Regulation of Yeast CTP Synthetase Activity by Protein Kinase C*

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CTP synthetase (EC 6.3.4.2, UTP:ammonia ligase (ADP-forming)) is an allosterically regulated enzyme in the yeast Saccharomyces cerevisiae. In this work we examined the regulation of CTP synthetase activity by S. cerevisiae protein kinase C (Pkc1p) phosphorylation. The results of labeling experiments with S. cerevisiae mutants expressing different levels of the PKC1 gene indicated that phosphorylation of CTP synthetase was mediated by Pkc1p in vivo. In vitro, Pkc1p phosphorylated purified CTP synthetase on serine and threonine residues, which resulted in the activation (3-fold) of enzyme activity. The mechanism of this activation involved an increase in the apparent V_{max} of the reaction and an increase in the enzyme’s affinity for ATP. In vitro phosphorylated CTP synthetase also exhibited a decrease in its positive cooperative kinetic behavior with respect to UTP and ATP. Phosphorylation of CTP synthetase did not have a significant effect on the kinetic properties of the enzyme with respect to glutamine and GTP. Phosphorylation of CTP synthetase resulted in a decrease in the enzyme’s sensitivity to product inhibition by CTP. Phosphorylation did not affect the mechanism by which CTP inhibits CTP synthetase activity.

CTP synthetase (EC 6.3.4.2, UTP:ammonia ligase (ADP-forming)) is an allosterically regulated enzyme that is essential for the growth and metabolism of cells. The product of its reaction, CTP, is required for the synthesis of RNA, DNA, phospholipids, and sialoglycoproteins (1). CTP synthetase catalyzes the ATP-dependent transfer of the amide nitrogen from glutamine to the C-4 position of UTP to form CTP (2, 3). CTP activates the reaction by accelerating the formation of a covalent glutaminyl enzyme catalytic intermediate (3, 4). Genes encoding for CTP synthetase have been isolated from Escherichia coli (5), Chlamydia trachomatis (6), Bacillus subtilis (7), Saccharomyces cerevisiae (8, 9), and human cells (10). The deduced amino acid sequences of the cloned CTP synthetase genes have a relatively high degree of identity, including a conserved glutamine amide transfer domain characteristic of glutamine amidotransferases (5-10).

CTP synthetases have been purified to apparent homogeneity from E. coli (3, 11), S. cerevisiae (12, 13), and rat liver cells (14). Purified CTP synthetases exist as dimers that oligomerize to tetramers in the presence of their substrates UTP and ATP (3, 12–15). CTP synthetases from E. coli (3, 4, 16–19) and S. cerevisiae (12, 13) exhibit positive cooperative kinetics with respect to UTP and ATP and negative cooperative kinetics with respect to glutamine and GTP. The positive cooperative kinetics toward UTP and ATP is attributed to the nucleotide-dependent tetramerization of the enzyme (3, 15).

A characteristic common to the pure CTP synthetases is the inhibition of their activities by the product CTP (3, 12–14). CTP inhibits CTP synthetase activity by increasing the positive cooperativity of the enzyme for UTP (12–14). Regulation of CTP synthetase activity by CTP inhibition plays an important role in vivo. For example, mutant mammalian cell lines with CTP synthetase activity insensitive to CTP inhibition exhibit abnormally high intracellular levels of CTP and dCTP (20, 21), resistance to nucleotide analog drugs used in cancer chemotherapy (22–25), and an increased rate of spontaneous mutations (23, 25, 26). In addition, elevated CTP synthetase activity is a common property of leukemic cells (27) and rapidly growing tumors found in liver (28), colon (29), and lung (30).

In S. cerevisiae, CTP synthetase is encoded by the URA7 and URA8 genes (8, 9). Comparison of the nucleotide and deduced amino acid sequences of the open reading frames of the URA7 and URA8 genes show 70 and 78% identity, respectively (8, 9). Biochemical characterization of the purified enzymes (12, 13) and phenotypic analysis of ura7 and ura8 mutants (9) have shown that the two CTP synthetases are not functionally identical. Our studies on the pure URA7-encoded enzyme have revealed that CTP synthetase is also regulated by protein kinase C phosphorylation (31). Protein kinase C is a transducer of lipid second messengers (32–34) and plays a central role in the regulation of a host of cellular functions including cell growth and proliferation (35–37). Rat brain protein kinase C phosphorylates the yeast CTP synthetase in vitro on serine and threonine residues, which results in an activation of CTP synthetase activity (31). In this study we demonstrated that CTP synthetase was a substrate for the S. cerevisiae protein kinase C (Pkc1p). Our data also demonstrated that this phosphorylation regulated CTP synthetase activity by changing the kinetic properties of the enzyme and its sensitivity to inhibition by CTP.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals were reagent grade. Growth medium supplies were purchased from Difco. Nucleotides, l-glutamine, phenylmethylsulfonyl fluoride, benzamide, aprotonin, leupeptin, pepstatin, nitrocellulose paper, phosphoamino acids, and bovine serum albumin were purchased from Sigma. Phosphatidylserine and diacylglycerol were purchased from Avanti Polar Lipids. Radiochemicals were purchased from DuPont NEN. Scintillation counting supplies were purchased from National Diagnostics. Protein assay reagent, molecular mass standards for SDS-polyacrylamide gel electrophoresis, and electrophoresis reagents were purchased from Bio-Rad. lG-Sepharose was purchased from Pharmacia Biotech Inc. DE53 (DEAE-cellulose) was purchased from Whatman, Inc. Cellulose thin layer sheets were from EM Science. Phosphocellulose papers for protein kinase C assays were purchased from Whatman.

**Strains and Growth Conditions**—CTP synthetase was purified from strain OK (MATα leu2 trpl ura3 Δ::TRP1 ura8) bearing the

*This work was supported by United States Public Health Service Grant GM-50679 from the National Institutes of Health, New Jersey State funds, and the Charles and Johanna Busch Memorial Fund. This is New Jersey Agricultural Experiment Station Publication D-10581-1. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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multicopy plasmid pFL44S-URA7 (8, 9). Plasmid pFL44S-URA7 directs a 10-fold overexpression of CTP synthetase (12). Cells were grown in complete synthetic medium (38) without uracil. Strain DL101 (MATa leu2-3, 112 ura3-52 trp1-1 his4 can1) was used as a representative wild-type for Pkc1p expression. Strain DL376 (MATa leu2-3, 112 ura3-52 trp1-1 his4 can1, clp3:LEU2) is a null allele pkc1 mutant defined by the disruption of Pkc1p. Strain DL105 is Dpkc1p bearing the PKC1 gene (40) on the multicopy plasmid Yep352. Strain DL688 is Dpkc1p bearing the plasmid pGY82. Plasmid pGY82 is a multicopy plasmid carrying a PKC1-ZZ fusion gene (41). This fusion gene carries two (ZZ) repeats of the 60-amino acid IgG-binding domain of Staphylococcus aureus protein A (41). The plasmids in strains DL105 and DL688 direct the overexpression of Pkc1p.1 DL101, DL376, and DL105 were grown in low phosphate medium (42) containing 1 mM sorbitol (39) and used to examine the effect of Pkc1p expression on the phosphorylation of CTP synthetase. DL688 cells were grown in YEPD medium (1% yeast extract, 2% peptone, 2% glucose) and used to purify ZZ-tagged Pkc1p. Cell numbers were determined by microscopic examination with a hemacytometer or by absorbance at 660 nm. All cells were grown at 30 °C. Strains DL101, DL376, DL105, and DL688 were obtained from David E. Levin (The Johns Hopkins University, Baltimore, MD).

In Vivo Labeling of CTP Synthetase—Exponential phase cells were labeled with [32P]orthophosphate (0.3 mCi/ml) and [3H]thymidine (5 μCi/ml) for 3 h. Nuclear localization was visualized with 0.5 mM Tris-HCl buffer (pH 8.0), 1 mM phenylmethylsulfonyl fluoride, 0.5 mM unlabeled with 0.5 M Tris-HCl buffer (pH 8.0). Pkc1p was stored in 50 mM glycine-HCl (pH 3.0). Fractions from the IgG-Sepharose column were diluted 5-fold, and CTP synthetase activity was measured spectrophotometrically as described below.

Enzyme Assays and Protein Determination—CTP synthetase activity was determined by measuring the conversion of UTP to CTP (molar extinction coefficients of 182 and 1520 M−1 cm−1, respectively) by following the increase in absorbance at 291 nm on a recording spectrophotometer (3). The standard reaction mixture contained 50 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 10 mM 2-mercaptoethanol, 2 mM L-glutamine, 0.1 mM GTP, 2 mM ATP, 2 mM UTP, and an appropriate dilution of enzyme protein in a total volume of 0.2 ml. Phosphorylated CTP synthetase was measured for 10 min at 30 °C by following the phosphorylation of the CTK1 Ser939 peptide with [γ-32P]ATP (3,000–4,000 cpm/pmol) as described previously (45). The reaction mixture contained 50 mM Tris-HCl buffer (pH 8.0), 0.2 mM EDTA, 0.2 mM 2-mercaptoethanol, 10 mM MgCl2, 10 mM 2-mercaptoethanol, 0.375 mM EDTA, 0.375 mM EGTA, 1.7 mM CaCl2, 20 μM diacylglycerol, 50 μM phosphatidylinerse, and 1.7 mM MgCl2. Reaction products were separated on 5% polyacrylamide gels containing 5% glycerol. The standard. A unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of product/min unless otherwise indicated. Protein was determined by the method of Bradford (49) using bovine serum albumin as the standard.

Analysis of Kinetic Data—Kinetic data were analyzed according to the Michaelis-Menten and Hill equations using the EZ-FIT Enzyme Kinetic Model Fitting Program (50). EZ-FIT uses the Nelder-Mead simplex and Marquardt/Nash nonlinear regression algorithms sequentially and tests for the best fit of the data among different kinetic models.

RESULTS

Phosphorylation of CTP Synthetase by Pkc1p—Our study was performed with protein kinase C (Pkc1p) from S. cerevisiae (41, 45). Pkc1p is the product of the PKC1 gene, which is required for progression through the cell cycle (41). Pkc1p has a substrate specificity similar to that of the γ, δ, and ε isoforms of mammalian protein kinase C (41, 45). To examine if CTP synthetase phosphorylation was mediated by Pkc1p in vivo, the extent of enzyme phosphorylation was measured in cells that expressed different levels of the PKC1 gene. Cells were incubated with [32P]orthophosphate to detect phosphorylated CTP synthetase and [3H]thymidine to normalize for the amount of CTP synthetase isolated. CTP synthetase was immunoprecipitated from wild-type cells, pkc1 mutant cells, and cells that overexpressed Pkc1p. The amount of each label incorporated into CTP synthetase was determined. The data shown in Fig. 1 are plotted as the ratio of the cpm of [32P]orthophosphate incorporated into CTP synthetase to the cpm of [14C]thymidine incorporated into CTP synthetase. If Pkc1p mediated phosphorylation of CTP synthetase in vivo, then the ratio of the labels found in CTP synthetase would be expected to change in response to Pkc1p levels. Indeed, this ratio was reduced in pkc1 mutant cells and elevated in cells that overexpressed Pkc1p when compared with cells that were wild-type for Pkc1p expression (Fig. 1). However, the fact that CTP synthetase was still phosphorylated to some extent in pkc1 mutant cells suggests that the enzyme was a substrate for other protein kinase(s) in vivo.

We examined if purified CTP synthetase was a substrate for

1 David E. Levin, personal communication.
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Fig. 1. Effect of Pkc1p expression on the phosphorylation of CTP synthetase. Wild-type cells, cells defective in the expression of Pkc1p (pck1Δ::LEU2), and cells that overexpress Pkc1p (YEp352 [pck1]) were labeled with 32P, and [32P(U)]-amino acids. CTP synthetase was immunoprecipitated from cells using anti-CTP synthetase antibodies. CTP synthetase was dissociated from the enzyme-antibody complex, and the amount of the label incorporated into CTP synthetase was determined by scintillation counting. The values are reported as the cpm of 32P incorporated into CTP synthetase relative to the cpm of 32C incorporated into CTP synthetase. The data are from an average of two independent growth studies.

Pkc1p in vitro. These studies were performed with a purified preparation of a ZZ-tagged Pkc1p (45). The ZZ-tag facilitates the purification of Pkc1p but does not alter the biochemical properties of the enzyme (41, 45). We examined the phosphorylation of CTP synthetase with Pkc1p under the conditions used to phosphorylate the enzyme with rat brain protein kinase C (31). Pkc1p catalyzed the incorporation of the γ-phosphate of [32P]ATP into CTP synthetase (Fig. 2A, lane 2). The omission of the protein kinase C cofactors calcium, diacylglycerol, and phosphatidylinositol from the phosphorylation reaction resulted in a 70% decrease in the phosphorylation of CTP synthetase (Fig. 2A, lane 4). CTP synthetase was phosphorylated on serine and threonine residues (Fig. 2B), and the stoichiometry of phosphorylation was 0.4 mol of phosphate/mol enzyme.

The effect of phosphorylation on CTP synthetase activity was measured. Phosphorylation of the enzyme resulted in a dose-dependent activation (3-fold) of CTP synthetase activity (Fig. 3). Maximum activation of activity occurred when the enzyme was measured with saturating concentrations of UTP and ATP (Fig. 3). When CTP synthetase activity was measured with saturating concentrations of UTP and ATP, Pck1p did not have a significant effect on CTP synthetase activity (Fig. 3). Overall, these results were similar to those reported for rat brain protein kinase C phosphorylation of yeast CTP synthetase (31). This indicated that the yeast Pck1p and the rat brain protein kinase C phosphorylated and activated yeast CTP synthetase in a similar manner.

Effect of Phosphorylation on the Kinetics of CTP Synthetase Activity with Respect to UTP and ATP—Kinetic analyses were performed to further characterize the effects of phosphorylation of CTP synthetase on its activity in vitro. The CTP synthetase that we purified was phosphorylated to some extent (31). We were unable to perform kinetic studies on the dephosphorylated form of CTP synthetase, however, because alkaline phosphatase treatment of the purified enzyme resulted in the loss of its activity (31). Previous studies have shown that yeast CTP synthetase exhibits positive cooperative kinetics toward UTP and ATP (12). Therefore, we investigated whether phosphorylation of the enzyme influenced these kinetic properties. These experiments were performed with saturating concentrations of glutamine, GTP, and magnesium ions. We first examined the effect of phosphorylation on the dependence of CTP synthetase activity on UTP using saturating and saturating concentrations of ATP. At the saturating ATP concentration, the phosphorylation of CTP synthetase altered the apparent V_{max} of the reaction and the positive cooperative kinetic behavior of the enzyme (Fig. 4A and Table I). Under these conditions, the apparent V_{max} of the phosphorylated enzyme was 2.75-fold greater than the apparent V_{max} of the native enzyme. Phosphorylation of CTP synthetase resulted in the loss of the enzyme’s positive cooperative kinetic behavior toward UTP when compared with the native enzyme (n = 1.1 and 1.4, respectively). At a saturating ATP concentration, phosphorylation had a small effect on the apparent V_{max} of the reaction.

2 Pck1p does not exhibit a cofactor dependence using the Bck1-Ser993 peptide as a substrate (45).
Phosphorylation of CTP synthetase did not affect the apparent $K_m$ values for UTP whether activity was measured with subsaturating or saturating concentrations of ATP (Fig. 4 and Table I).

We next examined the effect of phosphorylation on the dependence of CTP synthetase activity on ATP using subsaturating and saturating concentrations of UTP. At the subsaturating UTP concentration, the phosphorylation of CTP synthetase altered the apparent $K_m$ of the reaction and the positive cooperative kinetic behavior of the enzyme (Fig. 5 A and Table I). Under these conditions, the apparent $K_m$ value for ATP of the phosphorylated enzyme was 2.5-fold lower than the apparent $K_m$ value for the native enzyme. Phosphorylated CTP synthetase exhibited a decrease in its positive cooperative kinetic behavior toward ATP when compared with the native enzyme ($n = 1.4$ and $2.5$, respectively). At a saturating UTP concentration, phosphorylation of CTP synthetase resulted in a 5.3-fold reduction in its apparent $K_m$ value for ATP and a loss of its positive cooperative kinetic behavior with respect to ATP (Fig. 5 B and Table I). Phosphorylation of CTP synthetase resulted in a modest increase in the apparent $V_{max}$ of the reaction when activity was measured with subsaturating or saturating concentrations of UTP (Fig. 5 and Table I).

Effect of Phosphorylation on the Kinetics of CTP Synthetase Activity with Respect to Glutamine and GTP—GTP stimulates yeast CTP synthetase activity by increasing the $V_{max}$ of the reaction and decreasing the $K_m$ for glutamine (12). The enzyme also exhibits negative cooperative kinetics toward glutamine (12). We examined if phosphorylation influenced these kinetic properties. These experiments were performed with saturating concentrations of UTP, ATP, and magnesium ions. Phosphorylation of the enzyme did not have a significant effect on the apparent $V_{max}$ and $K_m$ values of the enzyme with respect to glutamine when measured in the absence (Fig. 6 A) or the presence (Fig. 6 B) of GTP (Table I). However, the phosphorylation of CTP synthetase abolished the negative cooperative behavior of the enzyme with respect to glutamine (Table I). The effect of phosphorylation on the activation of CTP synthetase activity by GTP was examined using saturating concentrations of all substrates (Fig. 7). Phosphorylation of CTP synthetase resulted in a small decrease in the apparent activation constant ($K_a$) for GTP (Table I).

Effect of Phosphorylation on the Inhibition of CTP Synthetase Activity by CTP—We questioned what effect phosphorylation of CTP synthetase had on the inhibition of CTP synthetase activity by CTP. The phosphorylated and native forms of CTP synthetase were inhibited by CTP in a dose-dependent manner (Fig. 8). When enzyme activity was measured using 0.5 mM ATP, phosphorylated CTP synthetase was less sensitive to CTP inhibition when compared with the native enzyme (Fig. 8 A and Table I). On the other hand, when CTP synthetase activity was measured using 1 mM ATP, the sensitivities of both enzyme

### Table I

| Substrate, activator, or inhibitor | Native CTP synthetase | Phospho CTP synthetase |
|-----------------------------------|-----------------------|------------------------|
|                                   | $V_{max(app)}$        | $K_m(app)$            | $n$ | $K_a(app)$ | $IC_{50}$ |
|                                   | units/mg              | mM                    |     | $\mu M$   | mg        |
| UTP$^{a}$                         | 0.92                  | 0.07                  | 1.4 | 2.53       | 0.07      | 1.1 |
| UTP$^{b}$                         | 2.53                  | 0.04                  | 1.0 | 3.00       | 0.04      | 1.0 |
| ATP$^{c}$                         | 1.15                  | 0.16                  | 2.2 | 2.30       | 0.17      | 1.8 |
| ATP$^{d}$                         | 2.14                  | 1.54                  | 2.5 | 2.53       | 0.60      | 1.4 |
| ATP$^{e}$                         | 2.53                  | 0.80                  | 1.7 | 3.00       | 0.15      | 1.0 |
| Glutamine$^{f}$                   | 1.09                  | 0.45                  | 0.8 | 1.10       | 0.37      | 1.0 |
| Glutamine$^{g}$                   | 2.53                  | 0.25                  | 0.9 | 3.00       | 0.24      | 1.0 |
| GTP                               | 15.7                  | 0.12                  | 0.12| 0.24       | 0.24      | 0.24 |
| CTP$^{h}$                         |                       |                       |     |            |           |     |
| CTP$^{i}$                         |                       |                       |     |            |           |     |

- $^{a}$ Kinetic constants determined with 0.5 mM ATP.
- $^{b}$ Kinetic constants determined with 2 mM ATP.
- $^{c}$ Kinetic constants determined with 1 mM ATP in the presence of 0.3 mM CTP.
- $^{d}$ Kinetic constants determined with 0.1 mM UTP.
- $^{e}$ Kinetic constants determined with 0.1 mM ATP.
- $^{f}$ Kinetic constants determined with 2 mM UTP.
- $^{g}$ Kinetic constants determined with 0 mM ATP.
- $^{h}$ Kinetic constants determined with 0.1 mM GTP.
- $^{i}$ Kinetic constants determined with 0.5 mM ATP.
forms to CTP were similar (Fig. 8B and Table I).

As described previously (12), the presence of CTP in the assay for the native enzyme caused an increase in the positive cooperativity toward UTP, an increase in the apparent \( K_m \) for UTP, and a decrease in the apparent \( V_{\text{max}} \) (Fig. 9). We questioned what effect phosphorylation had on the dependence of CTP synthetase activity on UTP in the presence of CTP. In these experiments the concentration of ATP was held constant at 1 mM, and the other substrates in the reaction were saturating. The apparent \( V_{\text{max}} \) value of the phosphorylated enzyme measured in the presence of CTP was greater than the apparent \( V_{\text{max}} \) value of the native enzyme measured in the presence of CTP (Fig. 9 and Table I). However, enzyme phosphorylation did not have a significant effect on the cooperative kinetic behavior of the enzyme toward UTP nor the apparent \( K_m \) value for UTP (Fig. 9 and Table I).

**DISCUSSION**

Phosphorylation/dephosphorylation is a major mechanism by which the activity of an enzyme is regulated (51, 52). The aim of this work was to examine the regulation of yeast CTP synthetase activity by protein kinase C. We demonstrated that purified CTP synthetase was a substrate for yeast Pkc1p and that Pkc1p mediated the phosphorylation of the enzyme in vivo. This does not, however, demonstrate that CTP synthetase is a substrate for Pkc1p in vivo. An alternate explanation could be that Pkc1p modulates the activity of another kinase that is responsible for phosphorylating CTP synthetase. We do know on the basis of our labeling experiments with pck1 mutant cells that another kinase phosphorylated the enzyme in vivo.

Phosphorylation of the enzyme in vitro resulted in the activation of CTP synthetase activity. This regulation of activity involved changes in the kinetic properties of the enzyme. Pkc1p phosphorylation of CTP synthetase activated the enzyme by increasing the apparent \( V_{\text{max}} \) of the reaction. The change in the apparent \( V_{\text{max}} \) was most evident when the kinetics of the enzyme toward UTP was measured using a subsaturating concentration of ATP. The phosphorylated enzyme also showed a greater affinity for ATP when compared with the native enzyme. This was reflected in the apparent \( K_m \) values for ATP. Another striking effect of Pkc1p phosphorylation of CTP synthetase was the decrease in the positive cooperativity toward

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**Fig. 5.** Effect of phosphorylation on the kinetics of CTP synthetase activity with respect to ATP. Phosphorylated (●) and native (○) CTP synthetase activities were measured as a function of the concentration of ATP using 0.1 (A) and 2 mM UTP (B). The concentrations of glutamine, GTP, and MgCl₂ were maintained at 2, 0.1, and 10 mM, respectively.

**Fig. 6.** Effect of phosphorylation on the kinetics of CTP synthetase activity with respect to glutamine. Phosphorylated (●) and native (○) CTP synthetase activities were measured as a function of the concentration of glutamine with 0 (A) and 0.1 mM GTP (B). The concentrations of UTP, ATP, and MgCl₂ were maintained at 2, 2, and 10 mM, respectively.

**Fig. 7.** Effect of phosphorylation on the activation of CTP synthetase activity by GTP. Phosphorylated (●) and native (○) CTP synthetase activities were measured in the absence and the presence of the indicated concentrations of GTP. The concentrations of UTP, ATP, glutamine, and MgCl₂ were maintained at 2, 2, 2, and 10 mM, respectively.
Phosphorylation did not affect the mechanism by which CTP synthetase activity was less sensitive to inhibition by CTP when activity was measured with a subsaturating concentration of ATP. However, enzyme activator (3, 4). Phosphorylated CTP synthetase was less sensitive to inhibition by CTP when activity was measured with a subsaturating concentration of ATP. However, enzyme phosphorylation did not affect the mechanism by which CTP synthetase activity by CTP. Phosphorylated (●) and native (○) CTP synthetase activities were measured with 0.1 mM UTP in the absence and the presence of the indicated concentrations of CTP using 0.5 (A) and 1 mM ATP (B). The concentrations of glutamine, GTP, and MgCl₂ were maintained at 2, 0.1, and 10 mM, respectively.

How could the phosphorylation of CTP synthetase by Pkc1p affect activity in vivo? The steady-state cellular concentrations of UTP (0.75 mM) and ATP (2.3 mM) are saturating for CTP synthetase activity (8, 12). Under these conditions Pkc1p phosphorylation of CTP synthetase would not be expected to affect activity in vivo. At the same time, enzyme phosphorylation would not affect the regulation of CTP synthetase activity by CTP inhibition. On the other hand, if the cellular concentrations of UTP and ATP were to decrease to subsaturating concentrations for the enzyme, Pkc1p phosphorylation would affect CTP synthetase activity. For example, the cellular concentration of ATP can vary between 0.57 to 2.3 mM depending on growth conditions (56). These concentrations fall within the range of the subsaturating to saturating ATP concentrations for CTP synthetase activity (12). It was the subsaturating ATP concentrations that had the major effect on the kinetic properties of the phosphorylated enzyme. In addition, CTP synthetase activity was less sensitive to product inhibition by CTP when ATP levels were subsaturating. The activation of CTP synthetase activity by Pkc1p phosphorylation may be a mechanism by which the cell regulates CTP synthetase when cellular ATP levels are limiting. At the present time it is not known what role phosphorylation plays in the function of CTP synthetase under different growth conditions. Future studies will address this question.

Our interest in CTP synthetase originates from the role CTP plays in the regulation of the pathways by which phosphatidylcholine is synthesized (57). Phosphatidylcholine is the essential end product of phospholipid synthesis and the major phospholipid found in S. cerevisiae (58, 59). CTP is required for the synthesis of phosphatidylcholine through the CDP-choline and CDP-diacylglycerol-based pathways (58, 59). In mammalian cells, phosphatidylcholine plays a major role in lipid signal transduction pathways (60). The diacylglycerol derived from the receptor-mediated hydrolysis of phosphatidylcholine is responsible for the sustained activation of protein kinase C (60). The phosphorylation and activation of CTP synthetase by Pkc1p in yeast may represent a mechanism by which lipid signal transduction pathways are coordinately regulated to CTP synthetase and cell growth.

Acknowledgments—We are grateful to David E. Levin for providing us with strains DL101, DL105, DL376, and DL868 and advice on Pkc1p. We also acknowledge Geri Marie Zeimetz for many helpful discussions during the preparation of this manuscript.

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