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REVIEW ARTICLE

Glutamate: a truly functional amino acid

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Abstract Glutamate is one of the most abundant of the amino acids. In addition to its role in protein structure, it plays critical roles in nutrition, metabolism and signaling. Post-translational carboxylation of glutamyl residues increases their affinity for calcium and plays a major role in hemostasis. Glutamate is of fundamental importance to amino acid metabolism, yet the great bulk of dietary glutamate is catabolyzed within the intestine. It is necessary for the synthesis of key molecules, such as glutathione and the polyglutamated folate cofactors. It plays a major role in signaling. Within the central nervous system, glutamate is the major excitatory neurotransmitter and its product, GABA, the major inhibitory neurotransmitter. Glutamate interaction with specific taste cells in the tongue is a major component of umami taste. The finding of glutamate receptors throughout the gastrointestinal tract has opened up a new vista in glutamate function. Glutamate is truly a functional amino acid.

Keywords Gamma-carboxyglutamate · GI tract · Glutathione · Insulin secretion · Neurotransmitter · Umami taste receptors

Why glutamate?

Although the primary focus of this paper concerns the functions of glutamate as a free amino acid, we must first enquire as to why glutamate is so abundant. Certainly, this follows from the fact that glutamate is one of the 20 canonical amino acids incorporated into proteins, but why should this be? Prominent features of glutamate that probably contributed to its role as a proteinaceous amino acid are: (1) that it is chemically stable, (2) that it may be metabolically produced and removed readily by virtue of its interconversion with α-ketoglutarate, a Krebs cycle intermediate, and (3) that it is negatively charged.

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Glutamate in proteins

In proteins, glutamate provides a negative charge which may be important in stabilizing the protein structure. For example, ion pairs involving glutamyl residues are important in stabilizing the leucine zipper structure of the transcription factor, GCN4 (Matousek et al. 2007). Charged residues, such as glutamate, are often found on the outer surface of globular proteins. The importance of polar residues on the surface is readily demonstrated by the devastating effect of a glutamate → valine mutation in position 6 of the β-chain of hemoglobin (HbS), to produce sickle-cell trait in heterozygotes with this mutation and sickle-cell disease in homozygous individuals (Ingram 1957). Deoxyhemoglobin has a hydrophobic pocket at the EF junction (phe B85, leu B88). Glutamate is permitted on the surface since it would be energetically unfavorable for it to interact with the hydrophobic pocket, so deoxyHbA remains soluble (Rotter et al. 2011). The valyl residue, however, which replaces it in HbS protrudes from the surface and easily fits into the hydrophobic pocket, causing the HbS molecules to stick together, resulting in long stiff fibers which distort the red blood cells (Rodgers et al. 1987).

Not only is glutamate incorporated into proteins as they are being synthesized, but it can be added as a posttranslational modification after synthesis, in the form of polyglutamyl tails. For example, polyglutamylation of tubulin is thought to affect its interaction with other proteins, such

as microtubule-associated proteins (MAPs) and molecular motors (Janke and Bulinski 2011).

In proteins, glutamate binds cations fairly weakly, but its affinity for calcium can be enormously increased by a vitamin K-dependent (VKD) carboxylation, which, post-translationally, introduces a γ-carboxylated glutamyl residue (gla) into proteins (Berkner 2005). This reaction is shown in Fig. 1. All VKD proteins contain a homologous amino acid sequence which targets the protein to the carboxylase. Carboxylation occurs at multiple glutamate residues within the "gla domain". VKD proteins include a number involved in hemostasis: prothrombin and factors VII, IX and X. Other VKD proteins are involved in bone morphogenesis (bone gla protein and matrix gla protein) (Berkner 2000). The inhibition of VKD carboxylase function is critical to coumarin-based anticoagulant therapy, since 4-OH coumarin analogs inhibit the vitamin K epoxide reductase (VKOR) that is required to reconvert the vitamin K epoxide to reduced vitamin K (Chu et al. 2006). These studies provide a clear illustration of one of the key roles played by glutamate residues, including their post-translational modification, in proteins.

The B vitamin, tetrahydrofolate (THF) with a glutamyl residue attached is absorbed from the intestine and transported in the blood. Once in cells, further glutamyl residues (up to 9) are added in isopeptide linkage. This form of THF is sequestered in cells and folate-dependent enzymes have a higher affinity for it (Stover and Field 2011).

Glutamate: nutrition and metabolism

Glutamate is classified as a non-essential amino acid, which means that it can be synthesized in adequate quantities, in vivo. In fact, it must be synthesized in vivo. This is evident as a result of careful balance studies across the intestines of a variety of animals, which reveal that dietary glutamate is almost quantitatively metabolized within the intestine, chiefly by enterocytes. This was first shown by Windmueller and Spaeth (1975, 1980) using the perfused rat intestine, as well as in vivo in the rat. Subsequently, work with piglets, preterm infants, and adult humans have shown that dietary glutamate is extensively metabolized by the intestine. Elegant work in post-absorptive humans by Matthews et al. (1993) and Battezzati et al. (1995) showed that 96 % of enteral glutamate was removed on first pass by the splanchnic bed. Indeed, dietary glutamate is a critical metabolic fuel, with much of it being totally oxidized to CO_2 (Burrin and Stoll 2009). Detailed studies in infant pigs revealed that only 5 % of enteral glutamate appeared in portal blood (Reeds et al. 1996). In premature human infants on enteral feeding, some 74 % of glutamate was removed in the first pass (Riedijk et al. 2007). One of the consequences of gut glutamate metabolism is that plasma glutamate levels are not particularly affected by dietary glutamate. Indeed, circulating glutamate is tightly maintained at rather low concentrations (Watford 2002). Gut metabolism of glutamate has a very important consequence: most of the body's glutamate needs to be synthesized endogenously. Glutamate may be synthesized in two separate ways. Firstly, it may be synthesized from α-ketoglutarate, by either glutamate dehydrogenase or by a variety of aminotransferases. Secondly, glutamate may be synthesized from other amino acids; the "glutamate family" of amino acids comprise glutamine, arginine, proline and histidine (Fig. 2). We have recently reviewed the regulation of glutamate metabolism including the many products of glutamate metabolism (Brosnan and Brosnan 2009).

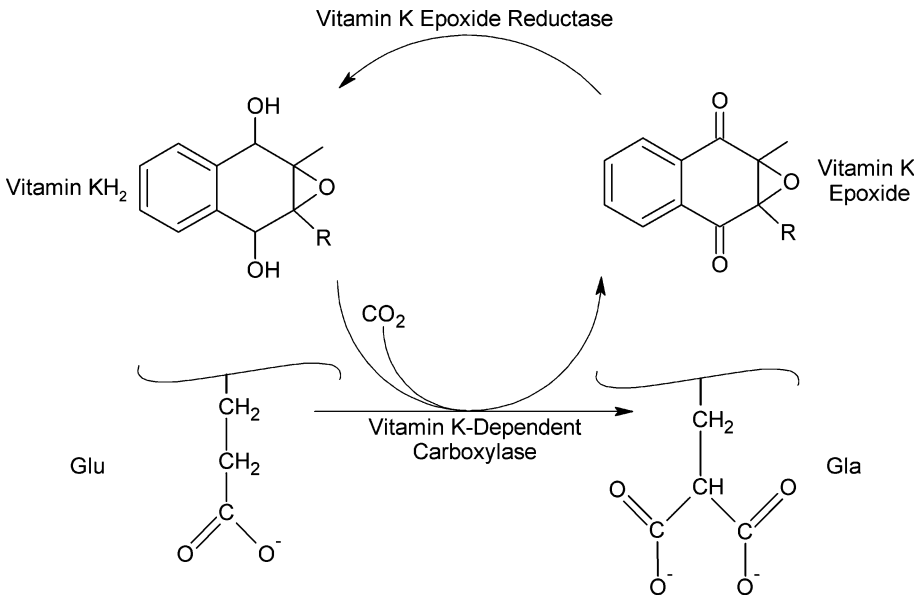

Fig. 1 Carboxylation of a glutamyl residue in a protein by vitamin K-dependent carboxylase to give a gamma-carboxylated glutamyl residue. The resulting vitamin K epoxide must be reduced to KH_2 by VKOR for the carboxylase to remain active

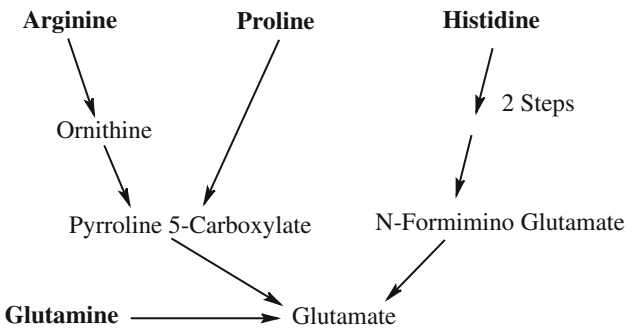

Fig. 2 Catabolism of the glutamate family of amino acids (from Brosnan and Brosnan 2009)

Glutathione

Glutathione (γ-glutamyl-cysteinyl-glycine) is a major intracellular antioxidant found in virtually all tissues. The first step in its synthesis is catalyzed by glutamate cysteine ligase which joins glutamate through its γ-carboxyl to cysteine's α-amino to give an isopeptide linkage. GSH synthase forms a peptide bond between γ-glutamyl-cysteine and glycine to give glutathione (for review, see Lu 2009). Reduced glutathione/oxidized glutathione (GSH/GSSG) form a redox pair that is important in maintaining intracellular redox balance. All cells are subjected to a certain level of oxidative stress; peroxides are reduced by GSH, catalyzed by GSH peroxidase, to protect the cell from oxidative damage. The GSSG formed will be reduced to GSH by GSH reductase so there is no loss of GSH.

Glutathione conjugates

GSH can be conjugated to toxic or waste compounds to give glutathionyl derivatives which can be excreted from the cell or the body. If the compound is electrophilic, it can interact directly with GSH but conjugation of many compounds can be catalyzed by GSH S-transferase (Meister and Anderson 1983). In leukocytes, arachidonic acid is converted to leukotriene A_4 (LTA_4) which is then conjugated to glutathione by LTC_4 synthase to give LTC_4, a cysteinyl LT shown in Fig. 3. LTC_4 can be subsequently converted to LTD_4 and LTE_4, the other cysteinyl LTs. These compounds are important causes of bronchoconstriction and other aspects of both the allergic response and asthma (Kanaoka and Boyce 2004). These latter reactions in which glutathione is conjugated to toxic materials or to leukotrienes do result in the loss of the glutathione molecule, necessitating de novo synthesis to replenish GSH levels.

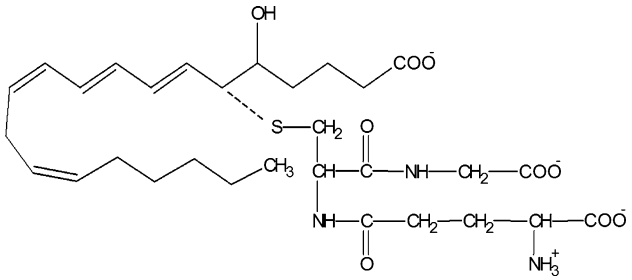

Fig. 3 Structure of leukotriene C_4 (LTC_4). Glutathione is conjugated through its cysteinyl SH group to C6 of LTA_4 by the enzyme, LTC_4 synthase

Glutamate as a signaling molecule

Despite the central position occupied by glutamate in amino acid metabolism (Brosnan 2000), it may be argued that the most important role played by glutamate is as a signaling molecule. Glutamate's signaling role may be illustrated by examples taken from the brain, pancreatic β cells, taste buds, and gut.

Brain

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. Proton-MRS studies of human brain reveal a very high (10–12 mM) glutamate concentration (Schubert et al. 2004), but this averaged out very different glutamate concentrations in different sub-cellular compartments, from about 1 μM in cerebrospinal fluid to 100 mM in secretory granules (Nedergaard et al. 2002). In addition, glutamate is the metabolic precursor, via glutamate decarboxylase, for γ-aminobutyrate (GABA), which is the principal inhibitory neurotransmitter in mammalian central nervous system. Glutamate exerts its actions in the CNS through two principal types of receptors; ionotropic glutamate receptors contain an ion channel which is directly activated upon glutamate binding, whereas metabotropic glutamate receptors activate ion channels via coupling to G-protein signaling systems, either indirectly through second messenger pathways or directly by $\beta\gamma$ subunits of the G-protein (Kew and Kemp 2005). There are many subtypes of each receptor. Although a detailed account of glutamate signaling in the brain is beyond the scope of this paper, mention should be made of its role in synaptic plasticity which underlies its role in cognitive functions such as learning and memory (Gécz 2010).

Pancreatic β-cells

Glutamate is part of an elegant regulatory system whereby ingestion of protein stimulates insulin secretion by β-cells.

Since the branched-chain amino acids are poorly metabolized in the liver, their blood levels increase after a protein-containing meal. Leucine activates glutamate dehydrogenase in the β-cell which increases glutamate oxidation; the consequent decrease in glutamate concentration leads to a deinhibition of glutaminase, so that glutamine becomes an important respiratory substrate for these cells, with a consequent increase in the cellular ATP/ADP ratio. Insulin secretion is very sensitive to this ratio, since an increased ratio closes the ATP-gated K^+ channel. This depolarizes the cell, activating a voltage-gated Ca^{2+} channel. The subsequent increase in cytosolic [Ca^{2+}] triggers the exocytotic release of insulin (Treberg et al. 2010). This mechanism is shown in Fig. 4.

Much of our knowledge of the role of glutamate dehydrogenase in insulin secretion comes from studies on children suffering from the hyperinsulinism/hyperammonemia (HI/HA) syndrome, the second most common cause of congenital hypoglycemia. These children present with periodic hypoglycemia, particularly after a high-protein meal. Hypoglycemia could also be elicited by leucine ingestion (Stanley 2004, 2009). Children with HI/HA have gain-of-function mutations in the glutamate dehydrogenase gene such that the enzyme is much less sensitive to inhibition by GTP (Stanley et al. 1998). The increase in glutamate oxidation within the β-cell increases the ATP/ADP ratio, with a consequent increase in insulin secretion.

Taste buds

Umami tastants, particularly glutamate, are thought to mediate appetitive responses to protein-rich foods. As such, they play a fundamental role in evaluating the nutritional value of foods.

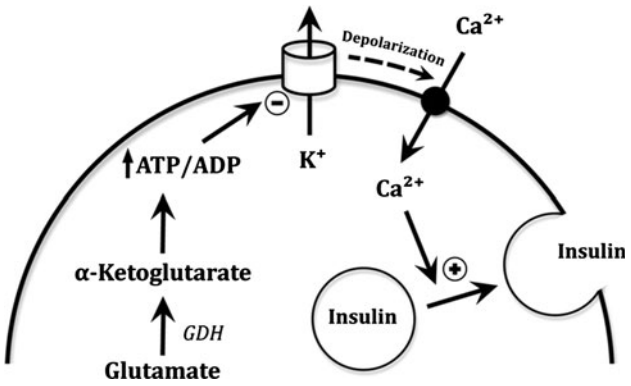

Fig. 4 GDH function in pancreatic β cells. Increased GDH activity results in increased glutamate oxidation and a consequent increase in ATP/ADP ratio. This in turn inhibits the ATP-gated K^+ channel, resulting in plasma membrane depolarization and influx of calcium, which activates the exocytosis of insulin granules. Reprinted from Treberg et al. (2009), with permission from Elsevier

Glutamate plays a critical signaling role by activating umami taste receptors. Activation of these taste receptors also require, as co-ligand, a 5′-ribonucleotide such as inosine monophosphate (IMP) or guanosine monophosphate. There is still some uncertainty as to the multiplicity of receptors required for the full expression of umami taste (Yasumatsu et al. 2009, 2011). Certainly, heterodimers of the G-protein-coupled receptors T1R1 and T1R3 are involved (Nelson et al. 2002). In addition, recent work, utilizing T1R3 knockout mice, has shown that these animals continue to discriminate between umami and other tastants, indicating a role for additional receptors. In particular, two metabotropic glutamate receptors, mGluR1 and mGluR4, may be important (Yasumatsu et al. 2011). Several of these receptors are shown in Fig. 5. In addition to the delineation of the role(s) of specific taste receptors, we are beginning to learn more as to how taste signals are conveyed to the brain as well as which brain regions respond to specific tastants. Recent work by Chen et al. (2011) employed sophisticated optical imaging techniques (such as two-photon calcium imaging) to locate neural responses to specific tastants. They identified discrete clusters of neurons in the mouse gustatory cortex that respond to salt, sweet, bitter, or umami stimuli. The umami response was found to be finely tuned to umami, rather than to the other taste qualities, and located in the insular cortex. Other L-amino acids activated the same neurons, but D-amino acids did not.

Gut

The demonstration by Höfer et al. (1996) of α-gustducin, the taste-specific G-protein, in the stomach and intestine led to the realization that taste-like cells occurred in the gut. It is now clear that taste-like glutamate receptors and cells occur both in the stomach and the small intestine. It is also clear that, via glutamate, they signal the presence of protein digestion in the GI tract. In this regard, they may be likened to the duodenal sensing of glucose (Kitamura et al. 2011). Both ionotropic and metabotropic glutamate receptors have been found in the stomach (Burrin and Stoll 2009) and it is clear that glutamate is the only amino acid that can stimulate afferent gastric vagal nerves. The intragastric infusion of glutamate stimulates specific forebrain regions including the limbic system and hypothalamus. It also stimulates gastric contractile activity (Sengupta et al. 2004). Glutamate, together with IMP, stimulates bicarbonate secretion in the rat duodenum, a possible protective effect in neutralizing the gastric acid produced during gastric protein digestion (Akiba and Kaunitz 2011). The requirement for both glutamate and IMP suggests the involvement of an umami-type receptor. The same group

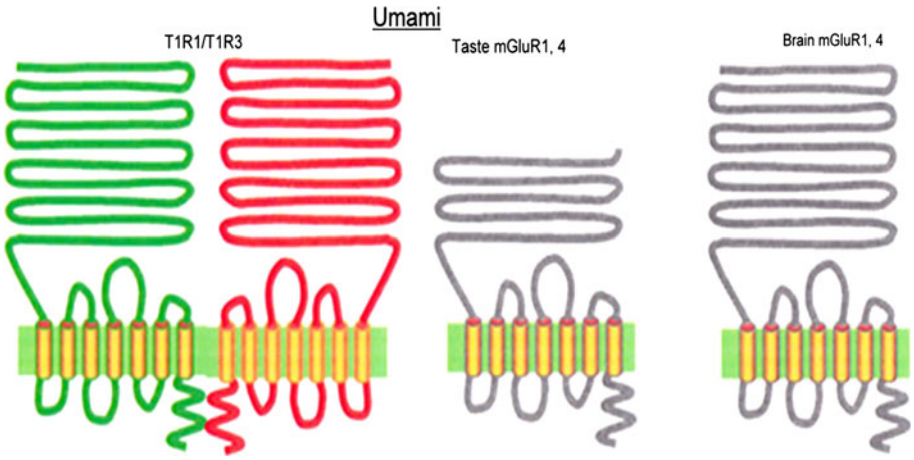

Fig. 5 Candidates for umami taste receptors are T1R1/T1R3, truncated metabotropic glutamate receptors 1 and 4 (taste mGluR1, 4) and brain-derived metabotropic glutamate receptor 1 and 4 (brain mGlu1, 4). Reprinted with permission from Yasumatsu et al. (2009)

has recently demonstrated an involvement of glucagon-like peptide-2 in mediating the increased bicarbonate secretion (Wang et al. 2011).

The entire field of the post-ingestive effects of glutamate is, in all likelihood, in its infancy. However, it greatly expands our understanding of the reach of this remarkable amino acid. Further advances may be expected.

Conclusions

Wu has defined a functional amino acid as one "that can regulate key metabolic pathways to improve health, growth, development and reproduction of animals and humans" (Wu 2010). This definition extends our horizons beyond the nutritional paradigm of nutritionally essential or non-essential amino acids. It also obliges a consideration of amino acids' function beyond their more familiar role as substrates for protein synthesis. To some, the concept of functional amino acids entails their provision as supplements, often in large amounts. Certainly, glutamate as monosodium glutamate is enjoyed by hundreds of millions of people because it imparts a savory taste to food. In addition, because of its critical roles in metabolism as well as in regulating a multitude of key physiological processes, glutamate may be numbered among the most functional of the amino acids.

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Conflict of interest None.

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