

Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance

Enzymes of Arginine Metabolism^{1,2}

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ABSTRACT In mammals, L-arginine is classified as a semiessential or conditionally essential amino acid, depending on the developmental stage and health status of the individual. It can be derived from proline or glutamate, with the ultimate synthetic step catalyzed by argininosuccinate lyase. L-arginine is catabolized by arginases, nitric oxide synthases, arginine:glycine amidinotransferase, and possibly also by arginine decarboxylase, resulting ultimately in the production of urea, proline, glutamate, polyamines, nitric oxide, creatine, or agmatine. There is considerable diversity in tissue-specific and stimulus-dependent regulation of expression within this group of enzymes, and the expression of several of them can be regulated at transcriptional and translational levels by changes in the concentration of L-arginine itself. Consequently, the interplay among these enzymes in the regulation of specific aspects of arginine metabolism can be quite complex. For example, nitric oxide production can be affected by the interplay between nitric oxide synthases, arginases, and argininosuccinate synthetase. This metabolic complexity can pose challenges for analyses of arginine metabolism not only because L-arginine is a substrate for several different enzymes but also because ornithine and citrulline, key products of arginine metabolism, can each be produced by multiple enzymes. This overview highlights key features of the arginine metabolic enzymes and their interactions. *J. Nutr.* 134: 2743S–2747S, 2004.

KEY WORDS: • arginine • ornithine • citrulline • nitric oxide • polyamines

L-Arginine is classified as a semiessential or conditionally essential amino acid because the ability of the body to synthesize sufficient quantities to meet its needs varies according to developmental age and the incidence of disease or injury (1–4). It is one of the more metabolically versatile amino acids, giving rise to nitric oxide (NO), urea, ornithine, citrulline, creatine, agmatine, glutamate, proline, and polyamines (Fig. 1) (5). It is therefore no surprise that its metabolism is complex and highly regulated (6). This complexity arises not only from the diversity of enzymes involved in metabolism of L-arginine and its metabolites but also from their cell-specific patterns of expression. These enzymes and their interrelationships are the primary focus of this brief review. The arginine transporters, which play an essential role in arginine metabolism and its regulation, are discussed in the article by Closs et al. (7) and therefore are not considered here. Additional aspects of arginine metabolism not discussed here can be found in recent reviews (5,6,8,9) and in accompanying articles in this volume.

Arginine catabolic enzymes

L-Arginine can be catabolized by 4 sets of enzymes in mammalian cells: nitric oxide synthases (NOS;⁴ EC 1.14.13.39), arginases (EC 3.5.3.1), arginine:glycine amidinotransferase (EC 2.1.4.1), and arginine decarboxylase (EC 4.1.1.19) (Fig. 2). With the exception of arginine decarboxylase, all these enzymes act on the guanidino group of L-arginine. Adding to the metabolic complexity is the fact that multiple isoforms exist for some of these enzymes—there are at least 3 distinct isoforms of NOS [neuronal NOS, inducible NOS (iNOS), and endothelial NOS] and 2 isoforms of arginase (types I and II; cytosolic and mitochondrial, respectively). Arginine deiminase (EC 3.5.3.6), the last enzyme listed in Figure 2, is not expressed by animal cells (10). Its inclusion here is based on the fact that it is expressed by some pathogenic microbes and thus can be present within the mammalian host, where it can disrupt host arginine metabolism. For example, the arginine deiminase activity of the human pathogen *Giardia lamblia* may provide protection from host cell defenses by consuming L-arginine, thus compromising the ability of host cells to produce NO (11).

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⁴ Abbreviations used: ADMA, asymmetric N^G,N^G-dimethyl-L-arginine; CAT-1, cationic amino acid transporter-1; DDAH, dimethylarginine dimethylaminohydrolyase; eIF2 α , eukaryotic initiation factor 2 α ; iNOS, inducible nitric oxide synthase; NO, nitric oxide; NOHA, N^G-hydroxy-L-arginine; NOS, nitric oxide synthase.

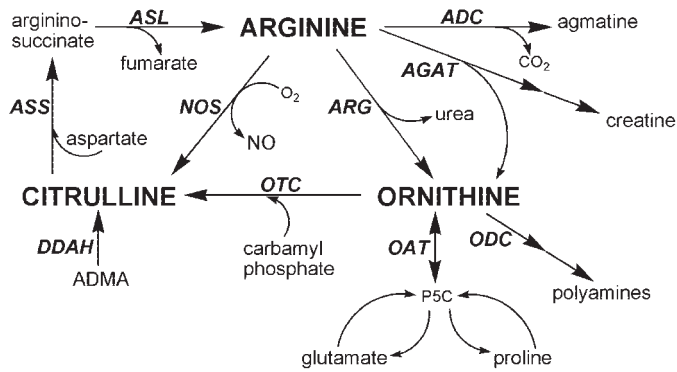


FIGURE 1 Metabolic sources and fates of L-arginine, citrulline, and ornithine. Only enzymes that directly use or produce these amino acids are indicated. L-Arginine is derived also from diet and turnover of cellular proteins (not shown). For the sake of clarity, not all reactants and products are shown. Abbreviations: ADC, arginine decarboxylase; AGAT, arginine:glycine amidinotransferase; ARG, arginase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; OTC, ornithine transcarbamylase; P5C, Δ^1 -pyrroline-5-carboxylate. Note: P5C is in chemical equilibrium with L-glutamate- γ -semialdehyde via a spontaneous nonenzymatic reaction (not shown).

Arginine:glycine amidinotransferase catalyzes the first and rate-controlling step in the synthesis of creatine, whose metabolism is discussed in more detail in a recent review (12) and in the article by Brosnan and Brosnan (13). Flux of L-arginine through arginine:glycine amidinotransferase is determined in part by dietary levels of creatine, which acts as a feedback repressor of this enzyme (12,14). Interestingly, there are structural similarities between arginine:glycine amidinotransferase, arginine deiminase, and dimethylarginine dimethylaminohydrolase [DDAH (EC 3.5.3.18), the enzyme that catabolizes asymmetric N^G, N^G -dimethyl-L-arginine (ADMA) and N^G -monomethyl-L-arginine to citrulline and di- or monomethylamine] (15), indicating that they represent members of a structural superfamily of enzymes.

Arginine decarboxylase converts L-arginine to CO_2 and agmatine, which can be catabolized further by agmatinase (16,17) to produce putrescine and urea, thus constituting a quantitatively minor pathway for polyamine synthesis in mammals. Although evidence of arginine decarboxylase activity has been presented for mammalian cells [e.g. (18–20)] and a putative human arginine decarboxylase clone has been reported (21), the existence of arginine decarboxylase in mammals is still somewhat controversial because a new report has challenged the conclusions of earlier studies (22). Interestingly, it is not clear that all mammals express a functional agmatinase (23). Evaluating the origin of agmatine in mammalian tissues is not straightforward because agmatine can be derived from dietary sources or generated by enteric bacteria. Although the physiologic roles of agmatine in mammals have not been conclusively defined (24), various studies have indicated that it may be a neurotransmitter (25), an inhibitor of cell proliferation (26), or an inhibitor of NOS (27–29).

The NOS and arginase enzymes are discussed in more detail in the articles by Stuehr (30) and Ash (31), respectively. Information regarding the regulation and function of these enzymes is too extensive to be considered in detail, so only a few points will be noted here. The NOS and arginase enzymes are expressed simultaneously under a wide variety of inflammatory conditions, resulting in cross-interactions whose con-

sequences are not always simple (32,33). Studies of these conditions have involved primarily the iNOS and arginase I isozymes. As both enzymes use the same substrate, one would anticipate that their simultaneous expression should result in competition for substrate. Thus, arginase activity in some—but not all (34)—circumstances has been shown to limit NO production by iNOS, apparently by limiting L-arginine availability [e.g. (35–39)]. The converse has not been conclusively demonstrated because synthesis of NO is accompanied also by release of N^G -hydroxy-L-arginine (NOHA), an intermediate in NO synthesis that is a potent natural inhibitor of arginase (40). Thus, inhibitory effects of NOS expression on arginase activity—particularly in the case of iNOS expression—are perhaps more likely to reflect the inhibitory action of NOHA than of substrate limitation (41). Elevated expression of arginases during inflammation or infection may not only reduce NO synthesis but also stimulate the synthesis of proline and polyamines via increased production of ornithine (34,42–44). Increased synthesis of polyamines and proline, in turn, can promote cell proliferation (42,45) and collagen synthesis, respectively—processes that play important roles in wound healing, fibrotic disorders, chronic inflammatory diseases, and infection.

Arginine synthetic enzymes

L-Arginine is synthesized from citrulline by the sequential action of the cytosolic enzymes argininosuccinate synthetase (EC 6.3.4.5) and argininosuccinate lyase (EC 4.3.2.1) (Fig. 1). This is energetically costly, as the synthesis of each molecule of argininosuccinate requires hydrolysis of ATP to AMP (not shown); i.e., 2 ATP equivalents. Citrulline can be derived from multiple sources: L-arginine via NOS, ornithine via catabolism of proline or glutamine/glutamate, or ADMA via DDAH (Fig. 1). It is important to note that the pathways linking L-arginine, glutamate, and proline are bidirectional. Thus, the net utilization or production of these amino acids is highly dependent on cell type and developmental stage (5). On a whole-body basis, synthesis of L-arginine occurs principally via the intestinal-renal axis, wherein epithelial cells of the small intestine, which produce citrulline primarily from glutamine and glutamate, collaborate with the proximal tubule cells of the kidney, which extract citrulline from the circulation and convert it to L-arginine, which is returned to the circulation (5). Consequently, impairment of small bowel or renal function can reduce endogenous arginine synthesis,

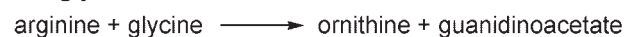
Nitric oxide synthases:



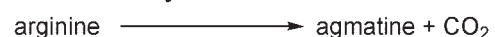
Arginases:



Arginine:glycine amidinotransferase:



Arginine decarboxylase:



Arginine deiminase:



FIGURE 2 Metabolic enzymes that use L-arginine as substrate.

thereby increasing the dietary requirement (8). Synthesis of L-arginine from citrulline also occurs at a low level in many other cells (46,47), and cellular capacity for arginine synthesis can be markedly increased under circumstances that also induce iNOS (48). Thus, citrulline, a coproduct of the NOS-catalyzed reaction, can be recycled to L-arginine in a pathway known as the citrulline-NO or arginine-citrulline pathway (32). This is demonstrated by the fact that in many cell types citrulline can substitute for L-arginine to some degree in supporting NO synthesis (32). However, recycling is not quantitative because citrulline accumulates along with nitrate and nitrite, the stable end-products of NO, in NO-producing cells.

Spatial relationships among arginine metabolic enzymes

To date, none of the arginine metabolic enzymes have been found together in any kind of complex that survives cell homogenization. However, this should not be taken as conclusive evidence for the absence of close associations among some of these enzymes within intact cells. The paradigm for such intracellular associations is the urea cycle, whose component enzymes are so closely organized within hepatocytes that intermediates generated within the pathway are immediately utilized and thus do not freely exchange with intracellular pools (49,50). This observation indicates that the mitochondrial and cytosolic enzymes of the urea cycle must therefore be tightly associated on either side of the mitochondrial membrane. Such an arrangement of tightly coupled enzymatic reactions in the absence of a physically identifiable multienzyme complex is termed a *metabolon* (51). Although firm evidence for other such associations among the arginine metabolic enzymes has not yet been reported, it is likely that they do exist. For example, it is reasonable to hypothesize a close association between argininosuccinate synthetase and argininosuccinate lyase in renal tubules and other nonhepatic cells in order to facilitate efficient conversion of citrulline to L-arginine and thus minimize the potential for cellular loss by diffusion of argininosuccinate, an energetically "expensive" metabolite whose synthesis requires the hydrolysis of 2 ATP equivalents.

Arginine as a regulator of protein expression

In addition to serving as a substrate for the enzymes discussed here, L-arginine itself selectively regulates, in a concentration-dependent manner, the expression of several enzymes of arginine metabolism and thus affects its own metabolic fate. For example, it has been known for 40 y that the activities of argininosuccinate lyase and argininosuccinate synthetase in several nonhepatic cell lines are repressed by L-arginine and increased when L-arginine is replaced by citrulline (52,53). Replacing L-arginine with citrulline increases transcription of the argininosuccinate synthetase gene (54), but the molecular mechanisms involved in this response have not been elucidated.

Expression of iNOS is inhibited when arginine availability is compromised by limitation of extracellular L-arginine, overexpression of arginase, or, in some cells, reduction of capacity for arginine uptake (55–58). Two studies have shown that translation of iNOS mRNA, relative to total protein synthesis, is preferentially inhibited at low levels of L-arginine (55,56). Translational inhibition involves increased phosphorylation of the translation eukaryotic initiation factor 2 α (eIF2 α), secondary to activation of the mammalian homolog of GCN2 kinase (55). However, different effects of arginine limitation

on stability of NOS protein were reported in the 2 studies, possibly reflecting differences in the cell types used or in experimental conditions (55,56). Neither of these studies found any effect of arginine limitation on levels of iNOS mRNA. In contrast, studies in our laboratory found that reduced arginine availability also selectively inhibits induction of iNOS mRNA in the RAW 264.7 macrophage cell line (unpublished observations, D. Kepka-Lenhart and S. M. Morris, Jr., 2004) indicating that there are multiple mechanisms whereby arginine availability regulates iNOS expression, possibly in a cell-type-specific manner.

Expression of at least one of the transporters involved in cellular arginine uptake also increases when L-arginine is limiting. Expression of cation amino acid transporter-1 (CAT-1) increases under conditions of general amino acid starvation (59) and also when individual amino acids, including arginine, are removed from the culture medium (60). This occurs not only via increased transcription of the CAT-1 gene (60) but also via increased translation initiation of CAT-1 mRNA at an internal ribosome entry site in a process that requires phosphorylation of the translation initiation factor eIF2 α (61,62).

Selective regulation of protein expression by arginine availability is not confined to proteins involved in arginine metabolism. For example, expression of the ζ chain of the T-cell antigen receptor is selectively reduced when L-arginine is limiting, via a reduction in half-life of the ζ -chain mRNA (63–65). Here too, as in the case of iNOS expression, arginine limitation results in impairment of the host defense system, thus supporting the notion that arginine supplementation may be beneficial in cases of injury or disease where circulating levels of L-arginine are significantly decreased.

Concluding remarks

Much remains to be learned about the complex and dynamic interrelationships between the arginine metabolic enzymes and how they relate to variations in nutritional requirements for L-arginine or in changes in biochemical markers in health and disease. For example, analyses of serum amino acids usually include values for ornithine and citrulline as well as for L-arginine. Levels of each of these amino acids can be significantly altered following injury and in a number of diseases. Investigators may therefore be tempted to infer changes in activities of specific arginine metabolic enzymes, based merely on changes in circulating levels of L-arginine, citrulline, and ornithine, but it should be clear from this discussion that care should be taken that any interpretations do not involve an oversimplified view of precursor-product relations. As levels of these 3 amino acids reflect the combined activities of multiple enzymes (Fig. 1) and arginine transporters that are expressed at different levels in a wide range of tissues, any such inferences should be considered speculative in the absence of additional information. For example, ornithine and citrulline can be derived from amino acids other than L-arginine, so it would not necessarily be correct to conclude that increases in circulating levels of ornithine or citrulline reflect increased metabolism of L-arginine via arginase or NOS, respectively.

Interest in arginine metabolism has increased greatly over the past 15 y or so, triggered primarily by the discovery of NO synthesis in mammals and its remarkable biological roles. This interest has not abated but has broadened as new and exciting information about other aspects of arginine metabolism has emerged, particularly with regard to expression and function of the arginases. Thus, new ideas for manipulating arginine me-

tabolism by nutritional regimens and other means for treatment of disease can be anticipated as our knowledge in this area continues to grow.

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