NOTE *Biochemistry*

Molecular Cloning and Tissue Distribution of Uncoupling Protein 1 (UCP1) in Plateau Pika (*Ochotona dauurica*)

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ABSTRACT. Uncoupling protein 1 (UCP1) is present exclusively in brown adipose tissue, and contributes to body temperature control during cold exposure. We cloned UCP1 cDNA of plateau pika (*Ochotona dauurica*), a small, non-hibernating, diurnal lagomorph that inhabits in relatively cold climates and at high altitudes in Mongolia and in northern China. The nucleotide sequence of pika UCP1 was highly homologous to UCP1 of other species, and the deduced amino acid sequence had some common domains for UCP, including six mitochondrial carrier protein motifs and a putative purine-nucleotide binding site. RT-PCR and Western blot analyses revealed that both UCP1 mRNA and protein were expressed exclusively in the interscapular adipose tissue. These results suggest that pika UCP1 contributes to heat production in brown adipose tissue, as do those in other species.
KEY WORDS: brown adipose tissue, plateau pika, uncoupling protein.

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Uncoupling proteins (UCPs) are members of the mitochondrial transporter family that dissipate the mitochondrial proton gradient as heat more than via ATP synthesis [12]. UCP1, a classical UCP, is present exclusively in brown adipose tissue (BAT), which is the major site of regulatory nonshivering thermogenesis in small rodents. BAT thermogenesis plays an important role in body temperature control during cold exposure. Cold stimulation activates sympathetic nerves to BAT, resulting in the activation of UCP1, and chronically induces UCP1 expression, mitochondrial biogenesis, and hypertrophy of BAT. It is now accepted that UCP1 is a key molecule for BAT thermogenesis during cold exposure [6]. Moreover, it is suggested that UCP1 may play a role in protection against reactive oxygen species and hypoxic states [2, 5].

Plateau pika (*Ochotona dauurica*) is a small, non-hibernating, diurnal lagomorph that inhabits alpine meadows in Mongolia and in northern China. Given the fact that they live in relatively cold climates and at high altitudes, this species may be useful to study the mechanisms of cold and/or hypoxic adaptation. In fact, it was reported that BAT mass, cytochrome oxidase (COX) activity, and UCP1 protein level were increased in cold environments in wild plateau pikas [8, 14]. It was also reported that oxygen utilization was increased in plateau pikas kept under hypoxic environment, whereas it was decreased in rats and in rabbits [4, 10]. Collectively, UCP1 may be one of the key molecules for adaptation to cold and hypoxic environments in plateau pika. However, there is few information about pika UCP1, including its molecular structure and expression pattern. In the present study, we cloned plateau pika UCP1 cDNA, and examined its mRNA and protein expression in various tissues.

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Daurian pikas (*Ochotona dauurica*), which were the first-generation offspring of pikas having been captured in Mongolia [9], were kept at 23°C for 22 months and given laboratory chow and water *ad libitum*. They were euthanized by intraperitoneal administration of an overdose of urethane, and various tissues (fat pads of interscapular, inguinal, perigonadal, and retroperitoneal regions, skeletal muscle, liver, brain, lung, stomach, kidney, spleen) were immediately collected. All experiments had been approved by the Animal Experiment Committee of Chukyo University.

Total RNA was isolated from interscapular fat pad using TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.A.), and reverse-transcribed (RT) using M-MLV reverse transcriptase (Invitrogen) and oligo-dT primer linked to an adapter sequence. To clone pika UCP1 cDNA, we first obtained two cDNA fragments corresponding to the open reading frame. After confirming the nucleotide sequence of the cDNA fragments, we employed 3'-RACE to determine the terminal sequence of the 3'-end. Polymerase chain reaction (PCR) was performed with a cDNA template and primers designed from the reported sequences of rat and rabbit UCP1, and also from the confirmed sequence of the cDNA fragments. PCR was conducted for 30 cycles of denaturation at 94°C (30 sec), annealing at 54–60°C (30 sec), and extension at 72°C (1 min). A final extension at 72°C was applied for 7 min and followed by rapid cooling to 4°C. The PCR product was gel-purified, ligated into a pGEM-T easy vector (Promega, Madison, WI, U.S.A.), cloned into *E. coli*, and then sequenced using an ABI PRISM 310 capillary

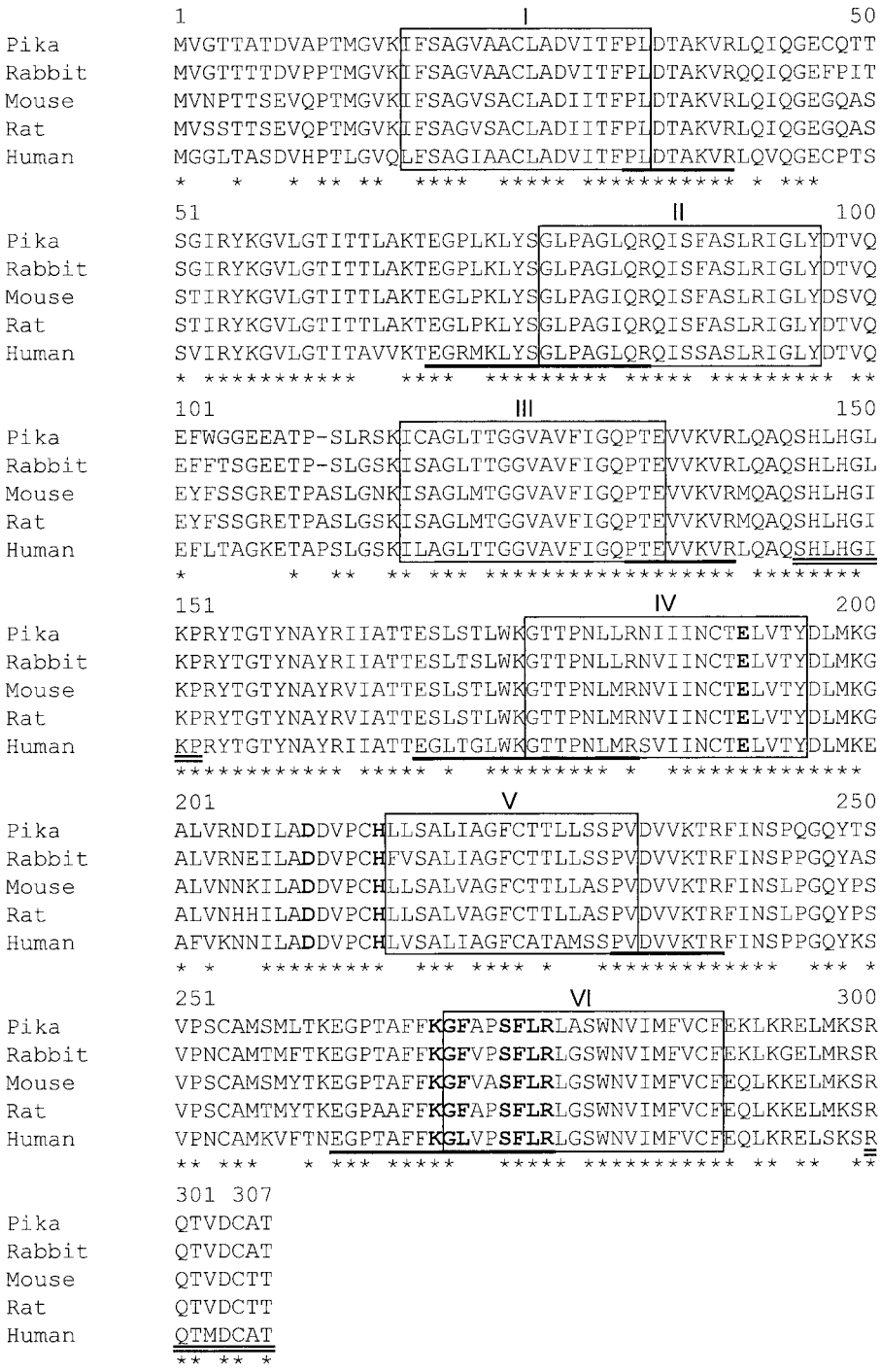

Fig. 1. Amino acid sequence of pika UCP1 and multiple sequence alignments among five mammalian species. The GenBank accession number of pika UCP1 gene is AB283043. The sequences included in the alignment are: rabbit UCP1 (GenBank Accession No. X14696), mouse UCP1 (GenBank Accession No. NM_009463), rat UCP1 (GenBank Accession No. NM_012682), and human UCP1 (GenBank Accession No. NM_021833). The sequences are presented in single letter code, and gaps introduced into the sequences to optimize alignments are illustrated with dashes. The six potential transmembrane domains are boxed and labeled by I-VI. The six mitochondrial carrier protein motifs are underlined. The putative purine-nucleotide binding site is indicated in bold letters. Two UCP1-specific sequences are double-underlined. Amino acid residues identical among the species are shown with asterisks.

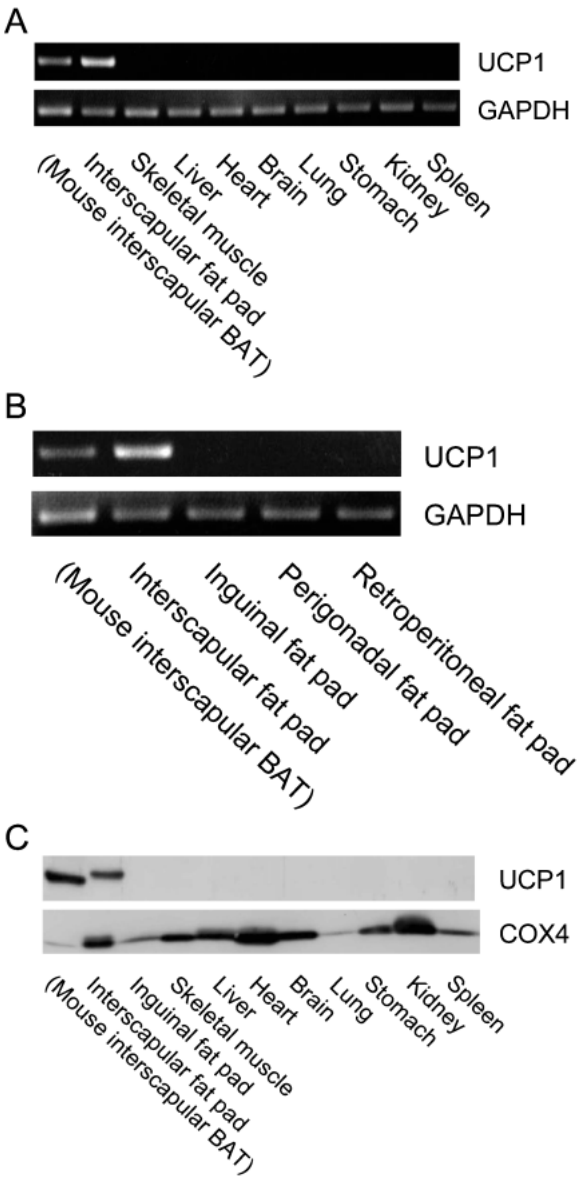

Fig. 2 Tissue distribution of pika UCP1. UCP1 mRNA (A, B) and protein (C) expression in various tissues of adult pikas were analyzed by RT-PCR and Western blotting, respectively. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and cytochrome oxidase subunit 4 (COX4) are used as internal control. For RT-PCR, total RNA (2 μg) extracted from individual tissues was reverse-transcribed and used for PCR analysis of UCP1 and GAPDH mRNA expression. For Western blotting, tissue specimen was homogenized and centrifuged, and the resulting supernatant (20 μg of total protein) was used for determining UCP1 and COX4 protein. Antibodies against rat UCP1 and bovine COX4 were provided from Drs. Teruo Kawada and Naohito Aoki (Kyoto University, Kyoto, Japan) and Molecular Probes (Eugene, OR, U.S.A.), respectively.

DNA sequencer (Applied Biosystems, Foster City, CA, U.S.A.).

Nucleotide sequence of the cloned cDNA revealed that the coding region of pika UCP1 consisted of 921 nucleotides, with 306 deduced amino acids, which were registered in DDBJ-GenBank-EMBL as accession number AB283043. The amino acid sequence of pika UCP1 showed some common domains for UCP [3, 7, 11], such as six transmembrane domains, six mitochondrial carrier protein motifs, and a putative purine-nucleotide binding site (Fig. 1). In addition, two UCP1-specific sequences, Ser^{144}-His-Leu-His-Gly-Ile-Lys-Pro and C-terminal sequence Arg-Gln-Thr-Xaa-Asp-Cys-(Thr/Ala)-Thr (Xaa is any amino acid) [1, 11], were also found. Figure 1 also shows that amino acid sequence was highly homologous to those of UCP1 of other species (mouse 84%, rat 85%, human 82%), especially of rabbits (91%). These results suggest that pika UCP1 has uncoupling activity, as do those of other species. Phylogenetic comparison of individual UCPs performed using the UPGMA method [13] revealed that pika UCP1 was genetically closer to that of rabbit than rodents, and far from UCP2 or 3 of other species (data not shown).

UCP1 has been confirmed as a specific molecule expressed exclusively in BAT. BAT exists in some particular regions, such as interscapular, perirenal, and cervical regions, but rarely in other regions. To examine the distribution of UCP1 in pika tissues, RT-PCR and Western blot analyses were performed (Fig. 2). Both UCP1 mRNA and protein were detected in the interscapular fat pad but not in other tissues examined, including inguinal, perigonadal, and retroperitoneal fat pads. We have examined the interscapular fat pad of totally seven pikas, all of which were rather pale and looked like white adipose tissue (WAT). However, histological observations revealed that it contained considerable number of multilocular adipocytes which were usually found in BAT (data not shown). In contrast, other fat pads consisted of unilocular adipocytes which were usually found in WAT. These observations are well consistent with the above finding of exclusive expression of UCP1 in the interscapular fat pad of pika.

It has been reported in small rodents that during cold acclimation UCP1 expression is not only up-regulated in BAT, but also induced in fat pads usually considered as WAT [15]. Up-regulation of UCP1 protein expression was also reported in interscapular fat pad of wild pikas captured in winter [14]. Since wild plateau pikas inhabit in cold environment, UCP1 is expected to be expressed also in other regions, such as inguinal, perigonadal, and retroperitoneal fat pads. In our study, however, we could not detect UCP1 in any fat pads except the interscapular region. This may be because our pikas had been kept warm at 23°C for about 2 years.

In summary, the molecular structure of pika UCP1 was quite similar to that of other mammals so far reported, suggesting that the uncoupling activity may also be similar. These results would be helpful to clarify the physiological roles of pika UCP1, and to study mechanisms of adaptation

to the specific plateau environments.

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