Negative Charge Correlates with Neural Expression in Vertebrate Aldolase Isozymes

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Abstract. Electrophoretic studies suggest that negatively charged neural proteins are a general feature of jawed vertebrates. In an apparent example of this, teleost fish express three aldolase isozymes, one of which is expressed predominantly in neural tissues and is more negatively charged than its more generally expressed paralogues. We characterized three aldolase isozymes from a single species of teleost fish, zebrafish (Danio rerio). These sequences indicated that the correlation of net negative charge and neural expression suggested in other species by gel electrophoresis was supported by sequence analysis. When aldolase sequences from the databases were included in phylogenetic analyses, the negative charge/neural expression phenomenon was observed across the gnathostome vertebrate sequences examined. We found no evidence for a period of positive Darwinian selection resulting in an accumulation of negatively charged amino acids during the evolution of the neural aldolase isozymes. This is likely attributable, however, to limitations associated with the age of the duplication responsible for the neural isozyme and the reconstruction of ancestral sequences.

Key words: Fructose bisphosphate aldolase — Gene duplication — Neural expression — Teleost fish

Introduction

Individual tissues often express unique sets of proteins. For example, the proteins of the vertebrate eye and brain are, as a group, more negatively charged than those proteins expressed in other tissues (Moore and McGregor 1965; Moore 1973). This charge bias has been well characterized in teleost fish, in which the isozymes [distinct forms of enzymes that catalyze identical chemical reactions (Markert and Moller 1959)] of the eye and brain of a number of gene families are more negatively charged than their paralogues expressed in other tissues (e.g., Fisher et al. 1980; Morizot and Schmidt 1990; Merritt and Quattro 2001). The apparent correlation between net negative charge and neural expression has led to the suggestion that the negative charge of neural proteins might be an adaptation to the neural environment (Fisher et al. 1980; Merritt and Quattro 2001).

We recently examined the evolution of a negatively charged, neurally expressed, isozyme of triosephosphate isomerase (TPI; EC 5.3.1.1) in teleost fish (Merritt and Quattro 2001). Two TPI proteins are expressed in teleost fish, including a neutral, generally expressed, isozyme (TPI-B) and a negatively charged, neurally expressed, isozyme (TPI-A) (Pontier and Hart 1981; Morizot and Schmidt 1990). All other
vertebrates express only a single TPI protein (Maquat et al. 1985; Old and Mohrenweiser 1988; Cheng et al. 1990; Straus and Gilbert 1985; Kuraku et al. 1999). The two teleost TPI isozymes are the products of separate loci (Pontier and Hart 1981) that result from a duplication event early in the radiation of teleost fish (Merritt and Quattro 2001). Importantly, the negative charge of the teleost neural TPI isozyme results from the biased accumulation of negatively charged amino acids during a period of positive Darwinian selection (Merritt and Quattro 2001).

Negative neural isozymes are not restricted to teleost fish but are found across gnathostome (jawed) vertebrates. For example, the vertebrate creatine kinase (Richardson et al. 1986; Morizot and Schmidt 1990) and enolase (Morizot and Siciliano 1984; Marangos and Schmechel 1987; Morizot and Schmidt 1990; Tracy and Hedges 2000) gene families include a negatively charged neural isozyme. The presence of negatively charged neural isozymes across a variety of vertebrate taxa suggests that the selective pressures favoring the accumulation of negative amino acids by teleost neural isozymes [e.g. TPI (Merritt and Quattro 2001)] might be present across all vertebrate taxa.

To address this possibility, we examined the evolutionary history of the aldolase (class I fructose-1,6-bisphosphate aldolase; EC 4.1.2.13) gene family, which includes a negatively charged neural isozyme common to gnathostome vertebrates (e.g., Penhoet et al. 1967; Phillip et al. 1979; Morizot and Schmidt 1990). Gnathostome vertebrates express three aldolase isozymes: aldolase A is expressed predominantly in muscle, aldolase B is expressed predominantly in the liver, and aldolase C is expressed predominantly in the brain and nervous system (Penhoet et al. 1967; Phillip et al. 1979; Morizot and Schmidt 1990). Gel electrophoresis indicated that both aldolase A and aldolase B are positively charged, whereas aldolase C is negatively charged (Penhoet et al. 1967; Phillip et al. 1979; Morizot and Schmidt 1990). Jawless vertebrates (agnathans; lamprey and hagfish) express two aldolase isozymes; neither is characteristically neural in expression and both are neutrally charged (R. Zhang et al. 1997). The three aldolase isozymes in gnathostomes and the two isozymes in agnathans share a single-gene ancestor early in the radiation of vertebrates (Kuraku et al. 1999); this suggests that the different number of isozymes in each group result from lineage-specific gene duplications.

All three aldolase isozymes have been sequenced from a number of tetrapod vertebrates [e.g., human (Sakakihara et al. 1985; Besmond et al. 1983; Rottmann et al. 1987) and rat (Mukai et al. 1986, 1991; Tsutsumi et al. 1984)]. Aldolase B (Llewellyn et al. 1995) and aldolase C (Berardini et al. 1997) have been described from teleost fish but, unfortunately, from two phylogenetically disparate groups. All three aldolase loci have not been described from a single taxonomic group of fish. Given that isozymes are known to vary in charge between even very closely related species (e.g., Pointer and Hart 1979, 1981), comparisons of currently available teleost aldolase sequences risk confounding between-locus patterns of enzyme diversification with between-species patterns. To circumvent the potential for confounding comparisons, we report the complete coding sequence of the three aldolase isozymes from a representative teleost fish, zebrafish (Danio rerio). We use these sequences and other aldolase sequences from GenBank in phylogenetic analyses to examine the evolutionary history of vertebrate neural aldolase proteins.

### Methods

**Danio rerio** (zebrafish) tissues were dissected from single fish purchased locally and processed immediately or stored at ~70°C for later processing. Total RNA was purified from tissues using RNasy (Qiagen). Complementary DNA (cDNA) was synthesized from total RNA using the Superscript Pre-amplification System (Gibco BRL).

### Aldolase Cloning and Sequencing

Oligonucleotide primers were designed for individual aldolase loci using alignments of zebrafish expressed sequence tags (ESTs) and other vertebrate aldolase sequences available from GenBank.

**Aldolase A.** Partial sequences of the 5′ and 3′ ends of the zebrafish Ald-A mRNA are available through GenBank (GenBank accession numbers AI436937 and AA606169, respectively). These sequences were used to design specific oligonucleotide primers that flank the aldolase A coding region.

DrALDA5′: 5′-ACG TGG TCG AGG CTG GTC G-3′
DrALDA3′: 5′-CAG GTG GAG TCA TTC C-3′

These two primers were used to amplify a 1230-base pair (bp) segment of the Ald-A mRNA from zebrafish muscle cDNA by the polymerase chain reaction (PCR) (Saiki et al. 1988). PCR was carried out for 40 cycles under the following conditions: denaturation at 95°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 1 min. PCR products were cloned into pGEM T-vector (Promega) and sequenced using a 377 automated Sequencer (Perkin Elmer). Dye terminator sequencing was performed with BigDye dye termination mix (Perkin Elmer Biosystems) using the manufacturer’s suggested protocol. At least three independent clones per PCR fragment were sequenced on both strands.

**Aldolase B.** EST sequences (GenBank accession numbers AI878510, AI629370, AI522695, AI477716, AI477428) provided partial 3′ sequences of the zebrafish Aldolase-B mRNA and were used to design an oligonucleotide primer specific to this region.

DrALDB3TR: 5′-ATA ACT TAC TTT GCA TCT TCA CTC-3′

An alignment of all vertebrate aldolase genes in GenBank was used to design a primer near the 5′ end of the aldolase coding region.

**ALD 44F:** 5′-CCN GAR CAR AAR AAR GA-3′ where N = A,G,C,T and R = A,G. Numbers refer to the amino acid position occupied by the 3′ base of the primer in the sea bream (Sparus aurata) aldolase B (Llewellyn et al. 1995). These two primers were used to amplify a 1142-bp product from zebrafish