

Review

# Critical Roles of Glutamine as Nitrogen Donors in Purine and Pyrimidine Nucleotide Synthesis: Asparaginase Treatment in Childhood Acute Lymphoblastic Leukemia

JOSEPH G. CORY and ANN H. CORY

*Department of Biochemistry and Molecular Biology, Brody School of Medicine,  
East Carolina University, Greenville, NC 27834, U.S.A.*

**Abstract.** *Asparaginase is a key component of the chemotherapy protocols used in the treatment of acute lymphoblastic leukemia (ALL). The current treatment protocols are remarkable in that childhood ALL cure rates are approaching 85%. As the name implies, asparaginase catalyzes the deamination of asparagine to aspartic acid. What is not generally realized is that asparaginase also catalyzes, essentially to the same extent, the removal of the amide nitrogen from glutamine to form glutamic acid. Glutamine is a required substrate for three enzymes involved in the de novo synthesis of purine nucleotides and two enzymes involved in the de novo synthesis of pyrimidine nucleotides. In this review, the specific roles of glutamine in the de novo synthesis of nucleotides are defined and an appropriate explanation for the cell cycle arrest and cytotoxicity induced in proliferating malignant lymphoblasts by asparaginase treatment is provided.*

A recent detailed study of the sera of 73 pediatric ALL patients treated with asparaginase showed that the serum levels of both asparagine and glutamine were reduced to same extent in the children undergoing treatment with asparaginase as one of the drugs in the protocol (1). Asparaginase is a critical component in the drug regimen for ALL treatment. There was an excellent correlation between the serum levels of asparaginase activity and the marked decreases in the levels of asparagine and glutamine. For the

*Correspondence to:* Joseph G. Cory, Ph.D., Department of Biochemistry and Molecular Biology, Brody School of Medicine, East Carolina University, Greenville, NC 27834, U.S.A. e-mail: coryjo@ecu.edu

**Key Words:** Acute lymphoblastic leukemia (ALL), asparaginase, glutaminase, glutamine in nucleotide syntheses, cell cycle, apoptosis, review.

entire group of children, the decreases in asparagine and glutamine levels ranged from 67% to 87% as a result of the asparaginase treatment. In a subset of this group of children, the levels of asparagine and glutamine were decreased in excess of 90%. The serum levels of other amino acids were also decreased but not nearly to the same extent (1). Other studies carried out in leukemia cells growing in tissue culture showed that asparaginase treatment caused the cells to arrest in G0/G1 and to undergo apoptosis (2, 3). In this review, the fact that the asparaginase treatment not only reduces the asparagine levels, but more importantly, reduces the levels of glutamine was emphasized. Glutamine is an essential substrate, being the nitrogen donor, for five enzymes involved in the *de novo* synthesis of purine and pyrimidine nucleotides that are required for RNA and DNA syntheses in rapidly proliferating cells (4).

## Synthesis of Purine and Pyrimidine Nucleotides

Glutamine is the nitrogen donor for N-3 and N-9 of the purine nucleobases, adenine and guanine, and for the amino group of guanine (Figure 1). The rate-limiting step in the *de novo* synthesis of purine nucleotides is catalyzed by glutamine 5-phosphoribosyl-1-pyrophosphate (PRPP) amidotransferase. In this reaction, the amino group that will become the N-glycosidic bond of the purine nucleotides is introduced. Likewise, glutamine is the co-substrate for the reaction in which 5-phosphoribosyl glycinamide is formed, introducing the nitrogen atom that will become N-3 of the purine ring. The enzyme catalyzing this reaction is 5'-phosphoribosylformylglycinamide synthetase. Lastly, the synthesis of guanosine 5'-monophosphate from xanthosine 5'-monophosphate requires glutamine as the amino donor for the reaction catalyzed by GMP synthetase (4).

The PRPP amidotransferase step is the rate-limiting and regulated step in the *de novo* pathway. The reaction is

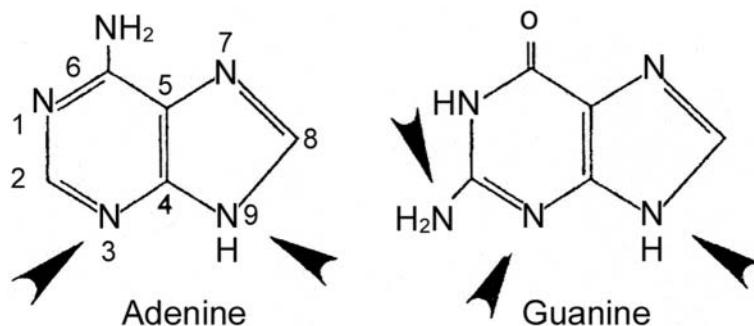


Figure 1. Structures of adenine and guanine nucleobases. Arrows indicate nitrogens that are donated by amide nitrogen of glutamine in glutamine-dependent enzyme-catalyzed reactions.

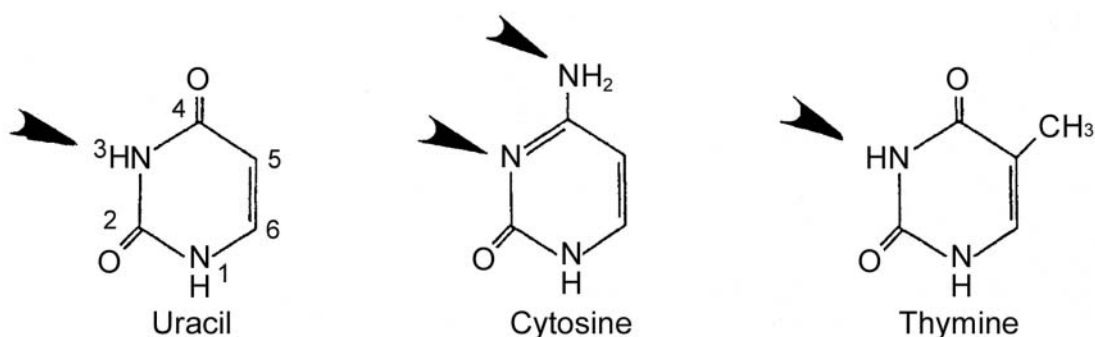


Figure 2. Structures of uracil, cytosine and thymine nucleobases. Arrows indicate nitrogens donated by amide nitrogen of glutamine in glutamine-dependent enzyme-catalyzed reactions.

allosterically activated by PRPP and inhibited by the products, IMP, AMP and GMP. Under normal conditions, the concentration of glutamine would not be considered to be rate-limiting in the reaction. However, in the asparaginase-treated patients in whom the serum concentrations are reduced 60 to 90%, glutamine could become a limiting substrate at a concentration that is less than saturating in this step (5, 6). The  $K_m$ 's reported for glutamine in the PRPP amidotransferase reaction (5, 6) range from: 0.5 mM (rat liver); 1.6 mM (human placenta); and 2.3 mM (rat hepatoma). Lowering the concentrations of glutamine 60 to 90% would definitely have an effect on the rate-limiting step of the *de novo* synthesis pathway (7).

The *de novo* synthesis of pyrimidine nucleotides requires glutamine as a co-substrate in two reactions. In Figure 2, the sources of nitrogen contributed by glutamine to the pyrimidine nucleobases, uracil, cytosine and thymine, are shown. In the reaction catalyzed by carbamoyl phosphate synthetase II (a cytosolic enzyme), glutamine is the required nitrogen donor (N-3). This is different from carbamoyl phosphate synthetase I (a mitochondrial enzyme) involved in urea synthesis which uses ammonium ions as the source for mitochondrial carbamoyl phosphate synthesis. It is

important to note that carbamoyl phosphate synthetase II catalyzes the rate-limiting step in pyrimidine nucleotide synthesis. The activity of carbamoyl phosphate synthetase II is allosterically regulated by UTP, the end product of the pathway, acting as an inhibitor. The synthesis of CTP from UTP via CTP synthetase also requires glutamine as the amino donor (4). CTP synthetase activity is dependent on the ratio of the cellular concentrations of UTP and CTP.

Another interesting point is that glutamine is also the amino donor for the synthesis of asparagine from aspartic acid in the reaction catalyzed by asparagine synthetase. It was reported that asparagine synthetase was up-regulated in asparaginase-treated cells, but that there was no attenuation of the cell cycle arrest (3). This would not be unexpected since glutamine would not be present to provide the amide group for the formation of asparagine from aspartic acid. There is no direct evidence that asparagine is the limiting factor in cell cycle transit.

Adequate cellular levels of asparagine and glutamine are required for normal protein synthesis. Glutamine, on the other hand, is also needed for asparagine synthesis and probably most importantly in its role as the nitrogen donor in the *de novo* pathways of nucleotide synthesis. It is

obligatory at the time of RNA and DNA syntheses in rapidly proliferating cells that the cellular levels of the nucleoside 5'-triphosphate substrates for RNA and DNA to be significantly increased. Therefore, the major impact of asparaginase treatment in patients, will be to decrease nucleotide synthesis due to the lack of glutamine in the cells. The cell cycle block reported for asparaginase-treated cells (2, 3) is consistent with this conclusion.

In conclusion, future studies relating to the biochemical bases for the cell cycle block, apoptosis and cytotoxicity induced by asparaginase treatment of ALL patients should focus on the effects of asparaginase treatment on the levels of nucleotide pools in the cells of the treated patients.

### References

- 1 Grigoryan RS, Panosyan EH, Seibel NL, Gaynon PS, Avramis IS and Avramis VI: Changes of amino acid serum levels in pediatric patients with higher-risk acute lymphoblastic leukemia (CCG-1961). *In Vivo* 18: 107-112, 2004.
- 2 Ueno T, Ohtawa K, Mitsui K, Kodera Y, Hiroto M, Matsushima A, Inada Y and Nishimura H: Cell cycle arrest and apoptosis of leukemia cells induced by L-asparaginase. *Leukemia* 11: 1858-1861, 1997.
- 3 Krejci O, Starkova J, Otova B, Madzo J, Kalinova M, Hruska O and Trka J: Upregulation of asparagine synthetase fails to avert cell cycle arrest induced by L-asparaginase in TEL/AML 1-positive leukemia cells. *Leukemia* 18: 434-441, 2004.
- 4 Cory JG: Purine and pyrimidine nucleotide metabolism. *In: Textbook of Biochemistry with Clinical Correlations* Devlin TM (ed.). Chapter 20, pp 789-822, Sixth Edition, Wiley-Liss, Hoboken NJ, 2006.
- 5 Wyngaarden JA: Regulation of purine biosynthesis and turnover. *Advan Enzyme Regul* 14: 25-42, 1976.
- 6 Weber G, Prajda N, Lui, MS, Denton JE, Aoki T, Sebolt, J, Zhen, Y-S, Burt ME, Faderan MA and Reardon MA: Multi-enzyme targeted chemotherapy by acivicin and actinomycin. *Advan Enzyme Regul* 20: 75-96, 1982.
- 7 Raivio RA and Seegmiller ER: Role of glutamine in purine synthesis and interconversions. *Clin Res* 19: 161-169, 1971.

*Received July 21, 2006*  
*Accepted August 18, 2006*