IDENTIFICATION OF UREA TRANSPORTER ISOFORMS

IN THE ELASMOBRANCH KIDNEY

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EXTENDED ABSTRACT ONLY – DO NOT CITE

In marine elasmobranch fishes, renal tubular urea reabsorption is, in part, responsible for the maintenance of a high plasma urea concentration**.** Furthermore, the regulation of tubular urea reabsorption is important in the renal response of euryhaline elasmobranchs to changes in environmental salinity. The mechanisms by which tubular urea reabsorption is regulated in the elasmobranch kidney have yet to be elucidated, but both passive and active mechanisms have been proposed. Passive reabsorption of urea occurs via facilitated urea transporter proteins (UT). A facilitated urea transporter (shUT), similar to that of the UT-A2 isoform in mammals, has been cloned from the kidney of the marginal elasmobranch, the spiny dogfish shark, *Squalus acanthias* (Smith and Wright, 1999). Interestingly, although only a single facilitated urea transporter isoform was identified, Northern analysis suggested that multiple isoforms may be expressed in the elasmobranch kidney. These findings indicate UTs may contribute to renal tubular urea reabsorption in elasmobranchs. We hypothesized that homologous urea transporters, similar to shUT, are also expressed in the kidney of euryhaline elasmobranchs and that these transporters play a role in mediating tubular reabsorption of urea in euryhaline elasmobranchs.

The initial step in testing this hypothesis was to identify UT isoforms expressed by a euryhaline elasmobranch. We utilized molecular cloning (PCR and 5'/3' Rapid Amplification of cDNA ends) and heterologous expression (urea uptake by *Xenopus* oocytes) techniques to identify and characterize cDNAs encoding facilitated UTs from the kidney of the euryhaline Atlantic stingray (*Dasyatis sabina*). The first stingray UT cDNA that we cloned was designated strUT-1. This cDNA is 2.7 kb in length, has an open reading frame (ORF) of 1296 nucleotides (nt) and encodes a 431 amino acid protein (Figure 1). The strUT-1 protein has a distinct carboxy-terminus compared to shUT, but aside from differences in the carboxy-terminus, the first 377 amino acids of strUT-1 were nearly 80% identical to the first 377 amino acids of shUT. By using RT-PCR and flanking primers within the 5' and 3' untranslated region of strUT-1 we also identified a second UT cDNA. This cDNA, which was designated strUT-2, is 3.6 kb in length, contains an open-reading frame of 1137 nt, and is predicted to encode for a UT of 379 amino acids (Figure 1). This shorter isoform is identical to the N-terminal 377 amino acids of strUT-1. Interestingly, even though the strUT-2 transcript was considerably larger than that reported for strUT-1 and shUT, this isoform is almost the same size, has a similar carboxy-terminus, and shares 79% sequence identity that of the shark UT. Both strUT-1 and strUT-2 include a double LP box signature sequence common to all UTs.

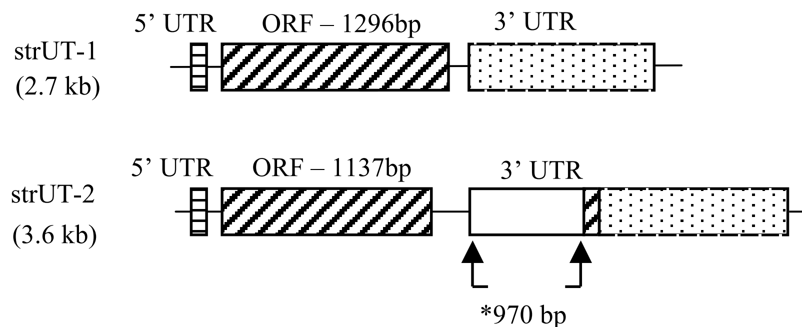

Figure 1. Schematic of strUT-1 and strUT-2 cDNA.
open-reading frame (ORF)
5' untranslated region (5'UTR)
3' untranslated region (3'UTR)
* strUT-2 contains a novel nucleotide cassette not present in strUT-1.
Retention of the unspliced cassette in strUT-2 results in an abbreviated ORF.

Characterization of both strUT-1 and strUT-2 in *Xenopus* oocytes demonstrated both isoforms increase [^{14}C]-urea uptake. This strUT induced [^{14}C]-urea uptake was inhibited by phloretin but was not altered by removal of sodium chloride from the medium. Northern analysis demonstrated transcripts of 2.7 kb and 3.6 kb corresponding to the predicted size of the message for strUT-1 and strUT-2, respectively. Interestingly, strUT expression was specific to the kidney. Further, Northern analysis indicated two other transcripts were expressed in stingray kidney. Following additional PCR optimization of our 5'/3' RACE reaction, we amplified two 3' RACE cDNAs 1.4 kb and 3.5 kb in size. The smaller 3'RACE product represents a 3'untranslated region (3'UTR) splice variant of strUT-1, whereas the larger transcript represents a 3'UTR splice variant of strUT-2. These cDNAs were designated strUT-1b and strUT-2b, respectively. The presence of 3'UTR splice variants is similar to that reported for UTs in the mammalian kidney (Bagnasco *et al*., 2000).

In the second series of comparative studies, we utilized the cloning techniques described above to identify facilitated urea transporters from the kidneys of two batoid species: a subtropical, marginal dasyatid stingray (*Dasyatis sayi*), and a temperate, stenohaline rajid skate (*Leucoraja ocellata*). A single UT cDNA has so far been cloned from the kidneys of both species. The cDNA from the skate (skUT) is 2.1 kb in length, with an open-reading frame of 1134 nt that encodes a 378 amino acid protein. The cDNA from *D. sayi* (UT) is 1.7 kb in length with an open-reading frame of 1140 nt that encodes a 380 amino acid protein. These urea transporters were similar in length and sequence identity to shUT and strUT-2.

We conclude that 1) orthologous facilitated urea transporters are expressed in the kidneys of phylogenetically distinct elasmobranchs, 2) multiple urea transporter isoforms are expressed in the kidneys of at least one species of elasmobranch, and 3) a common urea transporter isoform is expressed in the kidneys of elasmobranchs.

References

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