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The accumulation and synthesis of betaine in winter skate (*Leucoraja ocellata*)

Jason R. Treberg *, William R. Driedzic

Ocean Sciences Centre, Memorial University of Newfoundland, St. John's NL, Canada

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Abstract

The present study investigated aspects of betaine metabolism in an elasmobranch fish, the winter skate (*Leucoraja ocellata*). Based on the level of choline dehydrogenase (ChoDH) activity, the liver and kidney appear to be the major sites of betaine synthesis and the mitochondrial localization of ChoDH and betaine aldehyde dehydrogenase (BADH) indicates that the metabolic organization of betaine synthesis in winter skate is similar to other vertebrates. Food deprivation did not affect white muscle betaine content, and prolonged starvation (70 days) appeared to decrease the total hepatic betaine synthetic capacity. There was no decrease in ChoDH or BADH activity at the mitochondrial level with starvation, suggesting any decrease is due to catabolism of hepatic reserves rather than downregulation of betaine synthesis. Skates fed a high betaine diet (frozen squid ~ 55 μmol g^{-1}) had elevated white muscle betaine content compared to those fed a low betaine diet (frozen herring <2 μmol g^{-1}); however, high dietary betaine intake did not affect the activity of betaine synthesizing enzymes in liver. Acclimation to elevated salinity (120 and 130% seawater) did not result in an increase in white muscle betaine content. Taken as a whole, the present data suggest that diet is a major determinant of muscle betaine in the winter skate and that betaine is of marginal importance as an intracellular osmolyte in this species.

Keywords: Choline dehydrogenase; Betaine aldehyde dehydrogenase; Methylamines; Organic osmolyte; Elasmobranch; Osmoregulation

1. Introduction

Trimethylglycine, also referred to as glycine–betaine or simply betaine, is well established as an intracellular osmolyte in a number of animals (see reviews by Yancey et al., 1982; Dragolovich, 1994; Yancey, 1994; Craig, 2004). Betaine is synthesized in animals by the sequential and physiologically irreversible dehydrogenation of choline to betaine aldehyde and then to betaine by the enzymes choline dehydrogenase (ChoDH: EC 1.1.99.1) and betaine aldehyde dehydrogenase (BADH: EC 1.2.1.8).

Little is known about what role synthesis plays in relation to the regulation of betaine as an intracellular osmolyte in animal cells. In the mammalian kidney, betaine accumulation is due to *in situ* synthesis, but the liver may also provide betaine for uptake by renal cells (Craig, 2004). The metabolic regulation of betaine synthesis in response to osmotic challenge has been studied in invertebrates with the horseshoe crab heart and oyster gill being investigated in the most detail. In the horseshoe crab heart, mitochondrial choline uptake has been implicated as the rate limiting step of betaine synthesis (reviewed in Dragolovich, 1994), similar to the case in mammalian liver (Kaplin et al., 1993). Conversely, a number of data suggest that betaine synthesizing enzymes play an important part in the upregulation of betaine synthesis in oyster gill. Oysters from Chesapeake Bay accumulate far less betaine in their gills than Atlantic Ocean conspecifics, yet, the rate of mitochondrial choline uptake is similar between these oyster populations. However, these populations differ markedly in their capacity for betaine synthesis and accumulation in response to hyperosmotic challenge (reviewed in Dragolovich, 1994). Substrate and cofactor affinity is much higher in the Atlantic population of oysters for BADH, suggesting this enzyme may partially

* Corresponding author. Department of Biochemistry, Memorial University of Newfoundland, St. John's, Canada NL A1B 3X9. Tel.: +1 709 737 8547; fax: +1 709 737 2422.
E-mail address: jtreberg@mun.ca (J.R. Treberg).

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explain the differences in betaine synthesis (Perrino and Pierce, 2000b). Adding further support for a regulatory role of enzyme activities in betaine synthesis, there is no difference in substrate affinity for ChoDH between oyster populations, but mitochondrial activity in Atlantic oysters is double that of the Bay population (Perrino and Pierce, 2000a).

Many marine elasmobranchs (sharks, skates and rays) accumulate betaine, along with other methylamines including trimethylamine oxide (TMAO) and sarcosine, in muscle (Robertson, 1989; Withers et al., 1994; Stelle et al., 2005; Treberg et al., 2006b) as intracellular osmolytes (Yancey, 2001). Based on the activities of ChoDH and BADH in liver, elasmobranchs have significant capacity for the synthesis of betaine and enzyme activities in liver may be related to the degree of muscle betaine accumulation in marine species (Treberg et al., 2006b).

The synthesis of methylamines in elasmobranchs has received little experimental attention, with the majority of focus directed towards determining the species distribution for the synthesis of specific methylamines, namely TMAO. The study by Treberg et al. (2006b), which demonstrated that all species examined had the enzymes of betaine synthesis in the liver, presents the only data specifically on betaine synthesis in elasmobranchs. It was speculated that the freshwater stingray *Himantura signifer* may synthesize betaine in response to hyperosmotic challenge (Treberg et al., 2006b). If so, this would be the first demonstration of active synthesis of a methylamine in response to osmotic challenge in an elasmobranch. Treberg et al. (2006b) also speculated that diet may influence the methylamines accumulated in the muscle of marine elasmobranchs.

The present study was undertaken to provide currently lacking information on the synthesis and accumulation of betaine in elasmobranchs. First the metabolic organization of betaine synthesis was characterized in the winter skate, *Leucoraja ocellata*. The second goal of this study was to determine what effect diet, including food-deprivation, has on betaine accumulation and the levels of betaine synthesizing enzymes. Finally, we exposed skates to a hyperosmotic challenge to test if they would synthesize betaine in the face of osmotic challenge. In all cases, the other known major organic osmolytes (urea, TMAO, sarcosine, taurine and β-alanine) were also measured to determine if betaine acts in a manner consistent with a role as an osmolyte in this species.

2. Materials and methods

2.1. Animals and sampling

Skates were caught by divers in Conception Bay, Newfoundland, transported to the Ocean Sciences Centre, and housed in flow-through seawater tanks. Animals were maintained at 7–9 °C and fed chopped frozen herring for at least 4 weeks prior to use in experiments. When sampled, unless otherwise stated, fish were killed by a blow to the head. If bled, blood was collected into heparinized syringes by cardiac, or caudal (in >1 kg animals), puncture and centrifuged at 9000 ×*g* for 5 min at 4 °C to obtain plasma.

2.2. Experimental

2.2.1. Tissue distribution of choline dehydrogenase and betaine accumulation

An initial exploratory sampling was done to determine the tissues of interest with respect to the synthesis and accumulation of betaine. Skates, approximately 1.5–2.5 kg, were fasted for 6 days prior to sampling. Fish were killed by a blow to the head and tissues dissected out, frozen with liquid nitrogen and stored at −70 °C or colder until assayed. The tissues sampled included white muscle, red muscle, ventricle, liver, kidney, spleen and brain. All were assayed for ChoDH, the initial step of choline oxidation to betaine, as an indicator of betaine synthetic potential as well as analyzed for the major nitrogenous organic osmolytes used by elasmobranchs as described under the analytical section.

2.2.2. Subcellular distribution of betaine synthesis

Relatively large animals (>1.5 kg) were selected and killed by a blow to the head. Liver was dissected out and diced with scissors and homogenized for isolation of mitochondria by differential centrifugation as described in Treberg et al. (2006a). Fractions from the initial 600 ×*g* and 7000 ×*g* supernatants, as well as isolated mitochondria were frozen in liquid nitrogen and stored at −70 °C or colder for later analysis of mitochondrial and cytosolic marker enzymes along with ChoDH and BADH, as described below.

2.2.3. Food deprivation effects on betaine accumulation

For short-term food deprivation, winter skates (approximately 150–380 g) were held without feeding for up to 28 days. Of note, some of these animals were the same as those used in another study (Treberg and Driedzic, 2006), where animals were anesthetized with Eugenol and received a small (0.5 mL 100 g^{-1}) intraperitoneal injection of [^{14}C] TMAO after 6 days of food deprivation. Animals were anesthetized with Eugenol again immediately prior to sampling. There was no apparent difference between those used in the previous study and those only from the present experiment, indicating that the experimental procedure in Treberg and Driedzic (2006) was unlikely a confounding factor.

Large animals (>1.0 kg) were selected to compare long-term food deprivation (starvation treatment) effects on tissue betaine levels and the enzymes of betaine synthesis. Fish were either fed frozen herring muscle to satiation 2–3 times per week for 4 weeks and sampled or food was withheld for 10 weeks (70 days) prior to sampling. Liver mitochondria were isolated and frozen (as described previously) to determine if alterations at the mitochondrial level may be masked by the use of whole tissue homogenates for the assay of enzyme activities.

2.2.4. The effect of high dietary betaine intake: Herring compared to squid

To determine if high dietary betaine intake may influence tissue levels, winter skates were fed either frozen herring muscle (with low betaine content) or squid (~ 55 μmol g^{-1} betaine) to satiation 3–4 times a week for at least 4 weeks. Following this