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# Selective Assembly of V-ATPase Subunit Isoforms in Mouse Kidney

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The kidney plays vital roles in acid–base homeostasis, and the reabsorption of water, ions, and proteins. These processes are achieved through acidification of urine and endosomes of proximal tubule epithelial cells. Multisubunit vacuolar-type proton ATPase (V-ATPase) is one of the major acidification-machinery proteins that localizes to the apical or basolateral plasma membranes of intercalated cells in collecting ducts and the endosomal region at the base of brush border microvilli in proximal tubules. Multiple subunit isoforms of V-ATPase, which are expressed in kidney, have been identified. One obvious question is whether the pumps at different locations in the kidney have their own unique subunit identities. We have used a combination of methods to study this enzyme in kidney including immunocytochemical staining and immunoprecipitation analyses. The subunit isoforms of V-ATPase exhibited selective association/assembly in kidney: kidney-specific isoforms predominantly formed the intercalated cell proton pump, whereas the pump located in the brush border comprised ubiquitously expressed counterparts.

**KEY WORDS:** V-ATPase; acid-base homeostasis; subunit isoforms; kidney.

## INTRODUCTION

The kidney plays vital roles in acid–base homeostasis, by reabsorbing filtered bicarbonate and regenerating bicarbonate that is consumed during metabolic proton production. These processes are accomplished through renal proton secretion, which occurs primarily in the proximal tubules and collecting ducts (Gluck *et al*., 1995). Physiological studies have established that $Na^+/H^+$ exchange accounts for most of the proximal tubule acidification (Aronson, 1983), whereas in more distal segments, acidification is carried out by a multisubunit vacuolar-type proton ATPase (V-ATPase) that is localized to the plasma membranes of intercalated (IC) cells specialized for proton transport (Brown *et al*., 1988a,b, 1997).

At least two types of IC cell ($\alpha$- and $\beta$-cells) are present in the kidney cortex, and are involved in proton and bicarbonate secretion, respectively, in the distal nephrons and collecting ducts (Al-Awqati, 1996; Brown and Breton, 2000; Madsen and Tisher, 1986; Schuster, 1993). $\alpha$-Cells have V-ATPases on their apical plasma membranes and express the kidney homologue of the band 3, $Cl^-/HCO_3^-$ exchanger (AE1), on their basolateral plasma membranes (Alper *et al*., 1989; Schuster *et al*., 1991). $\beta$-cells contain an exchanger (AE4) on their apical membranes, and V-ATPases can be apical, basolateral, bipolar, and diffuse (Alper *et al*., 1989; Brown *et al*., 1988a; Schuster *et al*., 1991). In addition to V-ATPases located on the plasma membranes of IC cells, immunohistochemical studies have shown that the enzyme is also found in the brush border microvilli and subvilli invaginations (Brown *et al*., 1988b), where it is required for endosomal acidification that is essential for protein reabsorption. The same enzyme is also detected ubiquitously in intracellular organelles including late endosomes, lysosomes, and the Golgi apparatus (for reviews, see Futai

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*Abbreviations:* V-ATPase, vacuolar $H^+$-ATPase; IC cell, intercalated cell; PCT, proximal convoluted tubules; CD, collecting ducts.

*et al*., 2000; Nelson and Harvey, 1999; Nishi and Forgac, 2002).

V-ATPases are composed of two functional domains. The $V_1$ catalytic domain is a peripheral complex composed of eight subunits with molecular masses of 70–14 kDa (subunits *A*, *B*, *C*, *D*, *E*, *F*, *G*, and *H*), and the Vo domain is a membrane integral complex composed of five subunits with molecular masses of 100–16 kDa (subunits *a*, *d*, *c*, *c*′, and *c*″) that are responsible for proton translocation. Multiple subunit isoforms have been found for the largest subunit, *a*, of the Vo sector in nematode (Oka *et al*., 2001a), chicken (Mattsson *et al*., 2000), mouse (Nishi and Forgac, 2000; Oka *et al*., 2001b; Toyomura *et al*., 2000; Smith *et al*., 2001), and man (Peng *et al*., 1999). The $V_1$ sector also shows structural divergence (Murata *et al*., 2002; Sun-Wada *et al*., 2002, 2003b).

We have identified multiple subunit isoforms expressed predominantly in kidney in addition to *B*1 reported previously by Brown and colleagues (Brown *et al*., 1988a). *a*4 is specifically expressed in renal IC cells (Oka *et al*., 2001b; Smith *et al*., 2000, 2001). The *d* subunit as well as the *C*2 and *G*3 also contain kidney-specific isoforms (Smith *et al*., 2002; Sun-Wada *et al*., 2003b). These isoforms may contribute to targeting of V-ATPase to various destinations as well as regulation of proton secretion in different cell types.

Genetic studies of human diseases have revealed that V-ATPase is essential for acid–base homeostasis (Karet *et al*., 1999; Smith *et al*., 2000). Mutations in *a*4 and *B*1 cause renal tubular acidosis (RTA), indicating that these isoforms are essential for proton pumping from intercalated cells. One obvious question is whether *a*4 and *B*1 form an IC cell V-ATPase together with other kidney-specific isoforms. It would also be interesting to know whether the pumps at different locations have their own unique subunit identities.

## LOCALIZATION OF KIDNEY-SPECIFIC SUBUNIT ISOFORMS OF V-ATPASE

We have examined the localization of V-ATPase in kidney with isoform-specific antibodies. In the cortical collecting ducts, kidney-specific isoforms *d*2, *G*3, and *C*2 were localized to the apical and basolateral plasma membranes of type $\alpha$ and $\beta$ IC cells, respectively, which is similar to the case of previously characterized kidney-specific isoforms *a*4 and *B*1 (Brown *et al*., 1988b; Oka *et al*., 2001b; Smith *et al*., 2001; Sun-Wada *et al*., 2003a). The ubiquitously expressed subunit *A* and *E*2 isoforms were found to be concentrated at both the apical and basolateral domains of IC cells (Sun-Wada *et al*., 2003a). In addition, the plasma membranes of IC cells were also labeled by antibodies against the ubiquitously expressed isoforms including *G*1 (Sun-Wada *et al*., 2003a) and *d*1 (Sun-Wada, unpublished data).

In the proximal convoluted tubule (PCT), the V-ATPase is localized to the brush border microvilli and subvilli invaginations (Brown *et al*., 1988b). We found that antibodies against ubiquitous isoforms *A*, *B*2, *d*1, *E*2, *G*1, and *C*1 labelled this region (Sun-Wada, unpublished results). All four *a* subunit isoforms were found in the brush border region, although the localizations were slightly different. Other kidney-specific isoforms were not detected in PCT (Sun-Wada, unpublished results).

## SELECTIVE INTERACTIONS OF SUBUNIT ISOFORMS IN MOUSE KIDNEY

The IC cell-specific localization of kidney-specific isoforms prompted us to examine whether there is any selective association or assembly of the subunit isoforms. We examined the compositions of the enzyme complexes immunoprecipitated with anti-*B*1 (kidney-specific isoform) and *B*2 (ubiquitously expressed isoform) antibodies.

The *c* proteolipid, which is encoded by a single gene in the whole mouse genome (Hayami *et al*., 2001; Sun-Wada *et al*., 2000), was coimmunoprecipitated with either *B*1 or *B*2 (Sun-Wada *et al*., 2003a). The ubiquitously expressed *d*1 isoform was coimmunoprecipitated with both *B*1 and *B*2 (Sun-Wada *et al*., 2003a), whereas kidney-specific *d*2 was associated solely with the *B*1-containing complex. Meanwhile, ubiquitously expressed *a*1, *a*2, and *a*3 were coimmunoprecipitated with *B*2, not *B*1. Kidney-specific *a*4 was coprecipitated with both *B*1 and *B*2 (Sun-Wada *et al*., 2003a), which is consistent with its localization at both IC cells and brush border membranes (Oka *et al*., 2001b; Smith *et al*., 2001).

The $V_1$ sector contained two kidney-specific isoforms, *C*2 and *G*3. We found that *C*2 and *G*3 were associated with kidney isoform *B*1, whereas the ubiquitous counterparts *C*1 and *G*1 were coprecipitated with ubiquitous *B*2. The *G*1 isoform was also coprecipitated with *B*1, although the amount was significantly less than that with *B*2. The *E* subunit contained two isoforms, and *E*2 is the only isoform expressed in kidney. Immunoprecipitation revealed that *E*2 is associated with both isoforms of the *B* subunit (Sun-Wada *et al*., 2003a).

**Table I.** Activities of V-ATPase With the *B*1 and *B*2 Isoform

| Immunopurified V-ATPase with *B* subunit isoform | ATPase activity | |
|---|---|---|
| | $K_m^{ATP}$ ($\mu$M ATP) | $V_{max}$ (nmol/min/mg) |
| *B*1-type | 333 | 46 |
| *B*2-type | 364 | 40 |

## ENZYMATIC PROPERTIES OF V-ATPASE WITH THE *B*1 OR *B*2 ISOFORM

The solubilization and immunoprecipitation of V-ATPase were performed as described in Sun-Wada *et al*. (2003a). The amount of membrane protein bound to the beads was estimated as previously described (Gluck and Caldwell, 1987). ATPase activity was measured for the immunopurified enzyme (0.1 $\mu$g). Activity was measured with or without 1 $\mu$M concanamycin A as described in Tanabe *et al*. (2001), and the results shown represent the concanamycin A-sensitive fraction of the activity. $K_m$ and $V_{max}$ were calculated from Lineweaver–Burk plots of the reciprocal of initial ATP concentration *versus* the reciprocal of the initial rate of ATPase activity (nmol/min/mg).

The histochemical staining and immunoprecipitation analyses revealed that kidney-specific isoforms predominantly form the intercalated cell proton pump (*B*1-type), whereas the pump located in the brush border comprised ubiquitously expressed counterparts (*B*2-type). It was of interest to compare the enzymatic properties of the two types of pumps. The V-ATPase with *B*1 or *B*2 bound to affinity beads retained concanamycin A-sensitive ATPase activity. The $K_m^{ATP}$ values for the *B*1-type and *B*2-type enzyme were comparable (Table I), indicating that the *B*1- and *B*2-type enzymes exhibit no significant kinetic difference.

## CONCLUSIONS AND PROSPECTIVES

The subunit structure permutations of individual pumps in the kidney are limited. Our immunoprecipitation results showed that kidney-specific isoforms are selectively assembled to form a unique proton pump on the plasma membranes of cortical IC cells (Fig. 1). The ubiquitous isoforms, on the other hand, are associated in V-ATPases that are required for acidification of intracellular organelles and endocytic vesicles in proximal tubules. Meanwhile, minor combinations were still observed. For example, kidney-specific *a*4 is localized to the endosomal membranes in proximal tubules and forms an enzyme complex with ubiquitous subunit isoforms. In contrast, ubiquitous *G*1 was also observed, although at a low frequency when compared with *G*3, on the plasma membranes of IC cells.

Our results provide information about the structures of V-ATPase complexes functioning in different nephron domains. Determination of the physiological role of each specific isoform should facilitate understanding of the

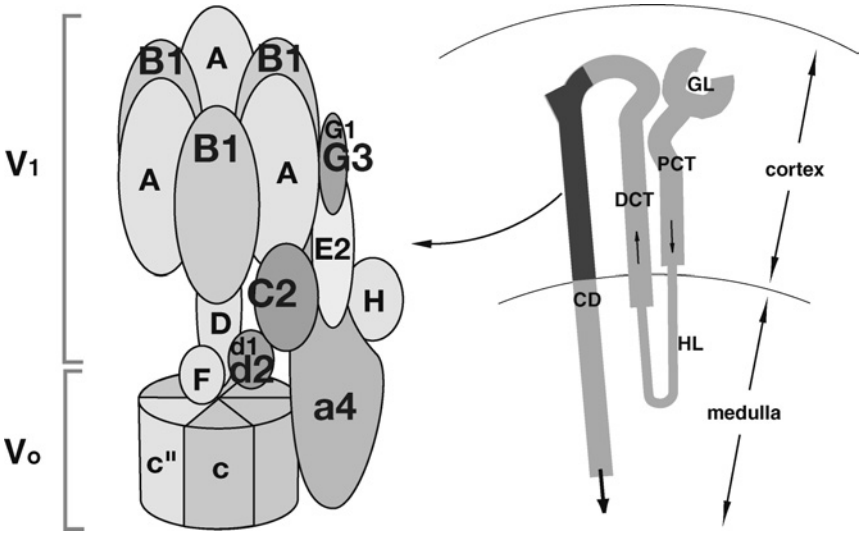


**Fig. 1.** Models representing the subunit isoform selectivity of V-ATPases in renal intercalated cells. CD, collecting duct; PCT, proximal convoluted tubule; DCT, distal convoluted tubule; HL, Henle loop; GL, glomeruli. The kidney-specific subunit isoforms are indicated by *large letters*.

mechanism that regulates the cellular localization of the proton pump in kidney. This knowledge may in future allow the development of therapeutic agents for the treatment of human diseases associated with the activity of a distinct type of ATPase.

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