Amino Acid Changes within Antenna Helix Are Responsible for Different Regulatory Preferences of Human Glutamate Dehydrogenase Isozymes*

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Human glutamate dehydrogenase (hGDH) exists in hGDH1 (housekeeping isozyme) and in hGDH2 (nerve-specific isozyme), which differ markedly in their allosteric regulation. Because they differ in only 16 of their 505 amino acids, the regulatory preferences must arise from amino acid residues that are not common between hGDH1 and hGDH2. To our knowledge none of the mutagenesis studies on the hGDH isozymes to date have identified the amino acid residues fully responsible for the different regulatory preferences between hGDH1 and hGDH2. In this study we constructed hGDH1(hGDH2390–448)hGDH1 (amino acid segment 390–448 of hGDH1 replaced by the corresponding hGDH2 segment) and hGDH2(hGDH1390–448)hGDH2 (amino acid segment 390–448 of hGDH2 replaced by the corresponding hGDH1 segment) by swapping the corresponding amino acid segments in hGDH1 and hGDH2. The chimeric enzymes by reciprocal swapping resulted in double mutations in amino acid sequences at 415 and 443 residues that are not common between hGDH1 and hGDH2 and are located in the C-terminal 48-residue “antenna” helix, which is thought to be part of the regulatory domain of mammalian GDHs. Functional analyses revealed that the doubly mutated chimeric enzymes almost completely acquired most of the different regulatory preferences between hGDH1 and hGDH2 for electrophoretic mobility, heat-stability, ADP activation, palmitoyl-CoA inhibition, and L-leucine activation, except for GTP inhibition. Our results indicate that substitutions of the residues in the antenna region may be important evolutionary changes that led to the adaptation of hGDH2 to the unique metabolic needs of the nerve tissue.

Glutamate dehydrogenase (GDH)3 is found in all organisms and catalyzes the oxidative deamination of glutamate to 2-oxoglutarate. Although this enzyme does not exhibit allosteric regulation in plants, bacteria, or fungi, its activity is tightly controlled by a number of compounds in mammals (1–6). All mammalian GDHs are homohexameric, exhibiting 32 symmetry, and the first 200 residues form the core “glutamate binding” domain. On top of this domain is a “NAD binding” domain that rotates about 20° during catalysis (7–9). Unique to the animal structures is a 48-residue antenna that protrudes above this NAD binding domain (7–9).

Various roles of GDH have been reported. Partial deficiency of GDH has been reported in some patients with cerebellar degeneration, suggesting that the enzymes are important in brain function (10). GDH has shown neuroprotective value in model systems where glutamate reuptake is inhibited (11). In addition, it has been reported that inhibition of GDH expression by antisense oligonucleotides was toxic to cultured mesencephalic neurons, with dopaminergic neurons being affected at the early stages of this inhibition (12). The existence of the hyperinsulinism-hyperammonemia syndrome further highlights the importance of GDH in the regulation of insulin secretion and indicates that GDH has an important role in regulating hepatic ureagenesis (13, 14).

In the human, GDH exists in a housekeeping isozyme (hGDH1) encoded by the GLUD1 gene and a neural and testicular tissue-specific isozyme (hGDH2) encoded by the GLUD2 gene (15). Although the two GDH isozymes are highly homologous (showing a 97% amino acid identity), they differ markedly in their regulatory properties such as heat stability and allosteric regulation by ADP, L-leucine, and GTP (3–6, 16, 17). Because the hGDH isozymes differ in only 16 of their 505 amino acids, these functional differences must arise from amino acid residues that are not common between hGDH1 and hGDH2. Reciprocally interchanging amino acids that are different between hGDH isozymes within the regulatory domain may reveal the residues important for preference in hGDH isozymes. Recent studies of structure-function relationships using site-directed mutagenesis of hGDH1 at single sites differing from hGDH2 showed that the R443S and the G456A change reproduced some but not all of the properties of hGDH2 (3–5,

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3 The abbreviations used are: GDH, glutamate dehydrogenase; hGDH, human GDH; HPLC, high performance liquid chromatography.
Our objective is to identify these critical residues by multiple mutagenesis. In this study we selected two such residues, Met-415 and Arg-443, because they are the only two amino acid residues that are different between hGDH1 and hGDH2 within the C-terminal 48-residue antigen region, which is thought to be part of the regulatory domain of mammalian GDH (7–9). Using cassette mutagenesis, we constructed hGDH1(hGDH2390–448)hGDH1 (amino acid segment 390–448 of hGDH1 replaced by the corresponding hGDH2 segment) and hGDH2(hGDH1390–448)hGDH2 (amino acid segment 390–448 of hGDH2 replaced by the corresponding hGDH1 segment) by swapping the corresponding amino acid segments between 390 and 448 regions in hGDH1 and hGDH2. The mutated cDNAs were expressed in E. coli DH5α (18) was purchased from Invitrogen and Genetic Stock Center, Yale University, New Haven, CT) lacked both GDH and glutamate synthase activities (19) and was used to test plasmids for GDH activity.

Expression and Purification of Chimeric Enzymes—The chimeric constructs were separately transformed into E. coli/DE3 for overexpression. Fresh overnight cultures of the E. coli/DE3 were used to inoculate 1 liter of LB medium containing 100 μg/ml ampicillin. The cell was grown at 37 °C until the reached 1.0, and then isopropyl 1-thio-β-d-galactopyranoside was added to a final concentration of 1 mM. After isopropyl 1-thio-β-d-galactopyranoside induction, each E. coli/DE3 was grown for an additional 3 h at 37 °C and then harvested by centrifugation. Cell pellets were suspended in 50 ml of 50 mM Tris-HCl, pH 7.2, 1 mM phenylmethylsulfonyl fluoride and lysed with a sonicator. Cellular debris was removed by centrifugation, and the crude extracts were loaded onto an 2,5-ADP-Sepharose column (1.5 × 5 cm) that was equilibrated with buffer A (50 mM Tris-HCl, pH 7.2, and 1 mM β-mercaptoethanol). The column was washed with buffer A until the breakthrough of protein had been eluted. The enzyme was then performed using a NaCl step gradient that went from 0 to 300 mM in 30 min at a flow rate of 1 ml/min. The fractions containing GDH activity were pooled, concentrated, and applied to a fast protein liquid chromatograph Resource-Q anion exchange column equilibrated with buffer B (50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, and 1 mM β-mercaptoethanol). The enzyme was then eluted using a linear gradient made with buffer B in increasing concentrations of NaCl up to 500 mM at 1 ml/min. The purified enzyme was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western blot analysis using monoclonal antibodies previously produced in our laboratory (22). Protein concentration was determined by the method of Bradford (23). Additional N-terminal residues (MIEGR) were removed by treatment with factor Xa (10 μg/ml of GDH) and purified by a HPLC gel filtration method as described before (4, 21). HPLC-purified proteins were subjected to automated amino acid sequencing.

Enzyme Assay and Kinetics—Steady state kinetic parameters were determined with the purified proteins unless otherwise indicated. Enzyme assays were performed by monitoring reduced coenzyme absorbance at 340 nm. Because E. coli only has an NADP+-dependent GDH (19), the enzyme assay was performed with NADH as a coenzyme. All assays were performed in duplicate, and initial velocity data were correlated with a standard assay mixture of 1 ml containing 50 mM triethanolamine, pH 8.0, 100 mM ammonium acetate, 0.1 mM NADH, and 2 mM EDTA, pH 8.0, at 25 °C. Enzyme assay was performed in the presence of 1 mM ADP unless otherwise indicated. The
reaction started with the addition of α-ketoglutarate to 2 mM final concentration. The $K_m$ values were calculated by linear regression analysis of double-reciprocal plots, and catalytic efficiency was estimated by use of the equation $v/[E]_0 = (k_{cat}/K_m)[S]$ (24). For the heat stability studies, the hGDH isoforms were incubated at 45 °C in the absence of ADP. At various times, aliquots were withdrawn to determine remaining activities by the addition of the standard assay mixture in the presence of 1 mM ADP.

**Regulation of Chimeric Enzymes**—Effects of allosteric regulators on GDH activities were examined by incubating the enzyme with the allosteric effectors at various concentrations in the assay buffer at 25 °C. At intervals after the initiation with the effectors, aliquots were withdrawn for the assay of GDH activity. Regulation of the hGDH isoforms and the chimeric enzymes by ADP or L-leucine was studied by adding ADP (0–1 mM final concentrations) or L-leucine (0–5 mM final concentrations) to the reaction mixture at various concentrations while keeping the other substrates constant. We also explored the effect of ADP and L-leucine used together at various concentrations on hGDH isoforms and chimeric mutants. ADP (0–1 mM) was added to the reaction mixture that contained L-leucine at various concentrations (0–5 mM). GTP inhibition and palmitoyl-CoA inhibition were studied by adding GTP (0–100 μM final concentrations) or palmitoyl-CoA (0–10 μM final concentrations) at various concentrations to the standard reaction mixture in the presence of 1 mM ADP. For the thermal stability studies, the wild-type and chimeric enzymes were incubated in 100 mM potassium phosphate buffer, pH 7.0 at 45 °C in the presence of 1 mM ADP. At various times, aliquots were withdrawn, and the remaining activities were assayed by the addition of the standard assay mixture.

**RESULTS**

Construction, Expression, and Purification of hGDH1-(hGDH2390–448)hGDH1 and hGDH2(hGDH1390–448)hGDH2—We have reported construction of synthetic hGDH1 and hGDH2 genes and expression of hGDH1 and hGDH2 isoforms from E. coli as soluble proteins (4, 21). Using cassette mutagenesis, the construction of hGDH1(hGDH2390–448)hGDH1 (amino acid segment 390–448 of hGDH1 replaced by the corresponding hGDH2 segment) and hGDH2(hGDH1390–448)hGDH2 (amino acid segment 390–448 of hGDH2 replaced by the corresponding hGDH1 segment) was carried out by swapping the corresponding amino acid segments in hGDH1 and hGDH2 (Fig. 1). The chimeric enzymes by reciprocal swapping resulted in double mutations in amino acid sequences at 415 and 443 residues that are not common between hGDH1 and hGDH2. In the wild-type hGDH isoforms, the 415 site is Met in hGDH1 and Leu in hGDH2, and the 443 site is Arg in hGDH1 and Ser in hGDH2. In hGDH1(hGDH2390–448)hGDH1, the 415 site is Leu and the 443 site is Arg (Fig. 1).

High level expression of the chimeric enzymes was achieved in E. coli strain DE3 upon induction with 1 mM isopropyl-1-β-D-thiogalactose at 37 °C for 3 h. Analysis of crude cell extracts by Western blot showed that expression levels of hGDH1(hGDH2390–448)hGDH1 and hGDH2-(hGDH1390–448)hGDH2 were almost identical to those of wild-type hGDH isoforms (data not shown). The expressed enzymes were purified by ADP-Sepharose column and fast protein liquid chromatograph Resource-Q column. To remove five additional N-terminal residues (MIEGR) that were introduced to create an initiation codon and a factor Xa recognition site, the purified enzymes were treated with factor Xa, purified by an HPLC Protein-Pak 300SW gel filtration column, and subjected to automated Edman degradation. N-terminal sequence analysis of the first eight amino acids was identical with the published sequence of the mature hGDH isoforms (15, 25). The purified wild-type hGDH2 was estimated to be 96% pure by SDS-PAGE (Fig. 2A). Compared with hGDH1, the hGDH2 protein is more basic and shows a slightly lower electrophoretic mobility (Fig. 2A). These differences are consistent with the electrophoretic characteristics of hGDH isoforms as reported by other investigators (15). Interestingly, when the amino acid segment 390–448 of hGDH1 was replaced by the corresponding hGDH2 segment to make hGDH1(hGDH2390–448)hGDH1, it was found to show the same electrophoretic mobility as hGDH2 (Fig. 2A). Likewise, when the amino acid segment 390–448 from the hGDH1 is spliced onto human GDH2, the electrophoretic mobility of hGDH2(hGDH1390–448)hGDH2 was almost identical to that of hGDH1. These results suggest that the amino acid differences at 415 and 443 sites within the antenna region are responsible for the electrophoretic mobility of the hGDH isoforms.

**Heat Stability of hGDH1(hGDH2390–448)hGDH1 and hGDH2(hGDH1390–448)hGDH2**—The relative resistance of hGDH2 to thermal inactivation was determined by incubation of the enzyme at 45 °C, pH 7.0. Heat inactivation proceeded faster for hGDH2 (half-life = 45 min) than for hGDH1 (half-life = 310 min) (Fig. 2B), supporting the previous observation that hGDH1 is the heat-stable form, and hGDH2 is the heat-labile form of human GDH (4, 16). Under these conditions, the hGDH1(hGDH2390–448)hGDH1 and
hGDH2(hGDH1390–448)hGDH2 chimeric enzymes showed the reciprocal changes in thermal stability of hGDH isozymes. hGDH2(hGDH1390–448)hGDH2 abolished the heat lability of hGDH2 and changed the half-life of hGDH2 from 45 to ∼280 min at 45 °C, which is comparable with that of hGDH1 (Fig. 2B). In contrast, hGDH1(hGDH2390–448)hGDH2 abolished the heat stability of hGDH1, and the result obtained with hGDH1(hGDH2390–448)hGDH1 was similar to that observed with hGDH2 (Fig. 2B). Previously, we reported that single replacement of Ser by Arg at hGDH2 position 443 abolished the heat lability of hGDH2 and changed the half-life of hGDH2 to 280 min at 45 °C, which is comparable with that of hGDH1 (Fig. 2B). In contrast, hGDH1(hGDH2390–448)hGDH2 abolished the heat stability of hGDH1 and the result obtained with hGDH1(hGDH2390–448)hGDH1 was similar to that observed with hGDH2 (Fig. 2B). Previously, we reported that single replacement of Ser by Arg at hGDH2 position 443 abolished the heat lability of hGDH2 and changed the half-life of hGDH2 to 280 min at 45 °C, which is comparable with that of hGDH1 (Fig. 2B). In contrast, hGDH1(hGDH2390–448)hGDH2 abolished the heat stability of hGDH1 and the result obtained with hGDH1(hGDH2390–448)hGDH1 was similar to that observed with hGDH2 (Fig. 2B). Previously, we reported that single replacement of Ser by Arg at hGDH2 position 443 abolished the heat lability of hGDH2 and changed the half-life of hGDH2 to 280 min at 45 °C, which is comparable with that of hGDH1 (Fig. 2B). In contrast, hGDH1(hGDH2390–448)hGDH2 abolished the heat stability of hGDH1 and the result obtained with hGDH1(hGDH2390–448)hGDH1 was similar to that observed with hGDH2 (Fig. 2B). Previously, we reported that single replacement of Ser by Arg at hGDH2 position 443 abolished the heat lability of hGDH2 and changed the half-life of hGDH2 to 280 min at 45 °C, which is comparable with that of hGDH1 (Fig. 2B). In contrast, hGDH1(hGDH2390–448)hGDH2 abolished the heat stability of hGDH1 and the result obtained with hGDH1(hGDH2390–448)hGDH1 was similar to that observed with hGDH2 (Fig. 2B). Previously, we reported that single replacement of Ser by Arg at hGDH2 position 443 abolished the heat lability of hGDH2 and changed the half-life of hGDH2 to 280 min at 45 °C, which is comparable with that of hGDH1 (Fig. 2B). In contrast, hGDH1(hGDH2390–448)hGDH2 abolished the heat stability of hGDH1 and the result obtained with hGDH1(hGDH2390–448)hGDH1 was similar to that observed with hGDH2 (Fig. 2B). Previously, we reported that single replacement of Ser by Arg at hGDH2 position 443 abolished the heat lability of hGDH2 and changed the half-life of hGDH2 to 280 min at 45 °C, which is comparable with that of hGDH1 (Fig. 2B). In contrast, hGDH1(hGDH2390–448)hGDH2 abolished the heat stability of hGDH1 and the result obtained with hGDH1(hGDH2390–448)hGDH1 was similar to that observed with hGDH2 (Fig. 2B). Previously, we reported that single replacement of Ser by Arg at hGDH2 position 443 abolished the heat lability of hGDH2 and changed the half-life of hGDH2 to 280 min at 45 °C, which is comparable with that of hGDH1 (Fig. 2B). In contrast, hGDH1(hGDH2390–448)hGDH2 abolished the heat stability of hGDH1 and the result obtained with hGDH1(hGDH2390–448)hGDH1 was similar to that observed with hGDH2 (Fig. 2B). Previously, we reported that single replacement of Ser by Arg at hGDH2 position 443 abolished the heat lability of hGDH2 and changed the half-life of hGDH2 to 280 min at 45 °C, which is comparable with that of hGDH1 (Fig. 2B). In contrast, hGDH1(hGDH2390–448)hGDH2 abolished the heat stability of hGDH1 and the result obtained with hGDH1(hGDH2390–448)hGDH1 was similar to that observed with hGDH2 (Fig. 2B). Previously, we reported that single replacement of Ser by Arg at hGDH2 position 443 abolished the heat lability of hGDH2 and changed the half-life of hGDH2 to 280 min at 45 °C, which is comparable with that of hGDH1 (Fig. 2B). In contrast, hGDH1(hGDH2390–448)hGDH2 abolished the heat stability of hGDH1 and the result obtained with hGDH1(hGDH2390–448)hGDH1 was similar to that observed with hGDH2 (Fig. 2B). Previously, we reported that single replacement of Ser by Arg at hGDH2 position 443 abolished the heat lability of hGDH2 and changed the half-life of hGDH2 to 280 min at 45 °C, which is comparable with that of hGDH1 (Fig. 2B). In contrast, hGDH1(hGDH2390–448)hGDH2 abolished the heat stability of hGDH1 and the result obtained with hGDH1(hGDH2390–448)hGDH1 was similar to that observed with hGDH2 (Fig. 2B). Previous...
sensitivity to inhibition by palmitoyl-CoA of hGDH1 and hGDH2.

Activation of hGDH1(hGDH2(390–448))hGDH1 and hGDH2-(hGDH1(390–448))hGDH2 by ADP—There were differences in the sensitivity to ADP between hGDH1 and hGDH2. Although hGDH1 was activated by ADP at a hyperbolic manner, the ADP stimulatory curves of hGDH2 showed a sigmoidal pattern typically found in allosteric regulation. When the segment 390–448 from the hGDH1 is spliced onto hGDH2 to make hGDH2(hGDH1(390–448))hGDH2, it was found to show almost identical sensitivity to ADP regulatory aspects of hGDH1 (Fig. 3B).

Similarly, replacing amino acid segment 390–448 of hGDH1 with the corresponding region of hGDH2 to produce chimeric hGDH1(hGDH2(390–448))hGDH1 resulted in the activation by ADP at a sigmoidal manner that is comparable with that of wild-type hGDH2 but substantially different from that of the wild-type hGDH1 (Fig. 3B). These results indicate that reciprocally switching amino acids at 415 and 443 sites influences preferences of the hGDH isozymes to ADP activation.

Inhibition of hGDH1(hGDH2(390–448))hGDH1 and hGDH2-(hGDH1(390–448))hGDH2 by GTP—Studies of the GTP inhibition on enzyme activity revealed that hGDH2 was resistant to
GTP inhibition, whereas hGDH1 was potently inhibited by this compound (Fig. 5). Interestingly, unlike the results in ADP or L-leucine activation studies, the reciprocal changes in the sensitivity to GTP inhibition were not observed when GTP inhibition studies were performed with the chimeric isoforms (Fig. 5). Therefore, the difference in the sensitivity to GTP inhibition between hGDH1 and hGDH2 is probably not due to the amino acid differences at 415 and 443 sites within the antenna region but due to the rest of the amino acids not common between hGDH1 and hGDH2.

DISCUSSION

There is evolutionary evidence that GLUD2 is an X-linked intronless gene and originated by retroposition from GLUD1 in the hominoid ancestor less than 23 million years ago (28). GLUD2 is an unusual example of a gene that emerged as an intronless duplicate of GLUD1 on chromosome 10 by retrotransposition of its mRNA, achieved specialized expression in the brain, and then acquired multiple amino acid changes to facilitate its functioning in the brain such as resistance to the high GTP levels and a markedly increased activity dependence on ADP and L-leucine. Burki and Kaessmann (28) have suggested that these features allow instant activation of the enzyme in the brain when there is high frequency firing of neurons. Previous studies showed that GDH activities were differentially altered in neurologic patients (29, 30), thus suggesting that these activities are under different genetic control. Because electoretinographic abnormalities have been detected in patients with spinocerebellar ataxia who have a selective deficiency of the heat-labile GDH isoforms (10, 31–34), studies on the molecular basis for the different molecular properties of the GDH isoforms may, thus, be of importance for understanding the biology of the human nervous systems and the genetic analysis of X-linked neurodegeneration. Previously, two types of mitochondrial glutamate carrier (GC1 and GC2) were identified differing in their $V_{\text{max}}$ and $K_m$ values and in their tissue distribution (35). The GC1 is expressed in all tissues, whereas the GC2 is mainly expressed in the brain and in the testis. This tissue distribution is almost identical to that of the housekeeping hGDH1 and neural and testicular tissue-specific hGDH2, respectively. Because the transport of glutamate into the mitochondria is linked to its metabolism by GDH, it may be assumed that both hGDH2 and GC2 have adapted to the particular metabolic needs of the neural and testicular tissues where they are expressed.

Because hGDH1 and hGDH2 differs in only 16 of their 505 amino acids, there may be differences in the secondary or tertiary structure that could explain the different molecular properties of the hGDH isoforms and that the functional differences must arise from amino acid residues that are not common between the two isoforms. Reciprocally interchanging amino acids that are different between hGDH isoforms within the regulatory domain may reveal the residues important for preference in hGDH isoforms. Recent studies of structure-function relationships by many investigators (3–6, 8, 17, 36) have revealed several important residues differing between hGD1 and hGDH2 by using site-directed mutagenesis, including S331T, M370L, M415L, R443G, G456A, R463A, R470H, and N498S. However, the mutagenesis studies at these sites provide only partial but not all of regulatory preferences of hGDH isoforms.

X-ray crystal studies (7, 8, 37, 38) have shown that the regulatory domain of mammalian GDHs includes the pivot helix and the antenna (residues 401–448), a protruding structure composed of an ascending 23-residue α-helix and a descending strand (residues 425–448) ending with a small α-helix (residues 439–448). The 48-residue antenna region extends from the top of each NAD binding domain and intertwines with the antenna of two adjacent subunits, which may play an important role in GDH allosteric regulation (7, 8, 37, 38). In the antenna region there are two amino acids that are not common between hGDH1 and hGDH2. The 415 site is Met in hGDH1 and Leu in hGDH2, and the 443 site is Arg in hGDH1 and Ser in hGDH2. Previously, functional analyses of the single mutagenesis revealed that substitution of Leu for Arg-443 had no effect on the catalytic or regulatory properties of the enzyme (36). We also have reported that L415M did not abolish the heat lability of hGDH2 (4). However, because Leu-415 and Arg-443 are located in the antenna region, we tested the possibility that the L415M substitution can act together with the R443S change to reproduce the unique properties of the isoforms. We have constructed hGDH1(hGDH2390–448)hGDH1 and hGDH2-(hGDH1390–448)hGDH2, by reciprocally switching the corresponding amino acid segments 390–448 in hGDH1 and hGDH2 (Fig. 1). Functional analyses revealed that the doubly mutated chimeric enzymes acquired most of the different regulatory preferences between hGDH1 and hGDH2, such as electrophoretic mobility (Fig. 2A), heat stability (Fig. 2B), palmitoyl-CoA inhibition (Fig. 3A), ADP activation (Fig. 3B), and L-leucine activation (Fig. 4).

Palmitoyl-CoA inhibition appears to also be dependent upon the antenna domain. Our results showed that hGDH2 was more sensitized to palmitoyl-CoA inhibition than hGDH1 (Fig. 3A). In contrast to plants and fungi, animals perform $\alpha$-oxida-
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tion of medium and long chain fatty acids mainly in the mitochondria, whereas the very long chain fatty acids are catabolized in the peroxisomes. Therefore, it has been proposed that the antenna evolved to link fatty acid and amino acid catabolism in the mitochondria (9). Previous studies have shown that mutations in the GTP and ADP binding regions do not affect palmitoyl-CoA inhibition (39). Although we do not yet know the location of the palmitoyl-CoA binding site, it is tempting to speculate that palmitoyl-CoA might bind to the antenna domain and be inhibited by affecting the conformational changes associated with the opening and closing of the catalytic cleft (7).

It is clear that ADP activation is the most primitive form of allosteric activation, and the antenna-fewer mutants clearly demonstrates that it is dependent upon the antenna (9). Our results suggest that substitutions of the residues in the antenna region may be important evolutionary changes that led to the adaptation of hGDH2 to the unique metabolic needs of the nerve tissue. The fact that hGDH1(hGDH2390–448)hGDH1 totally abrogated activation of hGDH1 by l-leucine in the absence of ADP (Fig. 4C) is consistent with the possibility that the introduction of the hGDH2390–448 segment favors the closed enzyme conformation. l-Leucine is thought to bind at the active site (39), and therefore, closure of the catalytic cleft will prevent l-leucine from entering this region. The finding that ADP sensitized hGDH1(hGDH2390–448)hGDH1 to the stimulatory effect of l-leucine provides additional evidence that opening of the catalytic site (acting allosterically) by ADP is necessary for l-leucine to act.

Because we previously identified Lys-450 as a site for GTP binding (26, 27), it was not unexpected that the double mutations at 415 and 443 sites within the antenna region of hGDH2 were not influenced to facilitate GTP inhibition. However, as the antenna deletion and a number of the hyperinsulinism-hyperammonemia mutations have shown (39–41), the antenna may have a function at least in part to communicate this inhibition. Previous studies showed that replacement of Gly by Ala made hGDH1 resistant to GTP (3). The Gly-450 site lies about a one-and-a-half α-helix away from Lys-450, with the side chain of the former amino acid residue protruding out of the α-helix in the opposite direction than the side chain of the latter according to the proposed structural models (7, 37, 38). It is interesting to note that the G450A substitution seems to be conserved during mammalian evolution. The bovine liver antenna isozyme contains Gly at position 450 of the human GLUD1 enzyme, contains Gly at position 452 (corresponds to Gly-456 in the human). Concerning the presence of Ala at this position in the human GLUD2, we separated two GDH isozymes from bovine brain and found by sequencing of isolated peptides that one of these isoforms contains Ala at position 452 (corresponding to Ala-456 in the human GLUD2) (26, 42).

Because GTP levels are generally higher in brain than in other tissues, GTP may not be a suitable modulator for hGDH2 activity in the nerve tissue. Taken together with the dependence on available ADP levels (3, 16), it will permit the recruitment of the enzyme under conditions of low energy charge (high ADP/ATP ratio), such as those occurring under intense glutamatergic neurotransmission (43). Therefore, hGDH2 may have evolved to function in the particular environment that prevails in glial cells after glutamate uptake.

In conclusion, except for different sensitivities to GTP inhibition, our results showed that R443S and L415M acting in concert are capable of reproducing most of the unique properties of hGDH2 almost completely. Therefore, we propose that the preference-determining region is mostly within the corresponding segments 390–448 in hGDH1 and hGDH2, and the amino acid changes at 415 and 443 sites within the antenna domain are responsible for most of the different regulatory properties between the hGDH isozymes.

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