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Threonine Deaminase from a Nonsense Mutant of *Escherichia coli* Requiring Isoleucine or Pyridoxine: Evidence for Half-of-the-Sites Reactivity

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The mutant IP7 of *Escherichia coli* B requires isoleucine or pyridoxine for growth as a consequence of a mutation in the gene coding for biosynthetic threonine deaminase. The mutation of IP7 was shown to be of the nonsense type by the following data: (i) reversion to isoleucine prototrophy involves the formation of external suppression at a high frequency, as shown by transduction experiments; and (ii) the isoleucine requirement is suppressed by lysogenization with a phage carrying the amber suppressor *su-3*. Cell extracts of the mutant strain contain a low activity of threonine deaminase. The possibility that this activity is biodegradative was ruled out by kinetic experiments. The mutant threonine deaminase was purified to homogeneity by conventional procedures. The enzyme is a dimer of identical subunits of an approximate molecular weight of 43,000 (Grimminger and Feldner, 1974), whereas the wild-type enzyme is a tetramer of 50,000-dalton subunits (Calhoun et al., 1973; Grimminger et al., 1973). The mutant enzyme is not inhibited by isoleucine and does not bind isoleucine, as shown by equilibrium dialysis experiments. Pyridoxal phosphate enhances the maximum catalytic activity of the mutant enzyme by a factor of five, whereas the wild-type enzyme is not affected. In wild-type and mutant threonine deaminase the ratio of protein subunits and bound pyridoxal phosphate is 2:1. The activation of threonine deaminase from strain IP7 is due to a second coenzyme binding site, as shown by (i) spectrophotometric titration of the enzyme with pyridoxal phosphate and by (ii) measurement the pyridoxyl phosphate content of the enzyme after sodium borohydride reduction of the protein. The observation of one pyridoxal phosphate binding site per peptide dimer in the wild-type enzyme and of two binding sites per dimer in the mutant strongly suggests that one of the potential sites in the wild-type enzyme is masked by allosteric effects. The factors responsible for the half-of-the-sites reactivity of the coenzyme sites appear to be nonoperative in the mutant protein.

In a previous paper we described mutant strains of *Escherichia coli* that possess a nutritional requirement either for L-isoleucine or for vitamin B_6 (9). The alternative growth requirement is caused by a genetic defect in biosynthetic threonine deaminase (L-threonine hydrolyase, deaminating, EC 4.2.1.16), the first enzyme of isoleucine biosynthesis. The mutant enzyme requires enhanced levels of the coenzyme pyridoxal phosphate for maximum catalytic activity. Similar mutants of *Salmonella typhimurium* recently were described by Guirard et al. (11).

Cell extracts of the mutant IP7 requiring isoleucine or vitamin B_6 contain a low threonine deaminase activity (8). The catalytic activity of the mutant enzyme is not affected by isoleucine, in contrast to the feedback inhibition of the wild-type enzyme by isoleucine (24).

Biosynthetic threonine deaminase of *E. coli* has a molecular weight of approximately 200,000 and consists of four probably identical subunits (4, 10). Threonine deaminase of mutant IP7 has a molecular weight of 85,000 and consists of two identical subunits equivalent to a molecular weight of approximately 43,000 (8). This report shows that the low subunit molecular weight of the IP7 threonine deaminase results from premature chain termination due to a nonsense mutation. The purified enzyme contains bound pyridoxal phosphate; the ratio of protein subunits and pyridoxal phosphate is 2:1 in the mutant threonine deaminase and in the wild-type enzyme. Our results show that the activation of strain IP7 threonine deaminase by pyridoxal phosphate is caused by a second bind-

ing site of low affinity. This site is not available in the wild-type enzyme due to "half-of-the-sites reactivity."

A preliminary account of some of this work has appeared previously (8).

MATERIALS AND METHODS

Organisms. Phage P1 was supplied by U. Henning. Phage θ80 *psu-3* was a gift from J. Smith. Mutant IP7 (Leu$^-$, Ile$^-$) was derived from the leucine-deficient strain *E. coli* BL3 (21) after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (1). For the isolation of revertants, minimal agar plates supplemented with 20 mg of L-leucine per ml were spread with cell suspensions of mutant IP7. Colonies appearing after 3 to 5 days of incubation at 37 C were picked and purified by streaking. IP7A (Leu$^-$, Ile$^-$, Rbs$^-$) was isolated after treatment of strain IP7 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

Growth of bacteria. The culture media used for growth of bacteria were previously described (9). For preparation of biodegradative threonine deaminase, the respective strains of *E. coli* were grown anaerobically in the medium described by Wood and Gunsalus (27).

Materials. Pyridoxal phosphate (Serva), $NaBH_4$ (Merck), and L-[^{3}H]isoleucine (Amersham Buchler) are commercial products.

Transduction. P1 transduction experiments were performed according to Lennox (16). Phage θ80 *psu-3* was used for the identification of amber mutants; cells of the bacterial mutants were plated onto plates onto which a lawn of a lysate of θ80 *psu-3* had been spread.

Threonine deaminase. Biosynthetic threonine deaminase of *E. coli* BL3 (15) and of mutant IP7 (8) was purified as described previously. The assay of biosynthetic threonine deaminase has been described (8). Crude extracts containing biodegradative threonine deaminase were prepared by ultrasonic treatment of cells in 0.2 M potassium phosphate (pH 8) containing 1 mM glutathione and 1 mM adenosine 5-monophosphate. The test solution for the assay of biodegradative threonine deaminase contained: 0.2 M potassium phosphate, pH 8, 25 mM L-threonine, 1 mM adenosine 5-monophosphate, and 1 mM glutathione. 2-Ketobutyrate formed was determined as described by Friedemann and Haugen (7).

Enzyme units are expressed as micromoles of product formed per minute.

Equilibrium dialysis. Experiments were carried out in 0.25-ml cells (Dianorm, Innovativ-Medizin, Zürich) at 4 C using Visking 20/32 tubing. When equlibrium was reached, the ligand concentration was determined by liquid scintillation counting in each cell compartment. Samples of 0.01 ml were dissolved in 10 ml of scintillation cocktail [toluene, 667 ml; Triton X-100, 333 ml; 2,5-diphenyloxazole, 5.5 g; 1,4-bis-2-(5-phenyloxazolyl)benzene, 0.1 g (19)]. Radioactivity was determined in a liquid scintillation counter (ABAC SL 40; Intertechnique, Paris).

Determination of pyridoxal phosphate. For microbiological determination, enzyme samples were hydrolyzed in 1 N H_2SO_4 for 2 h at 121 C. The solutions were adjusted to pH 7 by 2.5 N NaOH. Appropriate samples were assayed for vitamin B_6 with *Saccharomyces carlsbergensis* ATCC 9080 (18). For determination of the phenylhydrazone of pyridoxal phosphate (26), 0.03 ml of concentrated H_2SO_4 was added to 0.2 ml of enzyme solution containing 0.3 to 0.5 mg of protein. The samples were centrifuged, and 0.02 ml of phenylhydrazine reagent (26) was added to 0.18 ml of the supernatant. After 10 min at room temperature the absorbance was determined at 410 nm. Pyridoxal phosphate was used as standard.

Number of binding sites for pyridoxal phosphate. For spectrophotometric titration (13), samples of purified threonine deaminase (containing 1 molecule of pyridoxal phosphate per dimer) were allowed to react in the dark with increasing concentrations of pyridoxal phosphate; after incubation for 4 min at 20 C the absorbance was recorded at 420 nm. Saturation of the enzyme with pyridoxal phosphate is indicated by the change in the slope of the curve. For $NaBH_4$ reduction (14), to samples of strain JP7 threonine deaminase in 0.1 M potassium phosphate (pH 7.4) solid sodium borohydride was added to a final concentration of 0.5 mg per ml. The reaction mixtures were allowed to react at 4 C for 1 h in the dark. The reduced samples were then dialyzed against 0.1 M potassium phosphate (pH 7.4). The pyridoxyl phosphate content was determined by measuring the absorbance of the reduced samples at 325 nm (absorption maximum of pyridoxyl-ε-lysine). ϵ_{325} of pyridoxyl-ε-lysine is 10,000 (22). Treatment with $NaBH_4$ was performed in buffer without pyridoxal phosphate and in buffer containing 10^{-4} M pyridoxal phosphate, respectively.

Other methods. Protein was determined by the biuret method. Protein concentrations of pure preparations were determined spectrophotometrically at 280 nm. The extinction coefficient ($E^{1\%}_{280}$) of 8.4, determined for the wild-type enzyme on a refractometric basis (2), was used for strain IP7 threonine deaminase. It should be considered that the extinction coefficient of the mutant enzyme may differ slightly from the wild-type enzyme due to the decrease in molecular weight.

RESULTS

The specific activity of threonine deaminase in crude extracts of mutant IP7 grown under aerobic conditions is 0.003 units/mg of protein (0.3% of wild-type activity). Extracts from cells grown under anaerobic conditions in the medium of Wood and Gunsalus (27) have a specific activity of 0.2 units/mg due to derepression of biodegradative threonine deaminase. The possibility that the low enzyme activity observed in cells grown under aerobic conditions represents a low residual level of biodegradative threonine deaminase is ruled out by the different kinetic properties of the respective activities. (i) Biodegradative threonine deaminase requires adenosine 5-monophosphate for maximum activity (20), whereas threonine deami-

nase of strain IP7 shows no activation by this compound as shown in Fig. 1 for crude extracts of strain IP7; similar results were obtained with the purified strain IP7 enzyme. (ii) The specific activities of disk electrophoretically pure enzyme from mutant IP7 and of pure biodegradative enzyme from *E. coli* are 0.3 and 880 units/mg of protein (23), respectively.

Genetic characterization of mutant IP7. The different subunit molecular weights of biosynthetic threonine deaminase from wild-type and mutant IP7 suggested the involvement of a chain-terminating mutation in the *ilvA* gene of the latter strain. This was directly confirmed by the following experiments.

(i) Revertants of mutant IP7 were checked for the presence of external suppressors by transduction experiments by the method of Yanofsky and co-workers (28). Strain IP7A (Leu$^-$, Ile$^-$, Rbs$^-$) that is unable to utilize ribose was derived from strain IP7 subsequent to mutagenic treatment. Spontaneous revertants of mutant IP7 prototrophic for isoleucine were isolated as described above. The ribose marker is about 60% co-transducible with the *ilvA* gene as shown by transduction experiments with *E. coli* BL3 (wild strain) as donor (Table 1). Strain IP7A was transduced to Rbs$^+$ by P1 lysates of different revertants and plated on a mineral salt medium supplemented with leucine, isoleucine, and ribose. Rbs$^+$ recombinants were then replicated onto isoleucineless medium to select isoleucine-independent colonies. No cotransduction of ribose utilization and isoleucine independence was observed with the revertant strains IP7-1, IP7-2, and IP7-4 as donors; this indicates that reversion in these strains arose by an unlinked suppressor mutation. With the other revertants about 60% of Rbs$^+$ transductants were Ile$^+$, indicating that the reversion had occurred within the *ilvA* gene (Table 1).

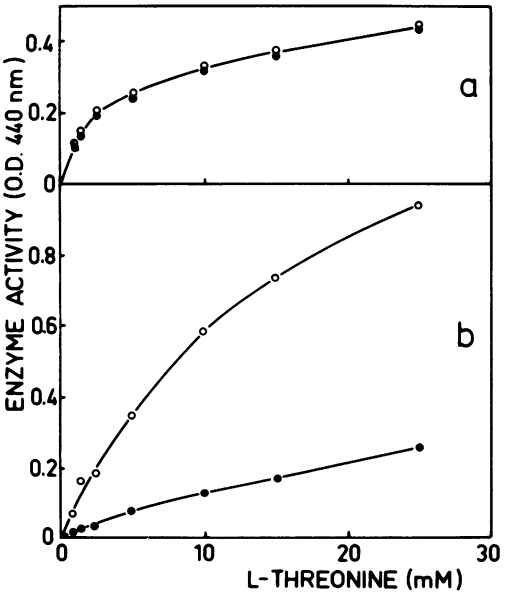

FIG. 1. *Substrate saturation curves of threonine deaminase in the presence (○) and absence (●) of adenosine 5-monophosphate. Crude extracts were prepared from strain IP7 grown with aeration in minimal medium supplemented with leucine and isoleucine (a) or without aeration in the medium of Wood and Gunsalus (27) (b). The crude extracts were dialyzed against the respective buffers. Protein concentrations used in the assays were 1.2 mg/ml (a) and 0.013 mg/ml (b).*

(ii) Phage θ80 *psu-3* was used to transduce an amber suppressor into mutant IP7. The suppressor *su-3* inserts tyrosine in place of the original amino acid coded by the wild-type gene. Cells of mutant IP7 were plated onto isoleucine-deficient medium onto which a lawn of a lysate of θ80 *psu-3* had been spread. Mutant IP7 grows well in the absence of isoleucine after lysogenization with this phage, thus indicating a mutation suppressible by an amber suppressor. In vitro the specific activity of threonine deaminase of IP7 θ80 *psu-3* does not exceed threonine deaminase activity of strain IP7; the enzyme produced by IP7 θ80 *psu-3* is not sensitive to inhibition by isoleucine.

Kinetic properties of the mutant enzyme. Kinetic experiments were performed with electrophoretically pure preparations with a specific activity of 0.32 units/mg. Figure 2 shows the activation of the mutant enzyme by pyridoxal phosphate; conversely, the wild-type enzyme is not affected by addition of the cofactor.

The influence of isoleucine is shown in Fig. 3 and 4. The mutant enzyme is not sensitive to

TABLE 1. *Genetic linkage of reversions to the ilv region*[a]

Donor	Rbs$^+$ recombinants selected	Isoleucine-independent prototrophs
E. coli BL3 (wild type)	150	90 (60%)
IP7-1	180	0
IP7-2	180	0
IP7-4	180	0
IP7-8	150	90 (60%)
IP7-9	150	84 (56%)
IP7-10	150	88 (59%)
IP7-13	150	92 (61%)
IP7-16	150	89 (59%)
IP7-126	150	94 (63%)
IP7-139	150	87 (58%)

[a] Strain IP7A (Leu$^-$, Ile$^-$, Rbs$^-$) served as recipient.

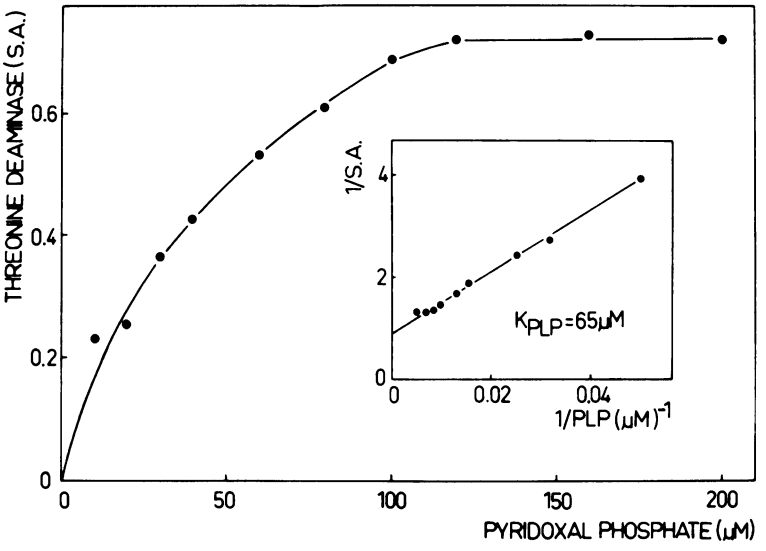

FIG. 2. *Catalytic activity of threonine deaminase of mutant IP7 as a function of pyridoxal phosphate (PLP) concentration. The enzyme was dialyzed exhaustively against 0.1 M potassium phosphate (pH 7.4) containing 1 mM mercaptoethanol and 1 mM ethylenediaminetetraacetate. S.A., Specific activity. Insert: Lineweaver-Burk plot.*

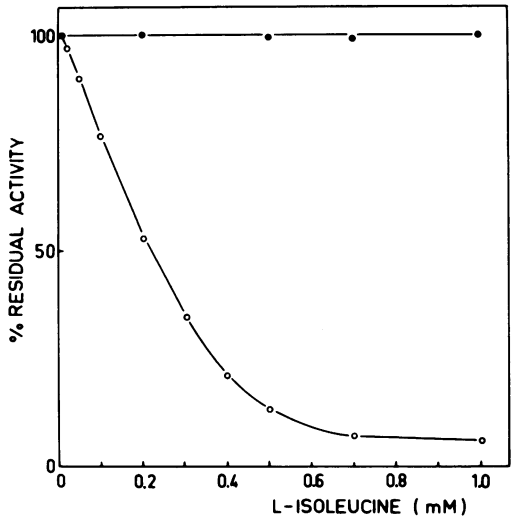

FIG. 3. *Catalytic activity of threonine deaminase of E. coli as a function of isoleucine concentration. Concentration of threonine was 25 mM. Symbols: ○, wild type; ●, IP7.*

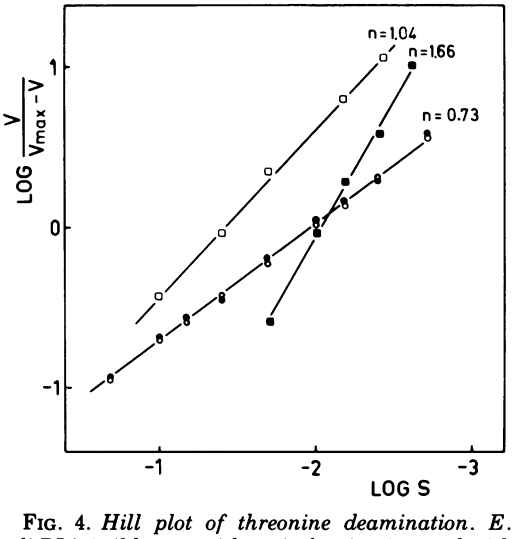

FIG. 4. *Hill plot of threonine deamination. E. coli BL3 (wild type) without isoleucine (□) and with 10^{-4} M isoleucine (■); mutant IP7 without isoleucine (○) and with 10^{-4} M isoleucine (●).*

inhibition by isoleucine, the allosteric inhibitor of threonine deaminase (24) (Fig. 3). In the absence of isoleucine the wild-type threonine deaminase shows hyperbolic substrate saturation kinetics (4, 12); the n_H value from the Hill plot is 1. Addition of isoleucine results in a sigmoidal substrate saturation curve (25); the n_H value is 1.6 at an isoleucine concentration of 10^{-4} M, indicating positive cooperativity of threonine sites (Fig. 4). On the other hand, n_H is 0.7 in the case of strain IP7 threonine deaminase, indicating negative cooperativity; isoleucine does not exert an effect on the substrate saturation kinetics of the mutant enzyme (Fig. 4). The same type of experiment was performed in the presence of 10^{-4} M pyridoxal phosphate; the Hill coefficient of strain IP7 threonine de-

aminase is 0.7, irrespective of the presence of the cofactor.

The binding of isoleucine to the purified IP7 enzyme was studied by equilibrium dialysis with [^{3}H]isoleucine. Strain IP7 threonine deaminase does not bind isoleucine (Fig. 5). Under the same conditions the wild-type enzyme binds two molecules of isoleucine per tetramer, with an association constant of 1.2×10^5/M (15).

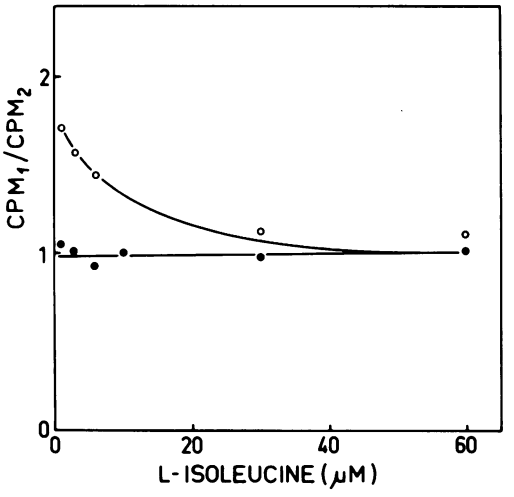

FIG. 5. *L-Isoleucine binding to biosynthetic threonine deaminase. Equilibrium dialysis experiments with [^{3}H]isoleucine were performed at 4 C. When equilibrium was attained, the radioactivity was determined in the enzyme chamber (counts per minute = cpm 1) and in the ligand chamber (cpm 2) by liquid scintillation counting. Enzyme concentrations were 0.5 mg/ml (wild type, ○) and 4 mg/ml (IP7, ●), respectively.*

Pyridoxal phosphate binding sites. Concentrated solutions of purified threonine deaminase of strain IP7 are yellow. The absorption spectrum shows a maximum at 420 nm, which is typical for pyridoxal phosphate-dependent enzymes (Fig. 6). The pyridoxal phosphate content of the purified enzyme was determined microbiologically and by measuring the phenylhydrazone of pyridoxal phosphate (Table 2). The microbiological determination yielded 1 pyridoxal phosphate per dimer. Chemical determinations yielded slightly lower values, presumably due to incomplete resolution of pyridoxal phosphate (6). The results indicate that

TABLE 2. *Pyridoxal phosphate content of threonine deaminase of mutant IP7*

Method	Pyridoxal phosphate content (mol/mol of threonine deaminase)
Microbiological (*Saccharomyces carlsbergensis*)	0.9 1.0 1.1
Absorbance of the phenylhydrazone of pyridoxal phosphate	0.65 0.7 0.7
$NaBH_4$ reduction	1.1 1.25
$NaBH_4$ reduction in 10^{-4} M pyridoxal phosphate	1.8 1.9
Spectrophotometric titration	2.0 1.8

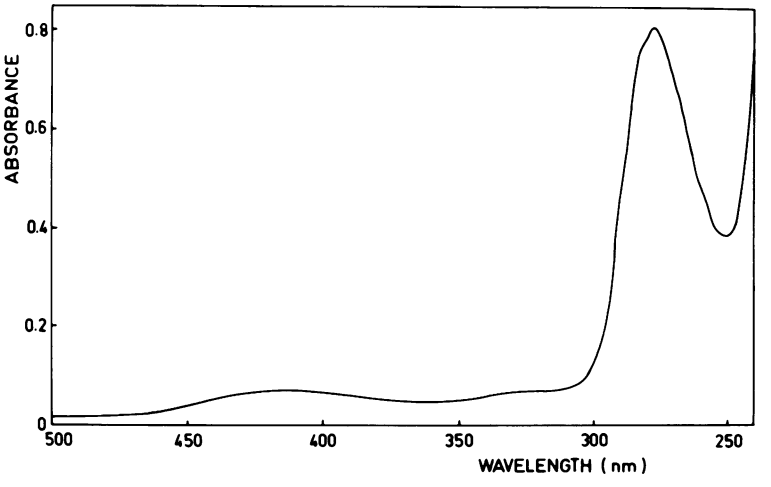

FIG. 6. *Absorption spectrum of threonine deaminase of mutant IP7. Native enzyme in 0.1 M potassium phosphate (pH 7.4) containing 1 mM mercaptoethanol and 1 mM ethylenediaminetetraacetate.*

threonine deaminase of strain IP7 contains 1/2 molecule of coenzyme per subunit; we have shown previously that the wild-type enzyme has the same ratio of bound pyridoxal phosphate and protein subunits (15).

These observations clearly rule out the hypothesis that activation of mutant IP7 threonine deaminase might be due to a decreased affinity of the enzyme for pyridoxal phosphate. The following data show that the mutant enzyme has a second pyridoxal phosphate binding site responsible for the observed activation. (i) Spectrophotometric titration of threonine deaminase with pyridoxal phosphate shows that the enzyme binds 1 additional mol of cofactor per 1 mol of enzyme (Fig. 7). Binding of the coenzyme was paralleled by enhancement of catalytic activity of the enzyme. (ii) The pyridoxyl phosphate content of the enzyme was determined by measuring the absorbance at 325 nm of the sodium borohydride-reduced enzyme. From these measurements 1 mol of pyridoxyl phosphate per 1 mol of IP7 dimer was obtained after reduction of the native enzyme. When the $NaBH_4$ treatment was performed in buffer containing 10^{-4} M pyridoxal phosphate, approximately 2 mol of pyridoxyl phosphate was found per 1 mol of threonine deaminase (Table 2). The same experiments as with the strain IP7 enzyme were performed with the wild-type threonine deaminase; under the described experimental conditions this enzyme did not show binding of more than two molecules of pyridoxal phosphate per tetramer by spectrophotometric titration and $NaBH_4$ reduction.

DISCUSSION

Biosynthetic threonine deaminase of *E. coli* is coded by the *ilvA* gene. The enzyme is derepressed in cells grown in minimal medium with aeration. When strain IP7 is grown under these conditions only a very low threonine deaminase activity is observed (as compared to the wild type). This indicates that strain IP7 carries a mutation in the *ilvA* gene. When *E. coli* is grown in a rich medium (27) under anaerobic conditions the biodegradative threonine deaminase is obtained; this enzyme is found in the respective extracts of mutant IP7 at the same level as in wild-type extracts. From some properties of the enzyme, which is present in mutant IP7 cells grown aerobically in minimal medium, it seemed possible that the low activity might be biodegradative in nature; biodegradative threonine deaminase has a subunit molecular weight of about 40,000 (23) and is not sensitive to inhibition by isoleucine (25). However, the possibility that strain IP7 enzyme might be biodegradative threonine deaminase was ruled out by the following data: (i) the enzyme does not need adenosine 5-monophosphate for maximum activity; (ii) the specific activities of purified IP7 threonine deaminase and of the biodegradative enzyme of *E. coli* are 0.32 (Table 3) and 880 (23), respectively. We conclude that the low threonine deaminase activity of the IP7 extracts represents the mutationally altered biosynthetic enzyme. Summarized data in Table 3 compare properties of threonine deaminase from wild-type and mutant strains.

Mutant IP7 is a nonsense mutant, as shown by several criteria. (i) The subunit molecular weight of IP7 threonine deaminase indicates that the mutant protein is a polypeptide fragment resulting from premature chain termination. (ii) P1 transduction studies using the ribose gene as selective marker have shown that some of the prototrophic revertants of IP7 arose by an unlinked suppressor mutation. (iii) The mutation is suppressible by the amber suppressor *su-3*, as shown by transduction studies with phage *θ80 psu-3*. However, no enhanced enzyme activities were observed in in vitro studies with strain IP7 *θ80 psu-3*. This may be due to instability of the suppressor-type enzyme.

Previous experiments with threonine deaminase of isoleucine-vitamin B_6 mutants of *E. coli* seemed to indicate that the affinity of the enzyme for pyridoxal phosphate might be reduced in the mutants (9). Whereas this hypothesis appears valid in the case of the isoleucine-vitamin B_6 mutants of *S. typhimurium* described by Guirard and co-workers (11), the present data show that threonine deaminase of *E. coli* IP7 contains one molecule of tightly bound pyridoxal phosphate per dimer. The wild-type threonine deaminase of *E. coli* contains two pyridoxal phosphate molecules per tetramer. Thus

TABLE 3. *Threonine deaminase of wild-type and mutant IP7 of E. coli*

Property	Wild type	IP7
Specific catalytic activity	230[a] (15)	0.32[a] (8)
Molecular weight	204,000 (4) 214,000 (15)	85,000 (8)
Subunit size	51,000 (4)	43,000 (8)
Activation of catalytic activity by pyridoxal phosphate	−	+
Pyridoxal phosphate content	2 per tetramer (15)	1 per dimer
Inhibition by isoleucine	+	−
Isoleucine binding sites	2 (15)	−

[a] Expressed as units per milligram of protein.

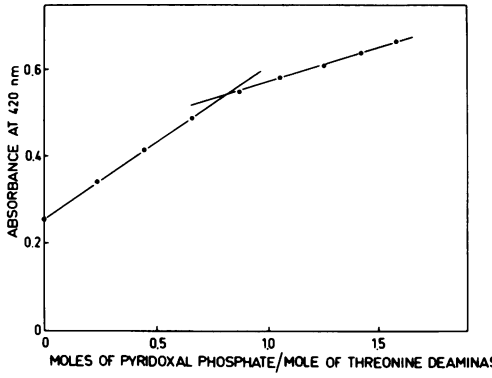

FIG. 7. *Spectrophotometric titration of mutant IP7 threonine deaminase with pyridoxal phosphate. Aliquots (0.05 ml) of 0.5 mM pyridoxal phosphate were added to 0.2 ml of enzyme solution (5 mg/ml in 0.1 M potassium phosphate [pH 7.4] containing 1 mM mercaptoethanol and 1 mM ethylenediaminetetraacetate). The absorbance at 420 nm was recorded after 5 min of incubation at room temperature.*

the ratio of pyridoxal phosphate and protein subunits is identical in wild-type and mutant enzyme.

Further studies showed that the observed activation of the mutant IP7 enzyme by pyridoxal phosphate is due to the binding of a second molecule of pyridoxal phosphate to the peptide dimer, as shown by photometric titration and $NaBH_4$ reduction. The hyperchromicity observed in photometric titration of the enzyme (Fig. 7) and $NaBH_4$ reduction in the presence of pyridoxal phosphate support the hypothesis that the coenzyme is bound to the second binding site by way of an azomethine bond. We hypothesize the involvement of the ϵ-amino group of lysine. Experiments to characterize the respective binding sites of both pyridoxal phosphate molecules are under way.

Saturation of the second binding site of strain IP7 threonine deaminase with pyridoxal phosphate results in about a fivefold enhancement of catalytic activity (Fig. 2). This may be explained by a conformational change or by high catalytic activity of the additional binding site. A decision is not possible on the basis of the present data.

The hypothesis of two pyridoxal phosphate sites and consequently of two active sites provides an explanation for the observed Hill coefficient of 0.7 (Fig. 4). We assume that these sites show negative cooperation with respect to threonine binding. Since the Hill constant is 0.7 in the presence and in the absence of pyridoxal phosphate, we suppose that the second binding site binds threonine irrespective of the presence of pyridoxal phosphate at this site.

The presence of an additional pyridoxal phosphate binding site in the nonsense protein is not easily explained. The new site might be generated de novo by conformational alterations due to the reduced chain length. This hypothesis appears rather unlikely, since pyridoxal phosphate is not only bound to the enzyme but also enhances the catalytic activity to a considerable extent. More likely, the second binding site may be masked in the wild-type enzyme and may be observable in strain IP7 as a consequence of the mutation; i.e., the wild-type enzyme—but not the mutant enzyme—shows half-of-the-sites reactivity.

The phenomenon of half-of-the-sites reactivity (17), in which one of two apparently identical subunits reacts with a substrate or a coenzyme, has been observed in a number of enzymes. Four possible mechanisms for the explanation of half-of-the-sites reactivity were discussed by Levitzki and co-workers (17): (i) involvement of nonidentical subunits; (ii) asymmetric association of two identical subunits to form a dimer with one of the sites deformed; (iii) steric hindrance of the second ligand molecule by the first due to adjacent sites; and (iv) ligand-induced conformational change that alters the vacant site (negative cooperativity). Levitzki and co-workers suggested that ligand-induced conformational change (negative cooperativity) may be the general mechanism for half-of-the-sites reactivity (17).

Decedue and co-workers studied the biosynthetic threonine deaminase of *S. typhimurium* (5). This enzyme contains two molecules of pyridoxal phosphate (3) and two isoleucine-binding sites; Decedue and co-workers (5) observed that apothreonine deaminase is capable of binding four rather than two isoleucine molecules and discuss the possibility that conformational changes obscuring potential cofactor sites also obscure two of the isoleucine sites.

The subunits of strain IP7 threonine deaminase represent about 80% of the wild-type enzyme. The loss of the COOH-terminal fragment involves considerable changes in the properties of the enzyme: (i) formation of a dimeric protein which does not aggregate to the tetrameric form; (ii) drop of catalytic activity to about 0.3% of wild-type activity; (iii) loss of feedback inhibition and of isoleucine binding; and (iv) binding of an additional molecule of pyridoxal phosphate (all-of-the-sites reactivity). This raises the question of whether the feedback resistance and the activation by pyridoxal phosphate might be a direct consequence of the dimeric state of the enzyme. The wild-type enzyme exists in a catalytically active dimeric state at pH 9.5; this dimer is not sensitive to isoleucine

inhibition (4). In view of this observation the dimer formation in strain IP7 might be responsible for the loss of feedback inhibition of the enzyme. On the other hand, preliminary results indicate that the wild-type enzyme binds isoleucine at pH 9.5 (K. Koerner, unpublished data). Furthermore, the wild-type enzyme is not activated by pyridoxal phosphate, even at basic pH values. Thus, there is no evidence indicating that the dimeric state of the mutant enzyme leads to feedback resistance and activation by pyridoxal phosphate.

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LITERATURE CITED

1. **Adelberg, E. A., M. Mandel, and G. C. C. Chen.** 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K 12. Biochem. Biophys. Res. Commun. 18:788-795.
2. **Babul, J., and E. Stellwagen.** 1969. Measurement of protein concentration with interference optics. Anal. Biochem. 28:216-221.
3. **Burns, R. O., and M. H. Zarlengo.** 1968. Threonine deaminase from *Salmonella typhimurium*. I. Purification and properties. J. Biol. Chem. 243:178-185.
4. **Calhoun, D. H., R. A. Rimerman, and G. W. Hatfield.** 1973. Threonine deaminase from *Escherichia coli*. I. Purification and properties. J. Biol. Chem. 248:3511-3516.
5. **Decedue, C. J., J. G. Hofler, and R. O. Burns.** 1975. Threonine deaminase from *Salmonella typhimurium*. Relationship between regulatory sites. J. Biol. Chem. 250:1563-1570.
6. **Dupourque, D., W. A. Newton, and E. E. Snell.** 1966. Purification and properties of D-serine dehydrase from *Escherichia coli*. J. Biol. Chem. 241:1233-1238.
7. **Friedemann, T. E., and G. E. Haugen.** 1943. Pyruvic acid. II. The determination of keto acids in blood and urine. J. Biol. Chem. 147:415-442.
8. **Grimminger, H., and J. Feldner.** 1974. Threonine deaminase from a mutant requiring isoleucine or pyridoxine: inability to form a tetrameric state. J. Bacteriol. 118:753-755.
9. **Grimminger, H., and F. Lingens.** 1969. Alternate requirement for pyridoxine or isoleucine in mutants of *Escherichia coli*. FEBS Lett. 5:225-226.
10. **Grimminger, H., I. Rahimi-Laridjani, K. Koerner, and F. Lingens.** 1973. Purification of threonine deaminase from *Escherichia coli*. FEBS Lett. 35:273-275.
11. **Guirard, B. M., B. N. Ames, and E. E. Snell.** 1971. *Salmonella typhimurium* mutants with alternate requirement for vitamin B_6 or isoleucine. J. Bacteriol. 108:359-363.
12. **Harding, W. M., J. A. Tubbs, and D. McDaniel.** 1970. Similar effects by valine and isoleucine on threonine deaminase. Can. J. Biochem. 48:812-815.
13. **Henderson, G. B., and E. E. Snell.** 1973. Crystalline L-histidinol phosphate aminotransferase from *Salmonella typhimurium*. Purification and subunit structure. J. Biol. Chem. 258:1906-1911.
14. **Johnson, G. S., and W. C. Deal, Jr.** 1970. Inactivation of tetrameric rabbit muscle pyruvate kinase by specific binding of 2 to 4 moles of pyridoxal 5'-phosphate. J. Biol. Chem. 245:238-245.
15. **Koerner, K., I. Rahimi-Laridjani, and H. Grimminger.** 1975. Purification of biosynthetic threonine deaminase from *Escherichia coli*. Biochim. Biophys. Acta 397:220-230.
16. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1:190-206.
17. **Levitzki, A., W. B. Stallcup, and D. E. Koshland, Jr.** 1971. Half-of-the-sites reactivity and the conformational states of cytidine triphosphate synthetase. Biochemistry 10:3371-3378.
18. **Morris, J. G., D. T. D. Hughes, and C. Mulder.** 1959. Observations on the assay of vitamin B_6 with *Saccharomyces carlsbergensis* 4228. J. Gen. Microbiol. 20:566-575.
19. **Patterson, M. S., and R. C. Greene.** 1965. Measurements of low energy beta-emitters in aqueous solution by liquid scintillation counting of emulsions. Anal. Chem. 37:854-861.
20. **Phillips, A. T., and W. A. Wood.** 1964. Basis for an AMP activation of "biodegradative" threonine deaminase from *Escherichia coli*. Biochem. Biophys. Res. Commun. 15:530-535.
21. **Rahimi-Laridjani, I., H. Grimminger, and F. Lingens.** 1973. Affinity chromatography of biosynthetic threonine deaminase of *Escherichia coli*. FEBS Lett. 30:185-187.
22. **Schnackerz, K. D., and E. A. Noltmann.** 1971. Pyridoxal 5'-phosphate as a site-specific protein reagent for a catalytically critical lysine residue in rabbit muscle phosphoglucose isomerase. Biochemistry 10:4837-4843.
23. **Shizuta, Y., A. Nakazawa, M. Tokushige, and O. Hayaishi.** 1969. Studies on the interaction between regulatory enzymes and effectors. III. Crystallization and characterization of adenosine 5'-monophosphate-dependent threonine deaminase from *Escherichia coli*. J. Biol. Chem. 244:1883-1889.
24. **Umbarger, H. E.** 1956. Evidence for a negative-feedback mechanism in the biosynthesis of isoleucine. Science 123:848.
25. **Umbarger, H. E., and B. Brown.** 1957. Threonine deamination in *Escherichia coli*. II. Evidence for two L-threonine deaminases. J. Bacteriol. 73:105-112.
26. **Wada, H., and E. E. Snell.** 1961. The enzymatic oxidation of pyridoxine and pyridoxamine phosphates. J. Biol. Chem. 236:2089-2095.
27. **Wood, W. A., and J. C. Gunsalus.** 1949. Serine and threonine deaminases of *Escherichia coli*: activators for a cell free enzyme. J. Biol. Chem. 181:171-182.
28. **Yanofsky, C., J. Ito, and V. Horn.** 1966. Amino acid replacements and the genetic code. Cold Spring Harbor Symp. Quant. Biol. 31:151-162.