Two distinct types of fatty acid-binding protein are expressed in heart ventricle of Antarctic teleost fishes

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This report provides the first evidence for the existence of two distinct types of fatty acid-binding protein (FABP) in cardiac tissue of vertebrates. Four species of Antarctic teleost fish (*Chaenocephalus aceratus*, *Cryodraco antarcticus*, *Gobionotothen gibberifrons* and *Notothenia coriiceps*) exhibited two FABP mRNAs of 1.0 kb and 0.8 kb, which we have termed H_h-FABP and H_{ad}-FABP (isolated from Heart tissue, with similarity to mammalian heart-type FABP or mammalian adipose-type FABP respectively). These FABP types appear to be products of distinct genes. Both FABP transcripts were abundant in cardiac and aerobic pectoral muscle. However, relative abundance of the two types varied distinctly among other tissues such as kidney, brain,

spleen and white muscle. Neither FABP type was expressed in liver or intestine. The coding regions of H_h-FABP and H_{ad}-FABP cDNAs from the same species are only ~60% identical with one another. However, homologues of each FABP species, which exhibit >98% identity to their respective types, were isolated from three other Antarctic teleosts. Phylogenetic analysis of aligned amino-acid sequences places H_h-FABP with other vertebrate heart-type FABPs, and H_{ad} with adipose/cutaneous FABPs. Expression of two distinct FABPs in cardiac tissue of Antarctic teleosts may be related to their ability to both utilize fatty acid as the primary metabolic fuel and to store lipid intracellularly.

INTRODUCTION

Intracellular fatty acid-binding proteins (FABPs) are a family of small (~15 kDa) polypeptides that are expressed at high concentrations in nearly all tissues. Since their first description [1,2], nine types of FABP have been identified in mammals, each of which is the product of a distinct gene and exhibits tissue-specific expression (for reviews see [3,4]). Although the primary sequence of these FABP types may differ considerably, the tertiary structure of the proteins is maintained as a 'clam shell' comprised of two orthogonal β -sheets connected by two α -helices. FABPs bind long-chain fatty acids and other hydrophobic ligands *in vitro*, but their function *in vivo* remains undefined. FABP types are expressed in a tissue-specific manner suggesting that each type may perform a unique function within the cell. Although several organs express more than one FABP type, only the H-type of FABP has been found in cardiac tissue of known vertebrates.

It has been proposed that, intracellularly, FABPs act as regulators of fatty acid metabolism by transporting long-chain fatty acids through the aqueous cytoplasm to the site of their oxidation, either mitochondria or peroxisomes, as an intracellular fatty-acid buffer to prevent micelle formation, as a regulator of ion-channel conductance and as a signal for cardiomyocyte hypertrophy (for a review see [3]). Of these diverse functions, a role for FABPs in regulating fatty acid metabolism is most strongly supported by circumstantial evidence. FABP concentration increases with treatments that increase fatty acid metabolism and is positively correlated with the ability of a tissue to metabolize fat.

The present paper reports results obtained from FABP-types expressed in both heart and oxidative skeletal muscle, which are

consistent with distinct physiological roles. The primary metabolic fuel of Antarctic notothenioid fish cardiac muscle is lipid [5] but, in contrast to mammals, heart tissue of Antarctic notothenioids also stores triglycerides intracellularly. We isolated two distinct FABP cDNAs from cardiac tissue of four Antarctic teleost fish, which may be related to these two possible metabolic fates of fatty acids. One form is consistent with that reported by Londraville and Sidell [6], and the second is a novel type with similarity to mammalian adipose FABP. To our knowledge, this is the first report of any vertebrate heart tissue expressing more than one FABP.

EXPERIMENTAL

Animals and tissues

Live specimens of *Chaenocephalus aceratus*, *Notothenia coriiceps*, *Parachaenichthys charcoti* and *Gobionotothen gibberifrons* were collected by Otter trawl off the south shore of Low Island and off the west coast of Brabant Island during the austral autumns of 1991, 1993 and 1995. *Lycodichthys dearborni* was caught south-east of Lavoisier Island in May 1993. Heart ventricle of *Cryodraco antarcticus*, caught in McMurdo Sound, was kindly given by Dr. Arthur DeVries. Tissues were dissected immediately after the fish were killed and were flash-frozen in liquid N₂. Samples were transported to the U.S.A. on solid CO₂ and stored at -70 °C until isolation of mRNA.

Isolation of mRNA

Total RNA was extracted from 0.5–1.5 g of heart ventricle, adductor profundus muscle and other tissues by the hot phenol

Abbreviations used: FABP, fatty acid-binding protein; H_h-FABP, FABP isolated from heart tissue with similarity to mammalian heart-type FABP; H_{ad}-FABP, FABP isolated from heart tissue with similarity to mammalian adipose-type FABP; RACE, rapid amplification of cDNA ends.

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The nucleotide sequences reported here appear in the EMBL and GenBank Nucleotide Sequence Databases under the accession numbers U92442, U92443, U92444, U92445, U92446, U92447, U792448, U92449, U92450 and U92451.

Table 1 Teleost FABP type-specific oligonucleotides used for PCR amplification and DNA sequencing

Oligonucleotide J is a degenerate sense-strand primer based on the partial peptide sequence of *Chaeno. aceratus* FABP isolated from aerobic pectoral adductor [6]. The oligo (dT)/*NotI* primer adaptor was from Promega. All other type-specific oligonucleotide primers were based on the nucleotide sequence of *Chaeno. aceratus* FABP types. Symbols: N = A, C, G or T; R = A or G; Y = C or T. → denotes a sense-strand primer, ← denotes an antisense-primer. Locations indicated are relative to the full-length cDNA sequences shown in Figure 1.

Primers	Sequence	Location
H_h-FABP		
J	5'd(GGNGTNGGNTTYGCNACNMG)3'	153 → 172
J8	5'd(GGTGTTGGCTTCGTACACG)3'	153 → 172
L8	5'd(TTCCGACCTGGCGTGTAGCG)3'	183 ← 164
M8	5'd(GTTGATGACAGGAAAGT)3'	306 → 322
N8	5'd(CAGGGAAGTCAACGGCAA)3'	398 → 415
A8N	5'd(TTGCGGTTGACTTCCCTG)3'	415 ← 398
P8	5'd(AGTCTGTCTCCTCCTCG)3'	1 → 20
Q8	5'd(CGTACCTGTCTCCCTCCA)3'	233 ← 213
H_{ad}-FABP		
J	5'd(GGNGTNGGNTTYGCNACNMG)3'	136 → 155
J6	5'd(GGTGTTGGGATTTGCGACCCG)3'	136 → 155
L6	5'd(CCTATCGTCTGCGGTGCTCTC)3'	302 ← 282
M6	5'd(GCAGACGATAGGAAGAC)3'	291 → 307
N6	5'd(GCTGGGATGGCAAGAGA)3'	356 → 373
A6N	5'd(TCTCTTTGCCATCCCAGC)3'	373 ← 356
P6	5'd(GTCACTTCACTGTTTATGTG)3'	20 → 41

method [7], with modifications for fish tissues as described by Vayda et al. [8]. A typical yield was 320 µg of total RNA/g fresh weight of heart ventricle and 200 µg/g fresh weight of skeletal muscle. Poly(A)⁺ RNA was isolated from 200 µg of total RNA by successive hybridization to two oligo(dT)-cellulose columns (Pharmacia). Typically, 5 µg of poly(A)⁺ was recovered per 200 µg of total heart ventricle RNA. RNA samples were stored at -70 °C until use.

cDNA synthesis and PCR amplification

First strand cDNA was synthesized from 0.2–0.5 µg of poly(A)⁺ RNA using Superscript II reverse transcriptase as described by the supplier (Gibco) and primed by 0.5 µM *NotI* primer adapter (Promega). PCR amplification was performed using the hot-start procedure, AmpliTaq (Perkin-Elmer) DNA polymerase and 2 µM sense and antisense primers. Oligonucleotide primers used to amplify and sequence the entire cDNA are listed in Table 1 and were synthesized by DNA International or Gibco Life Technologies. Reaction mixtures were subjected to 29 to 35 heating cycles using a thermocycler (M. J. Research).

Ligation-mediated PCR amplification was accomplished using the 5'-rapid amplification of cDNA ends (RACE) kit (Clontech). cDNA was synthesized from 2 µg of poly(A)⁺ mRNA primed with either A6N or A8N (Table 1). First strand cDNA was ligated to the 5'-RACE anchor oligonucleotide (5'-CACGA-ATTCACATCGATTCTGGAACCTTCAGAGG-3') (Clontech). Subsequent PCR amplification employed the antisense anchor primer (5'-CTGGTTTCGGCCACCTCTGAAGGTTCCAGAATCGATAG-3') and either FABP type-specific primer L6 or L8. Amplification products were purified for cycle sequencing by electrophoresis through a 1.8% (w/v) SeaPlaque GTG agarose (FMC Bioproducts) gel and subsequent gel digestion using β -agarase (Sigma).

RNA gel-blot hybridization

Equal amounts (5 µg) of total RNA were resolved by electrophoresis through 1% agarose-formaldehyde gels [7] and blotted to GeneScreen Plus nylon membranes (Dupont). Blots were probed by hybridization at 65 °C with either the J8-A8N PCR product or J6-A6N PCR product of *Chaeno. aceratus* heart cDNA, ³²P-labelled by the random primer method (Boehringer-Mannheim) using [α -³²P]dCTP (NEN-Dupont). Blots received two successive 30 min washes in 0.1 × SSPE [1 × SSPE = 0.15 NaCl/10 mM sodium phosphate (pH 7.0)/1 mM EDTA]/0.1% (w/v) SDS at 65 °C, and were exposed to Kodak X/AR 5 film with a tungsten intensifying screen for 1 day at -70 °C.

DNA sequencing

DNA sequence of amplified fragments was determined by the cycle sequencing method. DNA template (100 µg) was added to the PRISM Ready Reaction DyeDeoxy Termination Cycle Sequencing Kit (Applied Biosystems) with 10 µM of one of the primers shown in Table 1, and subjected to 25 cycles in a thermocycler (MJ Research). Sequence of the products were read by a 'stretch' automated DNA sequencer (373A; Applied Biosystems) at the University of Maine DNA sequencing facility.

Computer analysis and protein modelling

Phylogenetic relationships among FABP types were estimated by parsimony analysis of aligned sequences. H_{ad}-FABP and H_h-FABP Antarctic fish amino-acid sequences (deduced from cDNA sequences) were aligned with FABPs from other organs/taxa using CLUSTAL W [9]. Other taxa [taxon (accession number)] included: tapeworm (A48578), locust muscle (S43470), rat liver (M35991), bovine liver (X86904), pig liver (X77640), human liver (M10050), *Manduca sexta* 1 (M77754), *Manduca sexta* 2 (M77755), liver fluke (A44638), *Schistosoma mansoni* (M60895), *Schistosoma japonicum* (L23322), *Xenopus* intestinal (L19946), human intestinal (M18079), rat intestinal (K01180), shark liver (S20297), rat cutaneous (JC2201), bovine adipose (X89244), bovine brain (S06479), human muscle (X56549), mouse heart (X14961), rat heart (M18034) rat stomach (S06478), bovine heart (X12710), bovine mammary-derived growth inhibitor (X51933), mouse brain (U04827), rainbow trout heart (U95296) and chicken eye (A49184). The CLUSTAL W alignment was imported into PHYLIP [10], bootstrapped 500 × and subjected to parsimony analysis using the PROTPARS subroutine.

Protein structures were examined and modelled using RASMOL [25] and Kinemage [12] based on the known tertiary structures of human muscle FABP [13] and adipocyte FABP [14,15]. The model shown in Figure 6 was prepared using MOLSCRIPT software [16].

RESULTS

FABP cDNA was obtained by PCR amplification using the degenerate primer J (Table 1) and *NotI*/oligo(dT) (Promega). The primer J oligonucleotide was based upon the partial peptide sequence of *Chaeno. aceratus* aerobic skeletal muscle FABP [6]. Two distinct products of ~ 800 bp and ~ 600 bp were amplified from both *Chaeno. aceratus* heart ventricle and the highly aerobic pectoral muscle adductor profundus. Tissues from six *Chaeno. aceratus* individuals yielded the same result. Partial sequencing indicated that the two PCR products are derived from two

H_h-FABP	agtcgtgtctctcctcctcgctttacctctcacaccctcacacagtctgtgtatctttccacccctccactccgctgcaaac	80
H_{ad}-FABP	ttgggagagttgaacccagagtcacttcactgtttattgtcgaggatatacagaagctgcaaaa	63
ace FABP	M K K L G V G M V D V F V G T W N L K D S E K F D E Y M K K L G V G	
H_h-FABP	ATGGTCGATGTTTTCGTTGGCACCTGGAACCTGAAGGATAGTGAGAAGTTTGATGAGTACATGAAGAAGCTGGGTGTTGGC	161
H_{ad}-FABP	ATGGTTGAGAAATTTGTTGGGACGTGGAAGATGATTTCACGCATAACTTTGATGACTACATGAAAGCAATCGGTGTGGGA	144
	M V E K F V G T W K M I S S D N F D D Y K K A I G V G	
ace FABP	F A T R Q V G N V T K P T T I I S V E G D K V T L K F A T R Q V G N V T K P T T I I S V E G D K V T L K	
H_h-FABP	TTTCGTACACGCCAGGTGCGAAACGTGACCAAGCCACCACCATCATCTCAGTGGAGGGAGACAAG--GTGACGTTGAAG	239
H_{ad}-FABP	TTTTCGACCCGGCAGGTGGGAATCGCACCAAAACCACTTGGTAGTGAGCGTGGACGATCAAGGGTTTGTATGCTTGAAG	225
	F A T R Q V G N R T K P N L V V S V D D Q G F V C L K	
ace FABP	T Q S A I K N T E L S F K L D Q Q F D T Q S A I K N T E L S F K L D E E F D E T T A D D R K	
H_h-FABP	ACGCAGAGCGCCATCAAGAACACAGAGCTCTCCTTCAAGCTGGATGAGGAGTTTCGATGAGACCACCGCTGATGACAGGAAA	320
H_{ad}-FABP	TCTCAGAGCACTTTCAAACCACTGAGATCAAGTTCAAGCTAAACGAGCCATTTCGAAGAGACGACCGCAGACGATAGGAAG	306
	S Q S T F K T T E I K F K L N E P F E E T T A D D R K	
H_h-FABP	V K S F V T V D G G K L V H T Q K W D G K E T S L V R [*] GTCAAGTCCTTTGTAAACAGTTGATGGTGGGAAGTTGGTGCACACCCAGAAGTGGGACGGCAAAGAGACCAGCCTGGTCAGG	401
H_{ad}-FABP	ACCAAGACCGTGGTGACTCTGGAGAACGGGAACTTGTGCAGAAACAGAGCTGGGATGGCAAAGAGACAAATATCGAGAGG	387
	T K T V V T L E N G K L V Q K Q S W D G K E T N I E R [*]	
H_h-FABP	E V N G N N L T L T L K M D D V E S I R R Y V K A E * GAAGTCAACGGCAACAACCTCACACTGACACTAAAAATGGATGATGTCAATCCATACGTCGCTACGTTAAGGCAGAGTAA	482
H_{ad}-FABP	GAGATCGAAGATGGGAAATTTGGTAGCGAAATGCATTATGGGTGATGTGATCGCAGTGAGGACATACGTGATGGAGGCATGA	468
	E I E D G K L V A K C I M G D V I A V R T Y V M E A *	
H_h-FABP	atcccagcactgaaacatccctccagcatcttcttgcacagctaaagccctcgacccccaaactacaaccagaacaa	563
H_{ad}-FABP	ttggctcccttcagccactcggtggatacattgctggatgacaggctcagttcaatgagcggtataccaaactaaagtaa	549
H_h-FABP	taaaacctcgatggctgccgcagattccctcacactgtgctcagttataacatcactgcggaactgtaacatcattttct	644
H_{ad}-FABP	aggcgtgttttgccttactctttatcttcaactgttttgcacaaataaatccctgtgcctctgttctccaacatgacaca	630
H_h-FABP	ttatactgtggctccctatactgcaagaaacgtgaataattttctgatgtttgtgtgtttgtctcttaaagatccagt	725
H_{ad}-FABP	tttggtttcatttgaattgtgtgtgtgatttgAATAAAaatcaatgagaatttctc(A) _n	687
H_h-FABP	tttggtatgataattatttttagaaaatgttttctgcatggtgcctatgataaacttggttaagtgttggaatttcaacgg	806
H_h-FABP	ttgtctgttggcaacgtggaacaggcaAATAAAgtgtctacactgtgggacaaactgttaaacgg(A) _n	871

Figure 1 Comparison of H_h-FABP and F_{ad}-FABP cDNA sequences and deduced polypeptides

DNA sequences of the PCR amplification products were obtained by cycle sequencing and resolved on an automated DNA sequencer equipped with 'stretch' gel apparatus. Comparative alignment was accomplished using CLUSTAL W. The presumptive initiation codons (ATG), termination codons (TGA and TAA) and polyadenylation signals (AATAAA) are shown in boldface type. Amino-acid sequences were deduced from the cDNA sequences and are presented in the one-letter code. Partial polypeptide sequence of FABP protein (ace FABP) was isolated from *Chaeno. aceratus* pectoral muscle and reported by Londraville and Sidell [6].

distinct cDNAs. Both types exhibited similarity with mammalian heart-type FABP by BLASTN search, although one also exhibited similarity with mammalian adipose-type FABP. For this reason, we designated these species H_h-FABP and H_{ad}-FABP, in accord with the convention to name FABP species with respect to the first tissue of their isolation. Second generation, non-degenerate primers specific for each type (Table 1) were synthesized to obtain the complete sequence of the H_h-FABP and H_{ad}-FABP cDNAs. The extreme 5' termini were obtained by

ligation-mediated PCR using 5'-RACE and the antisense nested primers A6N/L6 and A8N/L8.

The complete sequences of H_h-FABP and H_{ad}-FABP are compared in Figure 1. Although these cDNAs encode related polypeptides of similar size, the nucleic acid sequences of these species are distinct. H_h-FABP contains an 80 nt 5' untranslated region, a 402 nt coding region and a 398 nt 3' untranslated region. In contrast, H_{ad}-FABP contains a 63 nt 5' untranslated region, a 405 nt coding region and a 219 nt 3'

Figure 2 Gel-blot hybridization of RNA isolated from *Chaeno. aceratus* tissues

Samples (5 µg) of purified RNA were resolved by electrophoresis through 1.4% (w/v) agarose formaldehyde gels as described previously [7]. Abbreviations: S, spleen; W, white skeletal muscle; R, aerobic pectoral adductor profundus muscle; H, heart ventricle; K, trunk kidney; B, brain; I, intestine; L, liver. (A) Blot probed with ³²P-labelled *Chaeno. aceratus* H_n-FABP cDNA PCR product. (B) Blot probed with ³²P-labelled *Chaeno. aceratus* H_{ad}-FABP cDNA PCR product. Size of hybridizing species shown is relative to mobility of RNA standards.

region. The two species share only 61.2% identity in the coding region, employ different termination codons (TGA and TAA respectively) and exhibit essentially no identity in the 5' and 3' untranslated regions. The deduced amino-acid sequences contain only 53.4% identical residues and 71.4% similar residues. Comparison of the *Chaeno. aceratus* FABP partial peptide sequence [6] with the predicted polypeptides encoded by the two cDNAs indicates that H_n-FABP was the protein isolated from *Chaeno. aceratus* pectoral muscle by Londraville and Sidell [6].

Tissue-specific differences in the steady-state levels of H_n-FABP and H_{ad}-FABP mRNA suggest that these species are not allelic variants of the same locus but are products of distinct genes. The 1.0 kb H_n-FABP mRNA has a widespread distribution, being most abundant in aerobic pectoral muscle, heart and brain, whereas the 0.8 kb H_{ad}-FABP mRNA has a narrower distribution and is much more abundant in kidney and glycolytic white trunk muscle than H_n-FABP mRNA (Figure 2). Neither type exhibits strong hybridization to mRNA isolated from liver or intestine. The cDNA sequence of H_n-FABP isolated from pectoral muscle is identical with H_n-FABP cDNA obtained from heart ventricle (results not shown). Similarly, the sequence of H_{ad}-FABP obtained from heart and pectoral muscle are identical (results not shown). These results suggest that H_n-FABP and H_{ad}-FABP are independently expressed in both muscle tissues.

Comparison of H_n-FABP and H_{ad}-FABP sequences from other Antarctic fishes confirms that these species are products of distinct genes. The H_n-FABP cDNA sequences from another Antarctic icefish, *Cryo. antarcticus*, and two red-blooded nototheniid relatives, *G. gibberifrons* and *N. coriiceps*, exhibit an

extremely high degree of sequence conservation (Figure 3). The four species exhibit 98.3 to 99.0% nucleotide identity in the coding region and 93.7 to 97.5% sequence identity overall, encoding polypeptides which differ only at two residues (Figure 3). The cDNA sequences of H_{ad}-FABP from these species are also highly conserved (Figure 4). *Chaeno. aceratus* and *Cryo. antarcticus* appear to encode identical H_{ad}-FABP polypeptides. *N. coriiceps* and *G. gibberifrons* exhibit an Arg⁸² → Lys transition and *G. gibberifrons* contains the additional change Ala¹³⁴ → Gly. To confirm that this extreme conservation was not due to PCR artifact, we isolated H_{ad}-FABP cDNA from a member of a third nototheniid family, *Parachaenichthys charcoti*, and from an Antarctic zoarcid, the eel pout *Lycodichthys dearborni*. The partial H_{ad}-FABP sequence from the latter unrelated species exhibits 89% nucleotide identity to the nototheniid species (Figure 4). The deduced eel pout polypeptide differed from *Chaeno. aceratus* H_{ad}-FABP by 12 amino acids; 17 other third-position changes were evident which did not alter the polypeptide sequence (Figure 4).

Sequence comparison and phylogenetic analysis (Figure 5) support the assertion that H_{ad}-FABP and H_n-FABP are separate types. Phylogenetic analysis of the deduced polypeptides indicates that the H_{ad}-FABPs and H_n-FABPs form distinct clades, uniformly composed of each type (Figure 5, upper panel). The H_n-FABP clade clusters with FABPs expressed in mammalian heart, brain and eye. This clade includes the only other known teleost heart-FABP from rainbow trout (GenBank accession number U95296). The H_{ad}-FABP clade clusters with mammalian adipose and cutaneous FABP. Phylogenetic analysis based on distance instead of parsimony results in a tree with very similar topology (results not shown). To further resolve the relationship of H_{ad}-FABP and H_n-FABP among known FABPs, we repeated the parsimony analysis excluding liver and intestinal FABPs except as outgroups. This analysis (Figure 5, lower panel) resolves H_{ad}-FABP and H_n-FABP with mammalian adipose and heart-type FABPs, respectively, in the majority of trees.

DISCUSSION

We have identified two distinct FABPs present in both heart ventricle and aerobic skeletal muscle of four Antarctic teleost fish. Although several mammalian organs express more than one FABP type, this is the first observation of two distinct FABPs expressed in a vertebrate heart. H_n-FABP corresponds to the *Chaeno. aceratus* FABP polypeptide previously described by Londraville and Sidell [6], and appears homologous with the form present in rainbow trout heart (GenBank accession number U95296). The protein isolated from icefish aerobic skeletal muscle clearly did not contain the H_{ad}-FABP type because the partial peptide sequence obtained was not a mixture of the H_n-FABP and H_{ad}-FABP sequences. H_{ad}-FABP mRNA is present in pectoral adductor profundus, albeit at a reduced abundance relative to H_n-FABP mRNA (Figure 2). Two peaks were evident in the DEAE chromatography of *Chaeno. aceratus* pectoral muscle FABP [6]. Londraville and Sidell [6], however, only characterized the more abundant fraction with the more basic pI. The extremely high degree of sequence conservation indicates operation of strong selective pressure on both the H_{ad}-FABP and H_n-FABP genes, and suggests that both gene products play key functional roles. We propose that H_{ad}-FABP is homologous to mammalian adipose FABP and H_n-FABP to mammalian heart FABP.

The modelled structure of teleost heart FABPs supports the conclusion that H_{ad}-FABP is homologous to adipose FABP and H_n-FABP to mammalian heart FABP. Of the 41 amino-acid

H_b-FABP		
C. ace	agtcgtgtctctcc-tcctcgctttacctctcacacccctcacacagtcgtgtatctttccaccctccactccgct	74
C. ant	c.....g.....c.c.....	75
G. gib	c.....g.t.....c.c.....c.....	75
N. cor	c.....g.....c.c.g.c.....t...	75
C. ace	gcaaac ATGG TCGATGTTTCGTTGGCACCTGGAACCTGAAGGATAGTGAGAAGTTTGATGAGTACATGAAGAAG	149
C. ant		150
G. gib		150
N. cor	 T	150
C. ace	CTGGGTGTTGGCTTCGCTACACGCCAGGTCGGAAACGTGACCAAGCCCACCACCATCATCTCAGTGGAGGGAGAC	224
C. ant		225
G. gib		225
N. cor		225
C. ace	AAGGTGACGTTGAAGACGCAGAGCGCCATCAAGAACACAGAGCTCTCCTCAAGCTGGATGAGGAGTTCGATGAG	299
C. ant		300
G. gib	 T	300
N. cor	.. A A	300
C. ace	ACCACCGCTGATGACAGGAAAGTCAAGTCCTTTGTAACAGTTGATGGTGGGAAGTTGGTGCACACCCAGAAGTGG	374
C. ant	 T T C.C A	375
G. gib	.. T G	375
N. cor	 A	375
C. ace	GACGGCAAAGAGACCAGCCTGGTCAGGGAAGTCAACGGCAACAACCTCACACTGACACTAAAAATGGATGATGTC	449
C. ant	 G	450
G. gib	 G	450
N. cor	 G	450
C. ace	GAATCCATACGTCGCTACGTTAAGGCAGAGTAAatcccagcactgaaacatccctccagcatcttcttgccacag	524
C. ant		525
G. gib		525
N. cor	 c	525
C. ace	ctaaagccctgcgaccccaaaactacaaccagaacaataaaacctcgtatggctgccgcagattccctcaca	599
C. ant		600
G. gib	 t	600
N. cor	 t t g	592
C. ace	ctgtgctcagtattaacatcactgcggactgtaacat-----cattttctttatactgtgggtccc	659
C. ant	 t-----	660
G. gib	 t cccttctctccctat	675
N. cor	 g .. cc .. t cccttctctccat t	667
C. ace	tatactgcaagaaacgctgaataatttctgatgattgtgtgtgtttgtctctctaaagatccagttttggatga	734
C. ant		732
G. gib	 a g	750
N. cor	 a	742
C. ace	taattatTTTTtagaaaatgTTTTctgcattggtgcctatgataaacttggttaagtgtggaatttcaacggttg	809
C. ant	 t	806
G. gib	 t t	825
N. cor	 c t	815
C. ace	tctgttggaacgtggaacaggca AATAAA gtgtctacactgtgggacaaactgttaaaccg-- (A) _n	871
C. ant	.. a t a -- (A) _n	865
G. gib	.. a a t agt (A) _n	886
N. cor	.. a a t a -- (A) _n	874

Figure 3 Comparison of H_b-FABP cDNA sequences among four Antarctic notothenioid teleosts

cDNA fragments were amplified and sequenced from *Cryo. antarcticus* (C. ant), *G. gibberifrons* (G. gib), *N. coriiceps* (N. cor) and aligned with that of *Chaeno. aceratus* (C. ace). Residues identical with the *Chaeno. aceratus* H_b-FABP sequence are indicated by (.), absence of a nucleotide in the sequence is indicated by (—).

differences between the mammalian heart FABP and teleost H_b-FABP, only two are residues which project side chains into the interior of the β -sheet structure (Figure 6). Both are iso-leucine/leucine substitutions on adjacent β strands within 4 Å (10⁻¹⁰ m) of each other. The remaining differences in amino-acid composition appear to have a non-random distribution localized

to the hydrophobic residues which comprise the 'conserved backbone' of FABPs [17] (Figure 6). By contrast, teleost H_{ad}-FABP differs from mammalian heart FABP at 59 residues, 14 of which occupy internal positions. Xu et al. [14] identified 18 internal residues of FABP which interact with bound fatty acid, three of which differ between the mammalian adipocyte- and

H_{ad}-FABP	
C. ace	ttgggagagtgaacccagagtcacttcactgtttatgtcgaggatatacagaagctgcaaac ATGG TTGAGAAATTTGTT 80
C. ant	(.....)
P. cha	(.....)
G. gib	a..... 80
N. cor	(.....a.....)
C. ace	GGGACGTGGAAGATGATTTCAGCGATAACTTTGATGACTACATGAAAGCAATCGGTGTGGGATTTGCGACCCGGCAGGT 160
C. ant	
P. cha	..A.....
G. gib	 160
N. cor	
C. ace	GGGGAATCGCACCAAAACCAACTTGGTAGTGAGCGTGACGATCAAGGGTTTGTATGCTTGAAGTCTCAGAGCACTTTCA 240
C. ant	
P. cha	
G. gib	 240
N. cor	G.....
L. dea	(.....G.....T.....A.G.....C.....G.C.....A.....C.....C.....)
C. ace	AAACCACTGAGATCAAGTTCAAGCTAAACGAGCCATTGGAAGAGACGACCGCAGACGATAGGAAGACCAAGACCGTGGTG 320
C. ant	
P. cha	T.....
G. gib	G..... 320
N. cor	G.....
L. dea	...T...A.....C.....T...G.....G.G.....
C. ace	ACTCTGGAGAACGGGAACTTGTGCAGAAACAGAGCTGGGATGGCAAAGAGACAAATATCGAGAGGGAGATCGAAGATGG 400
C. ant	
P. cha	TC.....
G. gib	 400
N. cor	T.....
L. dea	C.....C.....G.C.....ACG.....
C. ace	GAAATTGGTAGCGAAATGCATTATGGGTGATGTGATCGCAGTGAGGACATACGTGATGGAGGCAT TGA ttggctcccttca 480
C. ant	
P. cha	c.....
G. gib	G..... 480
N. cor	
L. dea	...G...A...C...A...C...TG...A.A...--...g.g...g
C. ace	gcccaactcgttggatacattgctggatgacaggctcagttcaatgagcgttat-accaaactaaagtaaaggcgtgtttt 539
C. ant	g.....--...aa...g
P. cha	g.....--...a.....c.....
G. gib	g.....-...c..... 539
N. cor	g.....-...c.....
L. dea	aaa...g.....cacia...g.....-...c.....
C. ace	gctcttact---ctttatcttcactgtttttgcaaaaataaatccctgtgcctctgttctccaacatgacacatttggtt 616
C. ant	.g.....-...c.....-...t.....)
P. cha	-...c.....t.....
G. gib	act.....c.....c..... 619
N. cor	t.....act.....a.....G.....
L. dea	.g...t..agt...-...cg.g...-...c...tt...cct..g.t...-...)
C. ace	tcatttgggaattgtgtgtgatttg AATAAA aatcaatgagaatttctc (A) _n 666
C. ant	
P. cha	(A) _n
G. gib	t...a.....(A) _n 669
N. cor	a.....(A) _n
L. dea	

Figure 4 Comparison of H_{ad}-FABP cDNA sequences among six Antarctic teleosts

cDNA fragments were amplified and sequenced from the channichthyid *Cryo. antarcticus* (C. ant), the bathydraconid *P. charcoti* (P. cha), nototheniids *G. gibberifrons* (G. gib) and *N. coriiceps* (N. cor) and the Antarctic eel pout *L. dearborni* (L. dea), and were aligned with that of *Chaeno. aceratus* (C. ace). Residues identical with the *Chaeno. aceratus* H_{ad}-FABP sequence are indicated by (.), absence of a nucleotide in the sequence is indicated by (-).

heart-FABP types. The arrangement of residues surrounding the fatty acid carboxy group in H_{ad}-FABP is similar to that of mammalian adipose FABP and distinct from that of the H_h-FABP/mammalian heart-type FABP. The presence of the threo-

nine-40 hydroxy group in H_h-FABP and mammalian heart FABP, and absence from the H_{ad}-FABP and adipose types, may affect hydrogen bonding of bound waters which surround the fatty acyl carboxy group. Conversely, H_{ad}-FABP and adipocyte

Figure 5 Phylogenetic relationships FABP sequences

Upper panel: parsimony tree of aligned FABP polypeptide sequences generated using PHYLIP [10]. Numbers at each node are the percentage of 500 bootstrapped trees that result in that node. Accession numbers of taxa/types are provided in the Experimental section. Lower panel: parsimony tree to resolve relationships between adipocyte and heart types, generated by omitting liver and intestine FABP types, maintaining bovine liver as an outgroup.

types contain cysteine at residue 117 and either alanine or valine at position 115, whereas leucine residues are found in at these positions in mammalian heart and H8-FABPs. Chemical modification has shown that the relative size of the residue at position 117 has a significant effect on fatty acid binding [18]. This

Figure 6 Differences between primary structure of teleost H_h -FABP and bovine H-type FABP

Each sphere represents the α -carbon atom of an amino acid which varies between the two species. Open spheres represent neutral amino acid substitutions, whereas black spheres represent amino-acid differences resulting in a charge difference between the two polypeptides.

distribution of internal residues suggests that H_{ad} co-ordinates the fatty acid in the binding pocket in a manner which is more similar to that of adipose FABP than mammalian heart FABP.

What is the physiological consequence of two FABP types in cardiac tissue? Tissues process fatty acids for different purposes. For example, mammalian heart primarily oxidizes fatty acids for aerobic energy production, whereas liver primarily esterifies fatty acids for storage in neutral lipid. Consequently, different FABPs may be needed among the tissues. With few exceptions [19–21], no significant difference in binding affinity has been found among FABP types or among fatty acids within a type [6,22–24]. However, the rate of transfer of FABP-bound fatty acids to acceptor membranes varies greatly among types [25–28] and may reflect the functional diversity of FABPs. In particular, heart-type FABP transfers fatty acids to acceptor membrane 50-fold faster than does liver-type FABP [29]. Heart and adipose FABPs both transfer FABPs at a ‘fast’ rate relative to liver FABP and both transfer fatty acids via similar collisional mechanisms [25,26], but the rate of fatty acid transfer is affected by pH, ionic strength, phospholipid head-group charge on acceptor membranes and surface charge on donating FABPs [25,26,28,30]. Accordingly, Herr et al. [30] proposed that FABPs may target fatty acid delivery to different intracellular compartments based on variation in organelle membrane lipid composition.

Fishes as a group tend to store neutral lipids in a manner that is distinct from fat deposition in mammals. Whereas mammals store fat primarily in adipose tissue and subcutaneously, fishes deposit neutral lipids in many tissues throughout the body and even in extracellular/mesenteric depots [31]. Antarctic teleost heart tissue does not contain fat cells and is composed almost entirely of spongy endocardium [32,33]. Within muscle cells, fish store neutral lipid in the form of non-membrane bound droplets,

predominantly triacylglycerol, dispersed throughout the cytoplasm [34–36]. Perhaps H_n -FABP and H_{ad} -FABP target fatty acids to different intracellular destinations within teleost muscle, such as acyl-CoA synthetase for eventual import and oxidation in mitochondria or peroxisomes, or phosphoglyceride acyl transferase and diacylglycerol acyl transferase for eventual incorporation of lipid into intracellular stores. Lipid accumulation increases with decreased body temperature, which may explain the presence of the H_{ad} -FABP type in Antarctic teleosts. Such metabolic targeting may explain why both H_{ad} -FABP and H_n -FABP are expressed in aerobic tissues but does not explain their abundance in brain and glycolytic muscle, two tissues with very limited abilities to oxidize fatty acids for generation of ATP. However, as excitable tissues, both brain and glycolytic (white trunk) muscle contain abundant ion channels. Free fatty acids have been proposed to alter ion-channel gating [37–39]. Thus the role of FABP in these tissues may be to maintain an extremely low specific-activity of free fatty acids, ensuring optimal function of ion channels.

Further study on the transfer kinetics of the H_{ad} and H_n FABP types and their expression in fishes with true adipose tissue may give unique insight into the structural and functional radiation of the FABP protein family.

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