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A comparative analysis of parvalbumin expression in pinfish (*Lagodon rhomboides*) and toadfish (*Opsanus* sp.)

Erich R. Schoenman [a], Joseph A. Chiaro [a], Ashley Jones [a], Loyd D. Bastin [b,c], David J. Coughlin [a,*]

[a] Widener University, Department of Biology, One University Place, Chester, PA 19013, USA
[b] Widener University, Department of Chemistry, One University Place, Chester, PA 19013, USA
[c] Widener University, Department of Biochemistry, One University Place, Chester, PA 19013, USA

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ABSTRACT

This study examines the role of a myoplasmic protein, parvalbumin, in enhancing muscle relaxation by fishes. Parvalbumin is thought to bind free Ca^{2+} during muscle contraction, thereby reducing intracellular [Ca^{2+}] in muscle and speeding muscle relaxation by reducing Ca^{2+} availability to the troponin complex. We hypothesized that parvalbumin expression is ubiquitously expressed in fish muscle and that its expression levels and role in muscle relaxation would depend on the activity level and the thermal environment of a given fish species. Muscle contractile properties and patterns of parvalbumin expression were examined in pinfish (*Lagodon rhomboides*) and two species of toadfish (gulf toadfish, *Opsanus beta*, and oyster toadfish, *Opsanus tau*). Unlike another sparid (sheepshead), the active swimming pinfish does not express parvalbumin in its slow-twitch red muscle. However, both sheepshead and pinfish have relatively high levels of parvalbumin in their myotomal white muscle. Gulf toadfish from the Gulf of Mexico expressed higher levels of parvalbumin and had faster muscle relaxation rates than oyster toadfish from more northern latitudes. The faster muscle of gulf toadfish also expressed relatively more of one parvalbumin isoform, suggesting differences in the binding properties of the two isoforms observed in toadfish swimming muscle. Parvalbumin expression and its role in muscle relaxation appear to vary widely in fishes. There are many control points involved in the calcium transient of contracting muscle, leading to a variety of species-specific solutions to the modulation of muscle relaxation.

1. Introduction

Swimming mode in fishes depends on the physiological properties and recruitment of the various myotomal muscle fiber types. Red, slow-twitch fibers provide the propulsive energy to propel fish at low undulatory swimming speeds, since they have limited oscillatory frequency (Rome et al., 1984). At higher swimming speeds, the pink and/or white muscle fibers are recruited (Coughlin and Rome, 1999). White fibers, which are recruited for faster swimming speeds, have a high power output but fatigue readily in most fish species (Webb, 1994).

Red, pink and white muscles represent fiber types that vary in terms of physiological, morphological and biochemical properties. This variability is responsible for the diversity found in the structure and function of muscles (Berchtold et al., 2000) and contributes to the diversity of fish swimming forms. Physiological properties of muscles such as contraction and relaxation are highly dependent on the types or isoforms of various muscle proteins (Berchtold et al., 2000). Different muscle fiber types display different physiological properties due to variations in the isoform(s) of myosin, troponin and other myofibrillar proteins. In fishes, variations can also be seen within a given muscle fiber type along the length of the myotomal swimming musculature (Coughlin, 2000, 2002). Subtle variations in the relative contribution of different isoforms of a given muscle protein appear to account for this intra-fiber type variation. For instance, Coughlin et al. (2005) showed that the relative expression of two isoforms of troponin T (TnT), a member of the troponin complex, varied along the length of the fish. The study demonstrated that there was a significant shift in the relative expression of two TnT isoforms from anterior to posterior in red muscle of rainbow trout (*Oncorhynchus mykiss*). In addition, variation in TnT expression correlated with muscle contraction properties along the length of the fish: the anterior muscle had faster rates of activation (Coughlin et al., 2005).

Fish commonly show longitudinal variation in the relaxation kinetics of their myotomal or swimming muscle — with the anterior muscle often displaying faster rates of relaxation from contraction (Coughlin, 2002). The present work focuses on a myoplasmic protein, parvalbumin, in aiding relaxation. Parvalbumin is a low molecular mass protein (9–11 kDa) that binds free Ca^{2+}, thereby reducing intracellular [Ca^{2+}] in muscle (see Arif, 2009, for a recent review of parvalbumin's function in muscle and nervous tissue). By binding

* Corresponding author. Tel.: +1 610 499 4025; fax: +1 610 499 4496.
E-mail address: djcoughlin@widener.edu (D.J. Coughlin).

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intracellular Ca^{2+}, parvalbumin aids relaxation from contraction in muscle and increases the rate of action potential firing by neurons. Each molecule of parvalbumin has two divalent ion binding sites. These sites have high affinity for Ca^{2+} and moderate affinity for Mg^{2+}. During the calcium transient, parvalbumin binds Ca^{2+} with a higher affinity than troponin C (TnC), but a lower affinity than the sarcoplasmic reticulum Ca^{2+}-ATPase pumps (Berchtold et al., 2000). As Ca^{2+} is pumped back into the sarcoplasmic reticulum by Ca^{2+}-ATPase pumps and the myoplasmic [Ca^{2+}] decreases, parvalbumin competes with TnC to bind to the sarcoplasmic Ca^{2+}, accelerating the relaxation of muscle. A wide range in total parvalbumin content in fish muscle, from zero to >1.5 mM, has been reported (Gillis, 1985). Greater parvalbumin content is typically associated with fast-twitch muscle of various vertebrates — muscle with high rates of relaxation (Heizmann et al., 1982; Hou et al., 1991; Berchtold et al., 2000).

In fish muscle, parvalbumin was reported until recently to be abundant only in fast-twitch muscle (Zawadowska and Supiková, 1992; Berchtold et al., 2000; Chauvigné et al., 2005). Fish muscle, at least white muscle, commonly expresses two to three isoforms of parvalbumin in a given fish (Sanuki et al., 2003). Some teleosts appear to express three to five isoforms of parvalbumin in their white muscle throughout development (Gillis, 1985; Chikou et al., 1997; Huriaux et al., 2002; Focant et al., 2003). Recently, Brownridge et al. (2009) reported eight isoforms of parvalbumin expressed in carp muscle (at the same time), although only 2–3 were expressed in relatively large amounts. We have demonstrated parvalbumin expression in the red and white muscle of various fish species, including sheepshead (*Archosargus probatocephalus*) and southern kingfish (*Menticirrhus americanus*) (Wilwert et al., 2006) and rainbow trout (*O. mykiss*) and brook trout (*Salvelinus fontinalis*) (Coughlin et al., 2007).

In fishes, there are correlations between parvalbumin expression and muscle relaxation. For instance, Thys et al. (1998, 2001) reported that faster relaxing anterior white muscle of cod (*Gadus morhua*) and bass (*Micropterus salmoides*) expresses higher levels of parvalbumin than posterior white muscle. We have shown that muscle with faster relaxation rates tends to express more parvalbumin *and* relatively more of a particular isoform (Wilwert et al., 2006; Coughlin et al., 2007). Brownridge et al. (2009) report similar results for carp (*Cyprinus carpio*) — differential expression of parvalbumin isoforms associated with variations in muscle relaxation rate. The goal of this work is to broaden the perspective of fish species in which patterns of both muscle relaxation and parvalbumin expression have been examined. We hypothesize that parvalbumin is ubiquitously expressed in fish myotomal muscle and that longitudinal variations in parvalbumin expression are associated with variations in muscle relaxation. First, we examined longitudinal patterns of muscle relaxation and parvalbumin expression in pinfish (*Lagodon rhomboides*). Pinfish are in the same family (Sparidae) as sheepshead. Sheepshead show a close correlation between muscle relaxation and total parvalbumin expression as well as relative expression of two parvalbumin isoforms in their myotomal muscle (Wilwert et al., 2006). Since pinfish are active swimmers similar to sheepshead, we predicted that pinfish will display patterns of muscle relaxation that correlate with parvalbumin expression.

Because most fishes are ectothermic and physiological properties of muscle are temperature sensitive (e.g. Johnston and Crockford, 1990; Rome et al., 2000), we also studied two species of toadfish from coastal habitats — New England and the Gulf of Mexico — that differ in their environmental temperatures but are otherwise similar in appearance and ecology. Oyster toadfish (*Opsanus tau*) experience temperatures of ~10–20 °C (Runge et al., 1999; Tang et al., 1999), while gulf toadfish (*Opsanus beta*) are found in shallow water bays in the Gulf of Mexico that can exceed 30 °C (Sogard et al., 1987; Tuckey and Dehaven, 2004). We predict that oyster toadfish will display a more significant role for parvalbumin in the modulation of muscle relaxation than the sub-tropical gulf toadfish. Toadfish communicate via sonic muscle in the walls of their swimbladder. Hamoir et al. (1980) reported that the oyster toadfish has high parvalbumin concentrations in its sonic muscle, a trait that Feher et al. (1998) and Rome (2006) suggest facilitates the high frequency oscillations necessary for sound production. Therefore, we included swimbladder muscle for comparison with the white myotomal muscle. The present studies on pinfish and toadfish are intended to shed some light on how parvalbumin enhances muscle relaxation, but they also show that parvalbumin expression is highly variable in fishes.

2. Materials and methods

2.1. Experimental animals

Pinfish (*L. rhomboides*) and gulf toadfish (*O. beta*) collected from the Gulf of Mexico were obtained from Gulf Specimen Marine Laboratory (Panacea, FL). Oyster toadfish (*O. tau*) collected from the coastal waters of Southeastern Massachusetts were obtained from the Marine Biological Laboratory (Woods Hole, MA). All handling of experimental animals was reviewed by the Widener University Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council.

A total of 10 pinfish were used in this study with a mean (±SD) mass of 38.5 ± 6.4 g and mean total length (TL) of 13.7 ± 1.0 cm. The mean mass and total length for the gulf toadfish ($n = 6$) were 81.5 ± 22.7 g and total length of 17.4 ± 1.3 cm, while mass and total length of the oyster toadfish ($n = 4$) were 358.0 ± 26.7 g and 26.6 ± 0.5 cm.

2.2. Isometric contraction kinetics

For pinfish, isometric contractile properties were determined for red and white muscle from anterior (ANT = 0.35 TL) and posterior (POST = 0.75 TL) body positions. For the toadfish, only the white muscle from the myotome was examined — again from ANT and POST. In addition, contractile properties were recorded from the swimbladder muscle.

To perform mechanics experiments, the fish were killed by spinal transaction and pithing. For pinfish, scales were removed and strips (~0.5 mm wide) of muscle with overlying skin were removed above and below the lateral line. Each strip contained red and white muscle. For toadfish, the scale-less thick mucous skin was removed and strips of white muscle with a very thin overlying layer of red muscle were extracted from lateral white myotome of the fish. Using a stereomicroscope, subsequent dissection was carried out in physiological saline at 20 °C (Coughlin et al., 2005). Live muscle bundles of one fiber type were prepared. Individual muscle bundles used in each experiment were the length of one myomere (4–5 mm) and had a cross-sectional area of 0.25–1.0 mm^2. For swimbladder, a sheet of muscle wraps from dorsal to ventral around each sides of the approximately 2 cm wide bladder. Strips of live muscle were cut from the sheet with connective tissue (wall of the bladder) at each end.

The muscle mechanics system was comprised of a servomotor (Cambridge Technology 300 S) and a force transducer (Cambridge Technology 404A). For all the experiments, the muscle bundles were tied to the system and maintained at an initial temperature of 20 °C. The physiological saline was aerated gently to supply oxygen and to induce circulation. Experimental control and data were carried out using a PC, a National Instruments input/output board and customized Lab-View software (National Instruments).

To obtain maximal tetanic force, the activation conditions (muscle length, pulse length and amplitude for twitch contractions, stimulus duration and frequency for tetanic contractions) for each bundle were optimized. For myotomal muscle, the typical tetanus stimulus period was 100–200 ms and was composed of 1.0–2.0 ms pulses at an amplitude of 6–10 V at a stimulation frequency of 200–400 Hz. Longer