sarcina lutea* as test microorganism) and of fatty acids (capillary GC-MS in the HP 5995 apparatus) have already been described [6].

4. RESULTS AND DISCUSSION

Table 1 shows the FA content and growth of *S. fradiae* under given conditions. In medium B with added L-threonine, the fraction of unbranched FA with odd carbon number increased; during biosynthesis of these FA propionyl-CoA serves as starting primer. The anteiso FA content, derived from 2-methylbutyryl-CoA (starting unit) produced by oxidative decarboxylation of 2-keto-methylvalerate, a direct precursor of isoleucine biosynthesis (Fig. 1), increased as well. Addition of L-threonine to medium A increased the unbranched FA with an even carbon number whilst other FA groups decreased pronouncedly. The total amount of FA groups decreased pronouncedly. The total amount of FA was constant under all cultural conditions and the quantity of lipids (determined as $CH_3OH : CHCL_3$ (1 : 1) -extractable material) did not vary.

The results obtained when studying the regu-

Table 1

Effect of L-threonine concentration on the composition of cell fatty acids (72-h culture) and growth of *S. fradiae*

Fatty acids [a]	Medium A (25 mM NH_4^+)				Medium B (25 mM L-glutamate)			
	0 [b]	1	3	10	0	1	3	10
Even straight	7.8	14.3	24.3	46.8	35.4	31.5	27.6	24.7
Odd straight	12.5	12.1	10.7	9.3	8.4	13.1	15.8	17.1
Even iso branched	20.2	18.4	11.9	9.7	24.5	18.3	15.7	13.4
Odd iso branched	20.5	18.0	15.8	7.1	6.3	5.1	4.4	3.9
Anteiso branched	39.0	37.2	37.3	27.1	25.4	32.0	36.5	40.9
Maximum growth (mg/ml)	3.5	3.7	4.0	4.5	3.8	3.9	3.9	4.2
Doubling time (h)	18.1	17.8	17.3	15.2	11.2	11.1	10.9	10.5

[a] Individual classes of fatty acids included the following fatty acids; even straight, 14:0, 16:0, 16:1, 18:0, 18:1, 18:2; odd straight, 15:0, 17:0, 17:1; even iso branched, i-14:0, i-16:0; odd iso branched, i-15:0, i-17:0; anteiso branched, ai-15:0, ai-17:0.
[b] Addition of L-threonine (mM).

lation of enzymes of threonine metabolism involved in the production of acetyl-CoA, propionyl-CoA and 2-methylbutyryl-CoA are presented in Table 2. Threonine dehydratase was partially repressed by ammonium ions (medium A). The induction by threonine was more significant in cells cultivated in the medium B. Synthesis of threonine aldolase was not influenced by nitrogen sources used and threonine induction was similar in both the media. Synthesis of transaminase for branched amino acids was induced only by threonine added to the medium with glutamate (medium B).

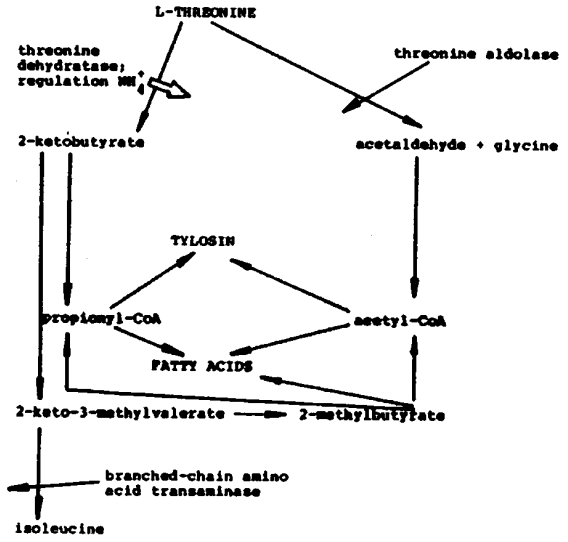

Fig. 1. Hypothetical scheme of L-threonine metabolism and its relationship with the biosynthesis of tylosin and fatty acids in *S. fradiae*.

Figure 2 illustrates the regulation of threonine dehydratase activity and threonine aldolase activity by NH_4^+ ions. Threonine dehydratase was significantly inhibited by NH_4^+ ions even when stimulated by AMP; threonine aldolase was slightly stimulated by NH_4^+ ions.

On the basis of changes in FA profile with nitrogen source and regulation of synthesis and activity of the enzymes involved, it can be assumed that threonine is metabolized to 2-ketobutyrate under conditions when threonine dehydratase is not either inhibited or repressed by ammonium ions. The latter compound can then be used for the synthesis of isoleucine or decarboxylated to propionyl-CoA. The assumption that decarboxylation is involved, is supported by a higher occurrence of unbranched FA with odd carbon number, whereas the increased amount of anteiso FA could be due to the 2-methylbutyryl-CoA produced. The existence of this metabolic pathway yielding 2-keto-3-methylvalerate, is also supported by the induction of transamination of branched-chain amino acids (Table 2).

When using NH_4^+ ions as a nitrogen source threonine dehydratase is inhibited and repressed and threonine is degraded by threonine aldolase to glycine and acetaldehyde which is further metabolized to acetyl-CoA. In this case the relative occurrence of unbranched FA with even

Table 2

Regulation of activity of threonine dehydratase, threonine aldolase and transaminase of branched-chain amino acids in media A and B (72-h culture)

Medium	Tylosin (μg/ml)	Threonine dehydratase (nkat/mg proteins)	Threonine aldolase (pkat/mg prot.)	Branched-chain amino acid transaminase (nkat/mg prot.)
A (25 mM NH_4^+)	89	8.1	26	0.23
A + 75 mM NH_4^+ (100 mM NH_4^+)	12	7.5	31	0.24
B (25 mM L-glutamate)	98	11.3	27	0.25
A + 10 mM L-threonine	112	11.4	52	0.26
B + 10 mM L-threonine	171	23.5	56	0.51

carbon number increased. However, threonine was found to stimulate tylosin production, irrespective of the metabolic pathway involved (Table 2). It is not clear, whether this stimulation involves only the production of acetyl-CoA and propionyl-CoA for the biosynthesis of tylactone or also the induction of branched-chain amino acid synthesis, as it is known that valine is a precursor of the tylosin butyrate unit [2,4]. Threonine aldolase activity might also be involved in the biosynthesis of the macrolide avermectins, where the starting unit can be derived from isoleucine or 2-methylbutyrate [14].

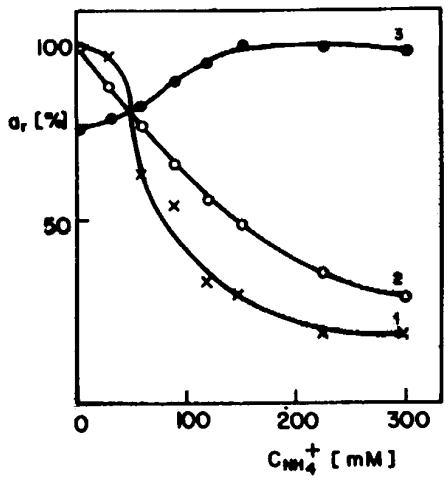

Fig. 2. Effect of NH_4^+ concentration on the activity of threonine dehydratase and threonine aldolase in a crude, desalted cell-free extract; Cells were cultivated in the medium B for 72 h and the cell-free extract was prepared as described in Section 3. a_r = relative activity; 1 = activity of threonine dehydratase without AMP; 100% a_r corresponds to the activity of 2.1 nkat/mg protein; 2 = activity of threonine dehydratase with AMP added to the assay mixture; 100% a_r corresponds to 11.3 nkat/mg protein; 3 = activity of threonine aldolase; 100% a_r corresponds to 27 pkat/mg protein.

Threonine dehydratase thus appears to be an enzyme regulating isoleucine synthesis and, in addition, the flow of carbon atoms in the catabolism of threonine and FA synthesis. In addition to valine dehydrogenase (EC 1.4.1.8), which is also repressed and inhibited by ammonium ions and is involved in the regulation of the biosynthesis of tylosin [15] and FA [16], the other enzyme which regulates the polyketide synthesis by means of regulation of branched-chain amino acid metabolism is threonine dehydratase. Its activity was neither inhibited nor stimulated by any branched amino acid. However, it was stimulated by AMP, ADP, ATP in decreasing order. Kinetic properties of the enzyme and its role in the regulation of tylosin biosynthesis in *S. fradiae* are being studied.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Z. Vaněk and Dr. V. Běhal for their valuable comments and for critically reading the manuscript.

REFERENCES

[1] Omura, S., Nakagawa, A., Takeshima, A., Miyazawa, J. Kitao, C., Piriou, F. and Lukacs, G. (1975) Tetrahedron Lett. 50, 4503–4506.

[2] Omura, S. Tsuzuki, K., Tanaka, Y., Sakakibara, H., Aizawa, M. and Lukacs, G. (1983) J. Antibiot. 36, 614–616.
[3] Pospíšil, S., Sedmera, P., Havránek, M., Krumphanzl, V. and Vaněk, Z. (1983) J. Antibiot. 36, 617–619.
[4] Omura, S., Tanaka, Y., Mamada, H. and Masuma, R. (1984) J. Antibiot. 37, 494–502.
[5] Tanaka, Y., Taki, A., Masuma, R. and Omura S. (1986) J. Antibiot. 39, 813–821.
[6] Dotzlaf, J.E., Metzger, L.S. and Foglesong, M.A. (1984) Antimicrob. Agents Chemother. 25, 216–220.
[7] Vančura, A., Řezanka, T., Maršálek, J. Křišťan, V. and Basařová, G. (1987) J. Basic Microbiol. 27, 167–171.
[8] Běhal, V., Hošťálek, Z. and Vaněk, Z. (1979) Biotechnol. Lett. 1, 177–182.
[9] Whitaker, J.R. and Granum, P.E. (1980) Anal. Biochem. 109, 156–159.
[10] Hatfield, G.W. and Umbarger, H.E. (1971) In: Methods in Enzymology, Vol. 17 B. (Tábor, H. and Taber, C.W., Eds.), pp. 561–566. Academic Press, New York, NY.
[11] Smith, L.V. and Gross, T. (1968) J. Biol. Chem. 243, 5651–5657.
[12] Mitruka, B.M. (1975) Gas Chromatographic Applications in Microbiology and Medicine, 1st edition, pp. 164–165. John Wiley and Sons, New York, NY.
[13] Coleman, M.S. and Armstrong, F.B. (1971) Biochim. Biophys. Acta 227, 56–66.
[14] Schulman, M.D., Valentino, D. and Hensens, O. (1986) J. Antibiot. 39, 541–549.
[15] Omura, S., Tanaka, Y., Mamada, H. and Masuma, R. (1983) J. Antibiot. 36, 1792–1794.
[16] Vančura, A. Řezanka, T., Maršálek, J., Vančurová, I., Křišťan, V. and Basařová, G. (1987) FEMS Microbiol. Lett., 48, 357–360.