JOURNAL OF BACTERIOLOGY, Dec. 1992, p. 8065–8072
0021-9193/92/248065-08$02.00/0

Vol. 174, No. 24

Functional and Structural Analyses of Threonine Dehydratase from *Corynebacterium glutamicum*

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Received 24 July 1992/Accepted 7 October 1992

Threonine dehydratase activity is an important element in the flux control of isoleucine biosynthesis. The enzyme of *Corynebacterium glutamicum* demonstrates a marked sigmoidal dependence of initial velocity on the threonine concentration, a dependence that is consistent with substrate-promoted conversion of the enzyme from a low-activity to a high-activity conformation. In the presence of the negative allosteric effector isoleucine, the $K_{0.5}$ increased from 21 to 78 mM and the cooperativity, as expressed by the Hill coefficient increased from 2.4 to 3.7. Valine promoted opposite effects: the $K_{0.5}$ was reduced to 12 mM, and the enzyme exhibited almost no cooperativity. Sequence determination of the *C. glutamicum* gene for this enzyme revealed an open reading frame coding for a polypeptide of 436 amino acids. From this information and the molecular weight determination of the native enzyme, it follows that the dehydratase is a tetramer with a total mass of 186,396 daltons. Comparison of the deduced polypeptide sequence with the sequences of known threonine dehydratases revealed surprising differences from the *C. glutamicum* enzyme in the carboxy-terminal portion. This portion is greatly reduced in size, and a large gap of 95 amino acids must be introduced to achieve homology. Therefore, the *C. glutamicum* enzyme must be considered a small variant of threonine dehydratase that is typically controlled by isoleucine and valine but has an altered structure reflecting a topological difference in the portion of the protein most likely to be important for allosteric regulation.

Threonine dehydratase (EC 4.2.1.16) catalyzes the conversion of threonine to 2-oxobutyrate, ammonium, and water. The anabolic enzyme initiates a sequence of five reactions leading to the ultimate formation of isoleucine. Furthermore, it is the only enzyme specific for isoleucine synthesis. Therefore, the dehydratase is an ideal target for the study of the flux control of isoleucine biosynthesis. Indeed, the enzyme of *Escherichia coli* was the first example of an enzyme at the beginning of a biosynthetic sequence that is subject to end-product inhibition (47). Work on this enzyme by Umbarger et al. (21, 48) and Changeux (6) has decisively influenced the allosteric regulation model of Monod et al. (33).

In all threonine dehydratases studied so far, isoleucine is the negative allosteric effector and valine is the positive allosteric effector. Isoleucine increases cooperativity, which is manifested as a sigmoidal dependence of activity in substrate saturation kinetics. Several aspects, however, are not well understood. Thus, without any effector for the enzyme of *E. coli*, sigmoidal dependence has been described (14, 48), whereas for the enzymes of *Salmonella typhimurium* (12), *Bacillus subtilis* (20), and yeast cells (4), hyperbolic kinetics have been noted. Threonine dehydratases are tetramers of identical subunits (8, 27), but it is not known how ligand binding promotes structural changes to effect substrate binding. Possibly, the number of binding sites, pyridoxal 5′-phosphate content, and the oligomeric state are involved, as shown for the enzyme of *S. typhimurium* (19, 23). There are several indications from mutant enzymes of *E. coli* and a homology comparison of the biosynthetic threonine dehydratase (*ilvA*) with its catabolic counterpart (*tdc*) that the carboxy-terminal portion is involved in allosteric regulation of the enzyme (10, 15, 45).

Since biosynthetic threonine dehydratase activity is tightly controlled, it is of course of major importance for the overproduction of isoleucine, as is our interest in using *Corynebacterium glutamicum*. This gram-positive bacterium is used for the manufacture of amino acids (26). In this bacterium, threonine dehydratase activity is also influenced negatively by isoleucine and positively by valine (32). We recently cloned the gene (7) and here report on the biochemical characteristics of the enzyme and its sequence, which might give new insights into the control of threonine dehydratase activity.

MATERIALS AND METHODS

Strains, plasmids, and growth. All bacterial strains and plasmids used are listed in Table 1. *E. coli* and *C. glutamicum* were grown on complex medium as described previously (43). When appropriate, kanamycin (25 μg/ml) or ampicillin (50 μg/ml) was added.

Genetic engineering. Plasmid DNA from *E. coli* was isolated by the method of Birnboim and Doly (2), and that from *C. glutamicum* was isolated by the variation described previously (43). *E. coli* was transformed by the RbCl method (18), and *C. glutamicum* was transformed by electroporation (28). For construction of pBM11, the 1,512-bp *Hin*dIII-*Eco*RI fragment of pBM1 was isolated, made blunt with Klenow polymerase, and ligated with pEKEx2, which had been cleaved with *Eco*RI, made blunt, and treated with calf intestine phosphatase. For construction of pBM10 and $pBM10_{inv}$, the 1,573-bp *Eco*RI fragment of deletion clone pBM1/Exo8, in which one *Eco*RI site originates from the pUC18 polylinker, was used. This *Eco*RI fragment was inserted in the *Eco*RI site of pEKEx2. Restriction enzymes, T4 DNA ligase, Klenow polymerase, and calf intestine phosphatase were obtained from Boehringer (Mannheim, Germany). Fragments used for ligation were separated on agarose gels and purified by use of the Geneclean kit from Dianova (La Jolla, Calif.).

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TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Relevant characteristic(s)	Source or reference
Strains		
E. coli DH5	F⁻ *supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	18
C. glutamicum ATCC 13032	Type strain	1
Plasmids		
pUC18	Cloning vector; Ap^r	49
pZ1	*E. coli-C. glutamicum* shuttle vector; Km^r	31
pEKEx2	*E. coli-C. glutamicum* expression vector; Km^r *tacP lacI*^{q}	13
pCC4	pHC79 containing *ilvA* from *C. glutamicum* as a *Sau*3A insert	7
pBM1 and pBM1$_{inv}$	pUC18 containing *ilvA* as a 2.85-kb *Hin*dIII insert from pCC4	This work
pBM1/Exo8	Exonuclease III deletion of pBM1	This work
pBM10 and pBM10$_{inv}$	pEKEx2 containing a 1,573-bp *Eco*RI insert from pBM1/Exo8	This work
pBM11	pEKEx2 containing a 1,512-bp *Hin*dII-*Eco*RI insert from pBM1	This work

DNA sequencing and sequence analysis. Plasmids pBM1 and pBM1$_{inv}$ were digested with *Kpn*I and *Bam*HI, and progressive unidirectional deletions were made by use of the 3′,5′-exonuclease activity of exonuclease III (Promega, Madison, Wis.). DNA sequence analysis was performed by the dideoxy chain termination method of Sanger et al. (42). Sequencing reactions were carried out with an AutoRead sequencing kit from Pharmacia. Electrophoretic analysis of the sequencing reactions was done with an automated laser fluorescence DNA sequencer from Pharmacia-LKB (Piscataway, N.J.).

Sequence data were compiled and analyzed by use of the HUSAR program package (release 2.0; EMBL, Heidelberg, Germany). Multiple alignments were carried out by use of the algorithm of Myers and Miller (35) with the CLUSTAL program package (22).

Enzyme assays. Cell extracts were prepared as described previously (43). Two threonine dehydratase assays were used. As a standard assay (i.e., to determine overexpression), the oxobutyrate formed was assayed as its semicarbazone derivative (30). The assay mixture contained, in a final volume of 0.8 ml, 0.1 M potassium phosphate (pH 8.2), 1 mM pyridoxal phosphate, 40 mM L-threonine, and crude extract. The assay mixture was incubated at 30°C, and 200-μl samples were taken. The reaction was terminated by the addition of 1 ml of reagent (1 g of semicarbazide plus 0.9 g of sodium acetate in 100 ml of H_2O). After incubation for 15 min at 30°C, 3 ml of H_2O was added, and the extinction was read at 254 nm. Appropriate blanks and standards were processed identically. For the second assay used for the kinetic studies, oxobutyrate formation was quantified with lactate dehydrogenase. First, the extract was gel filtered with 0.1 M potassium phosphate (pH 7)–1 mM dithiothreitol–0.2 mM pyridoxal phosphate–0.1 mM EDTA. The filtrate was stored unfrozen in ice. The assay mixture contained, in a final volume of 0.8 ml, 0.1 M phosphate buffer (pH 8.2), 30 μM pyridoxal phosphate, 30 μl of filtrate, and various amounts of L-threonine. The assay mixture was incubated at 30°C for 3 min, the reaction was terminated by the addition of 40 μl of trichloroacetic acid (36%), and the mixture was placed in ice for 6 to 15 min. Oxobutyrate was reduced by the addition of 40 μl of 2 M Tris–35 μl of 4 mM NADH–80 μl of H_2O–5 μl (8 U) of pig heart lactate dehydrogenase. After incubation for 15 min at 30°C, the assay mixture was centrifuged and the extinction was read at 340 nm to determine the NADH consumed.

Gel filtration. Gel filtration was done by fast protein liquid chromatography on Superose 12 (Pharmacia) in 0.1 M phosphate buffer (pH 7)–1 mM dithiothreitol–0.5 mM isoleucine–0.2 mM pyridoxal phosphate–0.1 mM EDTA. Standards (molecular weights) were catalase (232,000), aldolase (158,000), albumin (67,000), ovalbumin (43,000), lactate dehydrogenase (140,000), amylase (200,000), glucose 6-phosphate dehydrogenase (110,000), and alcohol dehydrogenase (150,000).

Nucleotide sequence accession number. The GenBank accession number for the nucleotide sequence for the *C. glutamicum* threonine dehydratase gene is L01508.

RESULTS

Steady-state kinetics. The first goal was to determine the kinetic properties of the enzyme of *C. glutamicum* by creating substrate saturation curves. For this purpose, a new, sensitive assay was employed to enable the direct use of a gel-filtered extract containing overexpressed enzyme (*C. glutamicum* ATCC 13032/pBM10$_{inv}$) to reduce any of the known effects on the stability of the protein (20). Figure 1, panel A1, shows that the activity of the *C. glutamicum* enzyme was sigmoidally dependent on the threonine concentration. With the more sensitive Eadie plot (Fig. 1, panel A2), an obvious maximum showing the positive homotropic cooperativity even more clearly was obtained (9). From this analysis, a V_{max} of 0.31 μmol/min/mg was derived, with a ($K_{0.5}$) of 21 mM. The Hill plot yielded a Hill coefficient of 2.4. The effect of isoleucine on the initial velocity of the enzyme is shown in Fig. 1, panel B1. At low threonine concentrations (ca. 20 mM), comparable to the $K_{0.5}$ in the absence of an effector, the enzyme had only very weak activity and high threonine concentrations were required to obtain maximum velocity. Analysis of these data yielded a V_{max} of 0.51 μmol/min/mg, a $K_{0.5}$ of 78 mM and, in the Hill plot, a slope of 3.7, indicating the increase in cooperativity due to the presence of the negative heterotropic effector isoleucine. In contrast, the effect of valine was to activate the enzyme, as has been described for threonine dehydratases from other sources (19, 48), but usually only in the presence of isoleucine (5, 19). For the *C. glutamicum* enzyme, the dependence of the initial velocity on the threonine concentrations was hyperbolic (Fig. 1, panel C1) and yielded a straight line in the Eadie plot (Fig. 1, panel C2). Accordingly, the Hill coefficient was 1.2, indicating no (or only very weak) cooperativity when valine was present. The $K_{0.5}$ was reduced to 12 mM, and the V_{max} was 0.45 μmol/

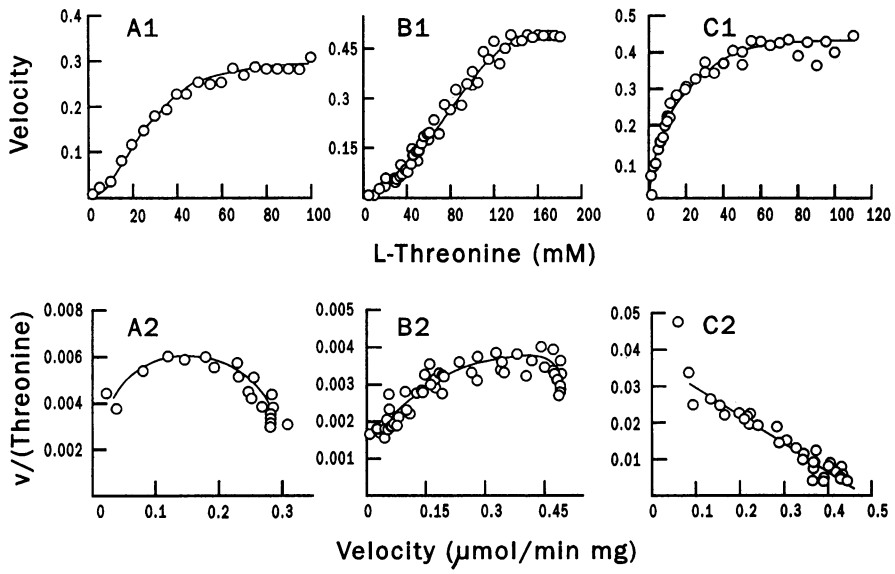

FIG. 1. Steady-state kinetics of threonine dehydratase. Panels A, B, and C contained no effector, 0.625 mM isoleucine, and 0.75 mM valine, respectively. Panels labeled 1 show saturation curves, and panels labeled 2 show the corresponding Eadie plots. In all assays, 77 μg of protein was used.

min/mg. These data show that the allosteric control of the enzyme of gram-positive *C. glutamicum* is comparable to that of the enzyme of *E. coli* when the recent data published by Umbarger (48) and Eisenstein (14) are used.

Molecular weight. For determination of the molecular weight of the native enzyme, an extract of *C. glutamicum* ATCC 13032/pBM10$_{inv}$ containing the cloned structural threonine dehydratase gene, *ilvA*, on a multicopy plasmid (7) was used. The molecular weight determined by gel filtration was 210,000 ± 20 000 (data not shown). This size is about the same as those reported for the enzymes in enterobacteria (19), *B. subtilis* (21), and *Brevibacterium flavum* (32), which is a close relative of *C. glutamicum*.

Sequencing and nucleotide sequence analysis. To make sequencing possible, we recloned a 2.85-kb *Hin*dIII fragment carrying *ilvA* from the original cosmid, pCC4 (7). It was inserted into *Hin*dIII-cut pUC18 in both directions to yield pBM1 and pBM1$_{inv}$. A detailed restriction map was made (Fig. 2) and, after preliminary analysis, a contiguous sequence of 1,925 kp for both strands was determined with overlapping subclones.

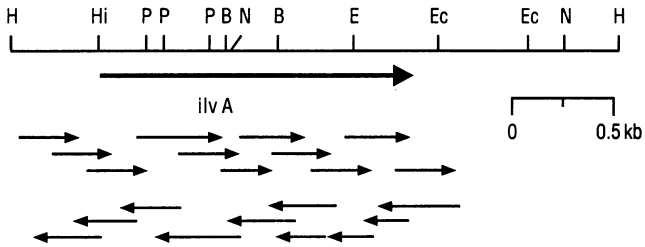

FIG. 2. Restriction map and sequencing strategy for the chromosomal *Hin*dIII fragment in pBM1 and pBM1$_{inv}$. The location and direction of transcription of *ilvA* are also shown. In pBM1, *ilvA* is transcribed in the same direction as *lacZ'*. The arrows indicate the extents and orientations of the nucleotide sequence determinations. H, *Hin*dIII; Hi, *Hin*dII; B, *Bgl*II; N, *Nco*I; Ec, *Eco*RI; E, *Eco*RV; P, *Pst*I.

The resulting nucleotide sequence is shown in Fig. 3. The coding region analysis (data not shown), based on a codon frequency table of 12 sequenced *C. glutamicum* genes, revealed an open reading frame with possible translational start sites at nucleotide (nt) 432, 447, 468, or 471. The stop codon is at nt 1740. However, only one authentic ribosome binding site (RBS) (5'-AGGAGAAGAT-3') is obviously present, at nts 408 to 417; the bases complementary to the 3' end of the *B. subtilis* 16S rRNA (34) are underlined. This sequence is spaced 20 nts in front of the ATG at nt 432, which is therefore the probable translational start site. In addition, a construct was made by taking advantage of a *Hin*dIII site between the assigned RBS and the start codon. The *Hin*dIII-*Eco*RI fragment from pBM1 (Fig. 2) was inserted into plasmid pEKEx2. This plasmid provides an inducible promoter functional in *C. glutamicum* but no RBS (13). The generated plasmid, pBM11, failed to result in expression of *ilvA* in *C. glutamicum*, although in similar experiments, e.g., with the structural glutamate dehydrogenase gene together with its RBS, overexpression was obtained (3). Besides the structural information, these data provide further evidence of a correctly assigned RBS and start codon.

Two more plasmids were constructed as follows. A deletion clone (pBM1/Exo8) used for sequencing and containing an *ilvA* fragment including 49 bp in front of the translational start codon was inserted in either orientation into plasmid pEKEx2. The resulting plasmids, pBM10 and pBM10$_{inv}$, both showed high levels of threonine dehydratase overexpression, with specific activities of 0.12 and 0.15 μmol/min/mg, respectively, without induction. These activities are 18- to 20-fold the single-copy activity (specific activity, 0.02 μmol/min/mg), indicating that the information for transcriptional initiation is restricted to the sequence specific to pBM10 and pBM10$_{inv}$. These data imply a close juxtaposition of the promoter and the translational initiation site, as has been found for a variety of *Streptomyces* genes (44). Finally, downstream of *ilvA*, centered around nt 1765, a region of dyad symmetry followed by several T residues was

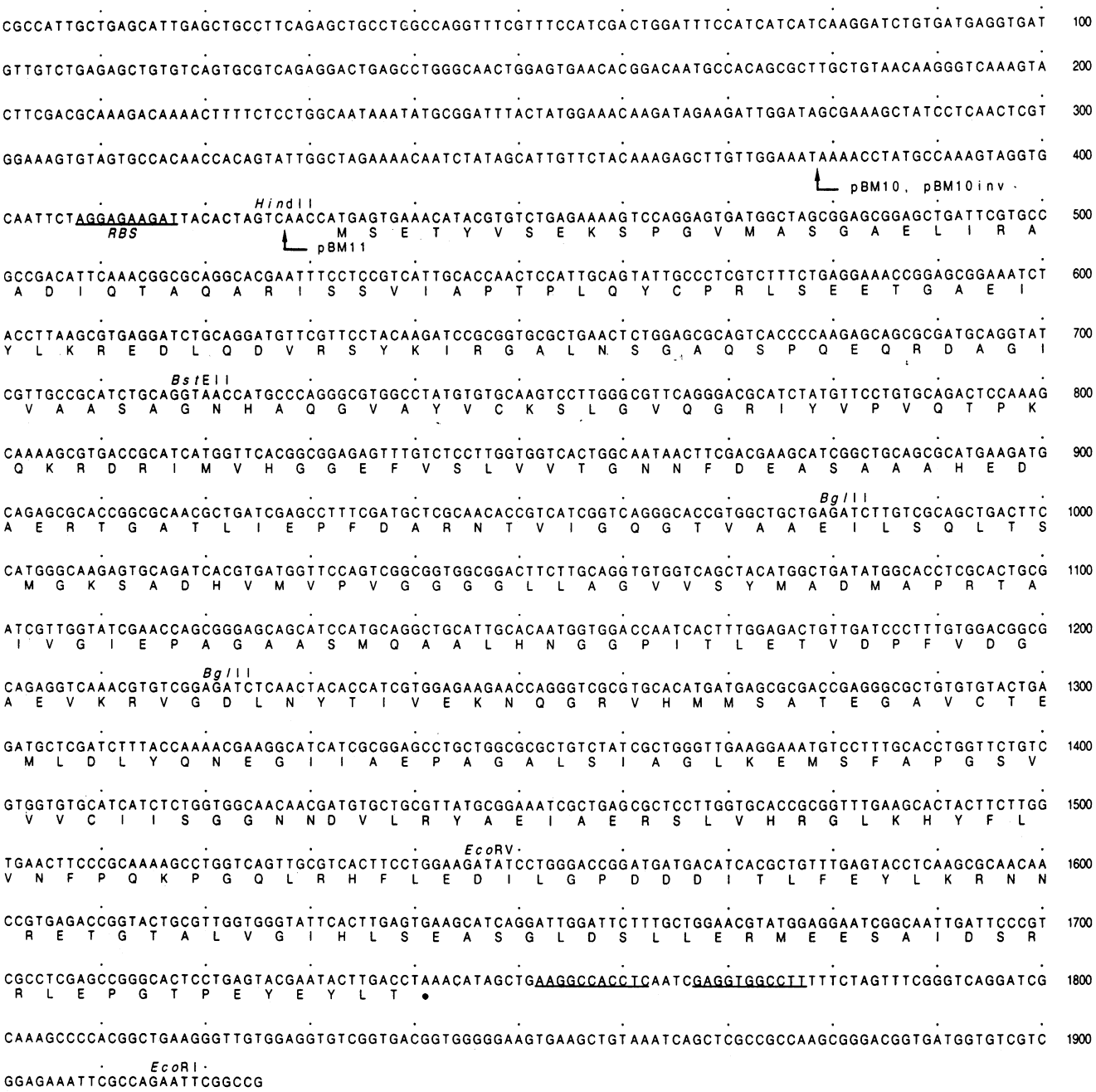

FIG. 3. Nucleotide sequence of *ilvA* and adjacent DNA and deduced amino acid sequence. Selected restriction enzyme sites are given above their recognition sequences. The putative RBS and potential transcription terminator are underlined. The arrows indicate the 5′ boundaries of inserts in deletion plasmids pBM10, $pBM10_{inv}$, and pBM11.

identified. The corresponding mRNA hairpin loop predicted from this sequence has a $\Delta G°$ (25°C) of −25.4 kcal/mol (ca. −106.3 kJ/mol) (50). The total structure is similar to those of rho-independent transcription terminators of *E. coli* (40), a result that is indicative of transcription termination of the *ilvA* message in *C. glutamicum* at this site.

Protein sequence analysis. The assigned open reading frame is 1,308 bp long. The predicted translation product of the *ilvA* gene is thus a polypeptide of 436 residues with a mass of 46,599 daltons. This information, together with the apparent M_r of the native threonine dehydratase determined by size fractionation, indicates that the enzyme of *C. glutamicum* is a tetramer with a calculated mass of 186,396 daltons. An identical subunit composition has been proven for the enzymes of *E. coli* (8, 27) and tomato (41).

The homology of the primary amino acid sequence over the total length of *ilvA* of *C. glutamicum* to the corresponding sequence of the gene of *E. coli* (8) is 26% when identical amino acids are considered. It is 54% when conservative amino acid changes are also included. Interestingly, however, the translation product of the *C. glutamicum* gene with its 436 amino acids (aa) is significantly smaller than that of

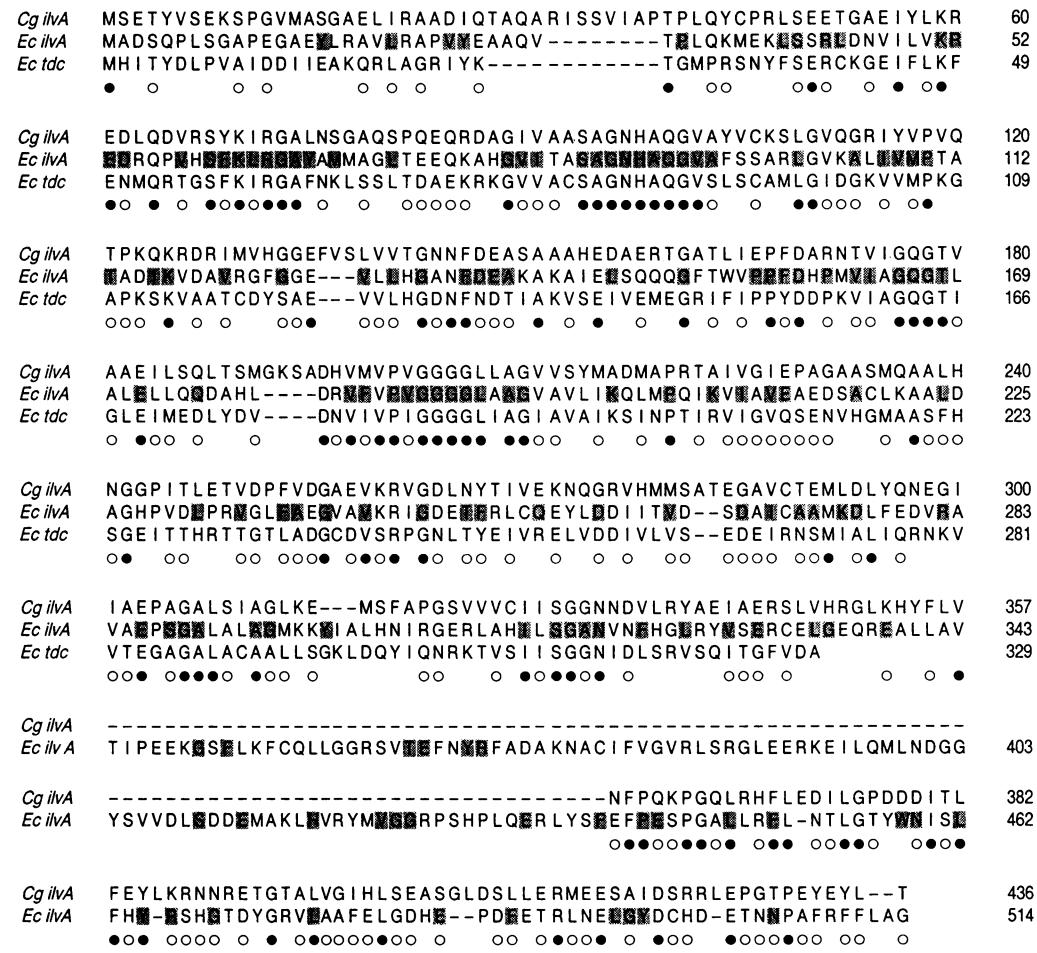

FIG. 4. Alignment of deduced amino acid sequences of *ilvA* from *C. glutamicum* (*Cg*) with those of *ilvA* and *tdc* from *E. coli* (*Ec*). Identical residues of the threonine dehydratases are designated by closed circles, and similar residues are designated by open circles. Similarity was determined by use of the Dayhoff PAM matrix (11), with a score of at least 8. Also included is a comparison of the enzymes of *Saccharomyces cerevisiae* and *Lycopersicon esculentum* (tomato), represented by the shaded areas within the *E. coli* sequence, which show identical amino acids of the enzymes. Broken lines indicate gaps introduced.

the *E. coli* gene (514 aa). To increase the homology between *ilvA* of *C. glutamicum* and the gene of *E. coli*, we had to introduce a large gap of 95 aa into the *C. glutamicum* sequence, separating it into amino- and carboxy-terminal portions. Therefore, it is reasonable to consider the amino- and carboxy-terminal portions individually. By doing so, we find that the homology (identical amino acids) is increased in the amino-terminal portion from 26 to 35%. It is this portion that also shares strong homology with the sequence over the total length of the catabolic threonine dehydratase gene (*tdc*) (Fig. 4), indicating directly the structural or catalytic importance of these amino-terminal structures (see Discussion). However, when the carboxy-terminal portion of the *C. glutamicum* sequence is considered, the situation is entirely different. A corresponding sequence is not present in *tdc*; this sequence is therefore suggested to be of relevance for allosteric regulation in the biosynthetic enzyme (*ilvA*) (45). In this carboxy-terminal portion of the *C. glutamicum* sequence, no distinct blocks of identical amino acids are conserved, as in the amino-terminal portion. Therefore, the primary sequence of the *C. glutamicum* enzyme is unique in its carboxy-terminal portion, although the enzyme is identical in function and comparable in control to the *E. coli* enzyme.

DISCUSSION

A sensitive assay and the availability of a gene overexpressing threonine dehydratase have allowed an initial study of the kinetics of the enzyme of *C. glutamicum*. The enzyme exhibits typical cooperativity in substrate saturation kinetics, even in the absence of the heterotrophic effector isoleucine. Only recently was this result definitely shown for the *E. coli* enzyme (14, 48). It is possible that the partially contradictory characterization of the enzymes is due to the type of assay used (21), the known lability of the enzyme (20), and the type of preparation (14). Because of the heterogeneity of the data (for the *E. coli* and *S. typhimurium* enzymes as well), it is difficult to trace kinetic differences between gram-negative and gram-positive bacteria. However, for gram-positive *B. flavum*, for which cooperativity has been demonstrated, a high $K_{0.5}$ can be deduced from the published curves (32). *C. glutamicum* is closely related to the latter organism, and we determined a $K_{0.5}$ of 21 mM.

Therefore, it is evident that the substrate affinities of the enzymes from these organisms are significantly lower than that of the *E. coli* enzyme (8 mM) (14).

An interesting feature of the *C. glutamicum* enzyme is the Hill coefficient. It is well known that this coefficient, expressing the degree of cooperativity, cannot exceed the number of binding sites (9). A value of 3.7 therefore suggests four functional sites in the *C. glutamicum* enzyme, a suggestion that is in good agreement with the subunit composition of the enzyme. With respect to the opposite changes promoted in the *C. glutamicum* enzyme by the heterotrophic effectors isoleucine and valine, the enzyme is comparable to other threonine dehydratases (5, 14, 20, 32). Given the functional identities of the various biosynthetic threonine dehydratases as well as their very similar regulatory characteristics, it is not surprising that their overall globular structures also appear similar. Thus, the *C. glutamicum* enzyme consists of four subunits with a total M_r of 186,396. Similar quarternary structures also have been proven for the enzymes of *E. coli* (5, 27) and the eukaryote *L. esculentum* (tomato) (41). These biochemical criteria suggest that the primary structures of the proteins are also comparable.

However, when the deduced amino acid sequences of *C. glutamicum* and *E. coli* are examined (Fig. 4), it is striking that a large gap had to be introduced between the carboxy- and amino-terminal portions of the *C. glutamicum* enzyme to obtain homology between the two proteins. This large gap of 95 amino acids accounts for most of the size difference between the two enzymes. Because of this gap, the *C. glutamicum* enzyme represents a new type of threonine dehydratase at the structural level. In addition, the *C. glutamicum* protein is only 54% homologous to the *E. coli* protein, whereas the *S. cerevisiae* and *L. esculentum* proteins are 67 and 63% homologous to the *E. coli* protein, respectively. These facts are illustrated in Fig. 4, in which homologous amino acids of the family represented by *E. coli*, *S. cerevisiae*, and *L. esculentum* are shaded. These regions are largely identical to the homology regions introduced by Taillon et al. (45). These authors compared anabolic threonine dehydratases with the catabolic threonine dehydratase of *E. coli* and noticed that the latter enzyme lacks a corresponding carboxy-terminal portion (Fig. 4), suggesting that amino-terminal regions are involved in catalysis and that carboxy-terminal regions are involved in regulation. Indeed, it is possible to attribute to certain regions at the amino-terminal portion specific functions required for catalysis, when the class of mechanistically related α,β-eliminases is considered. Thus, the second lysine in the motif K-X_8-S-(Y, F, or I)-K-X-R-G (aa 59 to 73) is the pyridoxal phosphate binding site. This motif is found in catabolic threonine dehydratases, for which it has been proven that pyridoxal phosphate is bound to the ε-amino group of this lysine (10). It is also present in D-serine dehydratases with bound pyridoxal phosphate (29, 36) and in threonine synthases (17, 37), which share with threonine dehydratases the common intermediate α-aminocrotonate (16, 38). In contrast, the motif I-X_3-D-X_4-V-I-X_5-A-X-E (aa 165 to 183), which is very similar in all the threonine dehydratases, also occurs in homoserine dehydrogenases (39), which are not α,β-eliminases but are oxidoreductases not containing pyridoxal phosphate. Common to both groups of enzymes is the fact that they recognize threonine, since homoserine dehydrogenase activity is regulated by this amino acid. Thus, in this case, the relationship among the enzymes is not a comparable mechanism but is the recognition of an identical amino acid. The motif is probably relevant for this relationship. Another block of homology is the string of G residues at aa 203 to 206. It is conserved (or similarly found) in many pyridoxal phosphate-containing enzymes (29). Invariant glycines are crucial for structural reasons, since glycine allows unusual main-chain conformations because of the specific dihedral angles of the peptide bond. Indeed, for the β-subunit of tryptophan synthase, which has α,β-eliminase activity, the homologous glycine-rich region was found in the active site pocket surrounding the phosphate group of pyridoxal phosphate (24). The *C. glutamicum ilvA* sequence after the G residue string up to aa 346 shares homology with the sequence of the non-feedback-regulated catabolic threonine dehydratase gene, *tdc*, of *E. coli* (Fig. 4). In addition, this sequence also exhibits weak homology to the D-amino acid aminotransferase of a *Bacillus* sp. (46). Therefore, in contrast to Taillon et al. (45), we associate this portion of the enzyme with the catalytic region.

Given the similarity of mechanistically related enzymes in the amino-terminal portion, it is reasonable to consider this portion to be the catalytic domain, which has in itself intrinsic activity and/or stability. In accordance with this idea, a mutant threonine dehydratase of *E. coli* lacks part of the carboxy-terminal portion but still has dehydratase activity (15). Therefore, it appears that structures of the carboxy-terminal portion are of major importance for allosteric regulation; this portion must then be considered the regulatory domain. The evidence for this idea is that (i) no structure involved in catalysis is attributed to this portion, (ii) the catabolic threonine dehydratase, which is not regulated by isoleucine, has no corresponding carboxy-terminal portion (Fig. 4), (iii) the one *ilvA* mutant of *E. coli* lacking a portion of the carboxy-terminal chain is insensitive to feedback regulation (15), (iv) one further mutant is known to be altered in feedback regulation because of point mutations in this carboxy-terminal portion (45), and (v) the *C. glutamicum* sequence itself is largely conserved in the amino-terminal portion but is different in the carboxy-terminal portion. Interestingly, there are several allosterically regulated proteins other than threonine dehydratases in which mutations affecting regulation are also located in the carboxy-terminal portion (25, 39). It is conspicuous that the carboxy-terminal portion of the *C. glutamicum* protein is very rich in charged amino acids, and it can therefore be expected that large areas are exposed to the surface. Because of the small size of the carboxy-terminal portion of the *C. glutamicum* protein, aa 347 to 436 probably represent the essence of a regulatory domain of threonine dehydratases. Therefore, this first example of a small regulated threonine dehydratase might be ideal for undertaking defined studies on the structural prerequisites necessary for regulation to provide new answers to the old question of the allosteric control of threonine dehydratases.

ACKNOWLEDGMENTS

We thank H. Cichorius and K. Krumbach for activity determination and gel filtration.

The work was part of a joint project with Degussa AG and was supported by grant 0319256A8 from the Bundesministerium für Forschung und Technologie.

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