Correlation between Generation Times and L-Threonine Dehydratase Activities in Isoleucine Revertants of *Serratia marcescens*

By S. KOMATSUBARA, M. KISUMI AND I. CHIBATA

*Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co.,
16-89 Kashima-3-chome, Yodogawa-ku, Osaka 532, Japan*

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INTRODUCTION

We have previously described the regulation of isoleucine–valine biosynthesis in *Serratia marcescens* (Kisumi, Komatsubara & Chibata, 1971*a*). In *S. marcescens* and other enteric bacteria, isoleucine biosynthesis is regulated by feedback inhibition and repression of L-threonine dehydratase (Umbarger, 1973). L-Threonine dehydratases from various sources were purified and their *in vitro* properties investigated (Umbarger, 1969). All the enzymes possessed two allosteric properties in common: feedback inhibition by isoleucine; and activation by valine or leucine. Mutants having feedback-resistant enzymes accumulated isoleucine in the medium, thus providing physiological confirmation of the feedback inhibition of L-threonine dehydratase (Kisumi *et al.*, 1972; Reh & Schlegel, 1969). However, little is known about the nature of feedback control of L-threonine dehydratase *in vivo*. This paper describes the correlation between generation times and enzyme activities in revertants derived from mutants of *S. marcescens*, lacking L-threonine dehydratase, which sheds some light on the *in vivo* feedback control of this enzyme.

METHODS

Bacteria. The bacterial strains used were *S. marcescens* strain 1 (wild type; Kisumi, 1962); an isoleucine auxotroph, strain 621 (L-threonine dehydratase-deficient) derived from strain 1; 20 spontaneous revertants derived from strain 621 by the method of Kisumi, Komatsubara & Chibata (1973); and strain 149 (L-threonine dehydratase-deficient) derived from strain AR130-1, an α -aminobutyric acid-resistant mutant of *S. marcescens* strain 1 (Kisumi *et al.* 1973).

Generation time. Bacteria were grown in minimal medium (MM) (Davis & Mingioli, 1950) modified by omitting citrate and increasing the glucose concentration to 0.5 % (w/v). Cultures in the late exponential growth phase were diluted into 500 ml shaking flasks containing 150 ml of the same medium. The initial absorbance at 660 nm was 0.05 (2.5×10^8 bacteria ml⁻¹). Cultures were incubated at 30 °C with reciprocal shaking (140 rev./min, 8 cm stroke) and their absorbances determined at hourly intervals. Bacteria grew exponentially until their absorbance reached 0.6. Generation times were calculated from the slopes of the linear portions of semi-log plots of the growth data.

Enzyme assays. Cell-free extracts were prepared from exponentially-growing bacteria. L-Threonine dehydratase and acetohydroxy acid synthetase activities were determined as described previously (Kisumi *et al.*, 1971*c*).

Fig. 1. Correlation between generation times and L-threonine dehydratase activities in the revertants derived from strain 621 (○) and in strain 1 (wild type) (●), cultured in minimal medium. Specific activity is expressed as $\mu\text{mol } \alpha\text{-ketobutyrate formed (mg protein)}^{-1} \text{ min}^{-1}$.

Table 1. *Generation times and isoleucine-valine biosynthetic enzymes of revertants derived from strain 621*

Enzyme activities were assayed using extracts prepared from bacteria cultured in minimal medium (MM). For growth of strain 621, the minimal medium was supplemented with 10^{-3} M L-isoleucine. The specific activities are expressed as $\mu\text{mol product formed (mg protein)}^{-1} \text{ min}^{-1}$.

Strain	Generation time (h)		L-threonine dehydratase			Specific activity of acetohydroxy acid synthetase
	MM	MM + 10 ⁻³ M L-isoleucine	Specific activity	Inhibition (%) by L-isoleucine:		
				3 × 10 ⁻⁴ M	10 ⁻³ M	
Wild type 1	1.0	1.0	0.15	68	95	0.053
Isoleucine auxotroph 621	∞	1.1	ND	—	—	0.042
Revertants 621-7	1.1	1.1	0.045	68	92	0.061
621-26	1.1	1.1	0.019	3	25	0.10
621-8	1.2	1.1	0.014	25	52	0.10
621-17	2.4	1.1	0.006	2	10	0.14

ND, Not detected.

RESULTS AND DISCUSSION

The 20 spontaneous revertants were isolated from colonies of different sizes on a minimal agar culture of strain 621. Their generation times and L-threonine dehydratase activities in minimal medium were measured and are shown in Fig. 1. Nine revertants with similar generation times (1.0 to 1.1 h) to the wild type possessed L-threonine dehydratase activities which were greater than 10 % of that of the wild type. Six other revertants grew more slowly in minimal medium (generation time less than 1.5 h), but grew at the same rate as the wild type in minimal medium plus isoleucine. These revertants had enzyme activities which were less than 10 % of that of the wild type. It was possible that the apparent low levels of L-

threonine dehydratase might be due to instability of the enzyme in cell-free extracts, but similar results were obtained with toluene-treated bacteria. In the revertants which had less than 10 % of the L-threonine dehydratase activity of the wild type, the enzyme was made relatively insensitive to feedback inhibition by isoleucine to varying extents (Table 1). Such revertants were derepressed for acetohydroxy acid synthetase, the second isoleucine-valine biosynthetic enzyme, suggesting that the intracellular isoleucine pools were limiting for normal growth; in other words, isoleucine biosynthesis is not subject to any feedback control in these strains. Thus, 10 % of the wild-type L-threonine dehydratase activity in the revertants allows sufficient *in vivo* production of α -ketobutyrate from threonine for normal growth.

To confirm that this low level of L-threonine dehydratase permits normal growth of the wild type in minimal medium, similar experiments were made with revertants of strain 149, an isoleucine auxotroph which had constitutive levels of the isoleucine-valine biosynthetic enzymes other than L-threonine dehydratase. As expected, an L-threonine dehydratase activity of more than 10 % of that of the wild type was necessary for growth at the same rate as that of strain AR130-1, the parent strain 149 (unpublished results).

Thus, based on *in vitro* assays, the wild type possessed an L-threonine dehydratase activity 10 times greater than the minimum necessary for normal growth. This suggests that about 90 % of this activity should be feedback-inhibited by isoleucine to avoid overproduction of the amino acid. This amount of *in vivo* feedback inhibition would be very severe, although repression may be relaxed. Mutants of *S. marcescens* resistant to α -aminobutyric acid, which were derepressed for L-threonine dehydratase and the other isoleucine-valine biosynthetic enzymes, did not accumulate isoleucine in a medium containing L-threonine (Kisumi, Komatsubara & Chibata, 1971*b*). Isoleucine hydroxamate-resistant mutants with a feedback-resistant L-threonine dehydratase produced large amounts of isoleucine from L-threonine (Kisumi *et al.*, 1971*d*). These facts also support the idea that feedback inhibition rather than repression acts as the primary mechanism for the control of the isoleucine biosynthesis.

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