Coordinated Slowing of Metabolism in Enteric Bacteria under Nitrogen Limitation: A Perspective

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Abstract

It is natural to ask how bacteria coordinate metabolism when depletion of an essential nutrient limits their growth, and they must slow their entire rate of biosynthesis. A major nutrient with a fluctuating abundance is nitrogen. The growth rate of enteric bacteria under nitrogen-limiting conditions is known to correlate with the internal concentration of free glutamine, the glutamine pool. Here we compare the patterns of utilization of L-glutamine and L-glutamate, the two central intermediates of nitrogen metabolism. Monomeric precursors of all of the cell’s macromolecules – proteins, nucleic acids, and surface polymers – require the amide group of glutamine at the first dedicated step of biosynthesis. This is the case even though only a minority (∼12%) of total cell nitrogen derives from glutamine. In contrast, the amino group of glutamate, which provides the remainder of cell nitrogen, is generally required late in biosynthetic pathways, e.g. in transaminase reactions for amino acid synthesis. We propose that the pattern of glutamine dependence coordinates the decrease in biosynthesis under conditions of nitrogen limitation. Hence, the glutamine pool plays a global regulatory role in the cell.
INTRODUCTION

Enteric bacteria are notable for their varying environment. Within a host they can experience conditions of high nutrient availability, high osmolarity, and high toxicity; outside a host, they can encounter the opposite. Evolution has adapted enteric bacteria to grow and divide, albeit at different rates, under both conditions. One can reasonably ask “How does the metabolism of these bacteria remain in balance under such different conditions?” Here we consider this question in relation to the response of *E. coli* and *S. typhimurium* to nitrogen availability.

We chose to consider nitrogen metabolism for several reasons. First, central metabolism of nitrogen is well studied and is relatively simple compared to carbon metabolism: there are only two central intermediates in nitrogen metabolism, the amino acids glutamate and glutamine, and these are synthesized from a single precursor, 2-oxoglutarate. In contrast, carbon metabolism entails the synthesis of 12 central intermediates. Second, all assimilated nitrogen is used for biosynthesis, so it is not necessary to consider storage forms or waste products. Finally, enteric bacteria have a strong nitrogen starvation response. It appears that transcription of up to 2% of the *E. coli* genome is activated by nitrogen regulatory protein C (NtrC) [1].

Enteric bacteria assimilate inorganic NH₃ into organic molecules by two means. In the reaction catalyzed by biosynthetic glutamate dehydrogenase, NH₃ is assimilated directly into glutamate. In the reactions of the glutamine synthase/glutamate synthase (GS/GOGAT) cycle, which is widely present in prokaryotes, NH₃ is assimilated first into glutamine and then into glutamate (Fig. 1). Under nitrogen-limited growth conditions, the major assimilatory pathway is the glutamine synthase/glutamate synthase (GS/GOGAT) cycle [2, 3]. If one traces biosynthetic nitrogen back to its last appearance in one of the two central intermediates, then only ~12% of cellular nitrogen derives from glutamine. The remainder comes from glutamate [4]. Nevertheless, enteric bacteria appear to perceive
external nitrogen limitation as internal glutamine limitation [5]. For a variety of nitrogen-limiting conditions, the growth rate of enterobacteria was found to correlate with the glutamine pool, which dropped by a factor of 10 or more. The glutamate pool remained high.

Motivated by the above observations, we explored whether the metabolic uses of glutamine might be better suited than those of glutamate to slowing biosynthesis in a coordinated manner. It is remarkable that glutamine is required at the first dedicated step in the biosynthesis of many of the cell’s major biosynthetic monomers: amino acids, purines and pyrimidines, and UDP-GlcNAc, a precursor of the cell-surface polymers murein and lipopolysaccharide. We consider the consequences of this pattern of glutamine utilization and propose that the uses of glutamine in metabolism, coupled to the drop in the glutamine pool, may be important in maintaining homeostasis under nitrogen-limiting conditions.

METHODS

To compare patterns of glutamine and glutamate utilization in metabolism, we made use of the following sources: *Escherichia coli* and *Salmonella typhimurium* edited by Neidhardt [6], and references contained therein; *The Enzymes of Glutamine Metabolism* edited by Prusiner and Stadtman [7]; and *The Amidotransferases* by Zalkin [10]. We made extensive use of original literature, particularly for $K_m$ values for the enzymes of glutamine and glutamate metabolism (Tables 1 and 2). Because these apparent $K_m$ values – concentrations required for half-maximal velocity – were measured *in vitro* under a variety of conditions, they can be regarded as only approximate guides to *in vivo* properties of the enzymes. Two easily accessible Web databases and portals that we utilized are EcoCyc, a database of metabolic pathways in *E. coli* ([http://ecocyc.pangeasystems.com/ecocyc/](http://ecocyc.pangeasystems.com/ecocyc/)), and KEGG, a database of metabolic pathways for a collection of organisms ([http://www.genome.ad.jp/kegg/](http://www.genome.ad.jp/kegg/)).
RESULTS

Glutamine and glutamate play different roles in biosynthesis. (i) Though the source of only 12% of cell nitrogen, the amide group of glutamine is required directly for the biosynthesis of monomeric units of all macromolecules (proteins, nucleic acids, and surface polymers). (ii) With the exception of the histidine biosynthetic pathway, glutamine is required at the first dedicated step of every pathway in which it is utilized. By contrast, glutamate is required more often than not at the end or in the middle of pathways. (iii) Glutamine-dependent reactions are generally coupled to ATP hydrolysis, and hence are essentially irreversible. More than half of the reactions involving glutamate are reversible transaminase reactions.

We first consider the utilization of glutamine. The enzymes of glutamine metabolism are listed in Table 1, along with their $K_m$ values for glutamine. Co-reactants for these enzymes and the conventional pathway designations are also listed. The $K_m$ values for glutamine of the enzymes fall in the range 0.2 - 1.7 mM. The glutamine pool concentration of *S. typhimurium* dropped from approximately 3-4 mM on ammonia, the optimal nitrogen source, to < 0.3 mM on growth-rate-limiting nitrogen sources, or in continuous culture with ammonia as the limiting nutrient. Comparison of pool sizes to $K_m$ values provides evidence that the velocities of many enzymes that utilize glutamine fall below half-maximal under nitrogen-limiting conditions. The fluxes in the corresponding biosynthetic pathways will be concomitantly reduced.

Figures 2-7 diagram the biosynthetic pathways that require glutamine. Glutamine is required directly for the biosynthesis of all of the major macromolecules of the cell: proteins (via the amino acids tryptophan, histidine, arginine, and glutamine itself), nucleic acids (via purines and pyrimidines), and cell-surface polymers (via glucosamine). Glutamine is also required for the biosynthesis of the cofactor folic acid. In all of these cases, glutamine acts as a “nitrogen carrier”, losing its amide group and hence yielding
glutamate as an immediate product.

In all but one case, the glutamine-dependent reaction is the first dedicated step in a biosynthetic pathway or the first dedicated step in synthesis of a parent compound of the pathway (e.g. carbamyl phosphate for the arginine and pyrimidine pathways). For example, as shown in Fig. 2, glutamine is a substrate for the first reaction unique to tryptophan biosynthesis. The other substrate, chorismate, is a precursor for many other aromatic compounds. Likewise, glutamine is required at the first dedicated step in purine biosynthesis. For this reaction, the other substrate, phosphoribosyl pyrophosphate (PRPP), is a common precursor for several pathways, including the histidine, tryptophan, and pyrimidine biosynthetic pathways. The fourth dedicated step of purine biosynthesis also requires glutamine, as does the biosynthesis of guanosine monophosphate (GMP) from xanthosine monophosphate (XMP).

Figure 4 shows the glutamine-dependent steps in the pyrimidine pathway. Glutamine is required for the biosynthesis of carbamyl phosphate. The latter is a parent compound for both the pyrimidines and for arginine. Glutamine is also required in the pyrimidine pathway at the final step from UTP to CTP.

The major intermediate in the biosynthesis of surface polymers is the compound UDP-GlcNAc. Initiation of the synthesis of peptidoglycan (murein), lipid A, O antigen, and enterobacterial common antigen all require UDP-GlcNAc [8]. As shown in Fig. 3, the synthesis of UDP-GlcNAc requires the amino sugar D-glucosamine 6-phosphate, which is a product of glutamine and the central metabolite D-fructose 6-phosphate [10]. Hence, glutamine is required at the first dedicated step in the biosynthesis of the cell-surface polymers.

Glutamine is also required for the biosynthesis of the folic-acid family of coenzymes. As shown in Fig. 4, glutamine enters at the first dedicated step of the folic-acid pathway. The other substrate is the common precursor chorismate.
The utilization of glutamine in histidine biosynthesis breaks with the above pattern. As shown in Fig. 7, glutamine is required at the fifth step of the dedicated histidine pathway. The enzyme which catalyzes this reaction has a $K_m$ for glutamine of 0.24 mM, the lowest of the enzymes of glutamine metabolism. This is below the concentration of glutamine measured by Ikeda et al. Therefore, the velocity of this reaction may not depend strongly on glutamine in the range of nitrogen limitation studied.

Finally, the amino acid asparagine is synthesized directly from glutamine, aspartate, and ATP. There is also an enzyme aspartate-ammonia ligase in E. coli that utilizes NH$_3$ in place of glutamine to synthesize asparagine. However, strains lacking the glutamine-dependent enzyme asnB cannot grow in nitrogen-limited media [9].

We consider now the utilization of glutamate. The enzymes of glutamate metabolism are listed in Table 2. They are divided into two classes: transaminase reactions and all others. The co-reactants and pathways are listed, along with the $K_m$ values for glutamate of the enzymes where available. Most of the measured $K_m$ values for glutamate are roughly 10 times as large as those for glutamine. This is consistent with the observation that the glutamate pool stays fixed in the range 15-25 mM, approximately 6 times larger than the glutamine pool of 3-4 mM under unlimited nitrogen [5].

In all the transaminase reactions, glutamate loses its amine group yielding 2-oxoglutarate as one product. For these reactions, glutamate plays the role of a nitrogen carrier, similar to the role played by glutamine. In contrast, the entire glutamate molecule is assimilated in the non-transaminase reactions.

In the interests of space, we have chosen to show only a few characteristic examples of metabolic pathways involving glutamate. Figure 8 shows the tyrosine pathway, starting from the common precursor chorismate. The reaction involving glutamate is a reversible transaminase reaction and occurs at the final step of the pathway. As shown in Table 2, there are twelve other pathways in which glutamate is involved via a transaminase
reaction. In nine of the twelve, the glutamate dependent step is later than the first dedicated step of the pathway. The cases in which glutamate is required for the first dedicated step are the aspartate pathway and the valine pathways, both of which are one-step pathways from common precursors, and the enterobacterial common antigen (ECA) pathway. In the ECA pathway, the co-substrate with glutamate, dTDP-4-dehydro-6-deoxy-D-glucose, is also an intermediate in the biosynthesis of rhamnose, a common constituent of gram-negative O-antigens [11].

Figure 9 shows the arginine pathway. Glutamate is the precursor compound and is also required for a transaminase reaction at the fourth step. Ornithine, an intermediate product in the arginine pathway, is also a precursor for the polyamines putrescine and spermidine [12].

As shown in Table 2, glutamate is required in seven non-transaminase reactions. Of these, the two in the folic-acid pathway occur later than the first dedicated step of the pathway. In contrast to the transaminase reactions, five of the non-transaminase reactions are coupled to ATP hydrolysis, and hence are expected to be irreversible in the cell.

Essentially all nitrogen in cell products derives ultimately from glutamine or glutamate. For example, amino groups of amino acids not listed in Table 2 still come indirectly from glutamate. Specifically, aspartate and serine provide nitrogens in the synthesis of several other amino acids, and their nitrogens are derived from glutamate. Unlike the reversible transaminase reactions involving glutamate, the amino-acid biosynthetic reactions involving aspartate or serine are either coupled to hydrolysis of ATP, or involve incorporation of the entire amino-acid molecule into the final product.

DISCUSSION

Several aspects of glutamine utilization appear to suit it to a general metabolic regulatory role. While the amide group of glutamine donates only 12% of cellular nitrogen (with the remainder derived from glutamate), glutamine dependent monomers are required to
initiate the syntheses of the cell’s macromolecules. Primary depletion of the free pool of glutamine under nitrogen-limiting conditions would therefore presumably slow fluxes into a number of biosynthetic pathways simultaneously. As a consequence of glutamine dependence at the first dedicated step in these pathways, no useless and/or toxic intermediates would accumulate. Instead, any accumulation would be of precursors for the pathways, i.e. substrates for their first enzymatic steps. These are substances like chorismate, PRPP, D-fructose 6-P, and HCO$_3^-$, which are common precursors for several pathways and are readily converted into other central metabolites (e.g. ribose 5-P and ATP from PRPP).

In addition, many of the glutamine-utilizing reactions are essentially irreversible because they are coupled to hydrolysis of ATP. Thus, these reactions cannot reverse to refill a glutamine pool depleted by a decrease in ammonia assimilation. Glutamine product pools can therefore be maintained high – thus preventing product starvation responses – even in the presence of a depleted glutamine pool. Finally, maintenance of a high glutamate pool under nitrogen-limiting conditions would insure that synthesis of biosynthetic monomers, once initiated, would be completed. There are transaminase-type reactions in both the purine and arginine pathways that utilize the amino group of aspartate rather than that of glutamate. All these reactions are coupled to hydrolysis of ATP or GTP, thus also assuring completion of monomer synthesis.

By contrast, if depletion of the glutamate pool were the primary consequence of decreased ammonia assimilation, the result would be the accumulation of useless and/or toxic intermediates in many biosynthetic pathways, e.g. folic-acid, histidine, iso-leucine, and lysine pathways. The presence of excess glutamine under these conditions would exacerbate the accumulation of such intermediates in pathways initiating with glutamine, e.g. the intermediate 7,8-dihydropteroate and its precursors in the folic-acid pathway. Moreover, the reversibility of the transaminase reactions utilizing glutamate would tend to replenish a depleted glutamate pool and would carry down the pools of its products. A
drop in product pools would induce product starvation responses, including upregulation of enzymes metabolizing glutamate, resulting in a futile tug-of-war with overexpressed enzymes competing for the depleted glutamate pool.

If the free pool of glutamine does indeed regulate biosynthetic fluxes, we believe it is critical to determine as directly as possible which pool or pools of glutamine-derived products are, like the pool of glutamine itself, actually depleted under nitrogen-limiting conditions. It is these secondary depleted pools which will directly limit the growth of the cell. Knowledge of which glutamine-product pools are depleted will give insight into the mechanisms the cell has adopted for slowing growth without disrupting, for example, the expression of proteins responsible for nitrogen assimilation. (The $K_m$ value for glutamine of glutaminyl-tRNA synthetase is measured to be 0.15-0.21 mM [13, 14], well below the glutamine concentrations measured for slow-growing cells [5], so direct glutamine-dependent slowing of protein synthesis can be discounted as a primary cause of slow growth.)

At least one glutamine-product pool must become depleted under nitrogen limitation or there would be no slowing of growth. However, it is unclear whether glutamine depletion propagates into depletion of multiple product pools, or into depletion of just a single product pool. The depletion of multiple product pools would provide a simple functional explanation for why so many biosynthetic pathways depend on glutamine. Moreover, a slowing of growth by depletion of multiple pools would likely be robust to fluctuations in product pool sizes.

If only a single glutamine-product pool is depleted, the slowing of growth would result in reduced demand for other cell products and, potentially, large and measurable increases in other product pools. In this scenario, the widespread dependence of biosynthesis on glutamine would function to reduce these accumulations by directly reducing biosynthetic rates. Insofar as growth rate is limited by the overall rate of nitrogen assimilation, no
obvious growth advantage would accrue from a mutation to increase the pool of the one depleted product.

**Nucleotides and Nucleic Acids** – Glutamine is a substrate for the first enzymatic steps in synthesis of ATP, GTP, UTP, and CTP. A significant drop in the pool concentrations of these nucleotides would presumably decrease rates of synthesis of both RNA and DNA. A global decrease in mRNA synthesis might, in turn, decrease protein synthesis. However, genes of the nitrogen-starvation response are known to be upregulated under nitrogen limitation [1], and would have to escape such a global decrease in mRNA synthesis. ATP and GTP are widely employed by the cell as energy carriers, so a significant drop in their pools would have a widespread effect on metabolic rates. CTP, which is a direct glutamine-product from UTP (Fig. 4), is also required in the biosynthesis of phospholipids [15] and coenzyme A [16].

**Cell-surface polymers** – Similar to its role in nucleotide synthesis, glutamine is required for synthesis of glucosamine from the common precursor D-fructose 6-P (Fig. 3). Glucosamine is the substrate for synthesis of UDP-GlcNAc and UDP-MurNAc (Fig. 10). The latter is used to initiate synthesis of the repeating unit of the osmotically-resistant layer of the cell envelope, murein [17]. UDP-GlcNAc is also used to initiate synthesis of two of the three portions of the lipopolysaccharide (LPS) component of the outer membrane, the lipid A and core portions [8]. The biosynthesis of lipid A begins with the fatty acylation of UDP-GlcNAc and later the core region is assembled using lipid A as a substrate. In *E. coli*, UDP-GlcNAc is also required to initiate synthesis of the third portion of LPS, the O-antigen. UDP-galactose is used in place of UDP-GlcNAc in O-antigen synthesis in *S. typhimurium*. Finally, as shown in Fig. 11, UDP-GlcNAc is used to initiate synthesis of the trisaccharide repeating unit of the enterobacterial common antigen (ECA), a glycolipid found in the outer membrane of enterobacteriaceae [18]. Depletion of glucosamine under nitrogen limitation would therefore potentially impact synthesis rates of the entire
suite of cell-surface polymers.

*Amino acids and proteins* – In comparison to the above, the roles of glutamine in synthesis of the amino acids tryptophan, histidine, arginine, and asparagine are less clear-cut, both in terms of the positions of the glutamine-dependent reactions in their biosynthetic pathways and in terms of the potential effects of depletion of these amino acids on growth. Although glutamine is required to initiate synthesis of tryptophan and is required for the synthesis of asparagine from aspartate, glutamine is utilized later in the arginine and histidine biosynthetic pathways. Given that ornithine, the co-substrate of the glutamine-dependent (or more specifically carbamyl phosphate-dependent) reaction in the arginine biosynthetic pathway (Fig. 9), is also a precursor of polyamines, the position of the glutamine-dependent reaction can be rationalized. As noted in Results, this is not the case for the histidine pathway, where glutamine is required at the fifth dedicated step (Fig. 7). However, the enzyme involved in histidine synthesis, imidazole glycerol-p synthase, has an unusually low $K_m$ (0.24mM) for glutamine, suggesting that this reaction is driven to completion even under nitrogen-limited conditions.

Although depletion of the tryptophan, histidine, arginine, and asparagine pools could certainly slow the rate of elongation of proteins, it would not be expected to slow their rate of initiation. Depletion of amino-acid pools therefore runs the risk of stalling protein elongation in the midst of synthesis. Interestingly, a requirement for glutamine by the enzyme that catalyzes the first committed step in folate biosynthesis might indirectly affect initiation of protein synthesis. This enzyme, aminodeoxychorismate synthase, has an unusually high $K_m$ (1.6mM) for glutamine (Table 1) so that flux into this pathway will be strongly reduced following glutamine depletion. The co-factor, folate, is required for one-carbon transfer reactions and hence is essential for both the synthesis of methionine and for the formylation of methionine that allows it to serve in the first step of protein synthesis. Though not in the context of nitrogen limitation, the possibility that initiation
of translation may be modulated by the folate pool has been noted by Gold [19].

As outlined above, primary glutamine depletion is expected to translate into secondary depletion of pools of one or more glutamine products. Under conditions of depletion, the specific regulatory systems for many of these product pathways (e.g. tryptophan and histidine) are known to increase activity and synthesis of pathway enzymes. Increased synthesis of these enzymes due to depletion of product pools would consume already scarce nitrogen without benefit and would result in a futile tug-of-war between specific regulatory mechanisms and our postulated general metabolic regulatory role for glutamine. Recent data from DNA microarrays indicate that transcription of the tryptophan and histidine biosynthetic operons is, in fact, slightly repressed under nitrogen-limiting conditions, and hence that this tug-of-war does not occur. For product pools that rise under nitrogen limitation, repression of enzyme expression is expected. For the specific product pools that deplete under nitrogen limitation, the regulatory mechanisms may be designed to avoid upregulation of enzymes under this condition. Interestingly, Rose and Yanofsky [20] described an uncharacterized “override mechanism” that allows repression of the tryptophan operon under conditions of slow growth, even when tryptophan pools are low.

To conclude, measurement of pools of glutamine products will shed light on how cellular homeostasis is maintained under nitrogen limitation. Comparative measurements between pool sizes under maximal and slow growth conditions, rather than absolute measurements, will suffice for this purpose. Bioassays may therefore be appropriate to measure relative pools of, e.g. folates [21], and other products for which absolute assays are not practical.

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Abbreviations used:
AICAR – aminoimidazole carboxamide ribonucleotide,
$C_{55}$-P – undecaprenyl monophosphate,
CoA – coenzyme A,
ECA – enterobacterial common antigen,
FGAM – 5’-phosphoribosyl-N-formylglycinamidine,
FGAR – 5’-phosphoribosyl-N-formylglycinamide,
Fuc4NAc – 4-acetamido-4,6-dideoxy-D-galactose,
GlcNAc – N-acetyl-D-glucosamine,
LPS – lipopolysaccharide,
ManNAcA – N-acetyl-D-mannosaminuronic acid,
MurNAc – N-acetyl-D-muramic acid,
PRFAR – phosphoribulosylformimino-AICAR-P,
PRPP – phosphoribosyl pyrophosphate,
THF – tetrahydrofolate,
THF(glu)$_n$ – folylpolyglutamate,
XMP – xanthosine monophosphate
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| Enzymes of Glutamine Metabolism | Enzyme | $K_M$ (mM) | Other Substrate(s) | Pathway(s) |
|-------------------------------|--------|------------|-------------------|------------|
| **Amino acids:**              |        |            |                   |            |
| Asparagine synthetase ($asnB$)| 0.66   | [22]       | Aspartate, ATP    | Asparagine |
| Glutamate synthase ($gltB,D$)| 0.4    | [23]       | 2-oxoglutarate    | Glutamate  |
| Imidazole glycerol-P synthase ($hisF,H$)| 0.24 | [24] | PRFAR              | Histidine  |
| Anthranilate synthase ($trpD,E$)| 0.5  | [25]       | Chorismate         | Tryptophan |
| **Nucleotide bases:**        |        |            |                   |            |
| Glutamine PRPP amidotransferase ($purF$) | 1.7 | [10, 26] | PRPP              | AMP, GMP   |
| FGAM synthetase ($purL$)      | 10.8   | [27]       | FGAR, ATP         | AMP, GMP   |
| GMP synthetase ($guaA$)       | 0.16 - 0.72 | [28] | XMP, ATP          | GMP        |
| Carbamyl-P synthase ($carA,B$)| 0.4   | [10, 29]   | HCO$_3^-$, ATP(2) | UTP, CTP, Arginine |
| CTP synthetase ($pyrG$)       | 0.16-1 | [30]       | UTP, ATP          | CTP        |
| **Amino sugar:**             |        |            |                   |            |
| Glucosamine 6-P synthase ($glmS$) | 0.4 | [31]       | D-fructose 6-P    | Glucosamine 6-P |
| **Coenzyme:**                |        |            |                   |            |
| Aminodeoxychorismate synthase ($pabA$) | 1.6 | [32]       | Chorismate        | Folic acid |

Table 1: Enzymes of glutamine metabolism. The $K_m$ for CTP synthetase ranges from 0.16 mM with a saturating GTP concentration to 1 mM in the absence of GTP [30]. FGAM synthetase is inhibited by glutamate according to $K_m \approx 0.06(1 + [\text{glutamate}]/[1.6\text{mM}])$ [27], in the table we have taken $[\text{glutamate}] = 20$ mM. GMP synthetase is sensitive to pH, with $K_m = 0.16$ mM at pH = 7.5 and $K_m = 0.72$ mM at pH = 8.2 [28].
### Enzymes of Glutamate Metabolism

| Enzyme                                                | $K_M$ (mM) | Other Substrate(s)            | Pathway(s)                      |
|-------------------------------------------------------|------------|-------------------------------|---------------------------------|
| **Transaminase (TA) reactions:**                      |            |                               |                                 |
| Branched-chain-amino-acid TA (ilvE)                    |            | 2-keto-isovalerate             | Alanine, Valine                 |
|                                                        |            | 2-keto-3-methyl-valerate      | Iso-leucine                     |
|                                                        |            | 2-keto-4-methyl-pentanoate    | Leucine                         |
| Acetylornithine TA (argD)                              | 24 [33]    | N-acetylglutamyl-phosphate     | Arginine                        |
| Aspartate TA (aspC)                                    | 3.9 [39]   | Oxaloacetic acid               | Aspartate                       |
| TDP-4-oxo-6-deoxy-D-glucose TA (rfaA)                  | 5.1 [34]   | dTDP-4-dehydro-6-deoxy-D-glucose | TDP-fucose (ECA)               |
| Histidinol-phosphate TA (hisF,H)                       | 10.4 [37]  | Phosphopyruvate                | Phenylalanine                   |
| Aromatic-amino-acid TA (tyrB)                          | 0.28 [31]  | p-hydroxyphenylpyruvate        | Tyrosine                        |
| Succinylaminopimelate TA (dapC)                        | 5.2 [37]   | N-succinyl-2-amino-6-ketopimelate | Lysine, Diaminopimelate        |
| Phosphoserine TA (serC)                                | 3-phospho-hydroxypyruvate | Serine                  |
| **Non-transaminase reactions:**                        |            |                               |                                 |
| N-acetylglutamate synthase (argA)                      | 5 [38]     | Acetyl-CoA                     | Arginine                        |
| Dihydrofolate synthase (folC)                          | 3.9 [39]   | 7,8-dihydropteroate, ATP      | Folic acid                      |
| Folylpyglutamate synthase (folC)                       | 0.3 [39]   | THF(ghu)$_n$, ATP             | Folic acid                      |
| Glutamine synthase (glnA)                              | 5.5 [40]   | NH$_3$, ATP                   | Glutamine                       |
| Glutamate-cysteine lygase (gshA)                       | 0.5-1.7 [11] [12] | Cysteine, ATP              | Glutathione                     |
| Glutamate racemase (murF)                              | 4 [43]     | Peptidoglycan                 |                                 |
| Glutamate 5-kinase (proB)                              | 7-10 [14] [33] [44] | ATP                           | Proline                         |

Table 2: Enzymes of glutamate metabolism. A putative transaminase acting directly from pyruvate to alanine has not been purified [9]. The histidinol-phosphate transaminase is competitively inhibited by imidazole acetal-phosphate; $K_m = 7$ mM at non-inhibiting concentrations [35]. N-acetylglutamate synthase $K_m = 5mM$ in the absence of arginine, and increases with arginine concentration [35].
Figure 1: Nitrogen central intermediates L-glutamine and L-glutamate. Under nitrogen-limited conditions, nitrogen is incorporated via the GS/GOGAT cycle as shown. The metabolism of glutamine is the source of only $\sim 12\%$ of the nitrogen content of the cell. Nevertheless, L-glutamine is required for the first dedicated step in the biosynthesis of monomeric precursors of all the cell’s macromolecules - purines and pyrimidines for nucleic acids, amino acids for proteins, and glucosamine for surface polymers. In contrast, L-glutamate is generally required late in biosynthetic pathways. For a more complete listing of reactions requiring L-glutamine or L-glutamate, see Tables 1 and 2, respectively.
Figure 2: Tryptophan biosynthetic pathway. The first dedicated step of tryptophan biosynthesis requires glutamine.
Figure 3: Purine biosynthetic pathway. The first dedicated step of purine biosynthesis requires glutamine. The fourth step also requires glutamine, as does the final step in GMP biosynthesis.
Figure 4: Pyrimidine biosynthetic pathway. Glutamine is required for the biosynthesis of carbamyl phosphate, which is a parent compound for both pyrimidine and arginine biosynthesis. The final step in CTP biosynthesis also requires glutamine.
Figure 5: Cell-surface polymer biosynthetic pathways. Glutamine is required for the first dedicated step of the biosyntheses of peptidoglycan (murein), lipid A, O antigen, and enterobacterial common antigen.
Figure 6: Folic-acid biosynthesis. The first dedicated step of folic-acid biosynthesis requires glutamine.
Figure 7: Histidine biosynthesis. Glutamine is required for the fifth dedicated step of histidine biosynthesis.
Figure 8: Tyrosine biosynthesis. Glutamate is required at the last step in the pathway for a transaminase reaction.
Figure 9: Arginine biosynthetic pathway. Glutamate is the precursor and is also required at the fourth step for a transaminase reaction. Ornithine is a precursor for both arginine and the polyamines putrescine and spermidine. The co-reactant with ornithine, carbamyl phosphate, is a direct product of glutamine (see Fig. 4)
Figure 10: Biosynthesis of UDP-MurNAc from UDP-GlcNAc. UDP-MurNAc is required to initiate synthesis of peptidoglycan.
Figure 11: Biosynthesis of enterobacterial common antigen (ECA). UDP-GlcNAc, a product of glutamine, is required to initiate ECA synthesis.