Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 312 (2003) 205–208
BBRC
www.elsevier.com/locate/ybbrc

Reappraisal of the 20th-century version of amino acid metabolism

Masayuki Katagiri* and Masahiko Nakamura

2-30-1 Kodatsuno, Kanazawa 920-0942, Japan
Institute for Protein Research, Osaka University, Suita 565-0871, Japan

Received 25 September 2003

Abstract

In this article, we advocate the radical revision of the 20th-century version of amino acid metabolism as follows. (1) Classic studies on the incorporation of [^{15}N]ammonia into glutamate, once considered to be an epoch-making event, are not distinctive proof of the ability of animals to utilize ammonia for the synthesis of α-amino nitrogen. (2) Mammalian glutamate dehydrogenase has been implicated to function as a glutamate-synthesizing enzyme albeit lack of convincing proof. This enzyme, in combination with aminotransferases, is now known to play an exclusive role in the metabolic removal of amino nitrogen and energy production from excess amino acids. (3) Dr. William C Rose's "nutritionally nonessential amino acids" are, of course, essential in cellular metabolism; the nutritional nonessentiality is related to their carbon skeletons, many of which are intermediates of glycolysis or the TCA cycle. Obviously, the prime importance of amino acid nutrition should be the means of obtaining amino nitrogen. (4) Because there is no evidence of the presence of any glutamate-synthesizing enzymes in mammalian tissues, animals must depend on plants and microorganisms for preformed α-amino nitrogen. This is analogous to the case of carbohydrates. (5) In contrast, individual essential amino acids, similar to vitamins and essential fatty acids, should be considered important nutrients that must be included regularly in sufficient amounts in the diet.

Keywords: Amino acid nutrition; Amino nitrogen; Aminotransferase; Essential amino acid; Glutamate; Glutamate dehydrogenase; Glutamate synthase; Glutamine synthetase; Nonessential amino acid

It is a privilege to be part of this issue honoring for Professor I.C. Gunsalus (Gunny) and to recall with gratitude the kindness and warm hospitality extended to me (M.K.) during my stay (September 1967 to March 1968) in his laboratory at the Department of Biochemistry, University of Illinois, Urbana, Illinois. I first met Gunny in May 1966 at the Symposium on the Biological and Chemical Aspects of Oxygenases held in Kyoto, Japan, under the auspices of The United States–Japan Committee on Scientific Cooperation. At that time, I was attracted to his camphor 5-*exo*hydroxylase system because it appeared to be more complicated and more closely related to mammalian steroid hydroxylase systems than our salicylate hydroxylase system, an FAD protein [1,2]. Dr. O. Hayaishi, one of the organizers of this symposium, made the necessary arrangements for me to work in Gunny's laboratory as a visiting scientist for 6 months during my sabbatical year. There, I participated in an ongoing project to elucidate the components of the camphor 5-*exo*hydroxylase system of *Pseudomonas putida*. This led to the revelation that in addition to the known non-heme iron protein, i.e., putidaredoxin, an NADH-dependent flavoprotein and a P-450 hemoprotein, referred to as putidaredoxin reductase and P-450$_{cam}$, respectively, are involved in the hydroxylation reaction [3,4]. After my return to my home laboratory in Japan, this experience led us to separate two distinctive steroid hydroxylating P-450 components, P-450$_{scc}$ and P-450$_{11\beta}$, from bovine adrenal cortex mitochondria in solubilized and purified forms. Fortunately, we were able to report those results in a volume honoring Gunny, *Experiences in Biochemical Perception*, which was published in 1982 [5]. In the same volume was an interesting statement by Sligar, "The Fourth Gunny Rule of Science is: *Always change your*

* Corresponding author. Fax: +81-76-261-6201.
E-mail address: m-katagiri@mtg.biglobe.ne.jp (M. Katagiri).

0006-291X/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2003.09.219

scientific direction and project every ten years" [6]. This encouraged me to contribute our recent opinion to this volume of *BBRC* dedicated to Gunny.

In 1985, I stated in my textbook that animals do not utilize ammonia for glutamate synthesis because they lack the enzymes capable of doing so [7]. Based on this view, we presented a hypothesis that animals are completely dependent on preformed α-amino nitrogen [8,9]. In the present article, we would like to reappraise an unsolved mystery in the 20th century regarding the origin of amino acids in life on Earth. In fact, in a survey of current biochemistry textbooks and relevant reviews, we found only common descriptions such as "animals can utilize ammonia for glutamate synthesis" or "mammalian glutamate dehydrogenase catalyzes glutamate synthesis" without any explanation or with only an ambiguous adjunct such as clearly, possibly, probably, or undoubtedly. Surprisingly, such claims appear to be widely accepted without further convincing proof, simply because the glutamate dehydrogenase-catalyzed reaction is reversible. Clearly, the reversible nature of an enzymatic reaction does not necessarily represent its synthetic role. Typical examples are the cases of such cleavage enzymes as glycogen phosphorylase, a series of fatty acid oxidizing enzymes, and polynucleotide phosphorylase, all at one time supposed to be extremely important synthesizing enzymes.

The first concept developed at the beginning of the 20th century, that animals can synthesize amino acids, may be due to misinterpretation of such reactions as those catalyzed by aminotransferases. Subsequently, Schoenheimer and his colleagues in 1939 and later Berl et al. showed the in vivo incorporation of a small but mass-spectrometrically significant amount of $^{15}NH_3$ into amino acids [10,11]. However, all of those experiments were performed on dying animals under unnaturally high and toxic levels of ammonium salt, namely, rats that consumed, instead of casein in normal diet, 0.1 mol of ammonium citrate/kg body wt/day for 5 days, and cats that were infused with 1 M ammonium acetate up to the time of death (for a period of 8 to 25 min). Consequently, the balance of the dynamic equilibrium of endogenous reactions might have been influenced without the net synthesis of amino acids. In fact, in the original report by Schoenheimer and colleagues, they stated: "However, unphysiological conditions employed in both series make it uncertain that we are dealing with a process which takes place under normal conditions" [10]. Unfortunately, this was absolutely correct. Cooper et al. performed short-term analyses of the order of seconds of the fate of truly tracer levels of ^{13}N-labeled ammonia in rat liver and brain [12–14] Their studies demonstrated that the role of glutamate dehydrogenase is included in the catabolism. Kinetic and binding analyses of mammalian glutamate dehydrogenase have indicated that its major allosteric inhibitors are GTP and NADH [15]. This pattern of regulation suggests that the main function of this enzyme may be the formation of 2-oxoglutarate and ammonia from glutamate. Note that the K_m values for NH_4^+ of a number of glutamate dehydrogenases, including those in plants and microorganisms, are high [16]. For bovine enzymes, this value is 3.2 mM, which is a lethal level in the cell, and therefore excludes possible biosynthetic role of this enzyme [17,18]. The equilibrium position of this reaction, which markedly favors glutamate formation, may be physiologically important [19]. Thus, we conclude that all the properties of glutamate dehydrogenase, including its allosteric nature, help prevent exhaustive discharge of the glutamate reservoir that must be maintained at appropriate levels in liver and other organs on the one hand and help maintain toxic ammonia at low levels on the other [9,19]. These conclusions are supported by studies on the hyperinsulinism/hyperammonemia syndrome, a form of congenital hyperinsulinism in which children have asymptomatic persistently elevated plasma ammonium levels [20]. This disease is caused by dominant mutations of glutamate dehydrogenase that impair its sensitivity to GTP, an allosteric inhibitor.

Rose and co-workers formulated a nutritional classification system for amino acids, calling them essential (or indispensable) and nonessential (or dispensable). Their experiments, together with a more recent study by Rogers and his group, seemed to have established the basic view that mammals, including humans, do not nutritionally require nonessential amino acids [21–23]. However, most of their experiments were deliberately performed in the presence of identical and sufficient amounts of total effective α-amino nitrogen. Consequently, their results only indicated whether animals synthesized the carbon skeletons of certain amino acids. Those problems may therefore be related to carbohydrate metabolism alone: many of the carbon skeletons of nonessential amino acids are intermediates of glycolysis or the TCA cycle. Since the primary importance of amino acid metabolism should be the means of obtaining α-amino nitrogen, we question the claim that animals can synthesize nonessential amino acids a priori. We propose that animals are completely dependent on the dietary supply of preformed α-amino nitrogen for the syntheses of proteins and other functional compounds, including hemes. In contrast, individual essential amino acids, similar to vitamins and essential fatty acids, are important nutrients that must be included regularly in sufficient and not excess amounts in the diet. Many nutritional studies have shown that body-weight gain and nitrogen retention are optimal in the presence of moderate amounts of both essential and nonessential amino acids [24,25]. The molar ratios of essential to total amino acids in egg and milk, presumably the optimally balanced nutritional sources for the embryo and the young, are approximately 0.5. Similar essential to

total amino acid ratios are found in many diets recommended for good nutrition, in normal transfusions, and in tissue culture media.

Two glutamate-synthesizing systems are known in plants and microorganisms. The first involves the participation of two enzymes present in all plants and in many microorganisms: (1) glutamine synthetase, which has a high affinity for ammonia and synthesizes glutamine from glutamate, ammonia, and ATP; and (2) glutamate synthase, which catalyzes the reductive amination of 2-oxoglutarate using glutamine to form two equivalents of glutamate [26,27].

Glutamine synthetase is highly regulated by cumulative feedback inhibition by the end products of glutamine metabolism, as shown by the enzyme of *Escherichia coli*. This activity is also controlled by adenylylation and deadenylylation by a series of regulatory cascades [28,29]. Incidentally, in *Experiences in Biochemical Perception*, Stadtman and co-workers presented an interesting study on the degradation of bacterial glutamine synthetase by P-450_{LM}- and P-450_{cam}-linked systems [30].

Glutamate synthase uses the amide nitrogen of glutamine for amination with reduced ferredoxin, NADH, or NADPH as a reducing equivalent [26,27]. There is considerable evidence that chloroplasts in the leaves of higher plants are the major sites of ammonia fixation and the reaction is light- and ferredoxin-dependent [26]. Thus, this process is analogous to CO_2 assimilation in the sense that it is characteristic of a light-dependent reaction in the biosphere. The coupling of glutamine synthetase with glutamate synthase provides an ATP-dependent and virtually irreversible synthesis of glutamate from 2-oxoglutarate and ammonia in a cyclic fashion.

The second glutamate-synthesizing system is that catalyzed by an NADPH-linked glutamate dehydrogenase [31]. This enzyme, found in some microorganisms that prefer ammonia as their source of nitrogen, is induced under high levels of ammonium salts. Differing from mammalian glutamate dehydrogenase (EC 1.4.1.3), this microbial enzyme (EC 1.4.1.4) catalyzes the primitive biosynthesis of glutamate, i.e., the direct amination of 2-oxoglutarate with ammonia. One interesting example is the bioindustrial production of glutamate from ammonium salts and molasses by the glutamate dehydrogenase of *Corynebacterium glutamicum* and other bacteria [32]. In those microorganisms, glutamate can also be synthesized by the evolved glutamine synthetase/glutamate synthase system described above.

In herbivores such as ruminants, similar roles played by symbiotic microbes in rumen in amino acid biosynthesis may be of great importance [33,34]. It is known that approximately 80% of rumen isolates can grow with ammonia as their sole nitrogen source. Hence, in breeding farms, part of the amino acid needs of cattle may be met by simple and low-cost nitrogen sources such as urea. Animal food products thus produced, such as meat and milk, may ultimately provide all the amino acids to meet our daily nutritional requirements. In human, the involvement of gastrointestinal microflora in the utilization of ammonia and the capacity of the large intestine to absorb amino acids have recently been investigated extensively [34–36].

It should be added that although animals do not have the ability to synthesize α-amino acids and hence proteins and porphyrins from ammonia, they can and must utilize ammonia to form carbamoyl phosphate via the action of carbamoyl phosphate synthetase I, and glutamine via the action of glutamine synthetase [37]. The two products are the most important sources of nitrogen for a number of biosynthetic pathways, and together play important roles in the formation of the nitrogen end product, urea, and many functional and informational compounds, e.g., purines (N3, N9, and 2-amino) and pyrimidines (N3 and 4-amino) [37,38]. However, mammalian glutamine synthetase differs from plant and microbial glutamine synthetases in molecular weight and the number of subunits [37]. In support of our assumption that animals cannot synthesize α-amino nitrogen, an equivalent to glutamate synthase has not been reported in animals [Medline Express, 1970–2002].

From the perspectives of basic biochemistry, physiology, medicine, ecology, and the world's major nutritional problems, we present the view that animals are completely dependent on plants and microorganisms for preformed α-amino nitrogen. This is analogous to the case of carbohydrates. Is it a coincidence that umami of glutamate is comparable to *amami* (or sweetness: the antonym of umami derived from Japanese), each comprising a signal for discrimination of food by its nutritive value [39]? Virtually all cells that require glutamate contain aminotransferases for the immediate transfer of preformed α-amino nitrogen to numerous oxo acids to form the corresponding amino acids. Mammalian glutamate dehydrogenase, in combination with aminotransferases, is known to play an important catabolic role in the metabolic removal of amino nitrogen and energy production from excess amino acids. Using $^{13}NH_3$, Cooper et al. emphasized the rapidity of nitrogen exchange in rat liver and brain via linked aminotransferases [13]. Finally, it gives us great pleasure to conclude this article by referring to Gunny's research on aminotransferases in the 1940s [40].

References

[1] I.C. Gunsalus, P.W. Trudgill, D.W. Cushman, Components and reaction of ketolactonases and methylene hydroxylases, in: K. Bloch, O. Hayaishi (Eds.), Biological and Chemical Aspects of Oxygenases, Maruzen, Tokyo, 1966, pp. 339–343.

[2] M. Katagiri, S. Takemori, K. Suzuki, H. Yasuda, The role of flavin in salicylate hydroxylase, in: K. Bloch, O. Hayaishi (Eds.),

Biological and Chemical Aspects of Oxygenases, Maruzen, Tokyo, 1966, pp. 315–326.
[3] M. Katagiri, B.N. Ganguli, I.C. Gunsalus, A soluble cytochrome P-450 functional in methylene hydroxylation, J. Biol. Chem. 243 (1968) 3543–3546.
[4] I.C. Gunsalus, C.A. Tyson, J.D. Lipscomb, Cytochrome P-450 reduction and oxygenation systems, in: T.E. King, H.S. Mason, M. Morrison (Eds.), Oxidases and Related Redox Systems, University Park Press, Baltimore, 1971, pp. 583–603.
[5] M. Katagiri, K. Suhara, Purification and characterization of adrenal P-450 cytochromes, in: L.N. Ornston, S.G. Sligar (Eds.), Experiences in Biochemical Perception, Academic Press, New York, 1982, pp. 267–278.
[6] S.G. Sligar, From stars to organic chemistry via cytochrome P-450, in: L.N. Ornston, S.G. Sligar (Eds.), Experiences in Biochemical Perception, Academic Press, New York, 1982, pp. 253–265.
[7] M. Katagiri, Amino acid metabolism, in: M. Katagiri (Ed.), Kiso Seikagaku (in Japanese), Kyoritu Shuppan, Tokyo, 1985, pp. 203–216.
[8] M. Katagiri, M. Nakamura, Is there really any evidence indicating that animals synthesize glutamate? Biochem. Educ. 27 (1999) 83–85.
[9] M. Katagiri, M. Nakamura, Animals are dependent on preformed α-amino nitrogen as an essential nutrient, IUBMB Life 53 (2002) 125–129.
[10] G.L. Foster, R. Schoenheimer, D. Rittenberg, Studies in protein metabolism, V. The utilization of NH_4^+ for amino acid and creatine formation in animals, J. Biol. Chem. 127 (1939) 319–327.
[11] S. Berl, G. Takagaki, D.D. Clarke, H. Waelsch, Metabolic compartments in vivo, ammonia and glutamic acid metabolism in brain and liver, J. Biol. Chem. 237 (1962) 2562–2569.
[12] A.J.L. Cooper, J.M. McDonald, A.S. Gelbard, R.F. Gledhill, T.E. Duffy, The metabolic fate of ^{13}N-labeled ammonia in rat brain, J. Biol. Chem. 254 (1979) 4982–4992.
[13] A.J.L. Cooper, E. Nieves, A.E. Coleman, S. Filc-DeRicco, A.S. Gelbard, Short-term metabolic fate of [^{13}N]ammonia in rat liver in vivo, J. Biol. Chem. 262 (1987) 1073–1080.
[14] A.J.L. Cooper, E. Nieves, K.C. Rosenpire, S. Filc-DeRicco, A.S. Gelbard, S.W. Brusilow, Short-term metabolic fate of ^{13}N-labeled glutamate, alanine, and glutamine (amide) in rat liver, J. Biol. Chem. 263 (1988) 12268–12273.
[15] T.J. Smith, P.E. Peterson, T. Schmidt, J. Fang, C.A. Stanley, Structure of bovine glutamate dehydrogenase complexes elucidate the mechanism of purine regulation, J. Mol. Biol. 307 (2001) 707–720.
[16] E.L. Smith, B.M. Austen, K.M. Blumenthal, J.F. Nyc, Glutamate dehydrogenases, in: P.D. Boyer (Ed.), The Enzymes, vol. XI, Academic Press, New York, 1975, pp. 293–367.
[17] E.E. Conn, P.K. Stumpf, The metabolism of ammonia and nitrogen-containing monomers, in: Outlines of Biochemistry, fourth ed., Wiley, New York, 1976, pp. 459–502.
[18] A. Meister, Biochemistry of glutamate, glutamine and glutathione, in: L.J. Filer Jr., S. Garattini, M.R. Kare, W.A. Reynolds, R.J. Wurtman (Eds.), Glutamic Acid: Advances in Biochemistry and Physiology, Raven Press, New York, 1979, pp. 69–84.
[19] K.L. Manchester, Glutamate dehydrogenase, a reappraisal, Biochem. Educ. 13 (1985) 131–133.
[20] C. MacMullen, J. Fang, B.Y. Hsu, A. Kelly, P. de Lonlay-Debeney, J.M. Saudubray, A. Ganguly, T.J. Smith, C.A. Stanley, Hyperinsulinism/hyperammonemia syndrome in children with regulatory mutations in the inhibitory guanosine triphosphate-binding domain of glutamate dehydrogenase, J. Clin. Endocrinol. Metab. 86 (2001) 1782–1787.
[21] W.C. Rose, M.J. Oesterling, M. Womack, Comparative growth on diets containing ten and nineteen amino acids with further observations upon the role of glutamic and aspartic acids, J. Biol. Chem. 176 (1948) 753–762.
[22] W.C. Rose, Amino acid requirement of man, Fed. Proc. 8 (1949) 546–552.
[23] T.P. Taylor, J.G. Morris, N.H. Willits, Q.P. Rogers, Optimizing the pattern of essential amino acids as the sole source of dietary nitrogen supports near-maximal growth in kittens, J. Nutr. 126 (1996) 2243–2252.
[24] A.E. Harper, Dispensable and indispensable amino acid interrelationship, in: G.L. Blackburn, J.P. Grant, V.R. Young (Eds.), Amino Acid Metabolism and Medical Applications, John Wright PSG Inc., Boston, 1983, pp. 105–121.
[25] P.J. Reeds, Dispensable and indispensable amino acid for humans, J. Nutr. 130 (2000) 18355–18405.
[26] P.J. Lea, B.J. Milfin, Alternative route for nitrogen assimilation in higher plants, Nature 251 (1974) 614–616.
[27] D.W. Tempest, J.L. Meers, C.M. Brown, Synthesis of glutamate in Aerobacter aerogenes by a hitherto unknown route, Biochem. J. 117 (1970) 405–407.
[28] R.J. Almassy, C.A. Janson, R. Hamlin, N.-H. Xuong, D. Eisenberg, Novel subunit-subunit interaction in the structure of glutamine synthetase, Nature 323 (1986) 304–309.
[29] E.R. Stadtman, The story of glutamine synthetase regulation, J. Biol. Chem. 276 (2001) 44357–44364.
[30] C.N. Oliver, R.L. Levine, E.R. Stadtman, Regulation of glutamine synthetase degradation, in: L.N. Ornston, S.G. Sligar (Eds.), Experiences in Biochemical Perception, Academic Press, New York, 1982, pp. 233–249.
[31] G. Gottschalk, Assimilation of ammonia, in: G. Gottschalk (Ed.), Bacterial Metabolism, second ed., Springer-Verlag, New York, 1986, pp. 40–42.
[32] M. Tesch, A.A. DeGraaf, H. Sahm, In vivo fluxes in the ammonium-assimilatory pathways in Corynebacterium glutamicum studied by ^{15}N nuclear magnetic resonance, Appl. Environ. Microbiol. 65 (1999) 1099–1109.
[33] R.L. Baldwin, M.J. Allison, Rumen metabolism, J. Animal Sci. 57 (Suppl. 2) (1983) 461–477.
[34] M.F. Fuller, P.J. Reeds, Nitrogen cycling in the gut, Annu. Rev. Nutr. 18 (1998) 385–411.
[35] A.A. Jackson, Salvage of urea-nitrogen and protein requirements, Proc. Nutr. Soc. 54 (1995) 535–547.
[36] C.C. Metges, Contribution of microbial amino acids to amino acid homeostasis of the host, J. Nutr. 130 (2000) 1857S–1864S.
[37] A. Meister, Glutamine synthetase of mammals, in: P.D. Boyer (Ed.), The Enzymes, vol. 10, Academic Press, New York, 1975, pp. 699–754.
[38] V.P. Young, A.M. Ajami, Glutamine: the emperor or his clothes?, J. Nutr. 131 (2001) 2449S–2459S.
[39] B. Lindemann, A taste for umami, Nat. Neurosci. 3 (2000) 99–100.
[40] H.C. Lichstein, I.C. Gunsalus, W.W. Umbreit, Function of the vitamin B_6 group, pyridoxal phosphate in transamination, J. Biol. Chem. 161 (1945) 311–320.