Biochemistry & Physiology: Open Access

West, Biochem Physiol 2018, 7:4
DOI: 10.4172/2168-9652.1000e160

Editorial Open Access

Cytidine 5'-triphosphate Synthetase: A Pyrimidine Biosynthetic Enzyme Critical to Cellular Synthesis and Cancer Chemotherapy

Thomas P West*

Department of Chemistry, Texas A&M University-Commerce, Commerce, TX, USA

***Corresponding author:** West TP, Department of Chemistry, Texas A&M University-Commerce, Commerce, TX, USA, Tel:+(903)886-5399; E-mail: Thomas.West@tamuc.edu

Received date: November 27, 2018; **Accepted date:** November 27, 2018; **Published date:** December 4, 2018

Abstract

The pyrimidine biosynthetic enzyme Cytidine 5'-triphosphate (CTP) synthetase has an important role in the biosynthesis of RNA, DNA and phospholipids. The synthetase has been found to be highly regulated at the level of enzyme activity and enzyme synthesis in both prokaryotes and eukaryotes including humans. The enzyme has been the target of inhibition by various drugs since cellular proliferation requires CTP synthesis. The design of new drugs targetting CTP synthetase activity in humans could prove important to cancer chemotherapies since it may allow new cancer treatment procedures to be developed.

Keywords: Cytidine 5'-triphosphate synthetase; Pyrimidine biosynthesis; Phospholipid synthesis; Nucleic acid synthesis; Cancer chemotherapy

Introduction

The enzyme Cytidine 5'-triphosphate (CTP) synthetase (EC 6.3.4.2) catalyzes a critical reaction in pyrimidine nucleotide biosynthesis as well as phospholipid formation [1-3]. The enzyme catalyzes the synthesis of CTP by the amination of Uridine 5'-triphosphate (UTP) involving an Adenosine 5'-triphosphate (ATP)-dependent phosphorylation of UTP where glutamine serves as the nitrogen donor [1,2]. The amino acid residues aspartate and leucine have been found promote the hydrolysis of glutamine [4]. The active enzyme is a homotetramer but requires the presence of UTP and ATP. The regulation of CTP synthetase in prokaryotes has been investigated. In the Gram negative bacterium *Escherichia coli*, CTP synthetase was purified and its activity was shown to be regulated by its product CTP as well as UTP, ATP, Guanosine 5'-triphosphate (GTP) or dTTP [1,2]. GTP has been reported as allosteric activator or inhibitor depending upon the *E. coli* cellular conditions [5,6]. The *E. coli* enzyme consists of an N-terminal synthetase domain and a C-terminal glutaminase domain. The latter domain cleaves ammonia from glutamine and the ammonia is transferred from the glutaminase to the synthetase domain by a tunnel mechanism [5,7]. The bacterial enzyme existed as an inactive dimer that aggregated to an active tetramer having a molecular weight of 210,000 daltons [2]. Positive cooperativity was noted towards the binding of the substrates ATP and UTP while negative cooperativity was observed for the binding of GTP and glutamine to the enzyme [8]. Recently, it has been determined that reduced Nicotinamide Adenine Nucleotide (NADH) or Nicotinamide Adenine Nucleotide Phosphate (NADPH) is a moderate inhibitor of the E. coli synthetase with the cofactors enhancing inhibition by CTP and regulation by GTP [9]. Regulation of the purified CTP synthetase from the Gram positive bacterium *Lactobacillus lactis* was found to be different from the *E. coli* enzyme since L. lactis synthetase was inhibited by ammonium ions in the absence of the nucleotides ATP and UTP [10]. It is interesting to note that the enzyme CTP synthetase forms filaments in *E. coli* as well as *Caulobacter crescentus.* In *C. crescentus,* the filaments not only have synthetase catalytic activity but control the curvature of the *C. crescentus* cell. The formation of filamentous structures by the enzyme has also been found in eukaryotes indicating the enzyme subunit polymerization is conserved [11]. The synthesis of the Gram negative bacterium *Salmonella typhimurium* CTP synthetase was repressed by a cytidine or a thymidine compound [12]. The pyrimidine ribonucleotide pool data indicated that likely a cytidine or thymidine nucleotide was involved in the repression of CTP synthetase synthesis in *S. typhimurium* [13]. It was also shown that CTP synthetase was regulated at the level of gene expression in the Gram positive bacterium *Bacillus subtilis.* Cytidine nucleotides repressed the *B. subtilis* CTP synthetase synthesis by interacting with an unidentified regulatory protein [14].

Regulation of CTP synthetase in eukaryotes has been explored. Two isozymes of CTP synthetase exist in humans with the isoforms having 74% similarity in amino acid sequence [15]. Moreover, the crystal structure of isozyme CTP synthetase 1 has been determined [16]. Further, CTP synthetase isozyme 1 has been shown to be regulated by covalent modification. The enzyme protein kinase A was found to phosphorylate serine residues in the synthetase which increased CTP synthesis [17]. This regulation by protein kinase A has also been observed for the yeast *Saccharomyces cerevisiae* CTP synthetase [18]. In humans, CTP synthetase can aggregate into an intracellular macrostructure called the cytoophidium which is thought to be related to the metabolic status of the cell. It has been shown that the presence of cytoophidia does exist in certain human cancer cells [19]. Human synthetase subunit polymerization increases catalytic activity and its filament structure in humans is important to explaining the allosteric mechanisms of the enzyme [20]. Similarly in *S. cerevisiae,* the destabilization of CTP synthetase tetramers to inactive dimers increased filament formation in the yeast [21]. It is known that CTP synthetase activity is increased in rat or human tumor cells [22]. In addition, an increase in CTP synthetase activity was observed with the malignant transformation of human lymphoblastic cells [15]. With CTP synthetase being a critical enzyme in nucleotide and phospholipid synthesis, it has been targeted in an attempt to arrest cancer cells by decreasing their available CTP concentration. The drugs that have

been developed targetting synthetase activity include 3-deazauridine, acivicin and cyclopentenyl cytosine. The effectiveness of these drugs upon tumor cells has varied [23-25].

Conclusion

In conclusion, the enzyme CTP synthetase has been widely studied in prokaryotes and eukaryotes due to its vital role in nucleotide formation, nucleic acid synthesis and phospholipid synthesis. Particularly for this reason, CTP synthetase has become a focus of cancer researchers. The inhibition of this pyrimidine biosynthetic enzyme by selected drugs in human cancer chemotherapy has shown promise in slowing cancer cell proliferation. Considering its growing importance to cancer chemotherapy, additional research on CTP synthetase needs to be done. New drugs that specifically target CTP synthetase in cancer cells could provide major advancements in human cancer cell treatments.

References

1. Long CW, Pardee AB (1967) Cytidine triphosphate synthetase of Escherichia coli B. I. Purification and kinetics. J Biol Chem 242: 4715-4722.
2. Long CW, Levitzki A, Koshland DE Jr (1970) The subunit structure and subunit interactions of cytidine triphosphate synthetase. J Biol Chem 245: 80-87.
3. Chang YF, Carman GM (2008) CTP synthetase and its role in phospholipid synthesis in the yeast *Saccharomyces cerevisiae*. Prog Lipid Res 47: 333-339.
4. Iyengar A, Bearne SL (2003) Aspartate-107 and leucine-109 facilitate efficient coupling of glutamine hydrolysis to CTP synthesis by *Escherichia coli* CTP synthase. Biochem J 369: 497-507.
5. Endrizzi JA, Kim H, Anderson PM, Baldwin EP (2004) Crystal structure of *Escherichia coli* cytidine triphosphate synthetase, a nucleotide-regulated glutamine amidotransferase/ATP-dependent amidoligase fusion protein and homologue of anticancer and antiparasitic drug targets. Biochemistry 43: 6447-6463.
6. MacDonnell JE, Lunn FA, Bearne SL (2004) Inhibition of E. coli CTP synthase by the "positive" allosteric effector GTP. Biochim Biophys Acta 1699: 213-220.
7. Lunn FA, Bearne SL (2004) Alternative substrates for wild-type and L109A E. coli CTP synthetases. Kinetic evidence for a constricted ammonia tunnel. Eur J Biochem 271: 4204-4212.
8. Levitzski A, Koshland DE Jr (1969) Negative cooperativity in regulatory enzymes. Proc Natl Acad Sci USA 62: 1121-1128.
9. Habrian C, Chandrasekhara A, Shahrvini B, Hua B, Lee J, et al. (2016) Inhibition of Escherichia coli CTP synthetase by NADH and other nicotinamides and their mutual interactions with CTP and GTP. Biochemistry 55: 5554-5565.
10. Willemoes M, Larsen S (2003) Substrate inhibition of Lactococcus lactis cytidine 5'-triphosphate synthase by ammonium chloride is enhanced by salt-dependent tetramer dissociation. Arch Biochem Biophys 413: 17-22.
11. Ingerson-Mahar M, Briegel A, Werner JN, Jensen GJ, Gitai Z (2010) The metabolic enzyme CTP synthase forms cytoskeletal filaments. Nat Struct Mol Biol 12: 739-746.
12. West TP, O Donovan GA (1982) Repression of cytidine triphosphate synthetase in *Salmonella typhimurium* by pyrimidines during uridine nucleotide depletion. J Gen Microbiol 128: 895899.
13. West TP, Herlick SA, O Donovan GA (1983) Inverse relationship between thymidylate synthetase and cytidine triphosphate synthetase activities during pyrimidine limitation in *Salmonella typhimurium*. FEMS Microbiol Lett 18: 275278.
14. Meng Q, Switzer, RL (2001) Regulation of transcription of the *Bacillus subtilis* pyrG gene, encoding cytidine triphosphate synthetase. J Bacteriol 183: 5513-5522.
15. van den Berg AA, van Lenthe H, Busch S, de Korte, Roos D, et al. (1993) Evidence for transformation-related increase in CTP synthetase activity in situ in human lymphoblastic leukemia. Eur J Biochem 216: 161-167.
16. Kursula P, Flodin S, Ehn M, Hammarstrom M, Schuler H, et al. (2006) Structure of the synthetase domain of human CTP synthetase, a target for anticancer therapy. Acta Cryst F62: 613-617.
17. Han GS, Sreenivas A, Choi MG, Chang YF, Shelley S, et al. (2005) Expression of human CTP synthetase in Saccharomyces cerevisiae reveals phosphorylation by protein kinase . A J Biol Chem 280: 38328-38336.
18. Yang WL, Carman GM (1996) Phosphorylation and regulation of CTP synthetase from *Saccharomyces cerevisiae* by protein kinase A. J Biol Chem 271: 28777-28783.
19. Chang CC, Jeng YM, Peng M, Keppeke GD, Sung LY, et al. (2017) CTP synthase forms the cytoophidium in human hepatocellular carcinoma. Exp Cell Res 361: 292-299.
20. Lynch EM, Hicks DR, Shepherd M, Endrizzi JM, Maker A, et al. (2017) Human CTP synthase filament structure reveals the active enzyme conformation. Nat Struct Mol Biol 24: 507-514.
21. Noree C, Monfort M, Shiau AK, Wilhelm JE (2014) Common regulatory control of CTP synthase enzyme activity and filament formation. Mol Biol Cell 25: 2282-2290.
22. Kizaki H, Williams JC, Morris HP, Weber G (1980) Increased cytidine 5'-triphosphate synthetase activity in rat and human tumors. Cancer Res 40: 3921-3927.
23. Hindenberg AA, Taub RN, Grant S, Chang G, Baker MA (1985) Effects of pyrimidine antagonists on sialic acid regeneration in HL-60 cells. Cancer Res 45: 3048-3052.
24. Fischer PH, Willson JKV, Risueno C, Tutsch K, Bruggink J, et al. (1988) Biochemical assessment of the effects of acivicin and dipyridamole given as a continuous 72-hour intravenous infusion. Cancer Res 48: 5591-5596.
25. Kang GJ, Cooney DA, Moyer JD, Kelley JA, Kim HY, et al. (1989) Cyclopentenylcytosine triphosphate. Formation and inhibition of CTP synthetase. J Biol Chem 264: 713-718.