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# Determination of Glutamate Dehydrogenase Activity and Its Kinetics in Mouse Tissues using Metabolic Mapping (Quantitative Enzyme Histochemistry)

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**Summary**
Glutamate dehydrogenase (GDH) catalyses the reversible conversion of glutamate into α-ketoglutarate with the concomitant reduction of $NAD(P)^+$ to NAD(P)H or vice versa. GDH activity is subject to complex allosteric regulation including substrate inhibition. To determine GDH kinetics in situ, we assessed the effects of various glutamate concentrations in combination with either the coenzyme $NAD^+$ or $NADP^+$ on GDH activity in mouse liver cryostat sections using metabolic mapping. $NAD^+$-dependent GDH $V_{max}$ was 2.5-fold higher than $NADP^+$-dependent $V_{max}$, whereas the $K_m$ was similar, 1.92 mM versus 1.66 mM, when $NAD^+$ or $NADP^+$ was used, respectively. With either coenzyme, $V_{max}$ was determined at 10 mM glutamate and substrate inhibition was observed at higher glutamate concentrations with a $K_i$ of 12.2 and 3.95 for $NAD^+$ and $NADP^+$ used as coenzyme, respectively. $NAD^+$- and $NADP^+$-dependent GDH activities were examined in various mouse tissues. GDH activity was highest in liver and much lower in other tissues. In all tissues, the highest activity was found when $NAD^+$ was used as a coenzyme. In conclusion, GDH activity in mice is highest in the liver with $NAD^+$ as a coenzyme and highest GDH activity was determined at a glutamate concentration of 10 mM. (J Histochem Cytochem 62:802–812, 2014)

**Keywords**
glutamate, enzyme cytochemistry, enzymes, enzyme inhibition, quantitation

## Introduction

Glutamate dehydrogenase (GDH, EC 1.4.1.3) is a metabolic enzyme that catalyses the reversible reaction of L-glutamate to α-ketoglutarate (α-KG), with the concomitant reduction of $NAD(P)^+$ to NAD(P)H or vice versa (Plaitakis et al. 2011; Mastorodemos et al. 2009, 2005). GDH is mainly located in the mitochondria, although recently it was shown that GDH is also present in the cytoplasm, endoplasmic reticulum and nucleus (Tiwari et al. 2014; Mastorodemos et al. 2009; Prisco et al. 1968). Yet, its functions outside the mitochondria are unknown.

GDH is a key enzyme in mitochondrial carbohydrate metabolism and catalyses the final step in glutaminolysis after the conversion of glutamine to glutamate by phosphate-activated glutaminase (PAG; Fig. 1). Via this pathway, α-KG can be shuttled into the tricarboxylic acid (TCA) cycle (DeBerardinis et al. 2008, 2007; Reitzer et al. 1979). This process is called anaplerosis, which can generate ATP by oxidation of α-KG, and raises the ATP:ADP ratio to induce insulin secretion (Treberg et al. 2010; Carobbio et al. 2009; Stanley et al. 2000, 1998). Alternatively, α-KG can be converted into citrate by IDH1 and IDH2, and aconitase for lipid synthesis via the formation of acetyl coenzyme A (AcCoA) (Brose et al. 2013; Filipp et al. 2012; Metallo et al. 2012; Collins et al. 2011; Mullen et al. 2011; DeBerardinis et al. 2007). On the other hand, glutamate can also be

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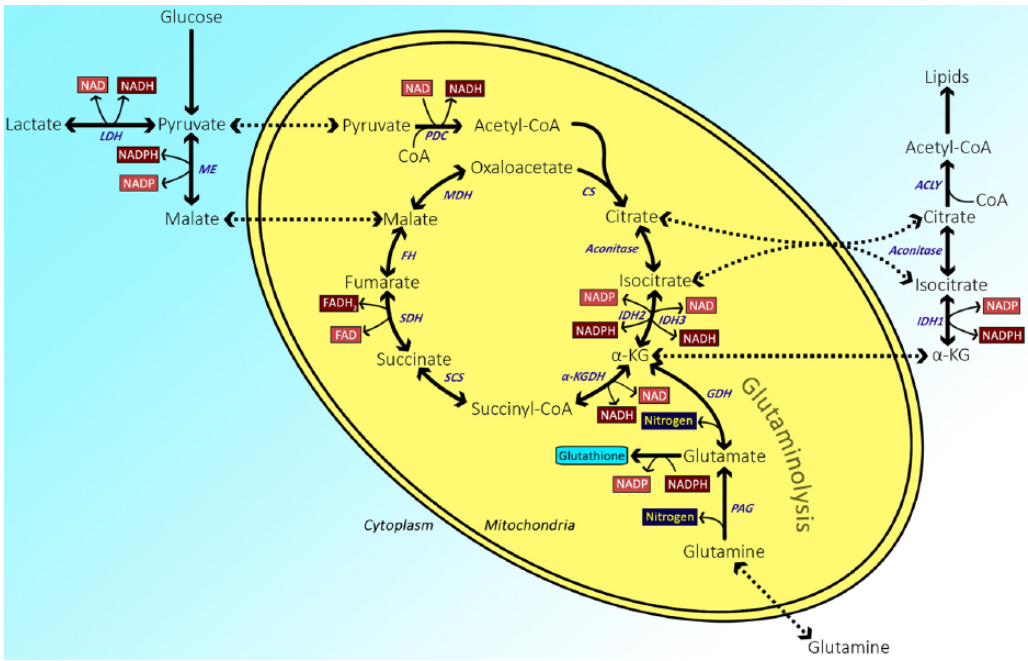


**Figure 1.** Cellular carbohydrate metabolism. Abbreviations: ACLY: ATP citrate lyase; α-KG: α-ketoglutarate; α-KGDH: α-ketoglutarate dehydrogenase; CS: citrate synthase; FH: fumarate hydratase; GDH: glutamate dehydrogenase; IDH: isocitrate dehydrogenase; LDH: lactate dehydrogenase; MDH: malate dehydrogenase; ME: malic enzyme; PAG: phosphate-activated glutaminase; PDC: pyruvate dehydrogenase complex. SCS: succinyl coenzyme A synthetase. SDH: succinate dehydrogenase.

converted into malate via the TCA cycle or into pyruvate by malic enzyme, generating NADPH required for cellular stress protection and for lipid and cholesterol metabolism (Koehler and Van Noorden 2003; Koh et al. 2004; DeBerardinis et al. 2007; Wise et al. 2008; Romero-Garcia et al. 2011; Lorin et al. 2013). Additionally, GDH activation by leucine facilitates the conversion of GDP to GTP, resulting in the activation of the small GTPase Rag, which activates mammalian target of rapamycin complex (mTORC), and results in cell growth and decreased autophagy (Lorin et al. 2013; Durán et al. 2012). Next to cellular carbohydrate metabolism, GDH is involved in ureagenesis, as the conversion of glutamate into α-KG also produces $NH_3^+$, which can be used to produce carbamoyl phosphate for the urea cycle (Spanaki and Plaitakis 2012; Treberg et al. 2010; Stanley et al. 2000, 1998; Boon et al. 1999; Nissim et al. 1992). Finally, GDH activity is increased in various brain pathologies, such as Parkinson's disease, schizophrenia and brain cancer (Plaitakis et al. 2010; Bao et al. 2009; Yang et al. 2009; Burbaeva et al. 2003). It is hypothesised that the increased GDH activity alters neuronal cell metabolism and increases the production of reactive oxygen species (ROS) through glutamate oxidation, which can damage neuronal cells (Plaitakis et al. 2010).

Two forms of GDH have been identified in humans, GDH1 and GDH2, which are encoded by the genes *GLUD1* and *GLUD2*, respectively (Shashidharan et al. 1994; Michaelidis and Tzimagiorgis 1993; Mavrothalassitis et al. 1988). *GLUD1* is expressed in many tissues with the highest expression in the brain and liver, whereas *GLUD2* is mainly expressed in retina, testis, and brain (Spanaki et al. 2010; Shashidharan et al. 1994; Mavrothalassitis et al. 1988). GDH2 activity is low and therefore glutamate is mainly converted into α-KG by GDH1 (Plaitakis et al. 2011; Mastorodemos et al. 2005). GDH has a lower $K_m$ for $NAD^+$ than $NADP^+$ and the $K_m$ for glutamate is higher when using $NADP^+$ instead of $NAD^+$, indicating that, at physiological glutamate concentrations, conversion of glutamate into α-KG is mainly $NAD^+$-dependent (Lee et al. 1999; Cho et al. 1995). Allosteric regulation of GDH activity is complex with many compounds affecting GDH activity. GDH is inhibited by GTP, GDP, palmitoyl-coenzyme A and $Zn2^+$ (Smith and Stanley 2008; Bell et al. 1987; Fahien and Kmiotek 1981; Dieter et al. 1981; Frieden 1965). L-leucine, L-isoleucine, L-valine and ADP activate GDH (Mastorodemos et al. 2005; McGivan et al. 1973; Markau et al. 1972). Additionally, GDH shows substrate inhibition at high glutamate concentrations through the formation of abortive complexes (Li et al. 2009; Smith and Stanley 2008; Bailey et al. 1982; Engel and Dalziel 1969). At last, phosphate affects GDH activity as GTP binds less tightly to GDH in the presence of phosphate (Dieter et al. 1981).

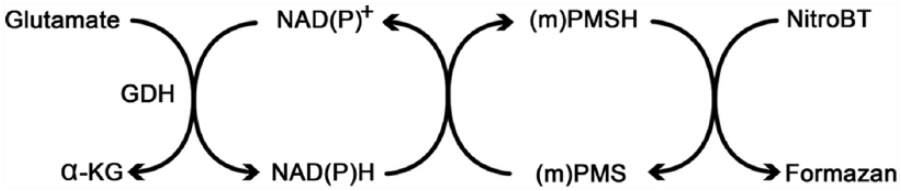


**Figure 2.** Principle of the tetrazolium salt method for metabolic mapping of the activity of dehydrogenases in general and GDH activity in particular. Abbrevations: (m)PMS: 1-(methoxy)phenazine methosulfate; PMS: phenazine methosulfate; NitroBT: nitro blue tetrazolium.

Because of the complex regulation mechanisms of GDH activity, GDH kinetics should be analyzed in intact tissues or cells using metabolic mapping (Chieco et al. 2013; Van Noorden 2010; Jonker et al. 1996; Van Noorden and Frederiks 1992). Metabolic mapping has been applied to determine GDH kinetics in liver, yet, these data were far from complete as the kinetics of GDH activity with $NAD^+$ or $NADP^+$ as a coenzyme in the presence of phosphate were not determined (Jonker et al. 1996; Maly and Sasse 1991).

The present study was performed because glutaminolysis emerged in recent years as a potential therapeutic target for primary brain tumors, such as glioblastoma (GBM) and, especially, secondary glioblastoma with an *IDH1* or *IDH2* mutation (Fig. 1) in which α-KG production may be essential for these cells (van Lith et al. 2014; Mohrenz et al. 2013; Seltzer et al. 2010). To facilitate functional metabolic studies on glutaminolysis, we determined the optimum metabolic mapping methodology to demonstrate GDH activity in mouse tissues.

## Materials & Methods

### *Mouse Samples*

Various tissues from male control wild-type C57BI/6J mice were obtained from the Animal Institute of the Academic Medical Center. Cerebrum, cerebellum, liver, kidney, pancreas, tongue, small intestine, colon, stomach, spleen, lung, heart and skeletal muscle tissues were snap-frozen in liquid nitrogen and stored at -80C. Animals were treated in accordance with the Institutional Standards for Human Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee approved the experiments. Unfixed cryostat sections of all tissues except the lung were cut with a nominal thickness of 7 μm at -20C and stored at -80C. The nominal thickness of lung cryostat sections was 8 μm. Prior to metabolic mapping, tissue sections were air dried for 30 min at room temperature.

### *Metabolic Mapping*

We used tetrazolium salts for the metabolic mapping of the activity of dehydrogenases (Fig. 2). In this method, the dehydrogenase, in this case GDH, reduces $NAD(P)^+$ to NAD(P)H. NAD(P)H reduces an electron carrier that is present in the medium and subsequently reduces the water-soluble slightly yellow nitro blue tetrazolium (NitroBT) into a water-insoluble blue formazan precipitate. The absorbance of the precipitated formazan at the site of GDH activity is therefore a direct measure of GDH activity (Chieco et al. 2013; Van Noorden 2010; Jonker et al. 1996; Van Noorden and Frederiks 1992). This methodology enables the assessment of GDH activity in its intact cellular microenvironment when unfixed cryostat sections are used. Chemical fixation affects (usually inhibits) enzyme activity. For proper localization of the enzyme activity by the generated formazan, macromolecules have to be kept in the tissue section during enzyme incubation (Van Noorden 2010; Van Noorden and Vogels 1989). One of the best methods to achieve this is addition of the water-soluble polymer polyvinyl alcohol (PVA) to the incubation medium. In PVA-containing media, small molecules, such as substrates and coenzymes, can diffuse freely, but large molecules, such as proteins, cannot. Additionally, PVA keeps the tissue morphology intact. This methodology ensures posttranslational modifications to the enzyme and its microenvironment are kept intact as much as possible.

For metabolic mapping of GDH activity, liver tissue sections of three mice were incubated for 30 min at 37C in an aqueous solution of 18% PVA (Sigma-Aldrich, St. Louis, MO) in 0.1 M phosphate buffer (pH 8; Merck, Darmstadt, Germany), containing 5 mM NitroBT (Sigma-Aldrich), 3 mM $NAD^+$ or $NADP^+$ (Roche, Basel, Switzerland), 2 mM ADP (Roche), 0.32 mM phenazine methosulfate (PMS; Serva, Heidelberg, Germany) and 0–50 mM L-glutamate (Sigma-Aldrich). Three sections from each mouse liver were used for each glutamate concentration tested. For all further experiments, 10 mM glutamate was used and one tissue section per mouse was used. Control reactions were performed in the absence of either glutamate, ADP or $NAD(P)^+$.

PMS stock solution was kept in the dark at 4C. All other stock solutions of $NAD^+$, $NADP^+$, ADP, NitroBT and glutamate were prepared freshly before incubation. NitroBT was dissolved by heating NitroBT in equal amounts of 100% ethanol and dimethylformamide each in a final concentration of 2% v/v (0.34 M ethanol and 0.26 M dimethylformamide).

After incubation, tissue sections were rinsed using 0.1 mM phosphate buffer, pH 5.3, at 60C for 30 min to immediately stop the enzyme reaction and to remove the viscous incubation medium directly from the sections. Afterwards, sections were rinsed with tap water and distilled water and dried on a warm plate. After drying, the tissue sections were embedded in glycerol jelly as mounting medium (Dako, Glostrup, Denmark).

### Image Analysis

Image cytometry was performed to quantify the amount of NitroBT formazan in specific areas of the tissue sections according to Chieco et al. (2013). Images of all tissues were obtained using ImageJ (NIH, Bethesda, MD) (Abràmoff et al. 2004; Schneider et al. 2012) on a Vanox-T microscope fitted with a 20× objective (Olympus, Tokyo, Japan) and a Scion cfw-1312 gray scale camera (Scion, Tucson, AZ). The following areas in the tissues were recorded: liver, periportal and pericentral areas of lobules; pancreas, exocrine tissue; kidney, cortex; cerebrum, cortex; cerebellum, molecular layer, granular cell layer, Purkinje cell layer and whiter matter; stomach, gastric glands; small intestines, villi; colon, crypts; skeletal muscle, muscle tissue; spleen, red and white pulp; lung, alveoli; heart, muscle tissue; tongue, skeletal muscle. One area per tissue section was measured (for small intestines, two areas were measured). Images were recorded at a resolution of 1360×1024 pixels. Samples were illuminated with white light and filtered by an infrared blocking filter (Chieco et al. 2013; Jonker et al. 1997). To record exclusively absorbance of formazan, a 585-nm monochromatic filter was used (Butcher 1978). Calibration was performed with a 10-step calibration glass slide before absorbance measurements were recorded (Chieco et al. 2013). Where possible, the same regions in serial sections were analysed. When this was not feasible, comparable regions were analysed. Regions of interest in the images were selected with the ImageJ plugin, ObjectJ, and the mean absorbance was determined as a measure of in situ GDH activity.

### Statistical Analysis

Mean absorbance values obtained with ImageJ of all samples were corrected for nonspecific staining in the control reactions and converted with Excel 2013 (Microsoft Corporation, Redmond, WA) to μmol/mL/minute using the law of Lambert-Beer (Van Noorden and Frederiks 1992). This law states that $A=\epsilon \cdot c \cdot d$, with A as absorbance, ϵ as extinction coefficient (16,000 at 585 nm), c as the formazan concentration, and d as light traveling distance (nominal thickness of the sections, 7 or 8 μm, with a standard deviation of 0.7 μm) (De Witt Hamer et al. 2006; Butcher 1978). Statistical analysis was performed with Graphpad Prism 6 (GraphPad Software, La Jolla, CA). Substrate inhibition curve fitting was performed using the Haldane equation within Graphpad Prism 6.

## Results

### GDH Kinetics in Mouse Liver

To determine GDH kinetics, mouse liver tissue sections were metabolically mapped for GDH activity with eight different glutamate concentrations (0, 0.4, 1.4, 2, 5, 10, 30 and 50 mM) with either $NAD^+$ (Fig. 3) or $NADP^+$ (Fig. 4) as the coenzyme. The $K_m$ values of GDH for glutamate were similar when $NAD^+$ or $NADP^+$ was used as coenzyme (Fig. 5; Table 1), whereas the $V_{max}$ of GDH with $NAD^+$ was 2.5-fold higher than with $NADP^+$. $V_{max}$ and $K_m$ were optimal at 3 mM $NAD^+$ or $NADP^+$ (data not shown). GDH showed substrate inhibition both in the presence of $NAD^+$ and $NADP^+$ as the coenzyme. The dissociation constants ($K_i$) of this glutamate inhibition were 12.2 mM and 4.0 mM when $NAD^+$ or $NADP^+$ where used as the coenzyme, respectively. These $K_i$ values indicate that GDH was less inhibited by its substrate glutamate when $NAD^+$ was used as the coenzyme than when $NADP^+$ was used, although this effect was not significant (Students *t*-test, $p=0.29$). Moreover, GDH activity was completely inhibited at higher glutamate concentrations when $NADP^+$ was used as the coenzyme, but not when $NAD^+$ was used as the coenzyme (Fig. 5). Glutamate did not affect the pH of the incubation medium. Therefore, the lower GDH activity at high glutamate levels was not caused by lower pH. Figure 5 shows that GDH $V_{max}$ was determined with a glutamate concentration of 10 mM in the incubation medium.

### Distribution of GDH Activity in Liver

Specific GDH activity was evenly distributed over periportal and pericentral zones of liver lobules. Both test and control reactions showed more formazan formation in pericentral zones (Figs. 3 and 4) and the specific test minus control reaction showed no differences in GDH activity between periportal and pericentral regions. Also, no differences in the localization patterns of formazan formation in periportal and pericentral zones of GDH activity were found when $NAD^+$ or $NADP^+$ were used as coenzymes (Figs. 3 and 4).

### GDH Activity in Different Mouse Tissues

GDH activity in various mouse tissues was determined with either $NAD^+$ or $NADP^+$ as coenzyme (Fig. 6). Liver had the highest GDH activity (at least 4.5-fold higher activity over other tissues when $NAD^+$ was used as coenzyme and at least 3.5-fold with $NADP^+$ as coenzyme). $NADP^+$-dependent GDH activity was only observed in the liver and pancreas. With $NAD^+$ used as a cofactor, activity in the cerebellum, small intestines and heart was also found.

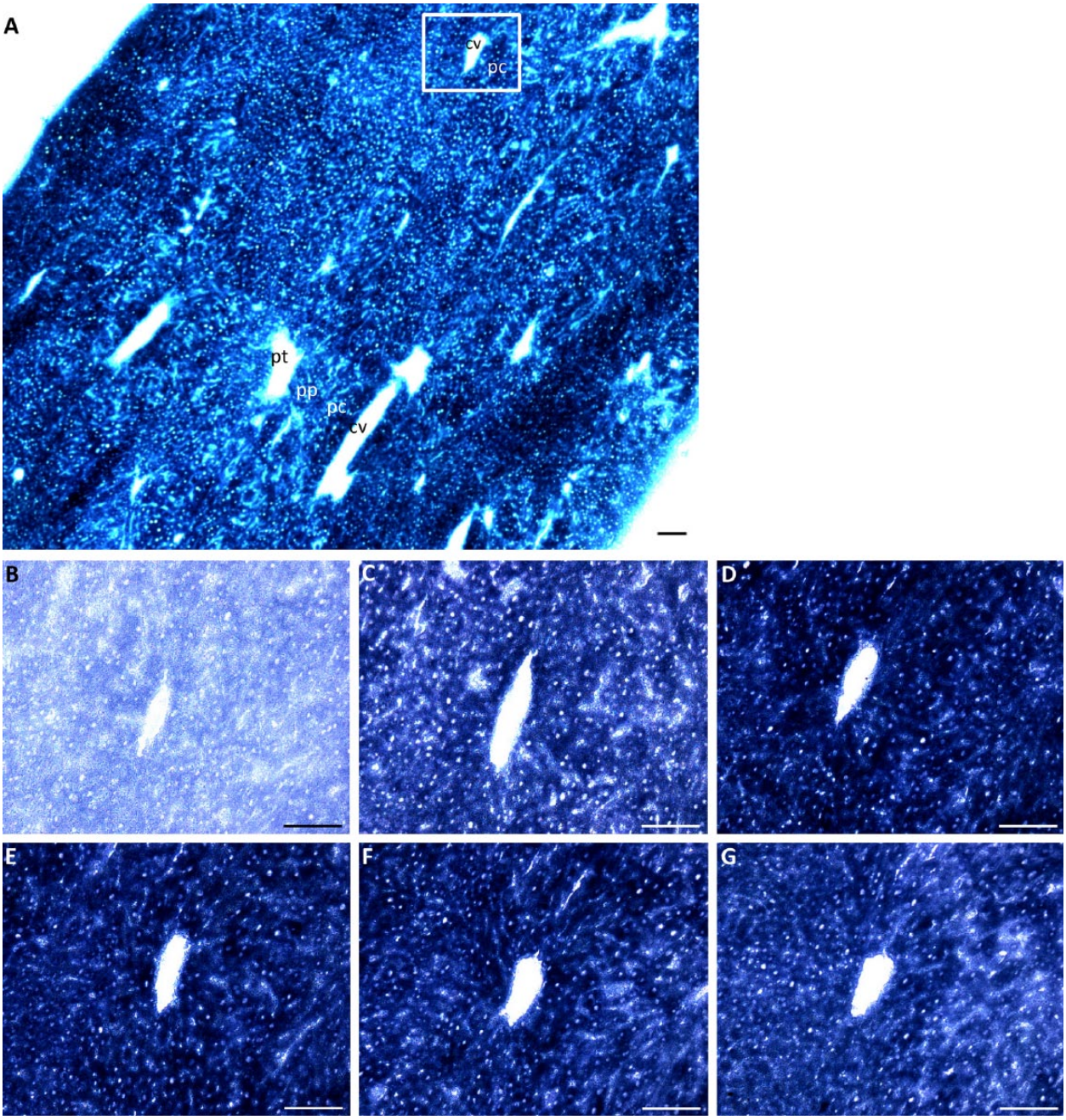


**Figure 3.** GDH activity staining with $NAD^+$ as coenzyme and various glutamate concentrations. (A) Overview of GDH activity in a mouse liver cryostat section as demonstrated in the presence of 10 mM glutamate. GDH activity was stained in the presence of (B) 0 mM, (C) 2 mM, (D) 5 mM, (E) 10 mM (F) 30 mM, and (G) 50 mM glutamate. Images (B–G) are of the same area in serial sections of (A), as indicated by the rectangle. Abbreviations: pt, portal tract; pp, periportal zone; pc, pericentral zone; cv, central vein. Scale, 100 µm.

## Discussion

In this study, we examined the effect of varying the glutamate concentration in the presence of the coenzymes $NAD^+$ and $NADP^+$ on GDH activity in the liver. With either coenzyme, substrate inhibition was found at glutamate concentrations higher than 10 mM, which is in accordance with previous findings (Li et al. 2009; Smith and Stanley 2008; Bailey et al. 1982; Engel and Dalziel 1969). With $NADP^+$ used as coenzyme instead of $NAD^+$, stronger substrate inhibition was found ($K_i$ values of 4.0 mM and 12.2 mM, respectively). The $K_i$ values of substrate inhibition

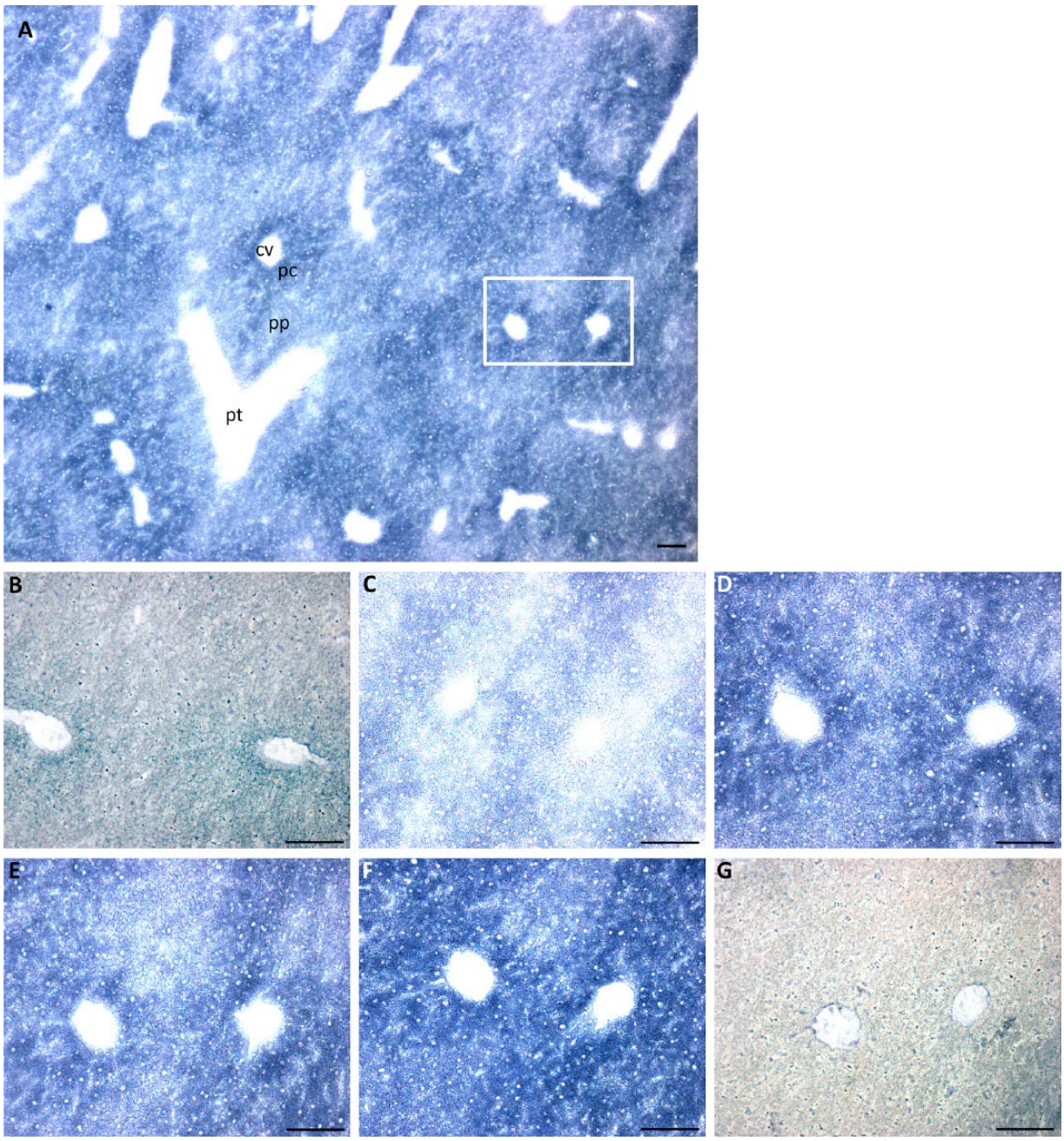


**Figure 4.** GDH activity staining with $NADP^+$ as coenzyme and various glutamate concentrations. (A) Overview of GDH activity in a mouse liver cryostat section as demonstrated in the presence of 10 mM glutamate. GDH activity was stained in the presence of (B) 0 mM, (C) 2 mM, (D) 5 mM, (E) 10 mM, (F) 30 mM, and (G) 50 mM glutamate. Images (B–G) are of the same area in serial sections of (A), as indicated by the rectangle. Abbreviations: pt, portal tract; pp, periportal zone; pc, pericentral zone; cv, central vein. Scale, 100 µm.

obtained with metabolic mapping are, to our knowledge, described for the first time here. Substrate inhibition of GDH occurs by the formation of an abortive complex of the enzyme with NAD(P)H and glutamate, which is destabilized by ADP (Bailey et al. 1982). Interestingly, the literature describes only substrate inhibition in the presence of phosphate (Li et al. 2009; Lee et al. 1999; Jonker et al. 1996; Geerts et al. 1996; Cho et al. 1995; Maly and Sasse 1991; Bailey et al. 1982; Engel and Dalziel 1969), indicating that the formation of these abortive complexes is

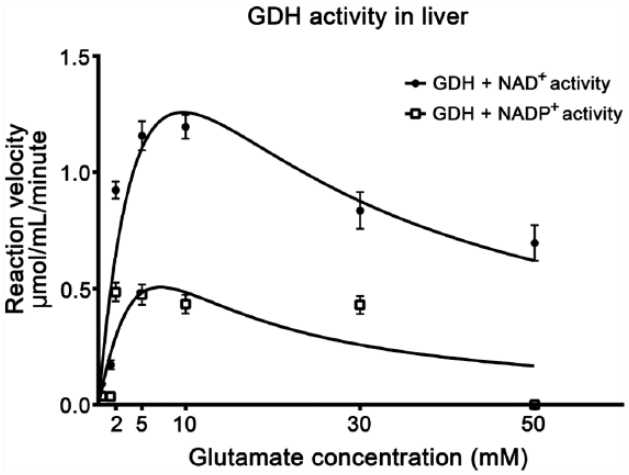


**Figure 5.** Kinetics of GDH activity in mouse liver against various glutamate concentrations in the presence of $NAD^+$ (●) or $NADP^+$ (□) as coenzyme. Error bars indicate SEM ($n$=3).

**Table 1.** GDH Kinetic Parameters.

| | GDH + $NAD^+$ | GDH + $NADP^+$ |
|---|---|---|
| $V_{max}$ | 1.26 | 0.51 |
| $K_m$ | 1.92 | 1.66 |
| $K_i$ | 12.2 | 3.95 |

$V_{max}$, maximal enzyme activity in µmol/mL/minute; $K_m$, Michaelis–Menten constant in mM glutamate; $K_i$, inhibitory constant of glutamate (mM).

mediated by phosphate ions by an unknown mechanism. Further, the biological function of substrate inhibition by these phosphate ions is unknown.

The $K_m$ values of GDH that we found with the use of metabolic mapping are 2- to 3-fold lower for $NAD^+$ and 5- to 13-fold lower for $NADP^+$ than previously reported for GDH purified from homogenates in vitro (Lee et al. 1999; Cho et al. 1995). Yet, this is in line with the higher concentrations of $NAD^+$ and ADP that we applied, which resulted in lower $K_m$ values. On the other hand, the $K_m$ value of GDH in the presence of $NAD^+$ (1.92 mM) is comparable to those previously found by metabolic mapping (2.5 mM; Jonker et al. 1996). The differences between GDH kinetics in intact tissue sections and homogenates indicate that the microenvironment and the microenvironmental conditions have significant effects on the activity of GDH.

$NAD^+$-dependent GDH $V_{max}$ was approximately 10-fold lower than previously found by metabolic mapping (Geerts et al. 1996), whereas the $V_{max}$ value of $NADP^+$-dependent GDH has not been determined before by metabolic mapping. This $V_{max}$ was determined in the presence of 100 mM glutamate in a phosphate-free medium, giving a higher $V_{max}$ value, as substrate inhibition does not occur in phosphate-free media. The lower $V_{max}$ found when $NADP^+$ was used as coenzyme instead of $NAD^+$ is in accordance with previous findings (Maly and Sasse 1991).

With $NAD^+$ as the coenzyme, GDH activity was mainly found in the mouse liver, where it is involved in ammonia homeostasis and urea genesis (Spanaki and Plaitakis 2012; Treberg et al. 2010; Stanley et al. 2000, 1998; Boon et al. 1999; Curthoys 1995). In our study, GDH activity was not different in periportal to pericentral zonation. This is partly in accordance with previous findings, which report both homogenous and heterogeneous distribution of GDH activity and mRNA levels (Boon et al. 1999; Maly and Sasse 1991; Sokal et al. 1989; Lamers et al. 1988). This is explained by the dynamic GDH expression, meaning that changes in the zonation of GDH activity can occur depending on diet, sex and endocrine activity (Boon et al. 1999; Lamers et al. 1988). Low $NAD^+$-dependent GDH activity was found in other tissues, such as the pancreas, cerebellum, small intestines and heart.

With $NADP^+$ as the coenzyme, GDH activity was found almost exclusively in the liver. This indicates that GDH metabolism is largely dependent on $NAD^+$. The produced NADPH by GDH can be used to reduce cytochrome P450, which metabolises xenobiotics and steroids (Pandey and Flück 2013; Frederiks et al. 2003). GDH serves as an additional pathway to generate reductive power in addition to the pentose-phosphate pathway and malic enzyme to maintain NADPH levels in liver (Frederiks et al. 2007, 2003; Kruger and von Schaewen 2003). Additionally, NADPH produced by GDH can be used for reductive power for lipid and cholesterol synthesis (DeBerardinis et al. 2007; Koh et al. 2004).

Surprisingly, GDH showed very low activity in mice cerebrum and only a modest activity in the cerebellum (Fig. 6). GDH activity has been metabolically mapped by Kugler and Baier (1992) in rat brain, where it showed an activity of 1 µmol/mL/min. Yet, Kugler and Baier (1992) used phosphate-free incubation media, which gives no substrate inhibition, resulting in higher enzyme activities at high glutamate concentrations, as previously described. We used phosphate-rich media to ensure stable GDH and it resembles better the in vivo conditions (Dieter et al. 1981; Frieden 1963). Our results are also in line with findings that deletion of *GLUD1* in the central nervous system does not affect mice, meaning that GDH is not essential in the brain (Frigerio et al. 2012). The differences in GDH activity between rat and mouse brain are in accordance with the mouse and rat gene expression profiles in the Gene Expression Atlas and BioGPS, showing that mice have a high GDH expression in liver only whereas rat has a ubiquitous expression in all tissues (Gene Expression Atlas (http://www.ebi.ac.uk/gxa); Keane et al. 2011; Wu et al. 2009; Lattin et al. 2008; Su et al. 2004). It is expected that

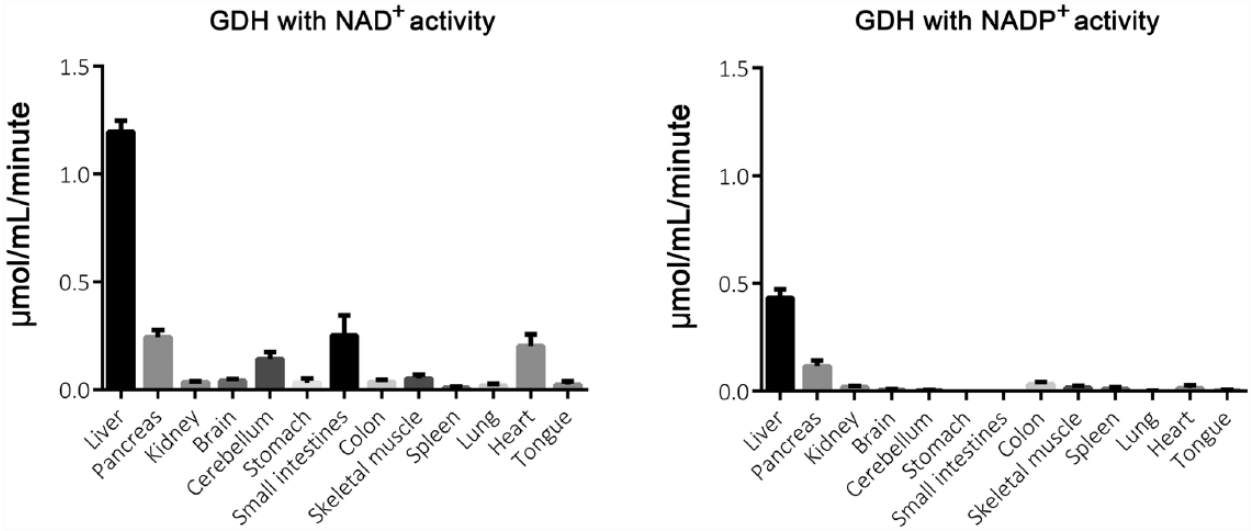


**Figure 6.** GDH activity in the presence of 10 mM glutamate and (A) $NAD^+$ or (B) $NADP^+$ as coenzyme after correction for nonspecific background staining in the absence of substrate. Activity is presented as mean $V_{max}$ ± SEM ($n$=3).

GDH activity in human brain is higher as GDH gene expression is relatively high in human brain (Gene Expression Atlas: http://www.ebi.ac.uk/gxa; Wu et al. 2009; Su et al. 2004). This is also confirmed by our measurements of GDH activity in human brain tissue (data not shown).

In conclusion, we showed that GDH kinetics are different when coenzymes $NAD^+$ or $NADP^+$ are used. With $NADP^+$ as the coenzyme, lower GDH activity was found. Yet, the $K_m$ values did not differ when either $NAD^+$ or $NADP^+$ were used as the coenzyme. Additionally, GDH showed substrate inhibition, which only occurs in the presence of phosphate ions. This inhibition was more prominent when $NADP^+$ was used as the coenzyme instead of $NAD^+$. Tissue-specific GDH activity determination in mice showed that GDH is predominantly active in liver and showed low activity in pancreas, cerebellum, small intestines and heart.

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## Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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