

Short sequence-paper

Cloning and unusual expression profile of the aldolase B gene from Atlantic salmon¹

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Abstract

A full-length clone of the aldolase B gene has been isolated from a cDNA library constructed from liver of Atlantic salmon (*Salmo salar*). Sequencing showed that the clone encodes a typical aldolase B, possessing a number of amino acid residues which are seen in aldolase B, but not in other aldolase isoforms. RT-PCR analysis showed that the gene is expressed in liver, kidney and intestine as expected. However, in contrast to mammalian and avian aldolase B, expression was also found in a number of other tissues. Levels of aldolase B mRNA in liver and kidney were not significantly altered during smoltification, the transformation of freshwater-dwelling salmon (parr) into saltwater-adapted salmon (smolts). © 1998 Elsevier Science B.V. All rights reserved.

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Aldolase (EC 4.1.2.13) catalyses the reversible cleavage of fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate [1]. Hence the enzyme participates in both glycolysis and gluconeogenesis. Mammals possess three related isoforms of aldolase [2], each representing the product of a separate gene and showing a distinct tissue distribution. The three isoforms are: aldolase A, found predominantly in muscle and associated with glycolysis; aldolase B, found in liver, kidney and intestine; and aldolase C, found in the brain. Aldolase B is associated with gluconeogenesis, and also with

fructose metabolism since it is able to use fructose 1-phosphate, in addition to fructose 1,6-bisphosphate, as a substrate. Hence, hereditary fructose intolerance in humans results from mutations within the aldolase B gene [3]. In rats, the aldolase B gene undergoes changes in expression during development [4]. The liver of a 14-day-old foetus produces considerably more aldolase A than aldolase B, but as development proceeds aldolase B expression increases dramatically with a concomitant decrease in the expression of the aldolase A gene, such that the livers of newborn rats predominantly express aldolase B. Transcription of the rat aldolase B gene is also influenced by hormones – it is stimulated by insulin and dexamethasone, but repressed by thyroxine and glucagon [5].

Much less is known about aldolase genes in lower vertebrates, though the same three aldolase genes appear to be present since aldolases A and C have

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Fig. 1. Nucleotide sequence of the salmon aldolase B cDNA clone and the putative amino acid sequence of the encoded protein. The bases underlined are those recognised by the primers used in RT-PCR analysis of aldolase B expression.

been cloned from *Xenopus laevis* [6,7], and aldolases B and C have been cloned from two different teleost fish, the gilthead sea bream [8] and the goldfish [9], respectively. The latter are the only fish aldolases to be described to date. It is particularly notable that no data have been published on aldolase genes from salmon because electrophoretic analysis of salmonid aldolases [2] suggested the possible existence of a fourth isoform, aldolase D. Furthermore, salmon undergo an unusual life-cycle event, known as smoltification, which transforms freshwater-dwelling salmon (known as parr) into saltwater-adapted fish (known as smolts) in preparation for their migration from river to sea [10]. Although it is difficult to compare developmental processes of fish and mammals, there are some parallels between smoltification and late foetal development, such as the activation of adult globin genes [11] and the induction of the serum albumin gene [12,13]. We therefore hypothesised that expression of the salmon aldolase B gene may change during smoltification. Consistent with this possibility is the fact that smoltification is influenced by hormones, with peaks of cortisol and thyroxine seen during the process [10,14]. Additionally, there are changes in metabolism during smoltification including decreases in glycogen synthesis and gluconeogenesis [15,16]. Consequently we have cloned the Atlantic salmon (*Salmo salar*) aldolase B gene, determined the tissue distribution of its transcript and compared its expression in parrs with that in smolts.

The Atlantic salmon aldolase B gene was isolated from a cDNA library constructed in the vector λ zap-II (Stratagene) using poly(A)⁺ RNA isolated from liver of adult salmon caught whilst returning to spawn. Thirty thousand recombinants from this library were probed at low stringency (final wash at 42°C in 1×SSC, 0.1% SDS) with a ³²P-labelled restriction fragment containing the complete coding sequence of the sea bream aldolase B gene [8]. One positive clone was plaque purified and converted to plasmid form using the λ zap automatic excision system. The insert from this plasmid was sequenced on a LiCor automated sequencer following the generation of appropriate subclones. Sequencing of this clone revealed it to contain a full-length insert encoding a putative protein of 362 amino acids with a predicted molecular weight of 39.2 kDa (Fig. 1). Da-

tabase searches showed that the protein encoded by the cloned gene was strongly related to aldolase B, showing about 83% amino acid identity with sea bream aldolase B and approximately 70% amino acid identity with goldfish aldolase C. An alignment of the amino acid sequence of salmon aldolase B and five other vertebrate aldolase sequences is shown in Fig. 2. The protein encoded by this gene appears to be typical of an aldolase B except for the deletion of two amino acids, between residues 65 and 66 of the salmon sequence.

The availability of fish aldolase B and C sequences, including the one described in this paper, allows more precise identification of residues which are characteristic of the various aldolase isoforms. An alignment of 16 vertebrate aldolase genes (not shown) revealed 12 amino acids which are conserved in all aldolase B proteins, and altered in all aldolase A and C proteins, so far described. These are, with respect to the salmon sequence, Phe-5, Thr-39, Gly-41, Phe-59, Ala-112, Leu-181, Ala-261, Lys-295, Ala-310, Lys-319, Thr-357 and Tyr-360. The presence of most or all of these 12 amino acids can hence be seen as defining a sequence as aldolase B. The location of these residues is indicated on Fig. 2. Studies of chimaeric aldolases, made by fusions between human aldolase A and B, have implicated the regions corresponding to residues 37–49 and 310–362 of the salmon sequence as being particularly important for aldolase B-specific functions, such as utilisation of fructose 1-phosphate [17]. Of the 12 aldolase B-specific amino acids identified above, six occur within these regions. These six residues would be good targets for future experiments, using site-directed mutagenesis, to further define the molecular basis for the differences in activity between aldolase B and the other aldolase isoforms.

We next analysed the expression of the salmon aldolase B gene using RT-PCR. RNA was prepared from tissues of salmon parr, early smolts and late smolts. Aliquots of 0.5 µg of total RNA were used in cDNA synthesis reactions which also included 0.5 µg random hexamers and 200 U MMLV reverse transcriptase in a total volume of 100 µl. Aliquots of 5 µl of the resulting cDNAs were used in PCR reactions with primers which would specifically amplify either aldolase B or, as a control, β -actin. The aldolase B primers used were TGACTACCCAGTTCC-

salalb	MTTQ [•] FPSLSPEQKKELSDIAQRIVAPGKGILAADESTG [•] TMG [•] KRLQKINVENNEENRRTFR	60
sbalb	**HQ*****F*N*****C**	60
hualb	*AHR**A*TQ*****E***S**N*****V***N**R**K***T*****Q**	60
goalc	**H*Y*A*TT*****Q*****S*A***NP*G***T*****LY*	60
hualc	*PHSY*A**A*****L*****V*S*A***SQ*G***T*****LY*	60
huala	*PY*Y*A*T*****H*****SIA***S*GT*T*****FY*	60
salalb	DLLFS--AVDPNCIGGIFFHETLYQMSDKGVLFPPQVIKDKGIVVGIKVDKGTAGLNGTD	118
sbalb	*I***TD*SIA**V*****K*SN*K*****V*****P*****	120
hualb	EI***VDSSINQS**V*****KDSQ*K**RNIL*E*****L*Q*G*P*A**N	120
goalc	Q***TADERMDK***V*****KA*D*TP*AKM***R*****VVP*A**N	120
hualc	QV***ADDRVKK*****KD*N**P*VRT*QD*****VVP*A**D	120
huala	Q**LTADDRVNP***V*L*****KA*D*RP*****S*G*****VVP*A**N	120
salalb	GETTTQGLDGLSERCAQYKKDGCDFAKWRCVLKISDACPSDLAIAENANVLARYASICQQ	178
sbalb	*****G**FA*****M	180
hualb	K***I*****V**G**A**R*A*Q***S***Q****A*****	180
goalc	G*****A*****S*****ETS**E**M*****	180
hualc	*****A**A*****ERT**A**L*****	180
huala	*****A*****GEHT**A**M*****	180
salalb	NGLVPIVEPEILPDGDHDLRLTQYVTEKVLAA [•] TYKALNDHHVYLEGTLLKPNMVTAGHSC	238
sbalb	*****Q*C**A*****V*****S*****P*****	240
hualb	*****VI*****EHC*****V*****A*	240
goalc	**I*****K*C*****C*****S*****	240
hualc	**I*****K*C*****V*****S*****P**A*	240
huala	**I*****K*C*****V*****S***I*****P**A*	240
salalb	PKKFT [•] PQEVGMATVTALRRTP [•] AAVPGITFLSGGQSEEEATQNLNAMNLTSLHRPWKLSF	298
sbalb	*****A*****S***C*****SIH***I*QVP*****T*	300
hualb	T**Y**EQ*A*****H*****C*****M**D**L***I**CP*PK*****	300
goalc	*T**SN**IA*****P**T*V*****SI***I*NCP*TK**A*T*	300
hualc	*I*Y**E*IA*****P***V*****SF***I*RCP*P**A*T*	300
huala	TQ**SHE*IA*****P**T*****SI***I*KCP*LK**A*T*	300
salalb	SYGRALQASALA [•] AWKGAANKQSAQDAFTSRAKSNGLASKGKYTAVSSDDQASM [•] QSLHTA	358
sbalb	*****Q**D***AAT*QV*VT***I*****E*KPSG*A***Q***Y**	360
hualb	*****V*****G*****EAT*E**MK**MA*CQ*A**Q*VHTG*SGA**T***F**	360
goalc	*****S**R*VKE*EKA*TEE*LK**EA***AQ***VSSGM*GS*G-***YV*	359
hualc	*****NA*R*QRD*AGA*TEE*IK**EV***AQ***EGSGE*GG*AA***YI*	360
huala	*****K**G**KE*LKA**EEYVK**LA*S**CQ***PSGQAGA**SE**FVS	360
salalb	N [•] YVY	362
sbalb	S***	364
hualb	C*T*	364
goalc	*HA*	363
hualc	*HA*	364
huala	*HA*	364

Fig. 2. Alignment of the amino acid sequence of salmon aldolase B (salalb) with sea bream aldolase B (sbalb), human aldolase B (hualb), goldfish aldolase C (goalc), human aldolase C (hualc) and human aldolase A (huala). Residues identical to the salmon sequence are indicated by an asterisk (*). Where necessary, spaces, indicated by a hyphen (-), have been inserted to maximise homology. Residues which are specific to aldolase B are indicated by a •.

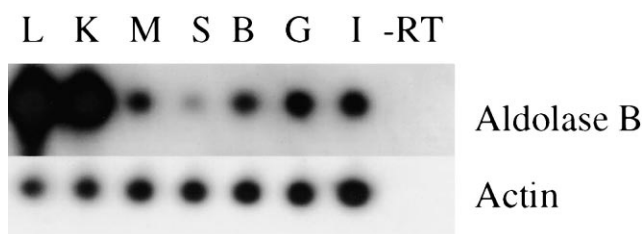


Fig. 3. RT-PCR analysis of the tissue distribution of aldolase B mRNA. cDNA was synthesised from RNA prepared from liver (L), kidney (K), muscle (M), skin (S), brain, (B) gill (G) and intestine (I) of salmon parr. Aldolase B and β -actin sequences were then selectively amplified by PCR. PCR products were detected by high stringency Southern blotting following electrophoresis through 2% agarose gels. The negative control (–RT) shows PCR amplification of a cDNA synthesis reaction, containing liver RNA, from which reverse transcriptase was omitted.

CATCC and CTGTGCCTTTGTCCACCTTA. The upstream aldolase B primer corresponds to the first six amino acids of the aldolase B protein (Fig. 1). As this region is poorly conserved between different aldolase isoforms (Fig. 2) it was anticipated that it would anneal to aldolase B cDNA, but not to cDNA derived from aldolase A or aldolase C. Primers for amplifying β -actin were designed from the sequence of a salmon β -actin clone (Genbank accession number AF012125) obtained by screening the salmon liver cDNA library with a fragment of the sea bream β -actin gene [18]. The sequences of β -actin primers were GTTGTGACAACGGATCC and AGCGTAACCCTCGTAGAT. PCR reactions, for the detection of both aldolase and β -actin, contained 2.5 mM $MgCl_2$, 10 pmol of each primer and 1.25 U Taq DNA polymerase. Amplification was performed by incubating for 17 (β -actin) or 20 (aldolase B) successive cycles at 95°C for 1 min, 58°C for 1 min and 72°C for 30 s. These numbers of cycles are many fewer than required to reach the plateau phase of amplification. PCR products were then fractionated on 2% agarose gels and detected by Southern blotting, using the appropriate ^{32}P -labelled probe, under high stringency conditions (final wash $0.1 \times SSC$, 0.1% SDS at 65°C for 1 h). No products were observed in negative control reactions from which reverse transcriptase was omitted.

Initially, expression of aldolase B was examined in a variety of parr tissues (Fig. 3). Aldolase B transcripts were, as expected, most abundant in liver

with slightly lower levels present in kidney. Aldolase B mRNA was also present in salmon intestine although, relative to expression in liver and kidney, expression of aldolase B in intestine of salmon appears much lower than that observed in the rat [19]. More surprisingly, we also detected low levels of aldolase B mRNA in each of the other tissues tested, namely brain, muscle, gill and skin. To determine the relative levels of aldolase transcripts in the tissues analysed, the regions of the Southern blot membrane which gave rise to the various bands shown on the autoradiograph in Fig. 3 were excised and the amount of probe bound to them determined by scintillation counting. This showed that liver contained approximately 1.4-fold more aldolase B mRNA than kidney, 10-fold more aldolase B mRNA than intestine and between 8- (gill) and 30- (skin) fold more aldolase B mRNA than the other tissues tested. Analysis of β -actin expression confirmed that the RNA prepared from each tissue was of similar quality and quantity. Aldolase B RT-PCR products from brain, muscle, gill, skin and intestine were subsequently subcloned and sequenced to confirm that they were derived from aldolase B, rather than arising by mis-priming from aldolase A or aldolase C transcripts. All sequences corresponded precisely to the aldolase B sequence shown in Fig. 1. The expression profile of aldolase B in salmon is clearly distinct from that of higher vertebrates [19,20]. It suggests a biological role for aldolase B in salmon tissues, and perhaps those of other fish, which is not seen in mammals and birds. Alternatively repression of the

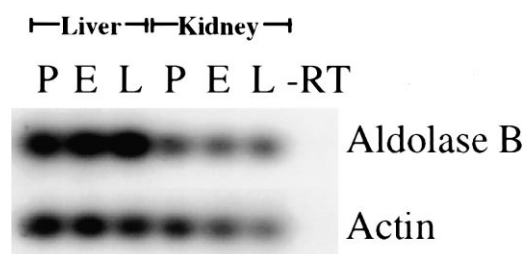


Fig. 4. Aldolase transcripts do not vary in abundance during smoltification. cDNA was synthesised from RNA prepared from liver and kidney of parrs (P), early smolts (E) and late smolts (L). Aldolase B and β -actin sequences were then selectively amplified by PCR. PCR products were detected by high stringency Southern blotting following electrophoresis through 2% agarose gels. The negative control (–RT) shows PCR amplification of a cDNA synthesis reaction, containing parr liver RNA, from which reverse transcriptase was omitted.

aldolase B promoter in tissues such as muscle and brain may be less efficient in salmon than in mammals and avians.

To determine whether expression of aldolase B in liver and kidney alters during smoltification, RT-PCR analysis of aldolase B expression was performed on RNA isolated from these tissues taken from parrs, early smolts and late smolts. We found that expression of aldolase B was essentially unaltered in these tissues during the smoltification process (Fig. 4). This suggests that the changes in carbohydrate metabolism involved in smoltification do not require modulation of aldolase B expression and that aldolase B expression is not influenced by the increased levels of plasma cortisol and thyroid hormones which accompany (and probably regulate) smoltification.

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