

Taurine: an Amino Acid Rich in Fish Meal

Subramanian Divakaran

Research Scientist¹, Oceanic Institute, 41-202 Kalaniana'ole Hwy.
Waimanalo, Hawaii 96795, USA.

¹Retired. Current address, 113 Wooddale, Euless, TX 76039, USA
E-mail: gramatophylum@comcast.net

Abstract

Taurine is a sulfur containing amino acid and is unique in that it is not linked to any protein by a peptide bond. Taurine is the most abundant free amino acid in any tissue. Taurine is found in greater concentration in aquatic foods than in land animal foods and consequently higher in fish meal than in meat meal. Taurine is a conditionally essential amino acid and is either derived from food/feed or biosynthesized in the liver. The most common pathway for taurine biosynthesis is by the conversion of cysteine to taurine mediated by the enzyme cysteine sulfinic acid decarboxylase. Physiological functions for taurine include neuromodulation, cardiac calcium ion modulation, hypolipemic and hypocholesteremic, bile synthesis, osmoregulation, detoxicant and many others. Very little information on taurine is available on the nutritional requirement and or its biosynthetic ability in aquatic species. Understanding taurine nutrition is essential to minimize or eliminate the need for fish meal in feeds, a byproduct whose world supply is constrained by demand from rapid aquaculture development. Knowledge of nutrition/biosynthesis in various aquatic species may play a key role in the future development of organic aquaculture farming and in the taxonomy of aquatic species.

Introduction

Taurine (2-aminoethanesulfonic acid, $^+\text{NH}_3\text{CH}_2\text{CH}_2\text{SO}_3^-$) is unique in that it is not linked to any protein by a peptide bond and taurine is not a constituent of any protein. Taurine is the most abundant free amino acid in many tissues. Physiological role of taurine has been extensively researched but source of taurine in various foods has been relatively neglected. Aquatic foods are a major source of taurine in human nutrition. Conches, blood clams and cuttlefish had taurine levels as high as 500-900 mg in 100 g of the fresh edible portion. Fish had varied taurine content, which were relatively high in rays and flat fish (280-320 mg/100 g fresh edible portion) relatively low in silver pomfret, yellow croaker and baby croaker (40-90 mg/100 g edible fresh edible portion). Natural and cultivated prawns had 100 mg in 100 g fresh edible portion. These authors (Zhao *et al.*, 1998) analyzed fresh edible portions of around 30 species of aquatic products for their taurine content as food. These authors found that sea foods contain 0.5 – 1% taurine compared to meats for e.g. Chicken legs contain only around 0.38% taurine. Strangely chicken legs contain 10 times more taurine than chicken breasts. Milk and vegetables contain practically little taurine and a species of beans is known to contain around 0.0005% taurine.

Taurine has been touted as a constituent of health food products, examples of which are the health drink Lipovitan, a taurine containing drink Red Bull is marketed as an aid to athletic performance, taurine containing products are marketed in North America for the treatment of epilepsy, hyperactivity (Calm-Kids) substance abuse (Crave-Away). Not an advertisement for any of these products is intended. Taurine is used as a nutritional supplement and aquatic foods are a major source of taurine in human nutrition (Zhao *et al.*, 1998).

Taurine biosynthesis

The fact that the animal kingdom, although not the bacterial or the plant retain large amounts of taurine from the oxidation of sulfur amino acids, whether an individual species makes it for itself or gets it from dietary source, implies that taurine confers an evolutionary advantage. There are other pathways of sulfur metabolism that provide clear advantages, yet the animal kingdom has "chosen" to retain plurality of metabolic routes (Huxtable 1999). Little information is available on taurine biosynthetic ability or inability in aquatic species

Mammals either biosynthesize taurine from cysteine, get it from diet or do both. Species with high dietary intake of taurine seem to have lost the ability for ready biosynthesis. The enzyme involved in the first step, cysteine sulfinatase decarboxylase (CSD) has now been well characterized and studied. The second step, the oxidation of hypotaurine to taurine, is still not well understood (Huxtable, 1999).

Taurine biosynthesis occurs essentially entirely via the formation and decarboxylation of cysteine sulfinatase. The conversion of cysteine to taurine (Figure 1) is unusual in that the activities of the two enzymes that determine its rate of synthesis in the liver are affected in opposite directions by excess of dietary protein. Cysteine dehydrogenase activity is elevated by an increase in dietary protein and where as CSD is decreased by an increase in dietary protein intake (Stipanuk *et al.*, 1994). The reduction in specific activity of CSD in rat liver hepatocytes fed

casein supplemented with DL-methionine and L-Methionine in diets is 32% and 54% respectively. Supplementation of diets with excess methionine favored high rates of taurine production, whereas supplementation of diets with excess protein favored high rates of sulfate production. Although dietary cysteine can lower the dietary requirement for methionine, there is an absolute requirement for methionine. It has functions in addition to transsulfuration is also needed for protein synthesis. These functions include methylation and polyamine synthesis. However, methionine is one of the most toxic amino acids. It causes growth retardation, tissue damage and iron deposition in the spleen. Tissue levels must be kept low, and a dietary load must be processed immediately (Huxtable, 1999). Relatively high levels of both Cysteine dioxygenase and CSD activities are needed for high rates of taurine production in hepatocytes (Bella and Stipanuk., 1996). Taurine production appears to be favored when cysteine availability is high, suggesting that taurine formation and excretion may be an important route of disposal of excess sulfur. These observations also suggest that the synthesis of taurine may not be conserved metabolically in the face of a limited supply of cysteine and that the diet may be the major source of taurine under such conditions (Stipanuk and Bagley, 1992).

Physiological role and requirement for taurine

Taurine is involved in a number of physiological processes such as neuromodulatory actions, cardiac Ca^{++} modulation, fat absorption by emulsification as taurine-conjugated bile acids, osmoregulation, reproduction and detoxification of metabolites and toxins to name a few. Due to its diverse physiological functions the β -amino acid taurine is indeed conditionally essential. Orally administered taurine has been reported to reduce lung oxidant damage from exposure to ozone, nitrogen dioxide, paraquat, amiodarone and bleomycin in animal models (Santangelo *et al.*, 2003).

Taurine plays an important role in conjugation of bile acids that are formed from cholesterol in the liver, suggesting that there is a close relationship between taurine and cholesterol metabolism. Taurine has a hypolipidemic effect and may stimulate hepatic bile synthesis (Ogawa 1996).

In studies with aquatic animals Takeuchi (2001) found that taurine was essential for growth and normal behavior of Japanese flounder, and determined the dietary requirement for that species and investigated its synthetic pathways. In a study of taurine requirement in mahi-mahi (*Coryphaena hippurus*), Divakaran *et al.*, (1992) found that this species cannot synthesize taurine to satisfy its nutritional requirement. Biosynthetic pathways for taurine in freshwater prawn (*Macrobrachium rosenbergii*) have been established (Smith *et al.*, 1987). King and Goldstein (1983) have shown that taurine is released from cells of marine fish in response to environmental dilution and these taurine pumps have been shown to be quiet potent.

One clear mammalian function of taurine is a metabolic one: that of bile salt synthesis. As well as varying in the use of taurine as a bile salt conjugator, mammalian species vary in their reliance on biosynthetic versus dietary taurine. As much of the clinical and nutritional interest in taurine stems from the significance of a dietary source, studies on species other than humans have to be carefully interpreted (Huxtable 1999). For taurine functions other than conjugation, there are a

number of major phenomena involving membrane effects. These include osmoregulation, calcium availability, channel regulation and phospholipoid methylation. In general taurine is high in species lacking cell walls (animals) and low in species with cell walls (Plants: bacteria). Taurine increase the retention of phosphatidylethanolomine and phosphatidylcholine. Taurine biosynthesis and dietary dependence in some mammals is summarized in Table 1.

Table 1: Taurine biosynthesis and dietary dependence in some mammals (Huxtable, 1999)

Species	Diet	Taurine synthesis	Dietary dependence on taurine
Guinea pig	Herbivore	High	None
Rat	Herbivore	High	Low
Monkeys (old world)	Omnivore	Poor	Moderate?
Monkeys (new world)	Omnivore	Poor	High
Human	Omnivore	Very poor	High
Cat	Carnivore	Very poor	Absolute

There is evidence that taurine protects neural cells from exotoxicity induced by excitatory amino acids. The increase in extra cellular levels of taurine in cell-damaging conditions may thus be an important endogenous protective mechanism particularly efficient in the immature brain (Saransaari and Oja 1996). In human subjects significantly lower neuroepinephrine excretion observed by taurine administration implies the suppression of the sympathetic nervous system (Mizushima et al. 1996). Taurine levels in the brain decrease and extra cellular levels increase in response to pathologic conditions such as ischemia, anoxia (Lombardini, 1992) and it is not unsafe to state in general taurine levels respond to conditions of stress.

Enantiostatic regulation has been defined as occurring when the effect of a change in physicochemical property of the internal milieu is opposed by a change in another physicochemical property. Such a regulation protects against the consequences of environmental change. Enantiostasis differs from homeostasis in that the function is maintained although physicochemical state is altered. Thus when taurine antagonizes calcium-induced alterations in cardiac contractility it is regulating enantiostatically, inasmuch as physiological normal contractility is re-established in the phase of physiologically abnormal calcium concentrations (Huxtable 1999). Taurine fits all requirements for an enantiomeric agent.

Future research

This brief review on taurine, its content in various foods and tissues, taurine biosynthesis and its physiological role and requirement emphasizes the need for further understanding of taurine in aquacultured animals. As stated earlier taurine is a conditionally essential amino acid and its nutritional requirements in aquatic animals seems little understood. Investigations on nutritional requirement for taurine becomes even greater as the supply and demand for fish meal and related byproducts such as fish solubles become constrained. Higher content of taurine in fisheries byproducts could be one of the many reasons for better growth performance compared to animal based by products. It is also seen from this review that taurine biosynthetic ability varies greatly from one species to another and research in this area is of immense importance in determining

Subramanian Divakaran. 2006. Taurine: An Amino Acid Rich In Fish Meal. En: Editores: L. Elizabeth Cruz Suárez, Denis Ricque Marie, Mireya Tapia Salazar, Martha G. Nieto López, David A. Villarreal Cavazos, Ana C. Puello Cruz y Armando García Ortega. Avances en Nutrición Acuícola VIII .VIII Simposium Internacional de Nutrición Acuícola. 15 - 17 Noviembre. Universidad Autónoma de Nuevo León,

Monterrey, Nuevo León, México. ISBN 970-694-333-5.

the nutritional need and dietary supplementation of taurine. It may not be inappropriate to say that ability or inability for taurine biosynthesis capabilities could lead to a taxonomic understanding of various aquatic species. Finally with the recent fascination and flare for organic aquaculture farming it may become essential to go for more regulated sources of taurine than those currently found in animal and fisheries byproducts.

NOTE: A Summary of procedure for determination of taurine biosynthesis in aquatic animals is given in Appendix 1.

References

- Bella D.L and M. H. Stipanuk. 1996. High levels of dietary protein or methionine have different effects on cysteine metabolism in rat hepatocytes. In, *Advances in Experimental Biology*, Volume 403. Taurine 2. Basic and clinical aspects. Ed. R.J. Huxtable, J. Azuma, K. Kuriyama, M. Nakagawa and A. Baba. Plenum Press, NY. Chapter 9, p 73- 84.
- Divakaran, S., S. Ramanathan and A.C. Ostrowski. 1992. Endogenous production of taurine in two teleost fish: *Coryphaena hippurus* and red hybrid tilapia. *Comparative Biochemistry and Physiology* 101B (3): 321-322.
- Huxtable, R.J. 1999. Expanding the circle 1975-1999; sulfur biochemistry and insights on the biological functions of taurine. In *Advances in Experimental Biology*, Volume 483. Taurine 4, Taurine and excitable tissues. L. D. Corte., R.J. Huxtable., G. Sparagli and K.F. Tipton. Kluwer Academic/Plenum Publishers. NY. p 1-25.
- King, P and L. Goldstein. 1983. Organic osmolytes and cell volume regulation in fish. *Molecular Physiology*, 4:53-66.
- Lombardini, J.B. 1992. Review, Recent studies on taurine in the central nervous system. In *Advances in Experimental Biology*, Volume 315. Taurine, Nutritional value and mechanics of action. Ed. J.B. Lombardini, S.W. Schaffer and J. Azuma. Plenum Press. NY. p. 245-450.
- Misushima, S., Y. Nara M. Sawamura and Y. Yamori. 1996. Effects of oral taurine supplementation on lipids and sympathetic nerve tone. In *Advances in Experimental Biology*, Volume 403. Taurine 2. Basic and clinical aspects. Ed. R.J. Huxtable, J. Azuma, K. Kuriyama, M. Nakagawa and A. Baba. Plenum Press, NY. Chapter 68, p 615 - 622.
- Ogawa, H. 1996. Effect of dietary taurine on lipid metabolism in normcholesterolemic and hypercholesterolemic stroke-prone spontaneously hypersensitive rats. In *Advances in Experimental Biology*, Volume 403. Taurine 2. Basic and clinical aspects. Ed. R.J. Huxtable, J. Azuma, K. Kuriyama, M. Nakagawa and A. Baba. Plenum Press, NY. Chapter 13, p 107-115.
- Saransaari, P and S. S. Oja. 1996. Taurine and neural cell damage. In *Advances in Experimental Biology*, Volume 359. Taurine in Health and Disease. Ed. R. J. Huxtable and D. Michalk. Plenum Press. NY. Chapter 52, p 481- 490.
- Smith, R.B., G.C. Miller, and R.W. Mead. 1987. Taurine tissue concentrations and salinity effect on taurine in the freshwater prawn *Macro brachium rosenbergii* (De mann). *Comparative Biochemistry and Physiology* 87A (4): 907-909.
- Stipanuk, M.H and P.J. Bagley. 1992. Metabolism of cysteine to taurine by rat hepatocytes. In *Advances in Experimental Biology*, Volume 315. Taurine, Nutritional value and mechanics of action. Ed. J.B. Lombardini, S.W. Schaffer and J. Azuma. Plenum Press. NY. p. 413-421.
- Stipanuk, M.H., P.J. Bagely., Y.C. Hsu., D.L. Bella., M.F. Banks and L.L. Hirshberger. 2004. Hepatic regulation of cysteine utilization for taurine synthesis. In *Advances in Experimental Biology*, Volume 359. Taurine in Health and Disease. Ed. R. J. Huxtable and D. Michalk. Plenum Press. NY. p 74-89.
- Takeuchi, T. 2001. A review of feed development for early life stages of marine finfish in Japan. *Aquaculture* 200:203-222.
- Zhao, X., J. Jia and Y. Lin 1998. Taurine content in Chinese food and daily taurine intake of Chinese men. In *Advances in Experimental Biology*, Volume 442. Taurine 3, Cellular and regulatory mechanisms. Ed. S. Schafer, J.B. Lombardini and R. J. Huxtable, Plenum Press, NY. p 501-505

Appendix 1: Summary of procedure for determination of taurine biosynthesis in aquatic animals.

Enzymatic preparation of L- [^{14}C] Cystine sulfinic acid

This study requires the radioactive Isotope L- [^{14}C] Cystine 50 μCi (1.85 MBq) and a scintillation counter.

Reagents

1. 0.04 M NAD in DW (neutralized with KOH) – 0.265g/mL, 0.0664 g/2.5 mL.
2. 0.05 M potassium phosphate buffer, pH 6.8, containing 0.3% triton X 100
3. 0.01 M $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6 \text{H}_2\text{O}$ in DW –made fresh – 0.392g/100 mL.
4. 0.10 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ in DW (neutralized with KOH) – 0.6950g/100 mL
5. 10% perchloric acid – 1 part 70% + 6 parts DW.
6. 12 N KOH – 67g/100 mL.
7. Potassium phosphate buffer, 0.20 M, pH 7.0 (27.2 g $\text{KH}_2\text{PO}_4/\text{L}$ + 34.8 g $\text{K}_2\text{HPO}_4/\text{L}$)
8. 0.022 M L-cysteine with L- [^{14}C] Cystine: In 0.111 M phosphate buffer, pH 6.5 prepared as follows (use a 10 ml volumetric flask)

For each 5 mL incubation mixture, dissolve 50- μmol cysteine free base in 1.00 mL (DW + L- [^{14}C] Cystine up to 1 mCu depending on desired amount sulfinic acid). Add 1.25 mL of 0.20 M phosphate buffer, pH 7 to the cysteine solution. Adjust pH to 6.5

9. 20 % liver supernatant: Homogenize liver (fish tilapia liver) in 4 volumes of 0.05 M potassium phosphate buffer with 3 g Triton X 100/liter. Centrifuge at 0-4°C and 20,000 g for 30 min. Use supernatant for assay.

Synthesis procedure for DL- [^{14}C] cysteine sulfenic acid (CSA)

1. Set two 15 mL screw cap centrifuge tube on ice.
2. Add to each tube
 - 2.0 mL of 0.022 M L- [^{14}C] Cystine in 0.11 M phosphate buffer pH 6.5.
 - 0.25 mL of 0.04 M NAD
 - 0.25 mL of 0.10 M hydroxylamine
 - 0.25 mL of 0.01 M $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$.
 - 2.0 mL of 20% liver homogenate supernatant
3. Incubate at 37°C shaking water bath for 2 hours
4. Terminate reaction by the addition of 1.0 mL of 10% perchloric acid (1M)
5. Cool and centrifuge 20,000 xg for 10 min.
6. Decant supernatant into a 15 mL test tube and neutralize with 0.01 mL 12 N KOH. To remove excess per chlorate. At this point the reactant supernatant can be stored Frozen until chromatography is performed.

Chromatography procedure

1. Adjust pH of the supernatant to pH 2 with 6 N HCl
2. Apply the entire volume to a Dowex 50 (H^+ form) column (0.9 X 15 cm). Prepare Dowex by first washing with 2 N HCl and then water to neutral pH.
3. Using 0.01 HCl as the eluant, collect the first 40 mL in 1.0 mL fractions. Take a 10 μL aliquot from each fraction and determine radioactivity. Using liquid scintillation Counter.
4. Pool fractions corresponding to **DL- [^{14}C] CSA** peak.
5. Check identity either using HPLC or TLC in a solvent system of n butanol: prop ionic Acid: DW (3:3:2) using CSA as control
6. Amino acid can be visualized by spraying dried plate with 0.5% ninhydrin in butanol. Heat plate. Compare control CSA blue spot with synthesized radioactive **DL- [^{14}C] C**

CSA spot.

7. Portions can then be freeze-dried, sealed in ampoules and stored at -70°C until used.

Study of Taurine synthesis from CSA by fish

Test samples: Tissue extracts from liver and kidney tissues of pre-feeding yolk sac larvae, fingerling, and adult fish.

Procedure for determining presence or absence of CSA decarboxylase in tissue samples.

Reagents:

1. 0.005 M Dithiothretol (DTT) in DW, pH 7.1 ((0.0077g/10 mL)
2. 0.015 M Pyridoxol phosphate (PALP) in DW. (0.0185g/5 mL)
3. 1.0 M potassium phosphate buffer, pH 7.10
4. 0.05 M potassium phosphate buffer, pH 6.8
5. 0.30 M L -cysteine sulfenic acid with **DL- $[\text{U}^{14}\text{C}]$ CSA (neutralized with KOH) 0.0460 g/mL**. Prepare such that about 50,000 – 1,000,000 dpm are added per flask, depending on required CSA. , pH 7.10.
6. Monoethanolamine: methyl cellosolve 1: 2 (V/V) Alkaline trap mix.
7. 10% Trichloroacetic acid (TCA)

Assay procedure:

1. Homogenize 2 g samples in 3 mL of 0.05 M potassium phosphate buffer, pH 6.8, to make a 40% homogenate. Centrifuge at $0-4^{\circ}\text{C}$ and $20,000\times g$ for 30 min. Use supernatant for assay. Determine protein content by Lowry
2. Label scintillation vial bottoms (see figure 2) and set on ice.
3. Add 0.1 mL or 0.2 mL tissue supernatant to each vial. Adjust total volume to 0.2 mL
With homogenization buffer.
4. Heat deactivates blanks by incubating 5 min in boiling water bath.
5. Add to each vial (a premix can be made **)
 - 0.025 mL of 0.015 M PALP, pH 7.1
 - 0.050 mL of 0.005 M DTT, pH 7.1
 - 0.125 mL of 1.0 M potassium phosphate buffer, pH 7.1
 - 0.050 mL of 0.30 M DL- $[\text{U}^{14}\text{C}]$ CSA
 Total volume is 5 mL.
6. Set up traps for CO_2 with alkaline trap mix.
7. Use 0.5 mL alkaline trap mix for each test sample.
8. Incubate at 37°C in a shaking water bath for 30 or 60 minutes.
9. Inject nitrogen into the trap to completely collect the C^{14}O_2 into the alkali trap mix.
10. Count radioactivity in the alkali trap mix using a liquid scintillation counter.

** A premix can be made as follows:

1. 0.10 mL PLP
2. 20.0 mL DTT
3. 50 mL 1 M phosphate buffer, 0.3 mL/vial
4. 2.20 mL **DL- $[\text{U}^{14}\text{C}]$ CSA**
5. 20 mL homogenization buffer

Take three $0.25\mu\text{L}$ aliquots of the premix and determine radioactivity.

Presence of radioactivity in the alkali trap mix due to the presence of C^{14}O_2 indicates taurine biosynthesis.

