Amino Acids (2012) 42:199–205
DOI 10.1007/s00726-011-0864-8

REVIEW ARTICLE

The transsulfuration pathway: a source of cysteine for glutathione in astrocytes

Gethin J. McBean

Received: 20 December 2010 / Accepted: 17 February 2011 / Published online: 3 March 2011

Abstract Astrocyte cells require cysteine as a substrate for glutamate cysteine ligase (γ-glutamylcysteine synthase; EC 6.3.2.2) catalyst of the rate-limiting step of the γ-glutamylcycle leading to formation of glutathione (L-γ-glutamyl-L-cysteinyl-glycine; GSH). In both astrocytes and glioblastoma/astrocytoma cells, the majority of cysteine originates from reduction of cystine imported by the x_c^- cystine-glutamate exchanger. However, the transsulfuration pathway, which supplies cysteine from the indispensable amino acid, methionine, has recently been identified as a significant contributor to GSH synthesis in astrocytes. The purpose of this review is to evaluate the importance of the transsulfuration pathway in these cells, particularly in the context of a reserve pathway that channels methionine towards cysteine when the demand for glutathione is high, or under conditions in which the supply of cystine by the x_c^- exchanger may be compromised.

Keywords Transsulfuration · Brain · Astrocyte · Cysteine · Cystathionine-γ-lyase · Cystathionine-β-synthase · Glutathione

Abbreviations

GSH	L-γ-glutamyl-L-cysteinyl-glycine (glutathione)
GSSG	Oxidised form of glutathione
DEM	Diethylmaleate
H_2S	Hydrogen sulfide
IL-6	Interleukin-6
JNK	c-Jun-*N*-terminal kinase
MAPK	Mitogen-activated protein kinase
NF-κB	Nuclear factorκB
SAPK	Stress-activated protein kinase
TNFα	Tumour necrosis factorα
xCT	Subunit of the x_c^- cystine-glutamate exchanger

G. J. McBean (✉)
UCD School of Biomolecular and Biomedical Science, Conway Institute, University College, Dublin 4, Ireland
e-mail: gethin.mcbean@ucd.ie

An overview of sulfur amino acid metabolism and the transsulfuration pathway

Sulfur amino acid metabolism is critically important in mammalian cells and is linked to provision of methyl groups for a large number of biochemical processes. Dietary methionine is activated by conversion to S-adenosylmethionine in an ATP-dependent reaction catalysed by methionine adenosyltransferase that, through action of methyltransferase, yields S-adenosylhomocysteine, followed by homocysteine (Fig. 1). Homocysteine can be either re-methylated back to methionine using a methyl group provided by methyl tetrahydrofolate, or irreversibly converted into cysteine via transsulfuration. In hepatic cells in particular, dietary cysteine acts in a methionine-sparing capacity and promotes re-methylation of homocysteine. Should the supply of cysteine be insufficient, homocysteine is channelled into the transsulfuration pathway by conjugation with serine to provide cystathionine, which is the immediate precursor of cysteine (Fig. 1). Cysteine is the source of both taurine and hydrogen sulfide (H_2S) besides being the essential amino acid constituent in the functional (CXXC) motif of the major cellular antioxidant families, which include GSH, glutaredoxins, thioredoxins and peroxiredoxins (Benight et al. 2009). It has been estimated that, in mammalian liver, approximately 50% of cysteine in GSH is derived from methionine via transsulfuration (Banerjee and Zou 2005). The focus of this article is to

review the current understanding of the transsulfuration pathway and its regulatory enzymes, and to assess the importance of this pathway in antioxidant defence in the brain and in astrocytes, in particular.

Regulatory enzymes of the transsulfuration pathway

The regulatory enzymes of the transsulfuration pathway are the pyridoxal-phosphate-dependent enzymes, cystathionine-β-synthase (L-serine hydro-lyase (adding homocysteine); EC 4.2.1.22) and cystathionine-γ-lyase (L-cystathionine cysteine-lyase; EC 4.4.1.1). Cystathionine-β-synthase is a heme-containing enzyme that is subject to regulatory control, as it catalyses the first committed step of the transsulfuration pathway (Aitken and Kirsch 2005; Banerjee and Zou 2005). Regulation of metabolic flux through the competing transmethylation and transsulfuration pathways occurs at several levels. S-adenosylmethionine is an allosteric activator of cystathionine-β-synthase that effectively channels excess sulfur towards metabolism when methionine levels are high. The presence of a heme cofactor suggests redox-sensitive regulation of enzyme activity and this has been verified by the observation that reduction of cystathionine-β-synthase to the ferrous state is accompanied by a twofold loss in activity that can be reversed on re-oxidation with ferricyanide (Banerjee and Zou 2005). Genetic defects of cystathionine-β-synthase are the most common hereditary causes of hyperhomocysteinemia in humans.

Cysteine is a competitive inhibitor of cystathionine-γ-lyase, which predicts that, at the physiological cysteine:cystathionine ratio in liver, the enzyme is inhibited under normal conditions (Martín et al. 2007). This would prevent accumulation of intracellular cysteine, which would likely undergo auto-oxidation to cystine and loss of redox balance. Cystathionine-γ-lyase in foetal hepatocytes is upregulated at both the mRNA and protein levels by mild oxidative stress and new-born infants and rat pups have a higher cystathionine-γ-lyase activity than fetuses (but not premature infants; Martín et al. 2007). Severe oxidative stress causes inhibition of the enzyme. Kidney cystathionine-γ-lyase is upregulated at the protein level following ischaemia/reperfusion injury in mice (Tripatara et al. 2009). A decrease in plasma homocysteine and upregulation of the enzyme in rat liver and lung following dietary docosahexaenoic acid administration has been noted (Huang et al. 2010).

A deficiency in the transsulfuration pathway leads to excessive homocysteine production, loss of cellular redox homeostasis and reduced GSH production (Rosado et al. 2007). Resulting changes in DNA methylation, coupled with DNA damage due to loss of antioxidant protection, are viewed as potential causes for tumour growth and expansion (Rosado et al. 2007).

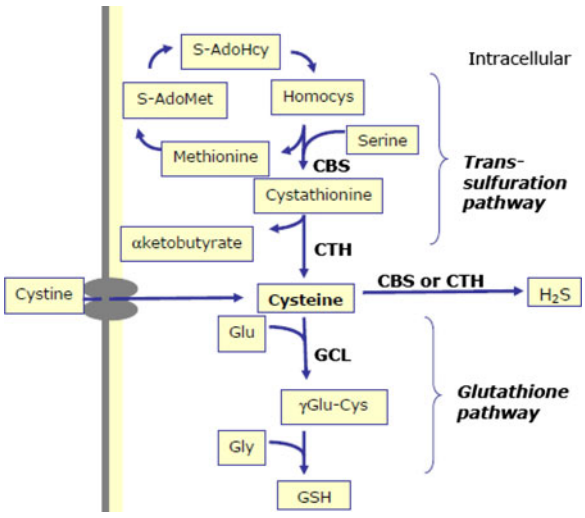

Fig. 1 Schematic representation of the transsulfuration pathway, showing cysteine as precursor of GSH and H_2S. *CBS* cystathionine-β-synthase, *CTH* cystathionine-γ-lyase, *CGL* glutamate cysteine ligase

H_2S production by cystathionine-β-synthase and cystathionine-γ-lyase

Much information on the activity and localisation of cystathionine-β-synthase and cystathionine-γ-lyase comes from the fact that both enzymes utilise cysteine or homocysteine to produce the gas H_2S (Fig. 1). H_2S is an endogenous anti-inflammatory, antioxidant and neuroprotective agent that may have therapeutic potential in a range of neurodegenerative diseases (Kimura 2010; Lee et al. 2009). There is therefore considerable interest in its mechanism of biosynthesis and several authors have provided details of this process in both nervous and non-nervous tissues. Cysteine is the preferred substrate for H_2S production by either enzyme and accounts for 70% of the total amount of the gas produced under normal conditions. Cystathionine-β-synthase generates serine as co-product of H_2S formation, whereas cystathionine-γ-lyase catalyses conversion of cysteine to pyruvate, H_2S and NH_3 (Lee et al. 2009). Singh et al. (2009) have established that the preferred reaction mechanism of cystathionine-β-synthase is by beta replacement of cysteine by homocysteine, as opposed to the alternative mechanisms of beta-elimination of cysteine to generate serine or condensation of two molecules of cysteine to cystathionine (Singh et al. 2009). In the reaction catalysed by cystathionine-γ-lyase, alpha, beta elimination of cysteine normally accounts for 70% of total H_2S, but in conditions such as hyperhomocysteinemia, the relative contribution of homocysteine to H_2S production increases to as much as 90% (Chiku et al. 2009).

Kinetic data have been used to predict the relative contribution of cystathionine-β-synthase and cystathionine-γ-lyase to H_2S production at physiologically relevant substrate concentrations. It is estimated that cystathionine-β-synthase would account for between 25–70% of the total amount of H_2S produced, depending on the level of activation of the enzyme by S-adenosylmethionine (Singh et al. 2009).

Localisation of cystathionine-β-synthase and cystathionine-γ-lyase

Cystathionine-β-synthase is abundant throughout the brain, and has been recorded as being particularly highly expressed in Purkinje cells and in the hippocampus (Li et al. 2009) and in cerebellar Bergmann glia and astrocytes (Kimura 2010 and references therein). Cystathionine-γ-lyase is expressed more restrictedly than cystathionine-β-synthase, and until recently was thought to be entirely absent from brain, which gave rise to the view that the transsulfuration pathway was not functional in this tissue (Grange et al. 1992).

Accordingly, studies using selective inhibitors for cystathionine-β-synthase and cystathionine-γ-lyase and gene knockout experiments promote the view that cystathionine-β-synthase is responsible for H_2S production in human brain, whereas cystathionine-γ-lyase is the dominant enzyme in the vasculature (Lee et al. 2009). For example, cystathionine-γ-lyase gene deletion in mice caused reduced H_2S production in serum, heart and aorta, but not brain. Similarly, immunolabelling of cystathionine-γ-lyase was observed in vessel walls in human brain, whereas strong staining for cystathionine-β-synthase was detected in astrocytes in the hippocampus and precentral cortex (Lee et al. 2009). Pong et al. (2007) recorded immunostaining for cystathionine-γ-lyase in mouse liver, but not brain, in confirmation of the fact that enzyme activity in liver is 100 times higher than in brain. However, there are conflicting views on the level of expression and activity of cystathionine-γ-lyase in the brain and not all studies concur with the view that the enzyme is confined to non-nervous tissue. Species differences may be important in this regard. For example, activity of the enzyme in human brain is 100 times that of mouse brain (Pong et al. 2007). In the salamander, expression of cystathionine-γ-lyase is detectable in retinal Müller cells, cerebellum and liver (Pong et al. 2007). Similarly, H_2S production in the porcine retina is blocked by the selective inhibitor of cystathionine-γ-lyase, DL-propargylglycine, signifying an active contribution of the enzyme in these cells (Opere et al. 2009). Functional activity of cystathionine-γ-lyase in the brain of Swiss albino mice was observed by Karunakaran et al. (2007), which was reduced by 68% 18 h after administration of DL-propargylglycine (Diwakar and Ravindranath 2007).

Cystathionine-β-synthase and cystathionine-γ-lyase in glial cells

The rate of synthesis of H_2S in human astrocytes isolated from surgically resected temporal lobe tissue was sevenfold higher than in microglial cells and tenfold higher than in neuronal cell lines and was significantly reduced in all these cell types by the cystathionine-β-synthase inhibitor, hydroxylamine, but not by DL-propargylglycine (Lee et al. 2009). Once again, there is conflicting data on the existence of cystathionine-γ-lyase in glial cells: Lee et al. (2006) identified expression of the gene in mouse microglial cells by reverse transcriptase-polymerase chain reaction (RT-PCR) and a corresponding reduction in endogenous H_2S following inhibition of cystathionine-γ-lyase with DL-propargylglycine and a second inhibitor of the enzyme, beta-cyano-L-alanine. However, primary rat astroglial cultures and glioma cells have demonstrable cystathionine-γ-lyase activity (Kandil et al. 2010; Kranich et al. 1998; Vitvitsky et al. 2006) and cystathionine can be used as an alternative to cysteine as a precursor for GSH synthesis (Kranich et al. 1998).

Whilst investigations into the variable distribution and activity of cystathionine-β-synthase and cystathionine-γ-lyase are informative in regard to regional selectivity in the mechanism of H_2S production, evidence of a functional transsulfuration pathway that is geared towards production of cysteine requires co-localisation and demonstrable activity of both cystathionine-β-synthase and cystathionine-γ-lyase. For this reason and because cystathionine-β-synthase is strongly expressed in brain, support for a transsulfuration pathway in this tissue relies on a demonstration of functionally active cystathionine-γ-lyase. Consequently, the lack of consensus in the literature in regard to the expression of this enzyme in brain has prompted the view that transsulfuration has little relevance in cerebral cysteine metabolism and production of GSH (Grange et al. 1992). However, more recent data supports the view that transsulfuration plays a physiological role in GSH production in the brain and in astrocytes in particular.

GSH synthesis in astrocytes—a role for the transsulfuration pathway?

As with the vast majority of cells, the rate of GSH biosynthesis in astrocytes must be regulated to compensate for loss of the thiol due to export following oxidation to GSSG, use in conjugation reactions, mercapturic acid production

and glutathionylation. In the brain, de novo synthesis of GSH in astrocytes occurs in a two-step process that is catalysed by γ-glutamate cysteine ligase (γ-glutamylcysteine synthase; EC 6.3.2.2.) and GSH synthase (EC 6.3.2.3), respectively. Both of these reactions require ATP. The rate of biosynthesis is controlled by cysteine availability, activity of γ-glutamate cysteine ligase, which is feedback inhibited by GSH and the level of expression of the enzyme. Astrocytes export GSH for use by neurones (Dringen et al. 1999). Since the first work by Bannai and co-workers (1984) almost 30 years ago, a large body of evidence has contributed to the view that cysteine for GSH is supplied to astrocytes in its oxidised form (cystine) from the extracellular medium by the electro-neutral cystine-glutamate (x_c^-) exchanger, which, on entry to the cystosol, is reduced to cysteine (Kato et al. 1993; McBean 2002; Sato et al. 2000). A full discussion of the x_c^- exchanger is made by Lewerenz et al. (2011) and Conrad and Sato (2011).

Nonetheless, several lines of evidence have challenged the traditional stance that the x_c^- exchanger is the exclusive mechanism for provision of cysteine for GSH in astrocytes. Kranich et al. (1998) observed that cystathionine could partially substitute for cysteine as precursor for synthesis of GSH in primary rat astroglial cultures, which could only happen if cystathionine-γ-lyase was active (although, oddly, methionine did not substitute for cysteine as GSH precursor in their experiments). More recently, Diwakar and Ravindranath (2007) showed depletion of GSH (38% at 6 h post-administration) in mouse brain following subcutaneous application of DL-propargylglycine that was accompanied by a significant reduction in the activity of brain cystathionine-γ-lyase. Vitvitsky et al. (2006) have provided additional evidence for an active transsulfuration pathway in mouse brain slices, cortical neuronal cultures and primary astrocytes. Astrocytes and neurones displayed depletion of GSH following incubation of the cells with DL-propargylglycine, which achieved a level of 40% of control after 10 h. Further evidence for the existence of a functional transsulfuration pathway in brain slices, astrocytes and neurones came from the observation that incubation with radiolabelled (^{35}S) methionine resulted in incorporation of the label into GSH. Once again, the only possible route for labelling of the S-atom in GSH from methionine is via transsulfuration to cysteine. Further confirmation of an active transsulfuration pathway in rat glioma cells and primary astrocytes has come from data showing a 23% reduction in GSH after 24 h incubation of the cells with DL-propargylglycine (Kandil et al. 2010). In all reports to date, the contribution of the transsulfuration pathway to GSH in brain slices, astrocytes or microglia is between 23 and 40%, which is less than the contribution observed in hepatic cells (approximately 50%; Beatty and Reed 1980; Mosharov et al. 2000). Thus, it can be concluded that under normal conditions, cysteine derived from the transsulfuration pathway is a minor, but significant, contributor to cellular GSH in brain.

Oxidative stress and the transsulfuration pathway

Oxidative stress is a positive regulator of the transsulfuration pathway. In a physiological context, oxidative stress activates cystathionine-β-synthase, thus promoting conversion of methionine to cysteine and enabling increased synthesis of GSH. This process involves endogenous reactive oxygen species-targeted proteolysis of the enzyme by a mechanism that is incompletely understood. At the transcriptional level, cystathionine-β-synthase is activated by glucocorticoids and inhibited by insulin, whereas its catalytic activity is regulated by binding of nitric oxide or carbon monoxide to the heme pocket of the enzymes (Li et al. 2009). There are conflicting reports on the response of cystathionine-β-synthase to oxidative stress in astrocytes. On the one hand, evidence has been presented that epidermal growth factor, cAMP and tumour necrosis factorα (TNFα), all cause upregulation of the enzyme via enhanced transcription (Kimura 2010). Conversely, Lee et al. (2009) have shown that inflammatory activation of microglia and astrocytes caused induction of nuclear factor kappaB (NF-κB), release of TNFα, interleukin-6 (IL-6) and nitrite ions that all resulted in down-regulation of cystathionine-β-synthase and inhibited production of H_2S. Interestingly, H_2S production in vascular cells, which use cystathionine-γ-lyase rather than cystathionine-β-synthase, was unaffected by inflammatory agents, signifying that different regulatory pathways control the activity of these enzymes in different cell types.

There is increasing evidence that the transsulfuration pathway in astrocytes may function in a reserve capacity that can be used to supply cysteine when provision of the amino acid via the cystine-glutamate exchanger is limited, or when oxidative stress places an increased demand on GSH synthesis. Experiments have shown that flux through the transsulfuration pathway increases during oxidative stress conditions, as achieved by exposure of astrocytes to *tert*-butylhydroperoxide for 10 h, and a concomitant increase in GSH synthesis (facilitated by rapid upregulation of γ-glutamate cysteine ligase; see Krejsa et al. 2010) (Vitvitsky et al. 2006). Similarly, the expression and functional activity of cystathionine-γ-lyase increases following depletion of GSH by diethylmaleate in rat glioma cells and in primary astrocytes (Kandil et al. 2010; Fig. 2). The same result was observed on depletion of GSH by blockade of the cystine-glutamate exchanger: in this case, the contribution of the transsulfuration pathway to GSH

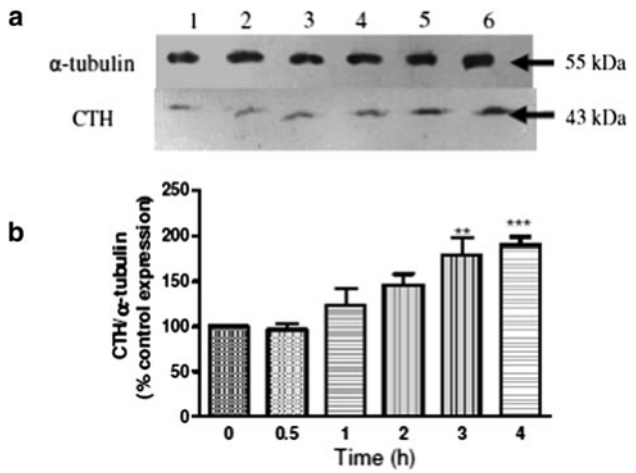

Fig. 2 Increase in cystathionine-γ-lyase (CTH) expression in rat primary cortical astrocytes by DEM. Cells were treated for 0–4 h with 100 μM DEM and the level of cystathionine-γ-lyase expression was normalised to the corresponding α-tubulin detected. (**a**) Representative Western blot of cystathionine-γ-lyase and α-tubulin expression in each sample, (**b**) data presented as a percentage of the cystathionine-γ-lyase expressed in the untreated group of $n = 3$ experiments. $^{**}p < 0.01$; $^{***}p < 0.001$ compared to untreated group, using one-way ANOVA

synthesis rose from 23 to 61% after 24 h and to 80% after 48 h (Kandil et al. 2010).

Further investigations have established that depletion of GSH in rat glioma cells by mild oxidative stress results in a c-Jun-*N*-terminal kinase (JNK) and p38-mitogen-activated protein kinase ($p38^{MAPK}$)-mediated increase in expression of cystathionine-γ-lyase and up-regulation of the transsulfuration pathway that promotes GSH synthesis (Kandil et al. 2010). A similar mechanism occurs in primary rat astrocytes (Kandil et al., unpublished observations). $P38^{MAPK}$ and JNK are two isoforms of MAPK that are activated by dual phosphorylation on both a tyrosine and a threonine and become activated by environmental stresses, such as irradiation, DNA-damaging agents, heat shock and inflammatory cytokines (Kyriakas and Avruch 1996). JNK phosphorylation takes place in the final step of the stress-activated protein kinase (SAPK) pathway that occurs in many cells as a prelude to apoptosis. It is likely that GSH depletion in astrocytes triggers activation of both pathways, thus stimulating the transsulfuration pathway and channelling methionine towards production of cysteine. A similar mechanism may take place in foetal hepatocytes, as moderate oxidative stress acts as a signal to up-regulate cystathionine-γ-lyase activity in these cells during the foetal-to-neonatal transition (Martín et al. 2007). Information on the mechanism of upregulation of cystathionine-γ-lyase by GSH depletion in astrocytes requires further investigation. However, it is worth noting that in murine hepatocytes, DEM-mediated cytosolic GSH depletion was associated with inhibition of TNFα-induced NF-κB transactivation of anti-apoptotic genes (including inducible nitric oxide synthase; Matsumaru et al. 2003). It was concluded that extra-mitochondrial GSH depletion alters the thiol redox state, leading to inhibition of NF-κB transactivation of survival genes and to sustained activation of JNK, both of which contribute to sensitisation of the cells to TNF-induced apoptosis.

Functional importance of the transsulfuration pathway in astrocytes

Notwithstanding the evidence that GSH depletion in astrocytes triggers up-regulation of the transsulfuration pathway that enables an increased supply of cysteine, further experimentation is required to establish fully the relative importance of this pathway, compared to the x_c^- exchanger, to intracellular cysteine during oxidative stress. Certainly, exposure of mouse retinal Müller cells to xanthine:xanthine oxidase for 6 h causes up-regulation of the x_c^- exchanger, accompanied by enhanced sodium-independent uptake of radiolabelled glutamate (a measure of exchanger activity) by up to 89% of control (Dun et al. 2006; Mysona et al. 2009). Similarly, DEM causes increased expression of the xCT subunit of the x_c^- exchanger in human vascular smooth muscle cells that leads to a restoration of GSH by 24 h (Ruiz et al. 2003). Both of these observations might argue against increasing flux though the transsulfuration pathway as a compensatory mechanism for augmenting GSH during oxidative stress. However, evidence suggests that up-regulation of the x_c^- exchanger in astrocytes drives a highly efficient cystine/cysteine redox cycle that, through export of cysteine, creates a reducing extracellular environment that effectively by-passes the need to export GSH (Banjac et al. 2008). If that is the case, then it is likely that intracellular GSH requires an alternative source of cysteine, such as could be provided by the transsulfuration pathway.

There may be situations in which import of cystine via the x_c^- exchanger is compromised and would therefore limit the capacity of the cell to synthesise GSH, if an alternative pathway was unavailable. Threats to neuronal survival, such as an increase in extracellular glutamate, promote GSH release from astrocytes in a presumed bid to provide protection to neurones (Frade et al. 2008). An excess of glutamate could out-compete cystine as a substrate for the x_c^- exchanger, whereas an increase in extracellular GSH would alter the redox balance in favour of cysteine. It is possible that both of these situations would damage uptake of cystine by the exchanger, to the extent that astrocytes would require provision of cysteine by the transsulfuration pathway.

Cancer cells have a high demand for GSH to promote cell growth and division. The requirement for cysteine is furnished via the x_c^- exchanger, which, as recorded in the case of gliomas, releases glutamate that damages surrounding neurones, thus providing space for tumour growth (Sontheimer 2008). Consequently, blockade of the exchanger is viewed as a potential target for cancer therapy (Chung and Sontheimer 2009; Lo et al. 2008; Savaskan et al. 2008) and has proven successful in experimental models as a means of limiting glutamate release. However, other evidence indicates that methionine uptake in gliomas is high and correlates positively with tumour viability (Kato et al. 2008), which may indicate a greater dependence on transsulfuration in these cells than in normal astrocytes. Additional research on the extent of upregulation of the transsulfuration pathway is required before one can be sure of the effectiveness of a strategy that is aimed at limiting GSH production by blockade of the x_c^- exchanger.

Conclusion

In summary, research has shown that the transsulfuration pathway in astrocytes is active and may be particularly important as a compensatory process that will supply cysteine for GSH synthesis as a response to mild or moderate oxidative stress, or when import of this amino acid by the x_c^- exchanger is limited. Further experimentation is required for a full understanding of how changes in activity of the regulatory enzymes of this pathway, cystathionine-β-synthase or cystathionine-γ-lyase operate to maintain a balance between the fluctuating demands for H_2S, cysteine and GSH in normal astrocytes and in astrocytoma/glioblastoma cells.

Acknowledgments The author's research in this area is supported by Science Foundation Ireland.

References

Aitken SM, Kirsch JF (2005) The enzymology of cystathionine biosynthesis: strategies for the control of substrate and reaction specificity. Arch Biochem Biophys 433:166–175

Banerjee R, Zou CG (2005) Redox regulation and reaction mechanism of human cystathionine-beta-synthase: a PLP-dependent hemesensor protein. Arch Biochem Biophys 433:144–156

Banjac A, Perisic T, Sato H, Seiler A, Bannai S, Weiss N et al (2008) The cystine/cysteine cycle: a redox cycle regulating susceptibility versus resistance to cell death. Oncogene 27:1618–1628

Bannai S (1984) Induction of cystine and glutamate transport activity in human fibroblasts by diethylmaleate and other electrophilic agents. J Biol Chem 259:2435–2440

Beatty PW, Reed DJ (1980) Involvement of the cystathionine pathway in the biosynthesis of glutathione by isolated rat hepatocytes. Arch Biochem Biophys 204:80–87

Benight N, Burrin DG, Stoll B (2009) Intestinal metabolism of sulfur amino acids. In: Masella R, Mazza G (eds) Glutathione and sulfur amino acids in human health and disease. Wiley, New Jersey, pp 47–72

Chiku T, Padovani D, Zhu W, Singh S, Vitvitsky V, Banerjee R (2009) H_2S biogenesis by human cystathionine gamma-lyase leads to the novel sulfur metabolites lanthionine and homolanthionine and is responsive to the grade of hyperhomocysteinemia. J Biol Chem 284:11601–11612

Chung WJ, Sontheimer H (2009) Sulfasalazine inhibits the growth of primary brain tumors independent of nuclear factor-κB. J Neurochem 110:182–193

Conrad M, Sato H (2011) The oxidative stress-inducible cystine/glutamate antiporter, system xc−: cystine supplier and beyond. Amino Acids (this issue)

Diwakar L, Ravindranath V (2007) Inhibition of cystathionine-γ-lyase leads to loss of glutathione and aggravation of mitochondrial dysfunction mediated by excitatory amino acid in the CNS. Neurochem Int 50:418–426

Dringen R, Pfeiffer B, Hamprecht B (1999) Synthesis of the antioxidant glutathione in neurons: supply by astrocytes of CysGly as precursor for neuronal glutathione. J Neurosci 19:562–569

Dun Y, Mysona B, Van Ells T, Amarnath L, Shamsul OM, Ganapathy V et al (2006) Expression of the cystine-glutamate exchanger (xc−) in retinal ganglion cells and regulation by nitric oxide and oxidative stress. Cell Tissue Res 324:189–202

Frade J, Pope S, Schmidt M, Dringen R, Barbosa R, Pocock J et al (2008) Glutamate induces release of glutathione from cultured rat astrocytes—a possible neuroprotective mechanism. J Neurochem 105:1144–1152

Grange E, Gharib A, Lepetit P, Guillaud J, Sarda N, Bobillier P (1992) Brain protein synthesis in the conscious rat using L-[^{35}S]methionine: relationship of methionine specific activity between plasma and precursor compartment and evaluation of methionine metabolic pathways. J Neurochem 59:1437–1443

Huang T, Wahlqvist ML, Li D (2010) Docosahexaenoic acid decreases plasma homocysteine via regulating enzyme activity and mRNA expression involved in methionine metabolism. Nutrition 26:112–119

Kandil S, Brennan L, McBean GJ (2010) Glutathione depletion causes a JNK and p38^{MAPK}-mediated increase in expression of cystathionine-γ-lyase and upregulation of the transsulfuration pathway in C6 glioma cells. Neurochem Int 56:611–619

Karunakaran D, Diwakar L, Saeed U, Agarwal V, Ramikrishnan S, Iyengar S et al (2007) Activation of apoptosis signal regulating kinase 1 (ASK1) and translocation of death-associated protein, Daxx, in substantia nigra pars compacta in a mouse model of Parkinson's disease: protection by alpha lipoic acid. FASEB J 21:2226–2236

Kato S, Ishita S, Suguwara K, Mawatari K (1993) Cystine/glutamate antiporter expression in retinal Müller glial cells: implications for DL-alpha-aminoadipate toxicity. Neuroscience 57:473–482

Kato T, Shinoda J, Oka N, Miwa K, Nakayama N, Yano H et al (2008) Analysis of ^{11}C-methionine uptake in low-grade gliomas and correlation with proliferative activity. Am J Neuroradiol 29:1867–1871

Kimura H (2010) Hydrogen sulfide: its production, release and functions. Amino Acids. doi:10.1007/s00726-010-0510-x

Kranich O, Dringen R, Sandberg M, Hamprecht B (1998) Utilization of cysteine and cysteine precursors for the synthesis of glutathione in astroglial cultures: preference for cystine. Glia 22:11–18

Krejsa CM, Franklin CC, White CC, Ledbetter JA, Schieven GL, Kavanagh TJ (2010) Rapid activation of glutamate cysteine ligase following oxidative stress. J Biol Chem 285:16116–16124

Kyriakas JM, Avruch J (1996) Sounding the alarm: protein kinase cascades activated by stress and inflammation. J Biol Chem 271:24313–24316

Lee M, Schwab C, Yu S, McGeer E, McGeer PL (2009) Astrocytes produce the antiinflammatory and neuroprotective agent, hydrogen sulfide. Neurobiol Aging 30:1523–1534

Lee SW, Hu YS, Hu LF, Lu Q, Dawe GS, Moore PK et al (2006) Hydrogen sulphide regulates calcium homeostasis in microglial cells. Glia 54:116–124

Lewerenz J, Maher P, Methner A (2011) Regulation of xCT expression and system xc− function in neuronal cells. Amino Acids (this issue)

Li L, Hsu A, Moore PK (2009) Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulfide in the cardiovascular system and in inflammation—a tale of three gases! Pharmacol Ther 123:386–400

Lo M, Wang YZ, Gout PW (2008) The x(c)− cystine/glutamate antiporter: a potential target for therapy of cancer and other diseases. J Cell Physiol 215:593–602

Martín JA, Pereda J, Martínez-López I, Escrig R, Miralles V, Pallardó FV, et al (2007) Oxidative stress as a signal to up-regulate gamma cystathionase in the fetal-to-neonatal transition in rats. Cell Mol Biol 53(Suppl):OL1010–OL1014

Matsumaru K, Cheng J, Kaplowitz N (2003) Mechanisms for sensitization to TNF-induced apoptosis by acute glutathione depletion in murine hepatocytes. Hepatology 37:1425–1434

McBean GJ (2002) Cerebral cystine uptake: a tale of two transporters. Trends Pharmacol Sci 23:299–302

Mosharov E, Cranford MR, Banerjee R (2000) The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes. Biochemistry 39:13005–13011

Mysona B, Dun Y, Duplantier J, Ganapathy V, Smith SB (2009) Effects of hyperglycaemia and oxidative stress on the glutamate transporters GLAST and system xc− in mouse retinal Müller glial cells. Cell Tissue Res 335:477–488

Opere CA, Monjok EM, Kulkarni KH, Njie YF, Ohia SE (2009) Regulation of [^{3}H]D-aspartate release from mammalian isolated retinase by hydrogen sulfide. Neurochem Res 34:1962–1968

Pong WW, Stouracova R, Frank N, Kraus JP, Eldred WD (2007) Comparative localization of cystathionine beta-synthase and cystathionine gamma-lyase in retina: differences between amphibians and mammals. J Comp Neurol 505:158–165

Rosado JO, Salvador M, Bonatto D (2007) Importance of the transsulfuration pathway in cancer prevention and promotion. Mol Cell Biochem 301:1–12

Ruiz E, Siow RCM, Bartlett SR, Jenner AM, Sato H, Bannai S et al (2003) Vitamin C inhibits diethylmaleate-induced L-cystine transport in human vascular smooth muscle cells. Free Radic Biol Med 34:103–110

Sato H, Tamba M, Kuriyama-Matsumura K, Okuno S, Bannai S (2000) Molecular cloning and expression of human xCT, the light chain of amino acid transport system xc−. Antioxid Redox Signal 2:665–671

Savaskan NE, Heckl A, Hahnen E, Engelhorn T, Doerfler A, Gansladt O et al (2008) Small interfering RNA-mediated xCT silencing in gliomas inhibits neurodegeneration and alleviates brain edema. Nat Med 15:629–632

Singh S, Padovani D, Leslie RA, Chiku T, Banerjee R (2009) Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H_2S biogenesis via alternative trans-sulfuration reactions. J Biol Chem 284:22457–22466

Sontheimer H (2008) A role for glutamate in growth and invasion of primary brain tumors. J Neurochem 105:287–295

Tripatara P, Patel NS, Brancaleone V, Renshaw D, Rocha J, Sepodes B et al (2009) Characterisation of cystathionine gamma-lyase/hydrogen sulphide pathway in ischaemia/reperfusion injury: an in vivo study. Eur J Pharmacol 606:205–209

Vitvitsky V, Thomas M, Ghorpade A, Gendleman HE, Banerjee R (2006) A functional transulfuration pathway in the brain links to glutathione homeostasis. J Biol Chem 281:35785–35793