Published in final edited form as:

Neurochem Int. 2011 September ; 59(4): 525–533. doi:10.1016/j.neuint.2011.07.003.

Glutamate dehydrogenase in brain mitochondria: do lipid modifications and transient metabolon formation influence enzyme activity?

Mary C. McKenna

Department of Pediatrics, School of Medicine, University of Maryland, Baltimore, MD 21201, USA

Abstract

Metabolism of glutamate, the primary excitatory neurotransmitter in brain, is complex and of paramount importance to overall brain function. Thus, understanding the regulation of enzymes involved in formation and disposal of glutamate and related metabolites is crucial to understanding glutamate metabolism. Glutamate dehydrogenase (GDH) is a pivotal enzyme that links amino acid metabolism and TCA cycle activity in brain and other tissues. The allosteric regulation of GDH has been extensively studied and characterized. Less is known about the influence of lipid modifications on GDH activity, and the participation of GDH in transient heteroenzyme complexes (metabolons) that can greatly influence metabolism by altering kinetic parameters and lead to channeling of metabolites. This review summarizes evidence for palmitoylation and acylation of GDH, information on protein binding, and information regarding the participation of GDH in transient heteroenzyme complexes. Recent studies suggest that a number of other proteins can bind to GDH altering activity and overall metabolism. It is likely that these modifications and interactions contribute additional levels of regulation of GDH activity and glutamate metabolism.

1. Introduction

The metabolism of glutamate, the primary excitatory neurotransmitter in brain, is complex and of paramount importance to overall brain function. Thus, understanding the regulation of enzymes involved in formation and disposal of glutamate and related metabolites is crucial to understanding glutamate metabolism. Our group has a long standing interest in the role of compartmentation and the regulation of key enzymes of brain metabolism including glutamate dehydrogenase (GDH). We have previously reported on the influence of small molecule effectors that can modulate the activity of mitochondrial aspartate aminotransferase (AAT), malate dehydrogenase (MDH) and malic enzyme (ME) in synaptic and nonsynaptic mitochondria from brain [1–10]. The concentration of many small molecule effectors which fluctuate during normal metabolism may change considerably in pathological conditions [6, 9]. Such effectors are of particular interest since small molecules greatly influence the formation of transient multienzyme complexes in brain which can influence metabolism in real time [6, 9].

© 2011 Elsevier Ltd. All rights reserved.

Correspondence to: Mary C. McKenna, Ph.D., University of Maryland School of Medicine, Department of Pediatrics, 655 W. Baltimore St., Room 13-019, Baltimore, MD 21201, USA, Phone: 410-706-1990, FAX: 410-706-0020, mmckenna@umaryland.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

This review summarizes both long standing and relatively new reports about the binding of other proteins to GDH and the participation of GDH in transient heteroenzyme complexes. Evidence regarding lipid modifications, including palmitoylation and acylation of GDH is also discussed.

2. Glutamate dehydrogenase catalyzes the oxidative deamination of glutamate to form α -ketoglutarate (α -KG)

Glutamate dehydrogenase (E.C. 1.4.1.3; L-glutamate: NAD(P)⁺ oxidoreductase, deaminating) catalyzes the deamination of glutamate to form α -KG and releasing NH₃ and forming NAD(P)H [11]. GDH can also catalyze the opposite reaction that requires energy, (e.g. converts NAD(P)H to NAD(P)⁺), the reductive amination of α -KG to form glutamate [11]. Studies by Cooper [12] and others [1, 2, 13–17] demonstrate that in rat brain the GDH reaction operates primarily in the direction of ammonia production as shown in Figure 1.

Two forms of GDH are found in human brain; many studies have reported that mammalian glutamate dehydrogenase (GDH1) is a widely expressed ‘housekeeping’ enzyme encoded by the GLUD1 gene [18–20]. The more recently identified enzyme GDH2, is robustly labeled in astrocytes and is more faintly labeled in neurons in brain [18]. Both proteins localize predominately to mitochondria which is consistent with the primary function of these proteins in oxidative deamination of glutamate in brain [21]. The structures of both GDH1 and GDH2 have been determined and specific differences that influence enzyme activity and kinetics have been found [21–28]. Changes in GDH activity and regulation have been reported in a number of conditions that lead to neurodegeneration [21, 23, 29–31]

2.1 Aminotransferase enzymes also mediate nitrogen transfer in brain

It should be noted that all transaminase enzymes transfer amino groups from glutamate to form α -ketoglutarate (α -KG), and catalyze the reverse reaction to form glutamate. Metabolism via the enzyme aspartate aminotransferase [L-aspartate:2-oxoglutarate aminotransferase, E.C. 2.6.1.1] also leads to formation and degradation of glutamate as this enzyme catalyzes the reversible transamination of oxaloacetate to aspartate in conjunction with the conversion of glutamate to α -ketoglutarate (α -KG) [11, 12]. Transamination via AAT requires pyridoxal-5'-phosphate as a cofactor, and involves multiple kinetically distinct steps [32]. In most tissues GDH and AAT act together in metabolism since GDH either removes an amino group from glutamate or adds an amino group to α -KG [1, 2, 13–17], whereas AAT (or other aminotransferase enzymes) transfers an amino group to or from glutamate to convert keto acids into amino acids [1, 2, 11, 13–17]. Both GDH and AAT have specific roles in neurons and astrocytes in brain [1, 13, 33–35]. The extent to which these enzymes work together in brain cells is not well defined. AAT has a lower K_m for α -KG, and higher K_m for glutamate than GDH [11]; however, protein modifications such as palmitoylation could modify these kinetic parameters [6]. The high affinity for α -KG is in keeping with the role of AAT in resynthesis of glutamate from TCA cycle intermediates in astrocytes [13]. Our group has reviewed the compartmentation and roles of GDH and AAT in brain [1, 4, 9, 14].

The enzymes branched chain amino transferase (BCAT) and alanine aminotransferase (AlAT) can also lead to degradation of glutamate via transamination with branched chain ketoacids (BCKA) or pyruvate, respectively [15, 36–47]. There is evidence that both BCAT and AlAT can facilitate the transfer of nitrogen between neurons and astrocytes in brain. The relative importance of these pathways is not completely understood; however, BCAT may contribute as much as 25% of the nitrogen for glutamate formation in brain [12].

2.2 Regulation of GDH in brain

The regulation of GDH is complex and has been intensively studied because of the importance of this enzyme in nitrogen metabolism in brain and other tissues [12, 21, 23, 48]. Both GDH1 and GDH2 associate into hexamers [18–20]. Activity of GDH1 is allosterically regulated and potentially inhibited by GTP, and is thus controlled by the need of the cell for ATP [18–20, 49]. The GDH2 from human brain is resistant to inhibition by GTP which gives this enzyme the ability to metabolize glutamate in the presence of high energy levels and an active TCA cycle [18]. Several manuscripts in this issue review the regulation of GDH [26, 28, 50].

Neuron specific overexpression of GDH1 in mice leads to increased glutamate release, altered synaptic plasticity and selective vulnerability of CA1 neurons [51]. Both gain of function mutations of GDH and hypofunction of GDH have been associated with hyperexcitability of brain and seizures [19, 26]. Mutations in the GTP binding site of GDH1 that lead to the rare hyperinsulinism-hyperammonaemia syndrome are associated with seizures and learning disabilities in children [52]. Central nervous system hyperexcitability is associated with such gain of function mutations in GDH1 [19, 26], also underscoring the importance of the regulation of GDH1 by GTP. In contrast, GDH activity was found to be significantly decreased in the temporal cortex in patients with medial temporal lobe epilepsy [53].

3. Compartmentation is the key to understanding metabolism in brain

It is essential to have knowledge of compartmentation to understand the regulation of brain metabolism since compartmentation contributes significantly to differences in neuronal and glial metabolism [1, 9] (and references therein). The first evidence of compartmentation came from studies using radiolabeled acetate, which showed that glutamine had a higher specific activity than that of its precursor glutamate; whereas, with glucose as precursor the specific activity of glutamate was higher than glutamine [54]. Such studies led to the concept that the pool of glutamate that gave rise to glutamine did not equilibrate with the rest of the glutamate in brain [54]. This understanding of how large and small pools of glutamate with different turnover rates could coexist in the brain [54, 55] led to the concept of the glutamate/glutamine cycle.

Studies using techniques including differential ultracentrifugation, ^{13}C -NMR spectroscopy and molecular tools for identifying transporters and enzymes have led to a more in depth understanding of the complexity of the cellular and subcellular compartmentation in brain [1, 10, 56–67]. Many studies have shown *intracellular* compartmentation of metabolism in both neurons and astrocytes [1, 2, 4, 5, 57, 65, 66, 68–71] (and references therein). Other reports have shown heterogeneity in the distribution of metabolic enzymes, *within* populations of neurons and astrocytes [1, 5, 72, 73], and differences between neurons and glia consistent with numerous compartments that have different metabolic capabilities [8, 9, 63, 74–79].

Compartmentation of transporters, enzymes and pathways is essential so that neurons and astrocytes can carry out highly specialized functions, and it mandates the trafficking of key metabolites between these cell types in brain [1, 77, 80]. A number of cellular and subcellular factors that contribute to compartmentation in brain are listed in Table 1.

3.1 Palmitoylation of proteins can dynamically influence compartmentation, localization and interactions of proteins, and signaling

In recent years there has been increasing interest in the importance of lipid modification, including acylation, which occur as post-translational modifications that influence the

insertion of proteins into intracellular and plasma membranes [81]. Each type of lipophilic group added confers unique properties to the modified protein, resulting in alterations in protein/protein interactions, membrane binding and targeting, and intracellular signaling [82].

Myristoylation and prenylation, are common posttranslational modifications which lead to formation of stable bonds with proteins and facilitate insertion into membranes [82]. Palmitoylation is a unique lipid modification of proteins because the 16-carbon fatty acid is usually attached via a labile thioester bond [81] (and references therein). This reversible modification allows proteins to shuttle between intracellular compartments (e.g. plasma membrane and golgi) and generally leads to a more rapid turnover of the protein [81]. A wide variety of important neuronal proteins have been recently shown to be palmitoylated including PSD95, SNAP25, GAD65 and GDH [81] (and references therein). AAT may also be a palmitoylated protein as studies with liver showed that AAT binds to the inner mitochondrial membrane, and must be transferred to lipids to become fully activated [83, 84]. Previous studies from our group demonstrated that palmitate significantly altered AAT activity in synaptic and non synaptic mitochondria [6].

Protein palmitoylation is the most common posttranslational lipid modification; its reversibility mediates protein shuttling between intracellular compartments [85]. Activation of glutamate receptors has been shown to increase palmitoylation of specific proteins and decrease palmitoylation of other proteins. Such activity mediated palmitoylation is thought to have a role in development and synaptic plasticity [81, 85]. Palmitoylation of proteins is closely regulated by a large family of specific palmitoyl acyltransferase enzymes (PATs), most of which contain a DHHC [asp-his-his-cys] motif, and by palmitoyl acyl thioesterases (PPTs) which mediate depalmitoylation [81]. The mechanisms that regulate these PATs and PPTs in a physiological context are not well understood [85]. It should be noted that palmitoylation can also occur spontaneously [81]. The extent to which this occurs in vivo is not known; however, spontaneous palmitoylation could modulate the activity of many enzymes, including GDH and AAT under physiological and/or pathological conditions. Mutations in the DHHC enzymes have been linked to a variety of neurological disorders including X-linked mental retardation, learning disabilities and schizophrenia [81, 86, 87] and references therein. Palmitoylation is of particular interest to our group since it regulates processing and targeting of proteins to precise microdomains and/or alters their conformation to regulate activity and/or facilitate interactions with other proteins [81].

3.2 Transiently formed multienzyme complexes influence metabolism

One of the least understood phenomena that influence metabolism is the dynamic association and dissociation of transiently formed multienzyme complexes (metabolons) that have a role in regulating metabolism. These transiently formed heteroenzyme complexes either associate or dissociate depending on fluctuations in the intracellular or intramitochondrial concentration of key metabolites in cells [1, 3, 6, 9, 83, 84, 88–90]. Thus formation of such transient complexes responds to highly localized changes in metabolite concentration and can theoretically influence cellular metabolism in “real time”. Characteristics of transiently formed heteroenzyme complexes are listed in Table 2.

Transiently formed enzyme complexes, mitochondrial binding proteins that interact with MDH and AAT [1, 83] and the high affinity of some enzymes can sequester small molecules can lead to what is essentially ‘channeling’ of metabolites through specific pathways [89–93]. Thus, the association or dissociation of these complexes have kinetic advantages and can greatly influence metabolism via multiple pathways.

Many studies have provided evidence of compartmentation of metabolites in the cytoplasm of brain cells [2, 4, 10, 68–70, 94]. The cytosol of cells is a very complex gel-like environment in which membranous surfaces and proteins may restrict the diffusion of even small molecules [95, 96], thus compartmentation within the cytoplasm can readily occur. The high density of proteins and enzymes in mitochondria, in conjunction with the higher protein/lipid ratio of ~ 3:1 in mitochondrial membranes [96] would restrict diffusion and make the existence of localized micro-environments the normal situation in mitochondria, rather than the exception [1, 97].

3.3 A number of factors influence the association and dissociation of transiently formed enzyme complexes in mitochondria

Several different transient multienzyme complexes may be formed between mitochondrial enzymes in the brain. Specific complexes are formed depending on (a) the binding affinities of enzymes for other enzymes, (b) the relative concentration of each enzyme in the mitochondrial matrix, (c) the affinity of binding to the mitochondrial membranes, (d) or to specific binding proteins in the mitochondrial membrane, (e) the order in which binding occurs, (f) the concentration of metabolites (e.g. citrate, oxaloacetate (OAA), α -KG, malate, aspartate, fumarate, etc.) and ions (e.g. Mg^{2+}) that influence binding of specific enzymes [1, 3, 6, 9, 83, 84, 88–90], (g) the form in which enzymes are found e.g., AAT and malate dehydrogenase (MDH) are dimers, GDH forms homohexamers when the concentration is sufficient, (h) the stoichiometry of enzyme binding, [e.g. α -ketoglutarate dehydrogenase complex (KGDHC) can associate with AAT and MDH with a ratio of 10 total AAT or MDH bound per KGDHC [83]], and (i) palmitoylation which alters protein conformation and allows proteins to associate and dissociate from specific subcellular microdomains [81], (e.g. palmitoyl-CoA has been shown to enhance the interactions between GDH and AAT [98]).

Data supporting the formation of transient multienzyme complexes comprised of different enzymes (hetero-enzyme complexes) in various tissues comes, for the most part, from in vitro studies (using isolated enzymes, purified enzymes, and purified mitochondrial membranes), that dealt with binding of enzymes to mitochondrial membranes or proteins, and/or to one or more other enzymes [1, 83, 84, 88–93, 99–101] (and references therein). Studies using green fluorescent protein (GFP) linked to mitochondrial enzymes reported an in vivo diffusion rate of ~ 4 Å/second for smaller mitochondrial enzymes such as the MDH dimer [102]. Photobleaching studies indicated that each of the four TCA cycle enzymes (MDH, CS, isocitrate dehydrogenase, succinyl CoA synthetase) [102] studied was present in two pools; an immobile pool that did not diffuse measurably in 14 sec, and a mobile pool that diffused 4 times slower than free GFP, which was consistent with a complex as large as the total molecular size of all of the TCA cycle enzymes [102]. These authors noted that some mitochondrial enzymes of the β -oxidation complex are partially or completely immobilized in mitochondria [102, 103], and that other reports suggested that mobility of glycolytic enzymes in cytoplasm is regulated by metabolic state [104]. Recent studies now provide evidence of heteroenzyme complexes in vivo [26, 28, 105].

A number of transient multienzyme complexes are known to form, some of which are described below. The influence of a variety of effectors that alter the binding and kinetics of binary, ternary and quaternary enzyme complexes between proteins has been reported [1, 83, 84, 88–93, 99, 106–109] (and references therein).

3.4 GDH can participate in a number of different transient heteroenzyme complexes

Both AAT and MDH can form complexes with GDH, and AAT has a higher affinity for binding than MDH [84, 98]. Since both enzymes can bind to GDH multiple complexes can

be formed. Under conditions used to study the formation of complexes in vitro, the molar ratio of enzymes bound was always >1 AAT per GDH homohexamer and the only complexes formed were the binary complex GDH-AAT, and ternary complexes consisting of GDH-MDH-(AAT)₂ and GDH-AAT-MDH [84]. Fumarase can bind to this ternary complex leading to formation of a quaternary complex [84]. Although there is no molecular crosslinking of the enzymes in these complexes, the physical association is so close that a crosslinker molecule with a bridge span of only 12 Å can link GDH and MDH or all three of these enzymes [84, 88]. Indeed, it is important to remember that even when enzymes that participate in these multienzyme complexes are dissociated, the diffusion of enzymes away from the membrane or each other would be slow in the viscous mitochondrial matrix [106, 110]. Photobleaching studies showing that the mitochondrial matrix is not contiguous over long distances is consistent with this concept [102]. With such close physical proximity it is easy to conceive how complexes could associate, dissociate when certain effectors are high, and then re-associate when the concentration of effectors again changes to favor association.

It is well known that allosteric effectors generally act through binding which induces conformational changes which have important effects on enzyme activity [93]. Substrate binding to AAT increases the free energy of the complex which facilitates the enzyme reaction [111]. A binary complex between MDH and AAT is not formed [84], yet the physical proximity of the enzyme in the ternary complex (AAT-GDH-MDH) is close enough to transfer the oxaloacetate from MDH to AAT [84]. A binary complex can form between AAT and GDH that facilitates the transfer of both enzymes and substrates to other enzymes when larger complexes are formed [83, 84]. This binary complex can associate with malic enzyme to other enzymes when larger complexes are formed and deliver substrates to malic enzyme which has a higher *K_m* for malate than mitochondrial MDH [8, 68]. In brain this association would facilitate conversion of TCA cycle metabolites into pyruvate and possibly increase the activity of the pyruvate recycling pathway. In liver palmitoyl CoA enhances the association of AAT and GDH into a complex [98]; whereas citrate enhances dissociation of AAT from GDH [88]. The estimated concentrations of mitochondrial enzymes are an order of magnitude higher than dissociation constants of the heteroenzyme complexes in liver [84]; however, such information has not been determined for brain.

Our studies of metabolism in cortical synaptosomes from rat brain are consistent with the possibility of a complex being formed between GDH and phosphate activated glutaminase that would facilitate the entry of glutamate, formed endogenously from glutamine, into a specific neuronal TCA cycle that is associated with GABA formation from glutamine [58]. Glutamate dehydrogenase has also been found to associate with the complex formed between pyruvate carboxylase and AAT, and MDH associated with this resulting ternary complex [112]. Known and expected interactions of GDH with other proteins are shown in Fig. 1.

3.5 Possible role of GDH in heteroenzyme complexes with metabolic enzymes

Very little is known about the role(s), importance and/or regulation of transiently formed enzyme complexes, many of which contain GDH, that contribute to compartmentation in brain. However, more than two decades ago Fahien and his coworkers postulated that “In the quaternary complex between glutamate dehydrogenase, the aminotransferases, malate dehydrogenase, and pyruvate carboxylase, glutamate dehydrogenase could play an organizational role similar to that of the α -ketoglutarate dehydrogenase complex [(KGDHC)]” [112]. They also noted that dissociation of NADH is the rate-limiting step in the GDH reaction, and that binding of MDH to GDH facilitated the removal of NADH from glutamate dehydrogenase. Since the larger mitochondrial enzymes (e.g., GDH or KGDHC) can fulfill a role in organizing MDH and AAT, the enzyme found in highest concentration in

the tissue or cell would likely perform the organization role [112]. Fahien et al. [112] noted that the two organizational enzymes would not compete with each other because the KGDHC can also associate with the AAT-GDH-MDH complex. In some tissues or cells (e.g. liver and brain) GDH may be present in sufficient concentration to fulfill such an organizational role [84, 112, 115, 116]. Rothe et al. [115, 116] reported that GDH could account for as much as 10% of the protein in mitochondrial matrix in astrocyte processes in brain. Immunolabeling of GDH is present but less abundant in neurons [115, 116]; however, evidence of high activity of GDH in neurons has been reported [1, 6, 113, 114, 117]. Fahien and Teller [84] have reported the relative concentration of mitochondrial enzymes in liver (AAT, 140 μ M; MDH, 140 μ M; GDH, 70 μ M; CS, 25 μ M; ALAT 4 μ M; KGDH, 2 μ M; PDH 0.3 μ M). The activity of brain enzymes has been reported by many groups [1, 6, 56, 113, 114]; however, little information is available on the relative concentration of enzymes in brain.

This organizational role for GDH would be quite important in metabolism because it provides a mechanism to enable enzymes that do not directly bind to each other (e.g. AAT and MDH) to be positioned in close enough proximity to transfer substrates and cofactors. In addition, if GDH does fulfill such an organizational role in tissue it would help to explain why mutations that cause conformational changes in GDH could lead to devastating neurological consequences.

4.0 Recent reports of interactions of GDH with other proteins

4.1 Binding and interaction of sirtuins with GDH

GDH has recently been identified as a novel substrate for mitochondrial sirtuins SIRT3 and SIRT4, enzymes that function in the control of basic mitochondrial processes including energy production, metabolism, apoptosis and intracellular signaling [118]. Sirtuins have two enzymatic activities in vitro: NAD⁺-dependent protein deacetylase activity and ADP-ribosyltransferase activity [118]. However, only ADP-ribosyltransferase activity has been described for SIRT4 as no acylated substrates have been identified [118]. Schlicker et al. [119] reported that human Sirt3 binds and deacetylates GDH in the mitochondrial matrix, leading to activation of the enzyme. In contrast, SIRT4 uses NAD to ADP-ribosylate GDH and thereby inactivates the enzyme [105]. Thus, Sirt4-dependent ADP ribosylation and Sirt3-dependent deacetylation inversely influence GDH activity [119]. SIRT4 regulates insulin secretion in pancreatic β cells by ADP-ribosylating and downregulating GDH activity and altering amino acid metabolism [105]. SIRT4 also binds to GDH in liver and brain and is thought to affect GDH activity in these tissues [105].

Wagner and Payne recently noted that [120] “reversible acetylation of mitochondrial proteins represents a new layer of protein regulation mediating acute and adaptive changes in mammalian metabolism”. It should be noted that both acetylation by SIRT3 and palmitoylation are two mechanisms that regulate GDH activity through lipid modifications [119].

4.2 GDH participates in a metabolon with branched chain aminotransferase (BCAT) and branched chain ketoacid dehydrogenase (BCKDH) in some tissues

In the brain and other tissues leucine and other branched chain amino acids (BCAA) serve primarily as nitrogen donors [121]. Hutson's group [39, 122] have recently demonstrated that the first two enzymes of branched chain amino acid catabolism, the mitochondrial branched chain amino acid transferase (BCATm) and branched chain ketoacid dehydrogenase (BCKDH) form a heteroenzyme metabolon. In rat liver and kidney tissue the metabolon also includes GDH1 [39, 121]. Bovine GDH1 binds to the pyridoxamine-5'-phosphate (PMP) form of the cofactor for mitochondrial form of the branched chain

aminotransferase enzyme in vitro [39, 121]. The protein complex they identified promoted BCAA oxidation and the transfer of nitrogen from the BCAT through glutamate as an intermediate and was facilitated by the binding of GDH1 to the complex which enhances BCKDH enzyme activity. It appears that GDH may have an organizational role in facilitating this complex.

It is not yet known if BCATm in brain, which is localized only in astrocyte mitochondria, forms such complexes with GDH. Indeed, such complexes may be possible in brain since the complex reported lead to deamination of glutamate (which is known to be partially mediated by GDH in astrocytes), followed by reamination with the amino group from the BCAA, which has been shown to be mediated by AAT or other aminotransferase enzymes, not by GDH in brain [2, 13, 123]. The complex between BCAT/BCKDH and GDH reported by the Hutson group [39, 121] was only formed with the mitochondrial form of the aminotransferase (BCATm), and thus would not be found in neurons which contain only the cytosolic form of this enzyme (BCATc) [37].

4.3 Binding of short chain fatty acyl dehydrogenase to GDH in pancreas

Stanley [26] recently reported that a recessive deficiency of short chain fatty acyl dehydrogenase (SCHAD) can lead to clinical hyperinsulinemia. Studies in SCHAD deficient mice suggest that the activation of GDH in SCHAD deficiency is due to a loss of a direct inhibitory protein-protein interaction between SCHAD and GDH [26].

5. Concluding remarks

Several years ago Marc Yudkoff [124] noted that "Flux through any pathway must be understood not only in terms of the K_m of the enzyme but also with regard to the presence of modulators of the reaction". There is compelling evidence that transient heteroenzyme complexes (metabolons) are formed in many tissues and that key metabolites have very specific effects on the association and dissociation of such complexes [83, 84, 125]. It is logical to assume that many of the same complexes identified in liver and other tissues may also be formed in neuronal and glial mitochondria. However, there is very little information about types of transient heteroenzyme complexes formed in brain [1]. It is important to note that many of the same metabolites that mediate metabolon formation in liver and other tissues have the ability to significantly influence the activity of key enzymes in brain [2–6, 10, 68, 94]. Little is known about the participation of GDH in heteroenzyme complexes in brain and what effect formation of GDH metabolons would have on metabolism in astrocytes or neurons. However, the dynamic formation and dissociation of these complexes in response to changes in metabolite concentrations provides a mechanism whereby mitochondria, and brain cells can respond to localized alterations in metabolism in real time.

The role of reversible palmitoylation reactions in influencing compartmentation and the association of GDH into enzyme complexes is an area that warrants more study. In addition, the opposing effects of SIRT3 and SIRT4 on GDH activity may provide additional levels of dynamic regulation of this key enzyme.

Smith and Stanley [49] recently observed that it is remarkable that the "tangled network of allosteric regulation" can adequately control GDH, a single enzyme that is crucial to insure appropriate levels of glutamate in the brain, ureagenesis in the liver and insulin secretion in the pancreas. It is likely that lipid modifications, protein binding, and formation of transient heteroenzyme complexes may contribute additional levels of regulation of GDH activity and glutamate metabolism.

Highlights

- This manuscript reviews evidence for binding of GDH to other proteins in brain.
- Participation of GDH in transient heteroenzyme complexes can influence metabolism in real time.
- Evidence for lipid modification of GDH by palmitoylation and acylation is reviewed.
- These modifications and interactions may contribute to the regulation of GDH activity and glutamate metabolism.

Acknowledgments

This work was supported in part by NIH grant HD016596.

Abbreviations

¹³C-NMR	¹³ C-Nuclear Magnetic Resonance spectroscopy
Acetyl CoA	acetyl coenzyme A
AIAT	alanine aminotransferase
AAT	aspartate aminotransferase
BCAA	branched chain amino acid
BCAT	branched chain aminotransferase
BCATm	mitochondrial branched chain aminotransferase
BCKA	branched chain ketoacid
BCKDH	branched chain amino acid dehydrogenase
CS	citrate synthase
DHHC	aspartate-histidine-histidine-cysteine motif in proteins
GABA	γ-aminobutyric acid
GDH	glutamate dehydrogenase
GDH1	glutamate dehydrogenase 1
GDH2	glutamate dehydrogenase 2
GAD	glutamic acid decarboxylase
GFP	green fluorescent protein
GLUD1	gene encoding GDH1
GTP	guanosine triphosphate
KGDH	α-ketoglutarate dehydrogenase
KGDHC	α-ketoglutarate dehydrogenase complex
α-KG	α-ketoglutarate
MDH	malate dehydrogenase
mME	mitochondrial malic enzyme

OAA	oxaloacetate
PAG	phosphate activated glutaminase
PATs	palmitoyl acyltransferases
PC	pyruvate carboxylase
PDH	pyruvate dehydrogenase
PMP	pyrodoxamine-5'-phosphate
PPTs	palmitoyl acyl thioesterases
PSD95	postsynaptic density protein 95
SCHAD	short chain fatty acyl dehydrogenase
SIRT3	sirtuin 3
SIRT4	sirtuin 4
SNAP25	Synaptosomal-associated protein 25
TCA	tricarboxylic acid cycle
? binding protein	unidentified protein that binds to AAT and to the MDH-CS complex

References

1. McKenna MC, Stevenson JH, Huang X, Hopkins IB. Differential distribution of the enzymes glutamate dehydrogenase and aspartate aminotransferase in cortical synaptic mitochondria contributes to metabolic compartmentation in cortical synaptic terminals. *Neurochem Int.* 2000; 37:229–241. [PubMed: 10812208]
2. McKenna MC, Sonnewald U, Huang X, Stevenson J, Zielke HR. Exogenous glutamate concentration regulates the metabolic fate of glutamate in astrocytes. *J Neurochem.* 1996; 66:386–393. [PubMed: 8522979]
3. Malik P, McKenna MC, Tildon JT. Regulation of malate dehydrogenases from neonatal, adolescent, and mature rat brain. *Neurochem Res.* 1993; 18:247–257. [PubMed: 8479597]
4. McKenna MC, Tildon JT, Stevenson JH, Huang X. New insights into the compartmentation of glutamate and glutamine in cultured rat brain astrocytes. *Dev Neurosci.* 1996; 18:380–390. [PubMed: 8940609]
5. McKenna MC, Tildon JT, Couto R, Stevenson JH, Caprio FJ. The metabolism of malate by cultured rat brain astrocytes. *Neurochem Res.* 1990; 15:1211–1220. [PubMed: 2129052]
6. McKenna MC, Hopkins IB, Lindauer SL, Bamford P. Aspartate aminotransferase in synaptic and nonsynaptic mitochondria: differential effect of compounds that influence transient hetero-enzyme complex (metabolon) formation. *Neurochem Int.* 2006; 48:629–636. [PubMed: 16513215]
7. McKenna MC, Sonnewald U. GABA alters the metabolic fate of [U-13C]glutamate in cultured cortical astrocytes. *J Neurosci Res.* 2005; 79:81–87. [PubMed: 15593283]
8. McKenna MC, Stevenson JH, Huang X, Tildon JT, Zielke CL, Hopkins IB. Mitochondrial malic enzyme activity is much higher in mitochondria from cortical synaptic terminals compared with mitochondria from primary cultures of cortical neurons or cerebellar granule cells. *Neurochem Int.* 2000; 36:451–459. [PubMed: 10733013]
9. McKenna MC. The glutamate-glutamine cycle is not stoichiometric: fates of glutamate in brain. *J Neurosci Res.* 2007; 85:3347–3358. [PubMed: 17847118]
10. McKenna MC, Tildon JT, Stevenson JH, Hopkins IB. Energy metabolism in cortical synaptic terminals from weanling and mature rat brain: evidence for multiple compartments of tricarboxylic acid cycle activity. *Dev Neurosci.* 1994; 16:291–300. [PubMed: 7768208]
11. Cooper, AJ.; Meister, A. Metabolic significance of transamination. In: Christen, P.; Meltzer, DE., editors. *Transaminases*. New York: Wiley; 1985. p. 500-580.edition.

12. Cooper AJ. (13)N as a tracer for studying glutamate metabolism. *Neurochem Int.* 2010
13. Westergaard N, Drejer J, Schousboe A, Sonnewald U. Evaluation of the importance of transamination versus deamination in astrocytic metabolism of [U-13C]glutamate. *Glia.* 1996; 17:160–168. [PubMed: 8776582]
14. McKenna MC, Tildon JT, Stevenson JH, Boatright R, Huang S. Regulation of energy metabolism in synaptic terminals and cultured rat brain astrocytes: differences revealed using aminooxyacetate. *Dev Neurosci.* 1993; 15:320–329. [PubMed: 7805585]
15. Yudkoff M, Nissim I, Nelson D, Lin ZP, Erecinska M. Glutamate dehydrogenase reaction as a source of glutamic acid in synaptosomes. *J Neurochem.* 1991; 57:153–160. [PubMed: 1675660]
16. Yudkoff M, Nissim I, Hertz L. Precursors of glutamic acid nitrogen in primary neuronal cultures: studies with 15N. *Neurochem Res.* 1990; 15:1191–1196. [PubMed: 2097513]
17. Wysmyk-Cybula U, Faff-Michalak L, Albrecht J. Effects of acute hepatic encephalopathy and in vitro treatment with ammonia on glutamate oxidation in bulk-isolated astrocytes and mitochondria of the rat brain. *Acta Neurobiol Exp (Wars).* 1991; 51:165–169. [PubMed: 1687972]
18. Spanaki C, Zaganas I, Kleopa KA, Plaitakis A. Human GLUD2 glutamate dehydrogenase is expressed in neural and testicular supporting cells. *J Biol Chem.* 2010; 285:16748–16756. [PubMed: 20194501]
19. Raizen DM, Brooks-Kayal A, Steinkrauss L, Tennekoon GI, Stanley CA, Kelly A. Central nervous system hyperexcitability associated with glutamate dehydrogenase gain of function mutations. *J Pediatr.* 2005; 146:388–394. [PubMed: 15756227]
20. Stanley CA. Hyperinsulinism/hyperammonemia syndrome: insights into the regulatory role of glutamate dehydrogenase in ammonia metabolism. *Mol Genet Metab.* 2004; 81(Suppl 1):S45–S51. [PubMed: 15050973]
21. Zaganas I, Kanavouras K, Mastorodemos V, Latsoudis H, Spanaki C, Plaitakis A. The human GLUD2 glutamate dehydrogenase: localization and functional aspects. *Neurochem Int.* 2009; 55:52–63. [PubMed: 19428807]
22. Mastorodemos V, Zaganas I, Spanaki C, Bessa M, Plaitakis A. Molecular basis of human glutamate dehydrogenase regulation under changing energy demands. *J Neurosci Res.* 2005; 79:65–73. [PubMed: 15578726]
23. Plaitakis A, Zaganas I. Regulation of human glutamate dehydrogenases: implications for glutamate, ammonia and energy metabolism in brain. *J Neurosci Res.* 2001; 66:899–908. [PubMed: 11746417]
24. Mastorodemos V, Kotzamani D, Zaganas I, Arianoglou G, Latsoudis H, Plaitakis A. Human GLUD1 and GLUD2 glutamate dehydrogenase localize to mitochondria and endoplasmic reticulum. *Biochem Cell Biol.* 2009; 87:505–516. [PubMed: 19448744]
25. Plaitakis A, Metaxari M, Shashidharan P. Nerve tissue-specific (GLUD2) and housekeeping (GLUD1) human glutamate dehydrogenases are regulated by distinct allosteric mechanisms: implications for biologic function. *J Neurochem.* 2000; 75:1862–1869. [PubMed: 11032875]
26. Stanley CA. Two genetic forms of hyperinsulinemic hypoglycemia caused by dysregulation of glutamate dehydrogenase. *Neurochem Int.* 2011
27. Smith TJ, Peterson PE, Schmidt T, Fang J, Stanley CA. Structures of bovine glutamate dehydrogenase complexes elucidate the mechanism of purine regulation. *J Mol Biol.* 2001; 307:707–720. [PubMed: 11254391]
28. Li M, Li C, Allen A, Stanley CA, Smith TJ. The structure and allosteric regulation of glutamate dehydrogenase. *Neurochem Int.* 2011
29. Plaitakis A, Berl S, Yahr MD. Neurological disorders associated with deficiency of glutamate dehydrogenase. *Ann Neurol.* 1984; 15:144–153. [PubMed: 6703655]
30. Plaitakis A, Berl S, Yahr MD. Abnormal glutamate metabolism in an adult-onset degenerative neurological disorder. *Science.* 1982; 216:193–196. [PubMed: 6121377]
31. Plaitakis A, Spanaki C, Mastorodemos V, Zaganas I. Study of structure-function relationships in human glutamate dehydrogenases reveals novel molecular mechanisms for the regulation of the nerve tissue-specific (GLUD2) isoenzyme. *Neurochem Int.* 2003; 43:401–410. [PubMed: 12742085]

32. Goldberg JM, Kirsch JF. The reaction catalyzed by *Escherichia coli* aspartate aminotransferase has multiple partially rate-determining steps, while that catalyzed by the Y225F mutant is dominated by ketimine hydrolysis. *Biochemistry*. 1996; 35:5280–5291. [PubMed: 8611515]
33. Yudkoff M, Daikhin Y, Nissim I, Horyn O, Lazarow A, Luhovyy B, Wehrli S. Response of brain amino acid metabolism to ketosis. *Neurochem Int*. 2005; 47:119–128. [PubMed: 15888376]
34. Moran J, Alavez S, Rivera-Gaxiola M, Valencia A, Hurtado S. Effect of NMDA antagonists on the activity of glutaminase and aspartate aminotransferase in the developing rat cerebellum. *Int J Dev Neurosci*. 1999; 17:57–65. [PubMed: 10219961]
35. Moran J, Rivera-Gaxiola M. Effect of potassium and N-methyl-D-aspartate on the aspartate aminotransferase activity in cultured cerebellar granule cells. *J Neurosci Res*. 1992; 33:239–247. [PubMed: 1453488]
36. Hutson S. Structure and function of branched chain aminotransferases. *Prog Nucleic Acid Res Mol Biol*. 2001; 70:175–206. [PubMed: 11642362]
37. Sweatt AJ, Garcia-Espinosa MA, Wallin R, Hutson SM. Branched-chain amino acids and neurotransmitter metabolism: expression of cytosolic branched-chain aminotransferase (BCATc) in the cerebellum and hippocampus. *J Comp Neurol*. 2004; 477:360–370. [PubMed: 15329886]
38. Hutson SM, Lieth E, LaNoue KF. Function of leucine in excitatory neurotransmitter metabolism in the central nervous system. *J Nutr*. 2001; 131:846S–850S. [PubMed: 11238772]
39. Islam MM, Nautiyal M, Wynn RM, Mobley JA, Chuang DT, Hutson SM. Branched-chain amino acid metabolism: interaction of glutamate dehydrogenase with the mitochondrial branched-chain aminotransferase (BCATm). *J Biol Chem*. 2010; 285:265–276. [PubMed: 19858196]
40. Yudkoff M, Zaleska MM, Nissim I, Nelson D, Erecinska M. Neuronal glutamine utilization: pathways of nitrogen transfer studied with [15N]glutamine. *J Neurochem*. 1989; 53:632–640. [PubMed: 2746241]
41. Yudkoff M. Brain metabolism of branched-chain amino acids. *Glia*. 1997; 21:92–98. [PubMed: 9298851]
42. Yudkoff M, Daikhin Y, Grunstein L, Nissim I, Stern J, Pleasure D, Nissim I. Astrocyte leucine metabolism: significance of branched-chain amino acid transamination. *J Neurochem*. 1996; 66:378–385. [PubMed: 8522978]
43. Yudkoff M, Nissim I, Daikhin Y, Lin ZP, Nelson D, Pleasure D, Erecinska M. Brain glutamate metabolism: neuronal-astroglial relationships. *Dev Neurosci*. 1993; 15:343–350. [PubMed: 7805588]
44. Bak LK, Johansen ML, Schousboe A, Waagepetersen HS. Among the branched-chain amino acids, only valine metabolism is up-regulated in astrocytes during glutamate exposure. *J Neurosci Res*. 2007; 85:3465–3470. [PubMed: 17497675]
45. Bak LK, Sickmann HM, Schousboe A, Waagepetersen HS. Activity of the lactate-alanine shuttle is independent of glutamate-glutamine cycle activity in cerebellar neuronal-astrocytic cultures. *J Neurosci Res*. 2005; 79:88–96. [PubMed: 15578733]
46. Waagepetersen HS, Sonnewald U, Larsson OM, Schousboe A. A possible role of alanine for ammonia transfer between astrocytes and glutamatergic neurons. *J Neurochem*. 2000; 75:471–479. [PubMed: 10899921]
47. Westergaard N, Sonnewald U, Schousboe A. Metabolic trafficking between neurons and astrocytes: the glutamate/glutamine cycle revisited. *Dev Neurosci*. 1995; 17:203–211. [PubMed: 8575339]
48. Cooper AJ, Plum F. Biochemistry and physiology of brain ammonia. *Physiol Rev*. 1987; 67:440–519. [PubMed: 2882529]
49. Smith TJ, Stanley CA. Untangling the glutamate dehydrogenase allosteric nightmare. *Trends Biochem Sci*. 2008; 33:557–564. [PubMed: 18819805]
50. Plaitakis A, Latsoudis H, Spanaki C. The human GLUD2 glutamate dehydrogenase and its regulation in health and disease. *Neurochem Int*. 2011; 59:495–509. [PubMed: 21420458]
51. Bao X, Pal R, Hascup KN, Wang Y, Wang WT, Xu W, Hui D, Agbas A, Wang X, Michaelis ML, Choi IY, Belousov AB, Gerhardt GA, Michaelis EK. Transgenic expression of Glud1 (glutamate dehydrogenase 1) in neurons: in vivo model of enhanced glutamate release, altered synaptic

- plasticity, and selective neuronal vulnerability. *J Neurosci*. 2009; 29:13929–13944. [PubMed: 19890003]
52. Bahi-Buisson N, Roze E, Dionisi C, Escande F, Valayannopoulos V, Feillet F, Heinrichs C, Chadeaux-Vekemans B, Dan B, de Lonlay P. Neurological aspects of hyperinsulinism-hyperammonaemia syndrome. *Dev Med Child Neurol*. 2008; 50:945–949. [PubMed: 19046187]
 53. Malthankar-Phatak GH, de Lanerolle N, Eid T, Spencer DD, Behar KL, Spencer SS, Kim JH, Lai JC. Differential glutamate dehydrogenase (GDH) activity profile in patients with temporal lobe epilepsy. *Epilepsia*. 2006; 47:1292–1299. [PubMed: 16922873]
 54. Berl S.; Clark, DD. Handbook of neurochemistry, Chemical and cellular architecture. New York: Plenum Press; 1969. Metabolic Compartmentation of glutamate in the CNS; p. 447-472.edition.
 55. van den Berg CJ, Garfinkel D. A stimulation study of brain compartments. Metabolism of glutamate and related substances in mouse brain. *Biochem J*. 1971; 123:211–218. [PubMed: 5164952]
 56. Lai, JC.; Clark, JB. Isolation and characterization of synaptic and nonsynaptic mitochondria from the mammalian brain. In: Boulton, AA.; Baker, GB., editors. *Neuromethods, Carbohydrates and Energy Metabolism*. Clifton NJ: Humana Press; 1989. p. 43-98.edition.
 57. Sonnewald U, Westergaard N, Hassel B, Muller TB, Unsgard G, Fonnum F, Hertz L, Schousboe A, Petersen SB. NMR spectroscopic studies of ¹³C acetate and ¹³C glucose metabolism in neocortical astrocytes: evidence for mitochondrial heterogeneity. *Dev Neurosci*. 1993; 15:351–358. [PubMed: 7805589]
 58. Sonnewald U, McKenna M. Metabolic compartmentation in cortical synaptosomes: influence of glucose and preferential incorporation of endogenous glutamate into GABA. *Neurochem Res*. 2002; 27:43–50. [PubMed: 11926275]
 59. Kolesnichenko IP, Dolgo-Saburova IS, Somova TV. [Changes in the biochemical composition of the cerebrospinal fluid in acute carbophos poisoning]. *Zh Nevropatol Psikhiatr Im S S Korsakova*. 1992; 92:95–99. [PubMed: 1355942]
 60. Choi IY, Lei H, Gruetter R. Effect of deep pentobarbital anesthesia on neurotransmitter metabolism in vivo: on the correlation of total glucose consumption with glutamatergic action. *J Cereb Blood Flow Metab*. 2002; 22:1343–1351. [PubMed: 12439292]
 61. Xu Y, Oz G, LaNoue KF, Keiger CJ, Berkich DA, Gruetter R, Hutson SH. Whole-brain glutamate metabolism evaluated by steady-state kinetics using a double-isotope procedure: effects of gabapentin. *J Neurochem*. 2004; 90:1104–1116. [PubMed: 15312166]
 62. Pierre K, Pellerin L. Monocarboxylate transporters in the central nervous system: distribution, regulation and function. *J Neurochem*. 2005; 94:1–14. [PubMed: 15953344]
 63. Hanu R, McKenna M, O'Neill A, Resneck WG, Bloch RJ. Monocarboxylic acid transporters, MCT1 and MCT2, in cortical astrocytes in vitro and in vivo. *Am J Physiol Cell Physiol*. 2000; 278:C921–C930. [PubMed: 10794666]
 64. Vannucci SJ, Simpson IA. Developmental switch in brain nutrient transporter expression in the rat. *Am J Physiol Endocrinol Metab*. 2003; 285:E1127–E1134. [PubMed: 14534079]
 65. Schousboe A, Westergaard N, Sonnewald U, Petersen SB, Huang R, Peng L, Hertz L. Glutamate and glutamine metabolism and compartmentation in astrocytes. *Dev Neurosci*. 1993; 15:359–366. [PubMed: 7805590]
 66. Waagepetersen HS, Sonnewald U, Larsson OM, Schousboe A. Synthesis of vesicular GABA from glutamine involves TCA cycle metabolism in neocortical neurons. *J Neurosci Res*. 1999; 57:342–349. [PubMed: 10412025]
 67. Waagepetersen HS, Sonnewald U, Larsson OM, Schousboe A. Multiple compartments with different metabolic characteristics are involved in biosynthesis of intracellular and released glutamine and citrate in astrocytes. *Glia*. 2001; 35:246–252. [PubMed: 11494415]
 68. McKenna MC, Tildon JT, Stevenson JH, Huang X, Kingwell KG. Regulation of mitochondrial and cytosolic malic enzymes from cultured rat brain astrocytes. *Neurochem Res*. 1995; 20:1491–1501. [PubMed: 8789613]
 69. Waagepetersen HS, Bakken IJ, Larsson OM, Sonnewald U, Schousboe A. Comparison of lactate and glucose metabolism in cultured neocortical neurons and astrocytes using ¹³C-NMR spectroscopy. *Dev Neurosci*. 1998; 20:310–320. [PubMed: 9778567]

70. Bakken IJ, White LR, Aasly J, Unsgard G, Sonnewald U. Lactate formation from [U-13C]aspartate in cultured astrocytes: compartmentation of pyruvate metabolism. *Neurosci Lett*. 1997; 237:117–120. [PubMed: 9453229]
71. Sonnewald U, Westergaard N, Schousboe A, Svendsen JS, Unsgard G, Petersen SB. Direct demonstration by [13C]NMR spectroscopy that glutamine from astrocytes is a precursor for GABA synthesis in neurons. *Neurochem Int*. 1993; 22:19–29. [PubMed: 8095170]
72. Oh YJ, Markelonis GJ, Oh TH. Immunocytochemical localization of mitochondrial malate dehydrogenase in primary cultures of rat astrocytes and oligodendrocytes. *J Histochem Cytochem*. 1991; 39:681–688. [PubMed: 1707906]
73. Cesar M, Hamprecht B. Immunocytochemical examination of neural rat and mouse primary cultures using monoclonal antibodies raised against pyruvate carboxylase. *J Neurochem*. 1995; 64:2312–2318. [PubMed: 7722517]
74. Lai JC, Murthy CR, Cooper AJ, Hertz E, Hertz L. Differential effects of ammonia and beta-methylene-DL-aspartate on metabolism of glutamate and related amino acids by astrocytes and neurons in primary culture. *Neurochem Res*. 1989; 14:377–389. [PubMed: 2569676]
75. Kvamme E, Svenneby G, Torgner IA, Drejer J, Schousboe A. Postnatal development of glutamate developing enzymes in hippocampus from mice. *Int. J. Dev. Neurosci*. 1995; 3:359–364.
76. Sonnewald U, Hertz L, Schousboe A. Mitochondrial heterogeneity in the brain at the cellular level. *J Cereb Blood Flow Metab*. 1998; 18:231–237. [PubMed: 9498839]
77. Schousboe A, Westergaard N, Waagepetersen HS, Larsson OM, Bakken IJ, Sonnewald U. Trafficking between glia and neurons of TCA cycle intermediates and related metabolites. *Glia*. 1997; 21:99–105. [PubMed: 9298852]
78. Sonnewald U, Westergaard N, Schousboe A. Glutamate transport and metabolism in astrocytes. *Glia*. 1997; 21:56–63. [PubMed: 9298847]
79. Yudkoff M, Daikhin Y, Nissim I. Acidosis and astrocyte amino acid metabolism. *Neurochem Int*. 2000; 36:329–339. [PubMed: 10733000]
80. Westergaard N, Sonnewald U, Petersen SB, Schousboe A. Glutamate and glutamine metabolism in cultured GABAergic neurons studied by 13C NMR spectroscopy may indicate compartmentation and mitochondrial heterogeneity. *Neurosci Lett*. 1995; 185:24–28. [PubMed: 7731547]
81. Fukata Y, Fukata M. Protein palmitoylation in neuronal development and synaptic plasticity. *Nat Rev Neurosci*. 2010; 11:161–175. [PubMed: 20168314]
82. Resh MD. Regulation of cellular signalling by fatty acid acylation and prenylation of signal transduction proteins. *Cell Signal*. 1996; 8:403–412. [PubMed: 8958442]
83. Teller JK, Fahien LA, Valdivia E. Interactions among mitochondrial aspartate aminotransferase, malate dehydrogenase, and the inner mitochondrial membrane from heart, hepatoma, and liver. *J Biol Chem*. 1990; 265:19486–19494. [PubMed: 2246239]
84. Fahien LA, Teller JK. Glutamate-malate metabolism in liver mitochondria. A model constructed on the basis of mitochondrial levels of enzymes, specificity, dissociation constants, and stoichiometry of hetero-enzyme complexes. *J Biol Chem*. 1992; 267:10411–10422. [PubMed: 1350279]
85. Noritake J, Fukata Y, Iwanaga T, Hosomi N, Tsutsumi R, Matsuda N, Tani H, Iwanari H, Mochizuki Y, Kodama T, Matsuura Y, Bredt DS, Hamakubo T, Fukata M. Mobile DHHC palmitoylating enzyme mediates activity-sensitive synaptic targeting of PSD-95. *J Cell Biol*. 2009; 186:147–160. [PubMed: 19596852]
86. Charych EI, Jiang LX, Lo F, Sullivan K, Brandon NJ. Interplay of palmitoylation and phosphorylation in the trafficking and localization of phosphodiesterase 10A: implications for the treatment of schizophrenia. *J Neurosci*. 30:9027–9037. [PubMed: 20610737]
87. Liu YL, Fann CS, Liu CM, Chang CC, Yang WC, Wu JY, Hung SI, Chan HY, Chen JJ, Hsieh MH, Hwang TJ, Faraone SV, Tsuang MT, Chen WJ, Hwu HG. HTF9C gene of 22q11.21 region associates with schizophrenia having deficit-sustained attention. *Psychiatr Genet*. 2007; 17:333–338. [PubMed: 18075473]
88. Fahien LA, Kmietek EH, Woldegiorgis G, Evenson M, Shrago E, Marshall M. Regulation of aminotransferase-glutamate dehydrogenase interactions by carbamyl phosphate synthase-I, Mg²⁺ plus leucine versus citrate and malate. *J Biol Chem*. 1985; 260:6069–6079. [PubMed: 3997814]

89. Fahien LA, Hsu SL, Kmiolek E. Effect of aspartate on complexes between glutamate dehydrogenase and various aminotransferases. *J Biol Chem.* 1977; 252:1250–1256. [PubMed: 14147]
90. Fahien LA, Kmiolek EH, MacDonald MJ, Fibich B, Mandic M. Regulation of malate dehydrogenase activity by glutamate, citrate, alpha-ketoglutarate, and multienzyme interaction. *J Biol Chem.* 1988; 263:10687–10697. [PubMed: 2899080]
91. Beeckmans S, Kanarek L. Demonstration of physical interactions between consecutive enzymes of the citric acid cycle and of the aspartate-malate shuttle. A study involving fumarase, malate dehydrogenase, citrate synthesis and aspartate aminotransferase. *Eur J Biochem.* 1981; 117:527–535. [PubMed: 7285903]
92. Beeckmans S, Van Driessche E, Kanarek L. Clustering of sequential enzymes in the glycolytic pathway and the citric acid cycle. *J Cell Biochem.* 1990; 43:297–306. [PubMed: 2398101]
93. Fahien LA, MacDonald MJ, Teller JK, Fibich B, Fahien CM. Kinetic advantages of hetero-enzyme complexes with glutamate dehydrogenase and the alpha-ketoglutarate dehydrogenase complex. *J Biol Chem.* 1989; 264:12303–12312. [PubMed: 2745445]
94. McKenna, MC.; Gruetter, R.; Sonnewald, U.; Waagepetersen, HS.; Schousboe, A.; Siegel, GJ.; Albers, RW.; Brady, ST.; Price, DL. *Basic Neurochemistry.* Burlington, MA: Elsevier Academic Press; 2006. *Energy Metabolism of the Brain*; p. 531-557.edition.
95. Jones DP. Intracellular diffusion gradients of O₂ and ATP. *Am J Physiol.* 1986; 250:C663–C675. [PubMed: 3010727]
96. Pollack, GH. *Cells, Gels and the Engines of Life*; A new, unifying approach to cell function. Seattle: Ebner and Sons; 2001. p. 320
97. Lopez-Beltran EA, Mate MJ, Cerdan S. Dynamics and environment of mitochondrial water as detected by 1H NMR. *J Biol Chem.* 1996; 271:10648–10653. [PubMed: 8631870]
98. Fahien LA, Kmiolek E. Complexes between mitochondrial enzymes and either citrate synthase or glutamate dehydrogenase. *Arch Biochem Biophys.* 1983; 220:386–397. [PubMed: 6824331]
99. Fahien LA, Teller JK, Macdonald MJ, Fahien CM. Regulation of glutamate dehydrogenase by Mg²⁺ and magnification of leucine activation by Mg²⁺ *Mol Pharmacol.* 1990; 37:943–949. [PubMed: 2359406]
100. Beeckmans S, Kanarek L. Enzyme-enzyme interactions as modulators of the metabolic flux through the citric acid cycle. *Biochem Soc Symp.* 1987; 54:163–172. [PubMed: 3332992]
101. Beeckmans S, Van Driessche E, Kanarek L. Immobilized enzymes as tools for the demonstration of metabolon formation. A short overview. *J Mol Recognit.* 1993; 6:195–204. [PubMed: 7917415]
102. Haggie PM, Verkman AS. Diffusion of tricarboxylic acid cycle enzymes in the mitochondrial matrix in vivo. Evidence for restricted mobility of a multienzyme complex. *J Biol Chem.* 2002; 277:40782–40788. [PubMed: 12198136]
103. Haggie PM, Brindle KM. Mitochondrial citrate synthase is immobilized in vivo. *J Biol Chem.* 1999; 274:3941–3945. [PubMed: 9933583]
104. Pagliaro L, Taylor DL. 2-Deoxyglucose and cytochalasin D modulate aldolase mobility in living 3T3 cells. *J Cell Biol.* 1992; 118:859–863. [PubMed: 1500428]
105. Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Weindruch R, Alt FW, Guarente L. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell.* 2006; 126:941–954. [PubMed: 16959573]
106. Srere PA. Complexes of sequential metabolic enzymes. *Annu Rev Biochem.* 1987; 56:89–124. [PubMed: 2441660]
107. Halper LA, Srere PA. Interaction between citrate synthase and mitochondrial malate dehydrogenase in the presence of polyethylene glycol. *Arch Biochem Biophys.* 1977; 184:529–534. [PubMed: 563705]
108. Moore GE, Gadol SM, Robinson JB Jr. Srere PA. Binding of citrate synthase and malate dehydrogenase to mitochondrial inner membranes: tissue distribution and metabolite effects. *Biochem Biophys Res Commun.* 1984; 121:612–618. [PubMed: 6732828]

109. Sumegi B, Srere PA. Complex I binds several mitochondrial NAD-coupled dehydrogenases. *J Biol Chem.* 1984; 259:15040–15045. [PubMed: 6439716]
110. Srere PA, Sumegi B, Sherry AD. Organizational aspects of the citric acid cycle. *Biochem Soc Symp.* 1987; 54:173–178. [PubMed: 3332994]
111. Hayashi H, Mizuguchi H, Miyahara I, Nakajima Y, Hirotsu K, Kagamiyama H. Conformational change in aspartate aminotransferase on substrate binding induces strain in the catalytic group and enhances catalysis. *J Biol Chem.* 2003; 278:9481–9488. [PubMed: 12488449]
112. Fahien LA, Davis JW, Laboy J. Interactions between pyruvate carboxylase and other mitochondrial enzymes. *J Biol Chem.* 1993; 268:17935–17942. [PubMed: 8349677]
113. Faff-Michalak L, Albrecht J. Aspartate aminotransferase, malate dehydrogenase, and pyruvate carboxylase activities in rat cerebral synaptic and nonsynaptic mitochondria: effects of in vitro treatment with ammonia, hyperammonemia and hepatic encephalopathy. *Metab Brain Dis.* 1991; 6:187–197. [PubMed: 1812392]
114. Salganicoff L, Derobertis E. Subcellular Distribution of the Enzymes of the Glutamic Acid, Glutamine and Gamma-Aminobutyric Acid Cycles in Rat Brain. *J Neurochem.* 1965; 12:287–309. [PubMed: 14336230]
115. Rothe F, Brosz M, Storm-Mathisen J. Quantitative ultrastructural localization of glutamate dehydrogenase in the rat cerebellar cortex. *Neuroscience.* 1994; 62:1133–1146. [PubMed: 7531302]
116. Rothe F, Brosz M, Storm-Mathisen J. Quantitative ultrastructural localization of glutamate dehydrogenase in the rat cerebellar cortex. *Neuroscience.* 1995; 64:iii–xvi. [PubMed: 7753371]
117. Erecinska M, Silver IA. Metabolism and role of glutamate in mammalian brain. *Prog Neurobiol.* 1990; 35:245–296. [PubMed: 1980745]
118. Verdin E, Hirschey MD, Finley LW, Haigis MC. Sirtuin regulation of mitochondria: energy production, apoptosis, and signaling. *Trends Biochem Sci.* 2010; 35:669–675. [PubMed: 20863707]
119. Schlicker C, Gertz M, Papatheodorou P, Kachholz B, Becker CF, Steegborn C. Substrates and regulation mechanisms for the human mitochondrial sirtuins Sirt3 and Sirt5. *J Mol Biol.* 2008; 382:790–801. [PubMed: 18680753]
120. Wagner GR, Payne RM. Mitochondrial acetylation and diseases of aging. *J Aging Res.* 2011; Volume:234875. [PubMed: 21437190]
121. Hutson S, Islam MM, Zaganas I. Interaction Between Glutamate Dehydrogenase (GDH) and L-Leucine Catabolic Enzymes: Intersecting Metabolic Pathways. *Neurochem Int.* 2011; 59:518–524. [PubMed: 21621574]
122. Islam MM, Wallin R, Wynn RM, Conway M, Fujii H, Mobley JA, Chuang DT, Hutson SM. A novel branched-chain amino acid metabolon. Protein-protein interactions in a supramolecular complex. *J Biol Chem.* 2007; 282:11893–11903. [PubMed: 17314104]
123. Sonnewald U, White LR, Odegard E, Westergaard N, Bakken IJ, Aasly J, Unsgard G, Schousboe A. MRS study of glutamate metabolism in cultured neurons/glia. *Neurochem Res.* 1996; 21:987–993. [PubMed: 8897461]
124. Yudkoff M, Daikhin Y, Nissim I, Lazarow A. Ketogenic diet, amino acid metabolism, and seizure control. *J Neurosci Res.* 2001; 66:931–940. [PubMed: 11746421]
125. Fahien LA, Kmietek EH, Marshall M. Interactions between carbamyl phosphate synthase-I-mitochondrial aspartate aminotransferase and palmitoyl-CoA. *Arch Biochem Biophys.* 1984; 230:213–221. [PubMed: 6712233]

Figure 1. Known and expected interactions of GDH with other mitochondrial proteins
 Schematic representation of GDH is localized in the mitochondrial matrix. The reactions catalyzed by GDH are described in the text. Enzymes and proteins known to interact with GDH are in purple ovals. Enzymes that interact with GDH only when it is complexed with other proteins are in green ovals. Enzymes that may interact with GDH (e.g. direct interaction has not been demonstrated but biochemical studies support interaction) are in orange ovals. *Abbreviations:* AAT, aspartate aminotransferase; BCAA, branched chain amino acid; BCAT, mitochondrial branched chain aminotransferase; BCKA, branched chain ketoacid; BCKDH, branched chain keto acid dehydrogenase; GDH, glutamate dehydrogenase; GAD, glutamic acid decarboxylase; KGDH, α -ketoglutarate dehydrogenase; α -KG, α -ketoglutarate; MDH, malate dehydrogenase; mME, mitochondrial malic enzyme; OAA, oxaloacetate; PAG, phosphate activated glutaminase; PATs, palmitoyl acyltransferases; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PPTs, palmitoyl acyl thioesterases; SIRT5, sirtuins; TCA, tricarboxylic acid cycle; ? binding protein, unidentified protein that binds to AAT and to the MDH-CS complex.

Table 1

Factors contributing to compartmentation in brain

<u>Regional and temporal differences:</u>	
Regional differences in neurons and glia	Cortex: GABAergic interneurons, glutamatergic neurons, cholinergic, dopaminergic; Cerebellum glutamatergic neurons Astrocytes from different regions have different properties
Cell types and populations develop at different times	Cortex develops earlier than cerebellum Neurons divide and differentiate prior to glial cells Metabolism changes during development
Cell migration, cell loss, and pruning and strengthening of connections	
<u>Cellular differences:</u>	
Differential types of brain cells	Neurons: cortical, cerebellar granule cells, purkinje cells, etc. Glial: astrocytes, oligodendroglia, microglia Other cells: ependymal cells, etc
Differential localization of transporters on brain cells	Glutamate, glutamine, glucose (GLUTs), monocarboxylic acid (MCTs; transport lactate and ketones)
Enzyme differences:	
Selective cellular localization	Astrocytes: pyruvate carboxylase and glutamine synthetase Neurons: glutamic acid decarboxylase (GABA formation)
Differential distribution of activity	Rates differ in astrocytes, neurons and synaptic terminals
Differential regulation of enzymes	Regulation of some enzymes e.g. GDH, MDH, AAT differs in astrocytes and neurons
Mitochondrial heterogeneity	Properties differ between neurons and astrocytes (e.g. aspartate glutamate carrier; complex I of Electron Transport Chain, etc).
<u>Subcellular:</u>	
Localized compartmentation	Occurs within mitochondria and in cytosol
Mitochondrial heterogeneity within cells	Enzymes, electron transport chain (ETC) complexes, etc: differ in mitochondria from synaptic terminals, neuronal cell bodies and astrocytes
Differential regulation of enzymes within cells	Regulation can differ in mitochondria from synaptic terminals and neuronal cell bodies
Formation of transient multienzyme complexes	Complex formation is modulated by the concentration of key intermediates (e.g. OAA, α -KG, malate, citrate)

Table 2**Characteristics of transiently formed hetero-enzyme complexes**

Transiently formed enzyme complexes associate and dissociate

Regulated by concentrations of metabolites -- Citrate, OAA, α -KG, malate, etc.

Some complexes bind to proteins on the inner mitochondrial membranes

- Some enzymes bind to unique binding proteins; others bind to Complex I
- Not all enzymes bind other enzymes; there are specific priorities and constraints that regulate binding
- There is competition for binding sites on the inner mitochondrial membrane

Binding to lipids affects activity of some enzymes

Association of enzymes into complexes can lead to channeling of metabolites and cofactors between specific reactions

Association and dissociation of transiently formed complexes is one of the least understood aspects of compartmentation and regulation of metabolism