Thr-431 and Arg-433 Are Part of a Conserved Sequence Motif of the Glutamine Amidotransferase Domain of CTP Synthases and Are Involved in GTP Activation of the Lactococcus lactis Enzyme

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A conserved sequence motif within the class I glutamine amidotransferase (GATase) domain of CTP synthases was identified. The sequence motif in the Lactococcus lactis enzyme is 429GGTLRLG435. This motif was present only in CTP synthases and not in other enzymes that harbor the GATase domain. Therefore, it was speculated that this sequence was involved in GTP activation of CTP synthase. Other members of the GATase protein family are not activated allosterically by GTP. Residues Thr-431 and Arg-433 were changed by site directed mutagenesis to the sterically similar residues valine and methionine, respectively. The resulting enzymes, T431V and R433M, had both lost the ability for GTP to activate the uncoupled glutaminase activity and showed reduced GTP activation of the glutamine-dependent CTP synthase reaction. The T431V enzyme had a similar activation constant, $K_A$ for GTP, but the activation was only 2–3-fold compared with 35-fold for the wild type enzyme. The R433M enzyme was found to have a 10–15-fold lower $K_A$ for GTP and a concomitant decrease in $V_{app}$. The activation by GTP of this enzyme was about 7-fold. The kinetic parameters for saturation with ATP, UTP, and $\text{NH}_4\text{Cl}$ were similar for wild type and mutant enzymes, except that the R433M enzyme only had half the $V_{app}$ of the wild type enzyme when $\text{NH}_4\text{Cl}$ was the amino donor. The mutant enzymes T431V and R433M apparently had not lost the ability to bind GTP, but the signal transmitted through the enzyme to the active sites upon binding of the allosteric effector was clearly disrupted in the mutant enzymes.

CTP synthase (EC 6.4.3.2) catalyzes the synthesis of CTP from UTP by amination of the pyrimidine ring at the 4-position. CTP synthase has three functionally distinct sites, i.e. the glutaminase site where glutamine hydrolysis occurs, the active site where CTP synthesis takes place, and the allosteric site where GTP binds. The reaction proceeds via phosphorylation of UTP by ATP to give an activated intermediate 4-phosphoryl UTP and ADP (1, 2). Ammonia then reacts with this intermediate, yielding CTP and $\text{F}_1$. Ammonia can either be utilized from the surrounding solution or generated by the hydrolysis of glutamine in a reaction activated by GTP (3, 4).

Sequence comparison and structure-function studies have suggested a role for several regions within the primary structure of CTP synthase. As such, the catalytic triad (5, 6) as well as the oxyanion hole (7) of the GATase domain have been identified (8). From mutations isolated from cells of various organisms that showed decreased sensitivity to the cytotoxic effects of cyclopentenylcytosine, the CTP/UTP site of CTP synthase has been identified because the mutant enzymes were less sensitive to CTP feedback inhibition (9–11). In addition, a region has been pointed out to be important for the structural integrity of the enzyme (12). Recently, analysis of a region of the Escherichia coli enzyme where selected residues between 102 and 118 were changed to alanine has been performed, and a possible role of aspartate 107 and leucine 109 in the coupling of glutamine hydrolysis to CTP synthesis has been identified (13).

We have characterized the CTP synthase from L. lactis as described in previous reports (3, 14). After gaining knowledge of the properties of the wild type enzyme with respect to enzyme kinetics and quaternary structure, we initiated a structure-function analysis of the L. lactis enzyme to increase the knowledge of the CTP synthases as a whole and also to be able to explain the quite different properties of this enzyme with respect to quaternary structure (3) and the mechanism of GTP activation (14) compared with those apparently common to other well characterized CTP synthases (4, 15–23). The lack of a three-dimensional structure of the enzyme complicates rational structure-function analysis of individual residues in CTP synthase. However, we set out to try and identify amino acid residues involved in GTP activation of the glutamine-dependent CTP synthase reaction. A region between residues 403 and 480 in the primary sequence of the GATase domain of CTP synthase, here represented by the L. lactis enzyme, shows three insertions compared with the consensus GATase domain (Fig. 1A). This region, which already shows weak overall sequence homology between the consensus and the CTP synthase domains together with the insertions in CTP synthase, suggests a high flexibility in the structure of the GATase domain. Except for a short stretch of conserved residues, the primary sequence in this region varies considerably within CTP synthases from various sources (Fig. 1A and B). In this work, we describe the effect on the kinetics of L. lactis CTP synthase mutant enzymes T431V and R433M, which were derived by changing the side chain of Thr-431 to valine and Arg-433 to methionine. Both side chains are part of the conserved sequence motif in the CTP synthase GATase domain shown in Fig. 1B.
EXPERIMENTAL PROCEDURES

Site-directed Mutagenesis and DNA Sequencing
—Site-directed mutagenesis in the coding region of the L. lactis pyrG gene was performed by the QuikChange method (Stratagene) using the complementary deoxyoligonucleotides LL5-T431V (CATGGGTGGAATTACGTCTTG) and LL3-T431V (CAAGACGTAATACCTCCACCGTG) for construction of the allele encoding the T431V enzyme, and the complementary deoxyoligonucleotides LL5-R433Ma (TGGAACATTAATGCTTGGACTTT) and LL3-R433Ma (AAAGTCCAAGCATATGTTCCA) for construction of the allele encoding the R433M enzyme. Letters in italics indicate the base changes introduced by the oligonucleotides. The plasmid pMW602 (3) was used as a template for mutagenesis. The mutations were verified by sequencing of the entire coding region using an ABI PRISM 310 DNA Sequencer as recommended by the supplier (PerkinElmer Life Sciences).

Protein Purification and Enzyme Assays
—All chemicals were purchased from Sigma. L. lactis wild type and mutant CTP synthases were produced and purified to homogeneity as judged by SDS-PAGE (24) (Fig. 2) by methods described previously for the wild type enzyme (3). Assays were performed at 30°C in 50 mM Hepes, pH 8.0, 2 mM dithiothreitol. For spectrophotometric measurement of CTP synthesis, the conversion of UTP to CTP with \( \Delta_{313} = 1338 \text{ cm}^{-1} \text{ M}^{-1} \) was recorded as described previously (3, 25). The isothermal titration calorimetry-based assay for CTP synthesis or glutamine hydrolysis was performed as described in detail elsewhere (14).

Analysis of Enzyme Kinetic Data
—Calculation of kinetic constants was performed by fitting the initial velocities to one of the four equations below using the computer program UltraFit (BioSoft, version 3.01). The reported standard errors are those calculated by the computer program. Equations 1 and 2 apply to hyperbolic and sigmoid substrate saturation kinetics, respectively. Equation 3 applies to hy-

| Enzyme   | Glutamine-dependent activity - GTP | + GTP   | NH4Cl-dependent activity |
|----------|-----------------------------------|--------|--------------------------|
| WT       | 0.096                             | 4.8    | 6.4                      |
| T431V    | 0.025                             | 0.065  | 5.2                      |
| R433M    | 0.050                             | 0.57   | 3.4                      |

**TABLE I**
Specific activity of wildtype and mutant CTP synthases
Assays were performed as described under "Experimental Procedures."

CTP synthase consensus GTP regulatory motif:

\[
\text{GG[T[S][ML]LG}
\]

**Fig. 1.** A conserved motif unique to CTP synthase is found within the GATase domain. A, comparison between L. lactis CTP synthase amino acid residues 303–529 and the consensus GATase domain (pfam00117, NCBI conserved domain data base). Residues in boldface are the cysteine, histidine, and glutamate residues of the catalytic triad. The GTP regulatory motif identified in this paper is underlined. B, consensus sequence for the GTP regulatory motif described in this paper based on a survey of CTP synthase sequences from the protein data base found in Entrez-PubMed.

**Fig. 2.** SDS-PAGE of wild type and mutant L. lactis CTP synthases. –3 μg of each enzyme was loaded on the gel. Lanes 1 and 5, low molecular weight marker. The molecular masses of the marker proteins are, from top to bottom, 97.4, 66.2, 45.3, 31.1, 21.5, and 14.4 kDa, respectively. Lane 2, wild type enzyme. Lane 3, T431V enzyme. Lane 4, R433M enzyme.

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peribolic activation kinetics. Equation 4 applies to cooperative substrate inhibition,

\[ v = \frac{V_{app}S}{(K_m + S)} \]  
(Eq. 1)

\[ v = \frac{V_{app}S^2}{(S_{max}^2 + S^2)} \]  
(Eq. 2)

\[ v = V_1 + V_2A/(K_A + A) \]  
(Eq. 3)

\[ v = \frac{V_{app}S}{(K_m + S + S/S_{max}^2)} \]  
(Eq. 4)

where \( v \) is the initial velocity; \( V_{app} \) is the apparent maximal velocity; \( S_{max} \) and \( A_{max} \) represent the concentration of a substrate \( S \) or activator \( A \), respectively, at apparent half-maximal velocity; \( K_m \) and \( K_A \) are the apparent Michaelis-Menten constants for substrate \( S \) or activator \( A \), respectively; \( n \) is the Hill coefficient; \( V_1 \) and \( V_2 \) are the apparent maximal velocities in the absence and presence of saturating concentration of activator, respectively; and \( I_{0.5} \) is the substrate concentration for half-maximal substrate inhibition. For equation 4, \( n \) was fixed at a value of 4 as determined from an analysis of initial velocity data obtained by varying \( \text{NH}_4\text{Cl} \) at several equimolar concentrations of ATP and UTP. Unless otherwise noted, all reported kinetic velocities are \( \mu \text{mol of CTP min}^{-1} \text{mg}^{-1} \).

RESULTS AND DISCUSSION

The work by Levitzki and Koshland (4) demonstrated that the glutaminase activity of the \( E. \text{coli} \) enzyme was activated by GTP in a manner that could account for the observed GTP activation of the overall glutamine-dependent CTP synthesis reaction. In agreement with this role of GTP in CTP synthase activation, CTP synthase that utilizes ammonia from the solution is not dependent on activation by GTP. However, in a recent investigation of the glutaminase reaction of the CTP synthase from \( L. \text{lactis} \), it was found that 4-phosphoryl UTP was likely to be a coactivator with GTP of the glutaminase reaction for this enzyme. GTP alone could not activate the glutaminase reaction to the same extent as the overall glutamine-dependent CTP synthase reaction, not even in the

Table II

| Enzyme   | ATP* | UTP* | NH4CT* |
|----------|------|------|--------|
|          | \( S_{0.5} \) | \( n \) | \( S_{0.5} \) | \( n \) | \( K_m \) | \( I_{0.5} \) | \( V_{app} \) |
|          | \( \mu M \) | | \( \mu M \) | | \( m M \) | | \( m M \) |
| Wild type| 137 ± 10 | 2.1 ± 0.2 | 74 ± 3 | 2.2 ± 0.2 | 57 ± 11 | 242 ± 11 | 8.7 ± 0.7 |
| T431V    | 154 ± 11 | 2.2 ± 0.2 | 86 ± 2 | 2.0 ± 0.08 | 95 ± 10 | 221 ± 6 | 10.8 ± 0.6 |
| R433M    | 142 ± 4  | 1.85 ± 0.06 | 98 ± 10 | 1.7 ± 0.2 | 51 ± 13 | 256 ± 15 | 4.4 ± 0.4 |

* Initial velocity data were fitted to Equation 2.

Table III

| Enzyme   | Glutaminase Reaction |
|----------|----------------------|
|          | − GTP | + GTP* |
|          | \( K_m \) | \( V_{app} \) | \( K_m \) | \( V_{app} \) |
|          | \( \mu M \) | \( \mu M \) | \( \mu M \) | \( \mu M \) |
| Wild type| 0.96 ± 0.06 | 0.143 ± 0.003 | 0.87 ± 0.03 | 0.361 ± 0.004 |
| T431V    | 1.02 ± 0.05 | 0.119 ± 0.002 | 0.97 ± 0.04 | 0.129 ± 0.002 |
| R433M    | 0.77 ± 0.03 | 0.204 ± 0.002 | 0.71 ± 0.02 | 0.228 ± 0.002 |

* The concentration of GTP when present was 1 mm.

![Fig. 3](https://example.com/fig3.png)

**Fig. 3.** Initial velocity dependence on the ammonium concentration for the synthesis of CTP by the wild type, T431V, and R433M enzymes. Experiments were performed as described under "Experimental Procedures." The initial velocity data were fitted to Equation 4, and the calculated kinetic constants are presented in Table II. Circles, wild type enzyme; squares, T431V enzyme; diamonds, R433M enzyme.

![Fig. 4](https://example.com/fig4.png)

**Fig. 4.** GTP activation of the glutamine-dependent CTP synthase reaction of the wild type, T431V, and R433M enzymes. Experiments were performed with the spectrophotometric assay for CTP synthesis as described under "Experimental Procedures." The initial velocity data were fitted to Equation 3, and the calculated kinetic constants are shown in Table IV. A, comparison of initial velocity data obtained for wild type CTP synthase (circles), T431V (triangles) and R433M (diamonds). B, detailed view of T431V. C, detailed view of R433M.
presence of UTP and an ATP substrate analog (14) as has been found for the E. coli enzyme (4).

Based on the work described above, it became clear that a difference existed between the E. coli and L. lactis CTP synthases with respect to the GTP activation of the glutamine-dependent CTP synthase reaction. We have initiated structure-function studies that aim at identifying residues involved in the GTP activation of CTP synthase. Because no tertiary structure model exists for CTP synthase, we started out by investigating the role of amino acid residues that are part of conserved sequence motifs in the primary structure of CTP synthase. Such a motif is shown in Fig. 1B. This motif seems to be absent in the primary structure of the GATase domains (Fig. 1A) of the other members of this enzyme family (8). This would be expected for sequence motifs where the amino acid residues are involved in GTP activation, because this activation is unique to CTP synthase. Within the sequence motif we picked two residues, Thr-431 and Arg-433, for mutational analysis because they were the only polar/charged residues and, therefore, were candidates for interaction with other residues or bound ligands.

The results shown in Table I, which present specific activities determined under standard assay conditions, immediately indicated that both the T431V and R433M enzymes were affected in GTP activation of glutamine-dependent CTP synthase. The activity of the NH4Cl-dependent reaction was similar to that of wild type enzyme in comparison with the effect of the mutations on the glutamine-dependent reaction.

For the L. lactis CTP synthase it is possible to control the oligomerization of the enzyme by varying the ionic strength. Increasing salt concentrations will dissociate the active tetramer into inactive dimers, and the substrate inhibition by NH4Cl can be explained by this property of the oligomer.2 The kinetics of saturation and inhibition with NH4Cl was similar for the wild type and mutant enzymes, except for the 2-fold reduced Vapp observed for the R433M enzyme (Fig. 3). Therefore we may also conclude that the stability of the mutant tetramers was similar to that of the wild type enzyme as indicated by Kd,50 for NH4Cl in Table II. Also, UTP binding and ATP binding of the mutant enzymes were unchanged compared with wild type enzyme as shown in Table II.

The kinetics for the uncoupled glutaminase activity in the absence of GTP was similar for mutant and wild type enzymes, but in the presence of 1 mM GTP this half-reaction was only stimulated at best to −1.1-fold for the mutant enzymes compared with the 2.5-fold observed for the wild type enzyme (Table III). The difference in the kinetic properties of mutant and wild type enzymes also became evident from the results obtained for GTP activation of the glutamine-dependent CTP synthesis (Table IV and Fig. 4). The T431V enzyme bound GTP with the same affinity as the wild type enzyme, but the activation by binding of GTP was only 2–3-fold compared with 35-fold for the wild type enzyme (Table IV). The GTP activation of the R433M enzyme displayed −10–20-fold lower Kd, but about a 15-fold lower Vapp (V1 + V2) than the wild type enzyme, and the GTP activation was only −7-fold (Table IV).

For both mutant enzymes, GTP binding had not been abolished by the side chain substitutions. In the case of the mutant enzyme T431V it seems that GTP binding was normal in terms of Kd, but the effect of binding the activator was greatly reduced. The fact that the glutamine-dependent CTP synthase reaction still retained a small degree of GTP activation (Table IV) compared with the wild type enzyme may indicate that some of the activation mechanism involving coactivation by 4-phosphoryl UTP and GTP was retained in the T431V mutant enzyme as discussed below. It is likely that the hydroxyl group of Thr-431 plays a role in the expected structural rearrangements in CTP synthase when GTP binding leads to activation of the enzyme, because only the GTP activation of the T431V enzyme differed from the kinetics of the wild type enzyme.

The interpretation of the altered kinetics resulting from the side chain substitution in the R433M enzyme is less straightforward, although in this case it is also evident that the GTP activation had been affected (Table IV). The concomitant decrease in V2 and Kd for glutamine-dependent CTP synthase resembles the wild type enzyme when GTP activation is studied at subsaturating concentrations of ATP and UTP that result in CTP synthesis at a rate below that of uncoupled glutaminase hydrolysis. Under these conditions, a substantial decrease in Kd for GTP is observed (14). The GTP activation proceeds up to a V2 in Equation 3 that depends on the concentration of ATP and UTP. In the range from below and up to the level of uncoupled glutaminase activity, the GTP activation observed cannot be explained by increasing the rate of glutamine hydrolysis. The GTP activation from below and up to the level of uncoupled glutaminase hydrolysis was interpreted in terms of combined mechanisms for GTP activation of the L. lactis enzyme that not only stimulate glutaminase hydrolysis but also the CTP synthase reaction (14). In the case of the R433M enzyme, the kinetics of GTP activation is not a consequence of subsaturation with ATP and UTP, because the Smax for these nucleotides was similar to that of the wild type enzyme (Table II). The part of the GTP activation mechanism of glutamine-dependent CTP synthase responsible for the observations with the wild type enzyme as outlined above may be the same as that responsible for the GTP activation remaining with the R433M enzyme.

Apart from GTP activation, the similar kinetic properties observed with both NH4Cl and glutamine as a substrate, together with the use of the same purification protocol, the similar stability during handling, and storage of the mutant en-

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### Table IV

| Enzyme         | GTP activation of the CTP synthesis reaction | ITC assay |spectrophotometric assay^a^ |^b^ |
|---------------|-----------------------------------------------|----------|----------------------------|---|
|               |                                            |          | Kd | V1 | V2 | Ka | V1 | V2 |
| Wild type     | 0.17 ± 0.04                                  | 0.21 ± 0.15 | 7.0 ± 0.6 | 0.187 ± 0.004 | N.A. | 8.00 ± 0.07 |
| T431V         | 0.18 ± 0.06                                  | 0.033 ± 0.002 | 0.067 ± 0.008 | 0.157 ± 0.005 | N.A. | 0.093 ± 0.001 |
| R433M         | 0.010 ± 0.004                                | 0.08 ± 0.06 | 0.55 ± 0.06 | 0.019 ± 0.004 | N.A. | 0.48 ± 0.02 |

^a^ Initial velocity data were fitted to Equation 1.

^b^ Initial velocity data were fitted to Equation 3.
zymes, suggest that no gross structural changes have been introduced by the side chain substitutions when compared with wild type enzyme.

In conclusion, the kinetic analysis of the T431V and R433M enzymes presented here further supports our previously suggested model on GTP activation of the *L. lactis* CTP synthase (14). This model can be described by GTP activation of the glutaminase reaction itself, as observed in the absence of ATP and UTP, but also by coordinating glutaminase activity with the synthesis of the reaction intermediate, 4-phosphoryl UTP. In this case, 4-phosphoryl UTP is thought to also act as a coactivator with GTP in further increasing the rate of glutamine hydrolysis.

The absent or greatly reduced GTP activation of the uncoupled glutaminase reaction for the two mutant enzymes described here is in agreement with a disruption in the enzyme of side chain interactions that are involved with GTP activation of this activity. This is also in accordance with the location of the investigated sequence motif in the primary structure within the GATase domain of CTP synthase (Fig. 1A). However, the observation that the glutamine-dependent CTP synthesis of the mutant enzymes was still activated by GTP, although to a much lesser extent than that of the wild type enzyme, is in agreement with a bifunctional role of GTP both as an activator of the glutaminase half-reaction and also as a coactivator with the 4-phosphoryl UTP intermediate in the overall reaction of *L. lactis* CTP synthase (14).

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REFERENCES
1. von der Saal, W., Anderson, P. M., and Villafranca, J. J. (1985) *J. Biol. Chem.* 260, 14993–14997
2. Lewis, D. A., and Villafranca, J. J. (1989) *Biochemistry* 28, 8454–8459
3. Wadskov-Hansen, S. L., Willemoes, M., Martinussen, J., Hammer, K., Neuhard, J., and Larsen, S. (2001) *J. Biol. Chem.* 276, 38002–38009
4. Levitzki, A., and Koshland, D. E., Jr. (1972) *Biochemistry* 11, 241–246
5. Weng, M., Makaroff, C. A., and Zalkin, H. (1986) *J. Biol. Chem.* 261, 5568–5574
6. Bearne, S. L., Hekmat, O., and Macdonnell, J. E. (2001) *Biochem.* 356, 223–232
7. Chittur, S. V., Klem, T. J., Shafer, C. M., and Davisson, V. J. (2001) *Biochemistry* 40, 876–887
8. Zalkin, H., and Smith, J. L. (1998) *Adv. Enzymol. Relat. Areas Mol. Biol.* 72, 87–144
9. Wyke, J. L., Wang, L. L., Tipples, G., and McClarty, G. (1996) *J. Biol. Chem.* 271, 15393–15400
10. Whelan, J., Phear, G., Yamauchi, M., and Meuth, M. (1993) *Nat. Genet.* 3, 317–322
11. Ostrander, D. B., O’Brien, D. J., Gorman, J. A., and Carman, G. M. (1998) *J. Biol. Chem.* 273, 18992–19001
12. Weng, M. L., and Zalkin, H. (1987) *J. Bacteriol.* 169, 3023–3028
13. Iyengar, A., and Bearne, S. L. (2003) *Biochem.* 369, 497–507
14. Willemoes, M., and Sigurskjold, B. W. (2002) *Eur. J. Biochem.* 269, 4772–4779
15. Levitzki, A., Stallcup, W. B., and Koshland, D. E., Jr. (1971) *Biochemistry* 10, 3371–3378
16. Levitzki, A., and Koshland, D. E., Jr. (1972) *Biochemistry* 11, 247–253
17. Long, C. W., Levitzki, A., and Koshland, D. E., Jr. (1970) *J. Biol. Chem.* 245, 80–87
18. Pappas, A., Yang, W. L., Park, T. S., and Carman, G. M. (1998) *J. Biol. Chem.* 273, 15954–15960
19. Yang, W. L., McDonough, V. M., Oziere-Kalogeropoulos, O., Adeline, M. T., and Carman, G. M. (1994) *Biochemistry* 33, 10785–10793
20. Thomas, P. E., Lamb, B. J., and Chu, E. H. (1988) *Biochem. Biophys. Acta* 953, 334–344
21. van Kuijlenburg, A. B., Elzinga, L., van den Berg, A. A., Slingerland, R. J., and Van Gennip, A. H. (1994) *Anticancer Res.* 14, 411–415
22. Anderson, P. M. (1983) *Biochemistry* 22, 3285–3292
23. Robertson, J. G. (1995) *Biochemistry* 34, 7533–7541
24. Laemmli, U. K. (1970) *Nature* 227, 680–685
25. Lang, C. W., and Pardee, A. B. (1967) *J. Biol. Chem.* 242, 4715–4721
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