ALTERNATE REQUIREMENT FOR PYRIDOXINE OR ISOLEUCINE IN MUTANTS OF ESCHERICHIA COLI

H. GRIMMINGER and F. LINGENS

Institut für Mikrobiologie und Molekularbiologie, Universität Hohenheim, 7 Stuttgart-Hohenheim, Germany

Received 8 October 1969

1. Introduction

Auxotrophs growing with either vitamin B_6 (= pyridoxine) or other substances were described previously [1, 2]. This report describes a mutant growing with either pyridoxine or isoleucine. Our experiments indicate that the alternate growth response of the mutant is a result of a mutation in the biosynthetic threonine deaminase (TD)gene. TD is the first enzyme of isoleucine biosynthesis.

2. Materials and methods

Escherichia coli K12 AB 287 requires threonine, leucine, histidine and thiamine for full growth. Minimal medium contained per liter of distilled water: $MgSO_4 \cdot 7\ H_2O$, 0.2 g; citric acid $\cdot\ H_2O$, 2.0 g; K_2HPO_4, 10.0 g; $NaNH_4HPO_4 \cdot 4\ H_2O$, 3.5 g; threonine, 0.1 g; leucine, 0.03 g; histidine, 0.02 g; thiamine, 1.0 mg. If necessary this medium was supplemented with pyridoxal (PAL, 0.1 mg/liter) or isoleucine (0.02 g/liter). Cultures were incubated at 37° C on a rotary shaker. Auxotrophs were induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) [3]. Cross-feeding tests were performed by the method of Dempsey and Pachler [4]. Pyridoxine was assayed after hydrolyzing the culture with H_2SO_4 using *Saccharomyces carlsbergensis* as tester strain [5]. Cells harvested from minimal medium containing isoleucine were disrupted by sonification. After centrifugation the supernatant was assayed for biosynthetic TD according to Umbarger and Brown [6]. Biodegradative TD was assayed in extracts of cells grown under anaerobic conditions in the medium described by Wood and Gunsalus [7].

3. Results and discussion

Following treatment with MNNG of *E. coli* AB 287 pyridoxine auxotrophs were isolated. Some of these do not only grow with PAL or pyridoxol (POL), but also with isoleucine or α-ketobutyric acid. In minimal medium supplemented with isoleucine or α-ketobutyric acid these mutants grow as rapidly as strain AB 287. With PAL however growth begins with a delay of about 20 hr in mutant NL 29 (table 1). The still remaining alternate requirement of the cells indicates absence of back mutation.

The production of pyridoxine was compared in strain AB 287, mutant NP 29 and mutant NP 562 which grows only with PAL or POL. In this experiment cells grown to logarithmic phase in minimal medium supplemented with PAL and isoleucine were centrifuged, washed and resuspended in minimal medium not containing PAL. Pyridoxine content of these suspensions (cells + medium) was measured immediately and after 6 hr incubation at 37° C (table 2). Pyridoxine production of mutant NP 29 equals that of strain AB 287, whereas NP 562 as a true vitamin B_6 mutant doesn't produce detectable amounts of the vitamin during 6 hr incubation. These results are consistent with our finding that mutant NP 29 feeds other pyridoxine auxotrophs blocked before pyridoxol.

E. coli cells grown under aerobic conditions in minimal medium contain only biosynthetic TD. The

Table 1
Growth (turbidity) of *E. coli* K 12 AB 287 and mutant NP 29 in minimal medium without and with isoleucine or PAL.

	AB 287			NP 29		
	Without addition	PAL	Isoleucine	Without addition	PAL	Isoleucine
20 h	1.36	1.34	1.36	0	0	1.39
40 h				0	1.4	

Table 2
Pyridoxine content (ng/ml) and growth (turbidity, in brackets) of washed cells suspended in minimal medium containing 20 mg isoleucine/l.

	0 hr	6 hr
AB 287	20 (1.3)	73 (1.76)
NP 29	20 (1.29)	60 (1.7)
NP 562	10 (1.25)	10 (1.72)

Table 3
Activity of biosynthetic threonine deaminase (μmoles keto acid formed per min per mg of protein $\times 10^3$) of *E. coli* K 12 AB 287 and mutant NP 29 with and without addition of pyridoxalphosphate.

	AB 287	NP 29
Without addition	24.3	0.12
10^{-4} M PALP	22.8	0.24

activity of this enzyme in NP 29 is only 0.5% compared with that in strain AB 287 (table 3). Following addition of pyridoxalphosphate (PALP) to cell extract of NP 29 activity of biosynthetic TD is raised twofold. In extracts of AB 287 an influence of PALP on TD activity is not observed (table 3). In contrast to the AB 287 enzyme that of mutant NP 29 is not inhibited by isoleucine.

Extracts of cells grown under anaerobic conditions [6] show similar activity of biodegradative TD in NP 29 and in AB 287.

From these results we conclude that the low TD activity of NP 29 causes the isoleucine requirement of the mutant. Since PALP increases activity of mutant TD *in vitro*, the growth effect of PAL *in vivo* may be explained by PALP induced activation of the altered enzyme. Probably the dissociation constant for PALP is higher in mutant TD than in TD of wild strain.

Acknowledgements

E. coli K 12 AB 287 was kindly supplied by Dr. U.Henning, Tübingen. We thank Dr. O.Oltmanns for helpful discussions. This work was supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie.

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