THE IMPORTANT ROLE OF TAURINE IN OXIDATIVE METABOLISM

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1. ABSTRACT

Several studies have demonstrated that especially high taurine concentrations are found in tissues with high oxidative activity, whereas lower concentrations are found in tissues with primary glycolytic activity. Based on such observations, we have studied if taurine is involved in mitochondrial oxidation. Several pieces of information have demonstrated taurine localisation in the mitochondria. We have developed a general biochemical model with preliminary data demonstrating the important role of taurine as mitochondrial matrix buffer for stabilising the mitochondrial oxidation. The model can have far-reaching perspectives, e.g., explaining the often-suggested anti-oxidative role of taurine, in contrast to the fact that taurine is very difficult to chemically oxidise. By stabilising the environment in the mitochondria, taurine will prevent leakage of the reactive compounds formed in the reactive mitochondrial environment and thus indirectly act as an antioxidant. Consequently, the model represents a new concept for understanding mitochondrial dysfunction by emphasising the importance of taurine for providing sufficient pH buffering in the mitochondrial matrix.

2. INTRODUCTION

Taurine is found in all animal cells typically in millimolar concentrations, e.g., 5-50 mM (Jacobsen and Smith, 1968), whereas the concentration in plasma and extracellular fluids is much lower, typically 50-200 μM. Several different actions have been ascribed to taurine, e.g. bile acid conjugation and as an intracellular osmolyte. However, despite the ubiquitous distribution and several reviews on the action of taurine in physiology and pathophysiology (Huxtable, 1992; Hansen, 2001, 2003), the overall role of taurine is still disputed.

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3. OXIDATIVE TISSUES AND TAURINE

When considering the tissue localisation of taurine, it is evident that the highest concentrations are found in highly energy-consuming tissue, like retina, nerves, kidney, heart, and oxidative muscle tissue in general (Jacobsen and Smith, 1968). Several studies exist on taurine distribution in muscles comparing oxidative and glycolytic muscle types or fibre types (e.g. Aristoy and Toldrá, 1998; Cornet and Bousset, 1999). In the reports from such studies, the differences in distribution of taurine, carnosine, and anserine are conspicuous (see Table 1).

Table 1. Carnosine, anserine, and taurine content in glycolytic and oxidative porcine muscles

Compound	**Glycolytic muscle** *Longissimus dorsi*	**Oxidative muscle** *Masseter*
Carnosine (β-alanyl-L-histidine)	10-15 µmol/g	1-2 µmol/g
Anserine (β-alanyl-L-1-methylhistidine)	1-3 µmol/g	1-3 µmol/g
Taurine	1-3 µmol/g	15-20 µmol/g

The concentrations are adapted from Aristoy and Toldrá (1998) and Cornet and Bousset (1999).

Carnosine and anserine are recognised as an intracellular buffer for buffering lactate formed by glycolysis and is obviously found in high concentration in the glycolytic muscle and low concentration in the oxidative muscle. On the contrary, taurine is found in high concentrations in the oxidative muscle and low concentration in the glycolytic muscle. Such preferential localisation of taurine in oxidative tissue indicates a possible importance for mitochondrial function.

4. SUBCELLULAR DISTRIBUTION OF TAURINE

The concentration gradient across the cellular membrane clearly demonstrates the action of the ATP-dependent taurine transporter. However, as seen from the reviews on taurine distribution, the taurine concentrations vary significantly among the different tissue types. Although the activity of the taurine transporter might depend on the specific tissue or the cell type within the tissue, it seems more likely to explain the variation in taurine distribution by applying a two- (or multi-) compartment model. In such a model different taurine concentrations are allowed in the subcellular compartments due to up-concentration in specific organelles. The total taurine concentration in the tissue will thus depend on the organelle distribution within the cells in the tissue. Such a multi-compartmental model requires subcellular taurine transporter activity, as found in a recent study (Voss *et al*., 2004) on the recognition of taurine transporter with primary antibodies. However, no association with any specific organelle was demonstrated.

5. MITOCHONDRIAL LOCALISATION

Immunocytochemical techniques have been developed to study tissue and subcellular distribution of taurine. Such studies demonstrated that taurine was distributed in all cellular subcompartments. However, in some organelles including the mitochondria increased immunoreactivity was reported indicating taurine localisation in the mitochondria (e.g. Ottersen, 1988; Terauchi and Nagata, 1993, Lobo *et al*., 2000).

Recent studies on mitchondrial tRNA have demonstrated the existence of taurine-modified uridine residues (Suzuki, 2002). Such t-RNA modification must be expected to be processed inside the mitochondrial matrix. The taurine modifications were not found in mutant tRNA from patients with mitochondrial encephalopathies, i.e., the study indicates the importance of taurine for having normal mitochondrial function. Possibly, taurine could be directly involved in the metabolic regulation of the glucose metabolism as indicated by the results on the interaction between taurine and pyruvate dehydrogenase phosphatase (Lombardini, 1997).

6. INTRACELLULAR BUFFERS AND MITOCHONDRIA

Any discussion on mitochondrial function and mitochondrial oxidation in modern biochemical textbooks is focused on the importance of the pH gradient between the cytosol and the mitochondrial matrix. Energy is stored electrochemically in the pH gradient in order to produce ATP by the ATP synthase enzyme system. However, as a result of the pH gradient the mitochondrial matrix is mildly alkaline (see Fig. 1).

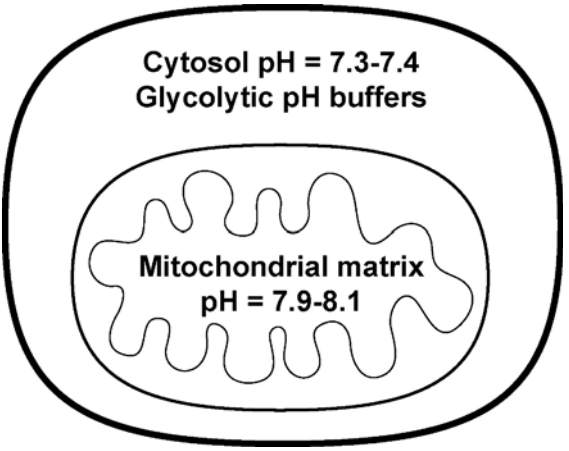

Figure 1. Schematic overview of cell indicating the pH gradient between the cytosol and the mitochondrial matrix. pH values adapted from Llopis *et al*. (1998).

The pH value of the matrix has been determined to about 8 by advanced measurements applying confocal microscopy and subcellular targeting of pH indicators based on green fluorescent protein (GFP) (e.g. Llopis *et al*., 1998).

Whereas the pH control of the cytosol by glycolytic buffers like carnosine and anserine has been described (e.g. Aristoy and Toldrá, 1998; Cornet and Bousset, 1999), no discussion on pH buffering of the alkaline pH in the mitochondrial matrix seems to exist. In Table 2 ionisation constants are presented for some compounds, which are involved in physiological pH regulation, and taurine.

Table 2. Ionisation constants for some intracellular pH buffers and taurine

Compound	pK value	Reference
Carbon dioxide/Bicarbonate (CO_2/HCO_3^-)	6.35 (25°C)	Beynon and Easterby, 1996
Dihydrogenphosphate/Hydrogenphosphate ($H_2PO_4^-/HPO_4^{2-}$)	7.21 (25°C)	Beynon and Easterby, 1996
Carnosine	6.8 (22°C)	Deutsch and Eggleton, 1938
Anserine	7.0 (22°C)	Deutsch and Eggleton, 1938
Taurine (amino group)	9.0 (25°C) 8.6 (37°C)	Hansen *et al*., 2005

It must be expected that pH buffering is required for the mitochondrial matrix due to the mildly alkaline environment inside. Existence of a low-molecular pH buffer will stabilise the mitochondrial pH gradient. Among the compounds presented in Table 2 taurine is the only candidate due its pK value. When comparing with other lists of physiological buffer, the lack of compounds with the pK values in the range 8–9 is conspicuous. In addition, the mitochondrial proteins are not expected to provide adequate buffering contribution, as the protein amino acid residues does not have pK values in this range either.

To summarise our analysis and observations, we now propose the following hypothesis on a very important cell physiological role of taurine:

Hypothesis: Taurine acts as a pH buffer in the mitochondrial matrix and thus stabilises the mitochondrial pH gradient

Preliminary experimental arguments for this hypothesis follow below. Additional thorough theoretical and experimental arguments can be found below and elsewhere (Hansen *et al*., 2005).

7. MITOCHONDRIAL MATRIX ENZYMES

In order to obtain some experimental evidence for the hypothesis proposed, the pH dependence of some enzymes localised in the mitochondrial matrix was studied. The pH dependence of isocitrate dehydrogenase from the tricarboxylic acid cycle has been determined with the results as shown in Table 3.

Table 3. pH dependence of the isocitrate dehydrogenase activity at 37°C comparing applicaton of Tris and taurine as buffer compounds

pH	Taurine	Tris
7.5	95.6	96.7
7.7	100.0	96.7
7.9	98.4	94.8
8.1	92.2	88.6
8.3	84.3	79.8
8.5	73.9	74.4
8.7	62.6	64.5
8.9	46.8	54.6
9.1	30.7	43.7
9.3	10.0	27.5
9.5	2.7	16.8

The measurement of isocitric dehydrogenase activity was performed in a final volume of 3.0 ml using a Shimadzu UV-1601 spectrophotometer. The assay was carried out as described (Bergmeyer, 1974) in a medium containing 80 mM Na_2SO_4, 0.44 mM DL-isocitric acid, 0.5 mM β-nicotinamide adenine dinucleotide phosphate, 0.2 mM manganese chloride, 0.015 U/ml isocitric dehydrogenase (from pig heart) (Sigma), and for pH buffering 40 mM of taurine or Tris. pH of the medium was adjusted to the indicated pH with H_2SO_4 or NaOH at 37°C. The activity was determined from the linear increase in absorbance at 355 nm (NADPH). Data are reported as mean of 6-8 determinations and normalised relatively to the maximum activity observed.

As seen from the data in Table 3 no significant difference is observed in enzyme activity using taurine instead of the traditional research buffer Tris. Actually, it seems that taurine inhibits enzyme activity at pH > 9 better than Tris.

Additional examples on important metabolic enzymes localised in the mitochondrial matrix are the acyl-CoA dehydrogenase enzymes (ACADs), which are primarily responsible for performing the fatty acid β-oxidation. The original mechanistic studies on these enzymes demonstrated strong pH activity dependence favouring mildly alkaline conditions at pH 8.0-8.5 (Reinsch *et al*., 1980, Schmidt *et al*., 1981) as seen in Fig. 2. In addition, to maintain a reasonably constant enzyme activity, the steep activity increase with pH immediately demonstrates a requirement for buffering the enzyme environment, i.e. buffering of the mitochondrial matrix.

Additional information on the ACADs can be found in recent studies (Ghisla and Thorpe, 2004, Hansen *et al.*, 2005). Several different ACADs were studied with different substrate specificity depending on fatty acid chain length. These studies demonstrated similar activity profiles to the profile in Fig. 2, i.e., mildly alkaline pH is required for having reasonable fatty acid β-oxidation activity. In case of insufficient pH buffering, reduced enzyme activity must be expected, and, consequently, impaired mitochondrial fatty acid oxidation will be observed.

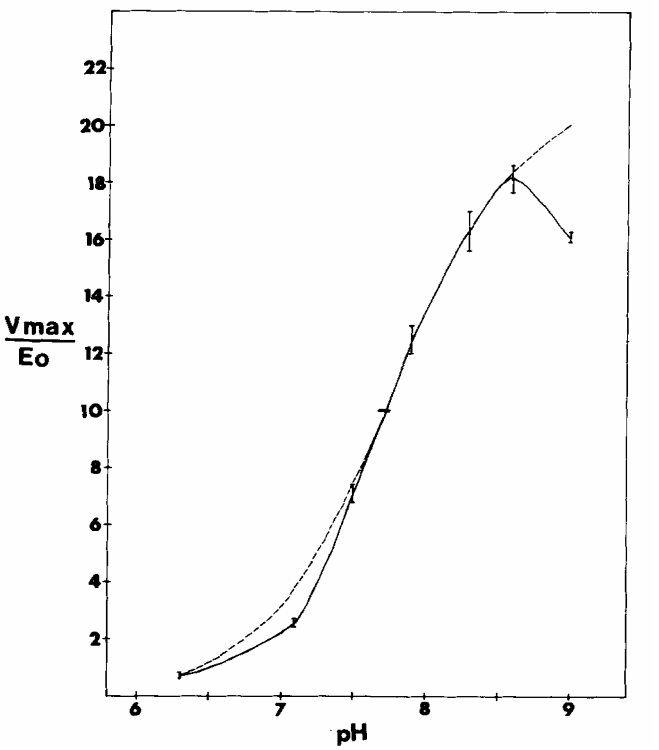

Figure 2. pH dependence of fatty acyl-CoA-dehydrogenase activity monitored through the reduction of electron transfer flavoprotein at 25°C. Phosphate buffer (pH 6.5 and 7.0) or Tris buffer (pH > 7.5) was used for the assay. The figure is reproduced from Schmidt *et al.* (1981), in which the experimental procedures are described in details.

8. ANTIOXIDATIVE ROLE OF TAURINE AND FURTHER PERSPECTIVES

Often taurine has been presented as an antioxidant despite the fact that the molecule is very stable and difficult to oxidise. However, by applying the hypothesis presented here, an indirect antioxidative role can be ascribed to taurine by maintaining mitochondrial oxidation and stabilising the oxidative environment and thus reduce the leakage of the reactive compounds formed inside mitochondria. On the contrary, taurine depletion will destabilise the oxidative environment with increased release of reactive oxygen species as a likely consequence. This situation will contribute to mitochondrial dysfunction.

Several clinical conditions have been reported as related to mitochondrial dysfunction, e.g., type 2 diabetes (Lowell and Shulman, 2005). Further studies are required pursuing the relationship between mitochondrial dysfunction and alterations in the cellular or mitochondrial composition like taurine depletion. However, the hypothesis presented focusing on the possible role of taurine as mitochondrial matrix buffer represents a new environmental concept for the understanding of mitochondrial dysfunction.

9. ACKNOWLEDGMENTS

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