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Short sequence-paper

The cDNA sequences of cytochrome *c* oxidase subunit VIa from carp and rainbow trout suggest the absence of isoforms in fishes [1]

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Abstract

The cDNAs of subunit VIa of cytochrome *c* oxidase from rainbow trout liver and carp heart are presented, revealing 82% identity of their deduced amino acid sequences. The two cDNAs are evolutionary equally distant from the livertype (VIaL) and heart-type (VIaH) of mammalian subunit VIa. The data suggest that in ectotherm fishes no isoforms of subunit VIa occur, and that the postulated tissue-specific mechanism of thermogenesis in mammals, based on interaction of ATP with subunit VIaH (Frank, V. and Kadenbach, B. (1996) FEBS Lett. 382, 121–124), is absent.© 1997 Elsevier Science B.V. All rights reserved.

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In mammals cytochrome *c* oxidase contains three mitochondrial coded and ten different nuclear coded subunits in 1:1 stoichiometric amounts, with tissue-specific isoforms for subunits VIa, VIIa and VIII [1,2]. The heart-type of subunit VIa (VIaH) is expressed in heart and skeletal muscle [3,4], but not in smooth muscle [5], while the liver-type (VIaL) is expressed in all other tissues. By using a monoclonal antibody reacting with subunit VIaH but not with VIaL, ADP (or ATP) was shown to bind to the matrix-oriented, N-terminal domain of subunit VIaH [6]. This binding site for ADP was confirmed in the crystal structure of cytochrome *c* oxidase from bovine heart [7]. At high intraliposomal ATP/ADP-ratios the H^+/e^--stoichiometry of reconstituted cytochrome *c* oxidase from bovine heart is decreased by 50%, and this decrease is prevented by preincubation of the enzyme with the monoclonal antibody to subunit VIaH [8]. The regulation of efficiency of energy transduction by the energy level in the enzyme from heart, but not from liver [9], was suggested to participate in thermogenesis in heart and skeletal muscle at rest (e.g., during sleep) [10]. This mechanism of thermogenesis is not expected to occur in ectotherm fishes. Therefore we investigated cytochrome *c* oxidase from hearts and livers of carp and rainbow trout and cloned the cDNAs for subunit

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[1] The sequence data in this paper have been submitted to the GenBank data library under the accession numbers: BankIt88656 U83980 (trout) and BankIt88644 U83907 (carp).

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VIa. In a previous publication on cytochrome *c* oxidase from rainbow trout [11] subunit VIa could not be found in the isolated enzyme, apparently due to loss of this subunit during the isolation procedure. The subunit composition of cytochrome *c* oxidase from tuna liver and heart revealed 13 subunits, like in mammals, with isoforms for subunits Va, VIc, VIIb and VIII, but not for subunits VIa and VIIa (Arnold et al., unpublished results).

Cytochrome *c* oxidase from carp hearts was isolated as previously described [11,12]. After chromatography on DEAE-sephacel and precipitation with ammonium sulfate the enzyme was separated into subunits by SDS-PAGE. A band in the region of subunit VIa was only seen with the enzyme precipitated at 37%, not after subsequent precipitation at 46% ammonium sulfate saturation, indicating loose binding of this subunit. The band was excised, tryptic digested, the peptides separated by HPLC, and the N-terminal amino acids sequenced as described by Eckerskorn and Lottspeich [13]. Three amino acid sequences were determined which were homologous to mammalian subunit VIa: SQGSHEGGAATWK, MQAHSHDPPEFVPYPHLRIRTK and PFPWSDGNHSLFHNK. The underlined sequences were used to design the two degenerative primers: TCfP1, (CARGGIAGYCAYGARGG) and TCfP2, (CAYGAYCCTCCIGARTTYGT).

Total RNA was prepared from heart and liver tissues from the carp *Cyprinus carpio* and the trout *Salmo gairdneri iredeus* by phenol/chloroform extraction [14]. Reverse transcription was carried out with a dT17-tailed oligonucleotide (QT-primer) containing two appended primer sequences (Q_{Inner} and Q_{Outer}) according to Frohman [15]. 3′RACE (rapid amplification of cDNA ends) was carried out as a touch down PCR with the degenerated gene specific forward primer TCfP1 derived from the underlined part of the first peptide (see Fig. 1) and the Q_{Outer}-primer which corresponds to the 5′-terminal sequence of the QT oligonucleotide. For all PCR amplifications the DNA polymerases of the Expand™ High Fidelity PCR System (Boeringer Mannheim) were used. Thirty cycles were used for all amplifications in which the annealing temperature of the initial cycles was reduced by 2°C every two cycles: denaturation for 60 s at 94°C; annealing for 30 s at 60–48°C; elongation for 45 s at 72°C. A further degenerated forward primer TCfP2, derived from the underlined part of the second peptide (see Fig. 1), was used for nested PCR with the Q_{Inner}-primer using the previous PCR-product as template. The final PCR products were cloned into pBluescript SK(−) and sequenced in both directions using the ^{T7}Sequencing™ Kit of Pharmacia Biotech. From the obtained nucleic acid sequences three gene specific reverse primers for 5′RACE-PCR [14] were designed for rainbow trout (TrP3, TrP4, TrP5) and carp (CrP3, CrP4, CrP5), respectively, as depicted in Fig. 1. 5′Reverse transcription was carried out with the outer gene specific primer (CrP3 and TrP3, respectively) followed by RNaseH treatment. A polyA tail was appended to the cDNA first strand with terminal desoxynucleotidyl transferase (USB). The cDNA second strand was generated with the QT-primer. PCR amplifications were carried out with the primers TrP4 for trout and CrP4 for carp, respectively, and the Q_{Outer}-primer, as described above. Nested PCR was performed using the primer TrP5 for trout and CrP5 for carp, respectively. The PCR fragments were cloned and sequenced in both directions as described above.

The DNA sequences and the deduced amino acid sequences of the cloned cDNAs for subunit VIa derived from the mRNAs of carp heart and trout liver are presented in Fig. 1. The coding regions of the two cDNAs show high homology.

In Fig. 2 the deduced amino acid sequences of the two cDNAs for subunit VIa from trout and carp are compared with the corresponding amino acid sequences of the liver- and heart-type of subunit VIa from human, bovine and rat at optimal alignment. The amino acid sequences of the mature proteins of the two fishes are 82% identical. Both cDNAs show a short precursor sequence similar to the heart-type of mammalian subunit VIa. However, the two subunits from fishes appear to be evolutionary equally distant from the mammalian heart-type (VIaH) and liver-type of subunit VIa (VIaL), as demonstrated in Fig. 3. This figure presents the percentage of identical amino acids between all eight sequences for subunit VIa. The three mammalian liver-types of subunit VIa are more than 90% identical, the heart-types are 80% identical. Between the heart- and liver-types of subunit VIa the identity is only 55–61%. The sequences from the fishes are 56–60% identical to mammalian liver-type and 46–60% identical to mammalian

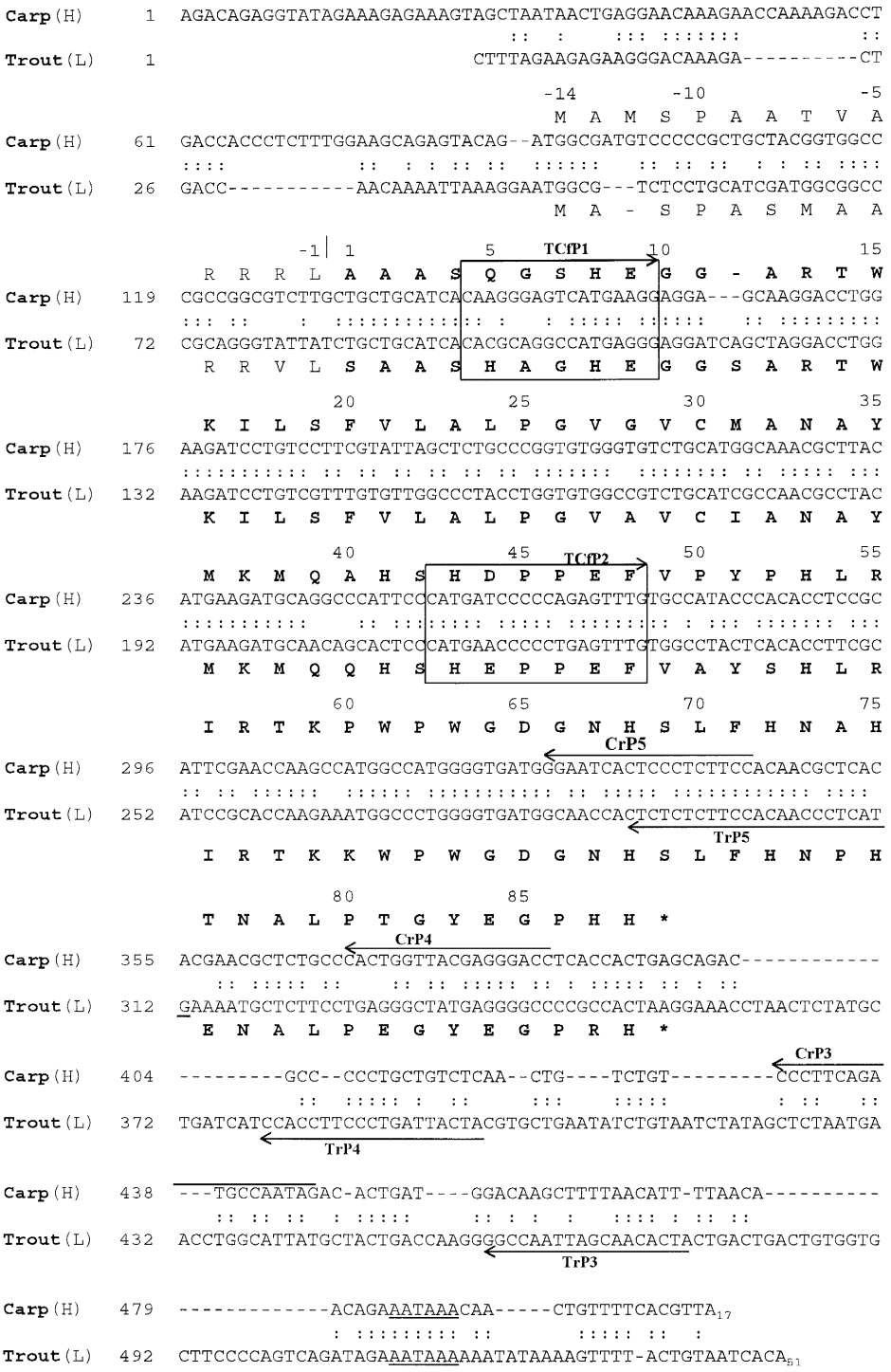

Fig. 1. cDNA sequences of COX subunit VIa from carp heart and trout liver. The two sequences were aligned by a computer program. The first amino acids of the mature proteins (shown in bold) of the deduced amino acid sequences were assumed in analogy to the mammalian sequences (see Fig. 2). The arrows indicate the 5′–3′ direction and the position of the used primers. The degenerated primers are boxed. The polyadenylation sites are underlined.

heart-type of subunit VIa. The same distance of the fish sequence to the liver- as well as the heart-type of mammalian subunit VIa suggests that only one isoform of subunit VIa occurs in fish. This is further supported by the fact that the trout cDNA was prepared from liver mRNA, the carp cDNA from heart mRNA, but their sequences are 82% identical compared to only 55–61% identity between mammalian liver-and heart-type of subunit VIa.

The apparent absence in ectotherm fishes of a

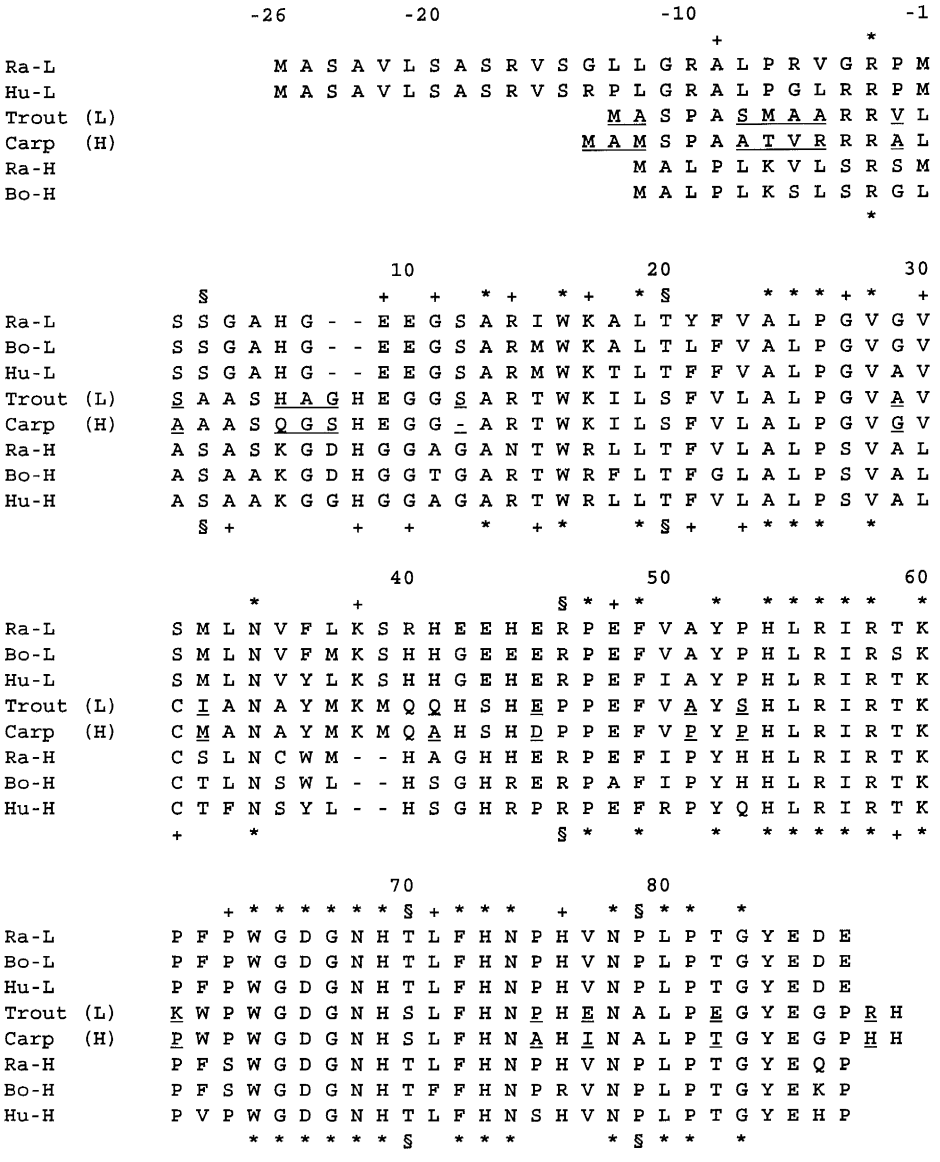

Fig. 2. Comparison of deduced amino acid sequences of cytochrome *c* oxidase subunits VIa from carp and rainbow trout with those from human, bovine and rat. The mammalian sequences were taken from [2]. The N-terminal amino acid of the mature subunits VIa from fish was deduced in analogy to mammals. Amino acids different in carp and trout are underlined. Asteriks above and below the sequences indicate identical amino acids in all species. Crosses above or below amino acids indicate identical amino acids between fish and the liver isoforms of mammals or the heart isoforms of mammals, respectively. Identical amino acids in all mammalian sequences which differ from fish sequences are indicated by a §. L and H indicate the liver- and heart-type of subunit VIa, from rat (Ra), bovine (Bo) and human (Hu); (L) and (H) indicate the origin of the cDNA from liver or heart, respectively.

	HuL	BoL	RaL	Trout	Carp	HuH	BoH	RaH
HuL	100	91	92	60	58	61	61	61
BoL		100	90	60	56	55	55	58
RaL			100	60	56	55	55	55
Trout				100	82	57	46	56
Carp					100	58	53	60
HuH						100	80	80
BoH							100	80
RaH								100

Fig. 3. Relationship between amino acid sequences of subunits VIa from mammals and fishes. The numbers indicate the percentage of identical amino acids of the mature proteins as aligned in Fig. 2. Compared are the sequences from human, bovine and rat liver-type (HuL, BoL, RaL) and heart-type (HuH, BoH, RaH) and of trout and carp (Tr, Ca), respectively.

different isoform of subunit VIa in heart as compared to liver supports the postulated thermogenic function of subunit VIaH in mammalian heart and skeletal muscle at rest (high ATP/ADP-ratios) [7–9]. Also in the partially endotherm tuna fish only one isoform of subunit VIa was found (Arnold et al., unpublished results). In ectotherm fishes this mechanism of thermogenesis via partial uncoupling of proton pumping from electron transport for maintaining the body temperature of mammals would not be appropriate.

A different binding site for ATP at the C-terminal domain of subunit VIa was identified in cytochrome *c* oxidase from yeast as well as from bovine liver (VIaL) and bovine heart (VIaH) [16]. The physiological function of subunit VIa in fishes and of subunit VIaL in mammals, however, remains to be investigated.

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