BIOCHEMISTRY OF THE RENAL V-ATPase

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Summary

In most eukaryotic cells, vacuolar H^+-ATPases (V-ATPases) are present primarily or exclusively in intracellular membrane compartments, functioning in the acidification of the endocytic and secretory vacuolar apparatus necessary for constitutive cell function. V-ATPases also participate in renal hydrogen ion secretion in both the proximal and distal nephron, residing at high concentrations on the plasma membrane, where they are regulated physiologically to maintain the acid–base balance of the organism. Recent experiments have begun to reveal how the kidney controls transcellular proton transport while still maintaining acidification of intracellular compartments. Control may occur by recruitment of proton pumps to or away from the plasma membrane. The proton-transporting plasma membrane of intercalated cells is a specialized apparatus that translocates the enzyme between an intracellular membrane pool and the plasma membrane in response to physiological stimuli. Regulation may also occur by changes in the kinetics of the V-ATPase. V-ATPases are a family of structurally similar enzymes which differ in the composition of specific subunits. Cytosolic regulatory enzymes present in renal cells may preferentially affect V-ATPases in selective membrane compartments.

Introduction

To maintain acid–base balance, the kidney reabsorbs all of the bicarbonate filtered by the glomerulus and excretes a quantity of acid equal to that produced by metabolic proton generation. Renal hydrogen ion excretion takes place in several nephron segments (Alpern, 1990). The proximal tubule reabsorbs 80% of the filtered bicarbonate; an additional 10% of the filtered bicarbonate is reabsorbed in the thick ascending limb (Borenstein *et al.* 1991; Good, 1990). The collecting duct is responsible for the final reabsorption of bicarbonate in the regulation of net acid excretion by the kidney (Schuster, 1990). In the collecting duct, hydrogen ion excretion is carried out by the intercalated cells, a specialized cell population rich in carbonic anhydrase (Brown, 1989; Madsen and Tisher, 1985) that employ a V-ATPase as a predominant means for hydrogen ion transport (Brown *et al.* 1988*a,b*). The renal V-ATPase has been isolated from bovine kidney, and its enzymatic and structural properties have been studied (Gluck and Caldwell, 1987). Like other V-ATPases, the kidney enzyme is a multimeric protein with a

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relative molecular mass of approximately 580×10^3; it is composed of several different polypeptide chains. The enzyme has been purified on monoclonal antibody immunoaffinity beads and remains enzymatically active, retaining the capacity for active proton transport when reconstituted in a phospholipid vesicle. SDS–polyacrylamide gels of the purified enzyme show polypeptides at M_r 70×10^3 (*A* subunit), a cluster at approximately 56×10^3 (*B* subunit) and polypeptides at 45×10^3, 42×10^3 (*C* subunit), 38×10^3, 33×10^3 (*D* subunit), 31×10^3 (*E* subunit), 15×10^3 (*c* subunit), 14×10^3 and 12×10^3 (Gluck and Caldwell, 1987, 1988; Wang and Gluck, 1990).

High-affinity polyclonal antibodies were used to localize the distribution of the enzyme in the mammalian kidney (Brown *et al.* 1988*a,b*). In the proximal tubule, V-ATPase staining was abundant on the brush-border microvilli in the initial portion of the proximal tubule and in the invaginations of the base of the apical microvilli throughout the proximal tubule. Moderate plasma membrane staining for V-ATPase was also observed in the thick ascending limb and the distal convoluted tubule. In the connecting tubule and collecting duct, heavy staining was observed in the proton-transporting intercalated cells. Rapid-freeze deep-etch micrographs confirmed that the V-ATPase resides on the luminal membrane of the acid-secreting cells (Brown *et al.* 1987). In these cells, proton pumps were present at a density of about $15\times10^3\,\mu m^{-2}$. V-ATPases are also present in many of the intracellular vacuolar compartments of eukaryotic cells (Yurko and Gluck, 1987), where they serve in the processing of membranes and intraluminal molecules in endocytosis and secretion. However, immunocytochemical studies suggest that the proton pump in these intracellular compartments is present at far lower densities than it is in the plasma membrane of proton-transporting cells (Rodman *et al.* 1991).

The V-ATPase in the plasma membrane of proton-transporting cells therefore displays several features distinct from V-ATPases of most other mammalian cells: cell-specific amplification, polarization to one or more plasmalemmal domains and physiological regulation to preserve acid–base homeostasis (discussed in more detail below). Work in our laboratory has focused on how proton-transporting cells maintain these highly specialized functions of the V-ATPase in the plasma membrane while still employing V-ATPases to acidify intracellular compartments as required for constitutive functions of the vacuolar system.

Properties of the V-ATPase in renal epithelial cells

Enzymatic properties

Several lines of evidence suggest that V-ATPases exist as a family of enzymes with differences in structure and function (Gluck, 1992; Simon and Burckhardt, 1990). V-ATPase isolated from bovine kidney microsomes by HPLC ion exchange contained two partially resolved peaks of ATPase activity which differed in their polypeptide composition (Gluck and Caldwell, 1987). V-ATPases isolated from separate membrane fractions from bovine kidney had different enzymatic and structural properties (Gluck, 1992; Wang and Gluck, 1990). V-ATPase was isolated from a purified brush-border membrane fraction, a kidney lysosomal fraction and from kidney microsomes (a mixture of membranes containing plasma membranes from intercalated cells) by immunoaffinity

chromatography on identical monoclonal antibody columns. V-ATPases isolated from these fractions differed in their pH optimum, in their ATP:GTP substrate selectivity, in the effects of added lipids on their activity, and in their sensitivity to divalent and trivalent cations (Gluck, 1992; Simon and Burckhardt, 1990; Wang and Gluck, 1990; Z.-Q. Wang and S. Gluck, in preparation). They also had subtle differences in structure that could be detected by immunoblotting on two-dimensional gels. The brush-border V-ATPase had a pH optimum of about 7.3, the approximate intracellular pH of the proximal tubule cell. The proximal tubule functions primarily in bulk bicarbonate reabsorption and is not the site for fine regulation of net hydrogen ion excretion. The pH optimum of the brush-border V-ATPase is, therefore, poised for the enzyme to function maximally at a normal intracellular pH. In contrast, the pH optimum of the microsomal enzyme is approximately 6.3, a 1 pH unit difference from the intracellular pH of intercalated cells. The V-ATPase of these cells may, therefore, respond with an increase in activity under conditions where intracellular pH decreases, such as a decrease in the extracellular fluid bicarbonate concentration. However, the magnitude of the change is only about 30% of the total activity between pH 6.3 and 7.3, and it is therefore likely that other regulatory mechanisms control the rate of transport. The pH optimum of the kidney lysosomal V-ATPase was approximately 6.7 (Gluck, 1992). 1 mmol l^{-1} Cu^{2+} inhibited the microsomal enzyme activity by 100%. A concentration of copper up to 5 mmol l^{-1} inhibited the brush-border V-ATPase by approximately 50%. The lysosomal V-ATPase was also relatively insensitive to copper, but was highly sensitive to 1 mmol l^{-1} aluminum ion; 5 mmol l^{-1} aluminum had no effect on the brush-border and microsomal V-ATPase. Thus, significant differences in enzymatic properties can be detected in V-ATPase from different kidney membrane fractions. Evidence suggests that the $70\times10^3 M_r$ subunit of the V-ATPase is the locus of the ATP hydrolytic site involved in catalysis (Forgac, 1989). Two-dimensional gels of the V-ATPase immunoaffinity-purified from different kidney membrane fractions have revealed only one $70\times10^3 M_r$ polypeptide (Wang and Gluck, 1990); only one gene encoding the subunit has been reported by cDNA cloning and genomic Southern blots (Marushack *et al.* 1992; Pan *et al.* 1991; Puopolo *et al.* 1991). A single type of $70\times10^3 M_r$ polypeptide would suggest that another subunit, probably the *B* subunit, which has an approximate M_r of 56×10^3 and has sequence homology with the regulatory α subunit of the F_1F_0 H$^+$-ATPases, may be a regulatory subunit affecting the overall enzymatic properties of the V-ATPases.

The renal V-ATPase had no requirement at all for monovalent anions or cations either for ATPase activity or for proton transport (Gluck and Caldwell, 1987, 1988; Wang and Gluck, 1990), as has also been reported for the coated-vesicle V-ATPase (Arai *et al.* 1989). Studies from other laboratories have suggested that mammalian V-ATPases are stimulated directly by chloride (Kaunitz *et al.* 1985; Moriyama and Nelson, 1987). In studies from our laboratory on V-ATPase isolated from bovine kidney, increasing concentrations of chloride decreased ATPase activity slightly. Other anions were found to have more profound effects on ATPase activity, as described in the fungal chromaffin granule V-ATPase, coated-vesicle V-ATPase and other V-ATPases (Arai *et al.* 1989; Bowman *et al.* 1989; Moriyama and Nelson, 1989). Nitrate inhibited the kidney V-ATPase activity at concentrations above 5 mmol l^{-1} through an uncompetitive type of

inhibition; dissociation of subunits from the cytoplasmic domain of the enzyme was observed only at concentrations greater than $150\,mmol\,l^{-1}$ nitrate (Wang and Gluck, 1990). Dissociation of the subunits of the catalytic sector at lower concentrations of nitrate has been observed in enzyme incubated at $4\,°C$ in the presence of ATP, the so-called 'cold inactivation' of the V-ATPase (Moriyama and Nelson, 1989). Sulfite stimulated ATPase activity at concentrations lower than $20\,mmol\,l^{-1}$; both sulfite and sulfate inhibited ATPase activity at concentrations above $20\,mmol\,l^{-1}$. These observations suggest that the kidney V-ATPase has an oxyanion binding site which regulates its activity. This anion binding site may function as a receptor for carbonate ion. The effects of different concentrations of bicarbonate on V-ATPase activity were tested under conditions of constant pH, and there was little effect (Z.-Q. Wang and S. Gluck, unpublished data), but under conditions where greater than $0.1\,mol\,l^{-1}$ carbonate ion was present, as estimated from the bicarbonate–carbonate proton K_d, a 20–30% inhibition of ATPase activity was observed. Carbonate might be present at significant concentrations in an alkaline cytosol or in the setting of a significant unstirred layer of bicarbonate ion surrounding the V-ATPase, as might occur following inhibition of cytosolic carbonic anhydrase. Carbonate inhibition might serve to inhibit further proton extrusion from the cell.

Structure

The microsomal, brush-border and lysosomal V-ATPases were compared on two-dimensional gels, and significant differences were observed in the structure of the $31\times10^3\,M_r$ subunit and the $56\times10^3\,M_r$ subunits among the different V-ATPase preparations (Wang and Gluck, 1990). The $31\times10^3\,M_r$ subunit had an isoelectric point of approximately 8.1 with a broad pH optimum. In the microsomal ATPase, the subunit appeared as a single polypeptide band at $31\times10^3\,M_r$. The $31\times10^3\,M_r$ subunit in the brush-border microvillar V-ATPase appeared as a predominant polypeptide at $31\times10^3\,M_r$ with several polypeptides at an identical pI, but of lower mobility (higher relative molecular mass; Wang and Gluck, 1990). A cDNA clone encoding the $31\times10^3\,M_r$ subunit was isolated and the complete amino acid sequence of the protein was deduced (Hirsch et al. 1988). Mice were immunized with a 10-residue peptide from the carboxy terminus, and a series of monoclonal antibodies reacting with the subunit were isolated and characterized (Hemken et al. 1992). Several of these reacted with the native $31\times10^3\,M_r$ subunit by immunoblot analysis. When these antibodies were examined by immunoblotting on two-dimensional gels and by immunocytochemistry, significant differences in reactivity were discovered (Hemken et al. 1992). Antibody E11 yielded a pattern of immunocytochemistry in rat kidney identical to the pattern described previously using rabbit polyclonal antibodies. E11 stained the brush-border microvilli in rat proximal tubules, the invaginations at the base of the microvilli, the apical and basolateral poles of cortical intercalated cells and the apical poles of medullary intercalated cells. On two-dimensional immunoblots, E11 reacted with a single $31\times10^3\,M_r$ polypeptide on the affinity-purified microsomal V-ATPase. However, E11 reacted with a series of polypeptides on two-dimensional immunoblots of the affinity-purified brush-border V-ATPase. The polypeptides had the same isoelectric point as the $31\times10^3\,M_r$ subunit, but

the additional polypeptides had a lower mobility, a pattern identical to that observed on the two-dimensional protein gels of the affinity-purified brush-border V-ATPase. A second antibody, H8, also reacted on immunoblots with the native $31\times10^3 M_r$ subunit from kidney microsomes. On immunocytochemical staining of rat kidney, antibody H8 stained the invaginations at the base of the microvilli and the intercalated cells with a pattern identical to that observed with antibody E11. However, H8 showed no staining of the brush-border microvilli. On immunoblots, this antibody stained the single $31\times10^3 M_r$ polypeptide in microsomal ATPase but had nearly absent immunoreactivity against the brush border enzyme. These results suggest that the structure of the $31\times10^3 M_r$ subunit differs in brush border; it has several polypeptide forms with a mobility lower than the most prevalent form of the subunit found in the kidney. The physiological role of the $31\times10^3 M_r$ subunit remains to be determined, but studies on the biosynthesis of the V-ATPase in LLC-PK$_1$ cells suggest that it controls the assembly of the enzyme on the membrane (J.-Y. Fu and S. Gluck, unpublished results).

The basis for the lower-mobility forms of the $31\times10^3 M_r$ subunit is unclear. A Southern blot was performed on human leukocyte DNA, cleaved with different restriction enzymes and probed with a PCR fragment spanning the carboxy terminal portion of the coding region of the $31\times10^3 M_r$ cDNA and a small portion of the 3' untranslated region. This probe hybridized to several fragments on Southern blots of leukocyte genomic DNA. A full-length human $31\times10^3 M_r$ cDNA was isolated from a human kidney library and used to screen a human genomic library. Several genomic clones were obtained. Two of these were purified and the lambda DNA was cleaved with the same restriction enzymes as those used for the genomic Southern blots; it was analyzed for hybridization to the same PCR fragment. The two clones accounted for all but one of the fragments observed on the Southern blots. Sequence analysis of the two clones showed that one was a true gene with a stretch of nucleotides identical to the human cDNA. The second genomic clone appeared to be a pseudogene, as it contained a stretch with a predicted protein sequence highly similar to human cDNA, but with several frame-shift changes (Hemken *et al.* 1992). The clone was also missing the stop codon found on the human $31\times10^3 M_r$ cDNA, and the carboxy terminal five amino acids were absent in all three reading frames. Nevertheless, this clone was used to perform an RNA hybridization on RNA from different tissues, and a transcript of 1.2 kb was detected uniformly in human kidney, heart, lung, liver, placenta, brain and other tissues. Hence, this clone and the unidentified DNA fragments hybridizing to the PCR-derived $31\times10^3 M_r$ cDNA fragment on the genomic Southern blot may represent isoforms of the subunit and might explain the presence of the lower-mobility immunoreactive polypeptides detected. It is also possible that the lower-mobility immunoreactive forms of the $31\times10^3 M_r$ subunit are the result of post-translational modifications. No evidence for glycosylation or phosphorylation was observed in V-ATPase purified directly from kidney brush border or from LLC-PK$_1$ cells (Hemken *et al.* 1992); the analysis of the sequence of the $31\times10^3 M_r$ subunit did not reveal potential sites for other post-translational modifications. The basis for the difference of structure in the subunit in the brush border remains unresolved.

The $56\times10^3 M_r$ subunit also showed differences in structure among the different V-ATPases isolated by immunoaffinity purification (Wang and Gluck, 1990). Two-

dimensional protein gels of both the brush-border and microsomal enzymes revealed a cluster of polypeptides at $58\times10^3 M_r$ and $56\times10^3 M_r$. In both V-ATPase preparations, the $58\times10^3 M_r$ subunit appeared as a single spot, whereas the $56\times10^3 M_r$ subunit appeared as a series of 4–6 spots with slightly different isoelectric points. The number of different $56\times10^3 M_r$ subunit polypeptides in the microsomal enzyme was consistently greater than in the brush-border enzyme. This difference may reflect the source of the microsomal ATPase; it was isolated from a more complicated mixture of membrane compartments than the brush-border V-ATPase. The lysosomal V-ATPase also contained a series of $56\times10^3 M_r$ polypeptides with slightly different isoelectric points on two-dimensional gels, but it did not contain the $58\times10^3 M_r$ polypeptide. cDNA cloning from our laboratory (Nelson *et al.* 1992) and others (Bernasconi *et al.* 1990; Puopolo *et al.* 1992; Südhof *et al.* 1989) has shown that there are at least two isoforms of the $56\times10^3 M_r$ subunit that are encoded by different genes. Comparison of the sequences of two human and bovine $56\times10^3 M_r$ isoforms shows that the middle portion of the coding region is highly conserved, but both the amino and carboxy terminal sequences of the two isoforms are entirely different (Nelson *et al.* 1992), and these differences are highly conserved in the corresponding isoform across species. RNA blots from different human tissues revealed that the kidney has the greatest levels of expression of the 'kidney' $(58\times10^3 M_r)$ isoform (isoform 1) of any tissue. Moderate levels were detected in the placenta and low levels were detected in the lung; hybridization was undetectable in poly-A+ mRNA from other human tissues. Similar results were found in RNA hybridization studies performed on total RNA from various bovine tissues. In contrast, RNA hybridization blots performed on human poly-A+ RNA with the 'brain' $(56\times10^3 M_r)$ isoform (isoform 2) showed nearly equal levels expressed in all tissues, but with somewhat higher levels of expression in brain (Nelson *et al.* 1992).

Immunoblots of microsomal membranes from various bovine tissues enabled the protein distribution of the 'kidney' isoform of the subunit to be determined; it has a M_r of 58×10^3 on SDS gels. The results were similar to those of the RNA blots: the subunit isoform protein was detectable only in kidney. Immunocytochemistry was performed on rat kidney sections with a polyclonal antibody to a peptide from the unique carboxy terminal sequence of the 'kidney' isoform to examine the cellular distribution of the isoform in kidney. As a control, sections were co-stained with a monoclonal antibody to the $31\times10^3 M_r$ subunit of the V-ATPase. The antibody to the $31\times10^3 M_r$ subunit exhibited a pattern of staining identical to that described above. Intense staining was found in the microvilli of the cells of the initial part of the proximal tubule and in the invaginations at the base of the microvilli of cells throughout the entire proximal tubule. Intense staining was also observed in the intercalated cells of the collecting duct. Moderate staining was present in several other segments. In the cortex, there are two functionally distinct types of intercalated cells: an H^+-secreting intercalated cell, or A cell, and a bicarbonate-secreting intercalated cell or B cell. We showed previously that the A cells stain predominantly in the apical pole. In contrast, the B cells stain in the basolateral membrane, or diffusely throughout the cytoplasm, or, in some cells, in both the apical and basal poles of the cell. In the medullary collecting duct, only the A-type intercalated cells

are present, and these intercalated cells showed staining with anti-V-ATPase antibody in the apical pole only.

In the kidney sections stained with the antibody to the 'kidney' isoform (the $58 \times 10^3 M_r$ subunit), intense staining was observed only in the intercalated cells. No staining was observed in any part of the proximal tubule or in the thick ascending limb. Moderate staining was observed in the apical membrane of the distal convoluted tubule, and weak staining was found in the apical membrane of the principal cells of the inner medullary collecting duct. In the cortical collecting duct, staining was observed in the apical or basolateral membranes of some intercalated cells, suggesting that the 'kidney' isoform of the subunit may contain structural information important to plasma membrane targeting, but apparently does not contain structural determinants for selective apical or basolateral polarization. Although the kidney isoform of the B subunit is amplified selectively in the intercalated cells, it is expressed in other tissues. Both the 'kidney' and 'brain' isoforms of the B subunit are expressed in three different cell lines, including the LLC-PK$_1$ and MDBK cell lines. Immunocytochemical staining of these cells with antibody to the 'kidney' isoform revealed staining of a perinuclear vacuolar membrane compartment which is currently unidentified. Thus, the current evidence supports the hypothesis that the proton-transporting apparatus of the intercalated cell represents an amplification of a vacuolar compartment present in general in eukaryotic cells, and which has been modified to serve in physiologically regulated transcellular hydrogen ion secretion. The specialized compartment of intercalated cells may be analogous to the amplified endoplasmic-reticulum-like compartment observed in the UT-1 cell line (Chin *et al.* 1982) that overexpresses HMG-CoA reductase.

Regulation of the V-ATPase in renal epithelia

In principle, regulation of renal H^+ secretion may occur by at least three different mechanisms: (1) by an increase in the kinetic activity of the V-ATPase without a change in the quantity or distribution; (2) by a change in the distribution of the V-ATPase, such that the enzyme is recruited to the plasma membrane from an intercellular pool or retrieved from the plasma membrane into an intercellular compartment; and (3) by an overall increase in the quantity of V-ATPase without a change in its kinetics or relative distribution between different membrane compartments. Studies from our laboratory indicate that changes in the kinetics and distribution of the V-ATPase participate in regulation.

Physiological studies provided evidence for kinetic changes in V-ATPase activity in the regulation of transepithelial hydrogen ion transport (McKinney and Davidson, 1987; Steinmetz, 1986). These physiological responses are probably not a direct consequence of changes in intracellular ionic composition. Although isolated microsomal V-ATPase had a pH optimum of 6.2, the difference in activity between pH 7.2 and pH 6.2 was only 30% (Gluck and Caldwell, 1987; Wang and Gluck, 1990). Thus, pH changes in the physiological range of intracellular pH do not produce a change in microsomal V-ATPase activity sufficient to be a principal direct regulator of activity in the intact cell. Physiological intracellular concentrations of other cations and anions had no significant

effect *in vitro* on the activity of the isolated V-ATPase (Gluck and Caldwell, 1987; Wang and Gluck, 1990).

We therefore investigated the possibility that the kinetic activity of the kidney V-ATPase was controlled by cytosolic regulatory proteins. Bovine kidney cytosol was found to contain both inhibitory and activating factors that directly modified the ATPase activity of the immunoaffinity-purified enzyme (Zhang *et al.* 1992*a,b*). The activator and inhibitor activities of cytosolic fractions were quantified by their effects on immunoaffinity-purified V-ATPase bound to monoclonal antibody beads. The difference in ATPase activity with and without cytosol was used to determine the degree of inhibition or activation.

An inhibitor was purified from cytosol by a sequential combination of ultracentrifugation, ammonium sulfate fractionation, acid precipitation, cation exchange chromatography, anion exchange chromatography and HPLC anion exchange chromatography (Zhang *et al.* 1992*b*). The precise degree of purification could not be determined because the starting cytosol contained activator activity which masked the inhibitor activity, but a 118-fold purification was achieved from the anion exchange column fractionation step. The isolated inhibitor was a heat-labile protein that appeared on SDS gels as a $63 \times 10^3 M_r$ polypeptide (Zhang *et al.* 1992*b*). The active fraction had an approximate relative molecular mass of 12×10^3 measured by gel filtration, suggesting that the active form of the inhibitor was a dimer. Inhibition was concentration-dependent and saturable, with a Hill coefficient of 1.46, consistent with a requirement for dimerization. The inhibitor required a 10 min preincubation with the V-ATPase to exert its maximal effect, and inhibition was not reversible by any washing procedures tested. Inhibition was not affected by a series of protease inhibitors, and was not reduced by addition of an excess of other proteins. The inhibitor produced a 50–60% reduction in the ATPase activity of the immunoaffinity-purified kidney microsomal V-ATPase at pH 6.3 and a 90% reduction in ATP-dependent proton transport in bovine kidney microsomal vesicles. The percentage of ATPase activity inhibited by the inhibitor increased from 50% at pH 7.0 to 70% at pH 8.1. Thus, under conditions representing a cytosolic pH in the high physiological range, the inhibitor was more efficient in suppressing V-ATPase activity. The inhibitor was highly specific for the V-ATPases with little or no effect on Na^+/K^+-ATPase or Ca^{2+}-ATPase, but inhibited the mitochondrial F_1F_0 H^+-ATPase by 0–12%. The isolated inhibitor had equal effects on the isolated microsomal, brush-border and lysosomal V-ATPases from kidney and, therefore, has a potential role in controlling H^+ transport in several cellular compartments (Zhang *et al.* 1992*b*).

V-ATPase activator was partially purified from bovine kidney cytosol by a sequential combination of ultracentrifugation, ammonium sulfate precipitation, acid precipitation, QAE anion exchange chromatography and aminohexylagarose chromatography, yielding a 75-fold purification with 27% recovery of activity (Zhang *et al.* 1992*a*). The activator was heat-stable, retaining 77% of its activity when heated to 75°C for 10 min, but retaining only 4% of its activity when boiled for 10 min; it was also sensitive to trypsin. The activator stimulated the immunoaffinity-purified V-ATPase, exerting a direct effect on the enzyme. Activation was concentration-dependent and saturable, with a Hill coefficient of 1.0 (Zhang *et al.* 1992*a*). 70–80% of the activating effect occurred within

1 min, and the time course for activation was not affected by the concentration of activator. The activator, therefore, appears to function by a non-enzymatic mechanism, probably by binding to the V-ATPase. Activation was reversible when the beads were washed, but the binding affinity of the activator was pH-dependent. At pH values below 7.5, the activator washed off the beads with a slower time course than at pH values of 7.5 or above. This property may be physiologically important, as a drop in cytosolic pH would promote binding of the activator to the V-ATPase and stimulate proton extrusion from the cell. The activator was highly specific for V-ATPases with no effect on the Na^+/K^+-ATPase or Ca^{2+}-ATPase and with only a 5–10% stimulation of the mitochondrial F_1F_0 H^+-ATPase. Unlike the inhibitor, the activator had a much greater effect on the isolated brush-border and microsomal V-ATPases than it did on lysosomal V-ATPase. Consequently, it may have a selective function in controlling V-ATPases residing on the plasma membrane involved in transcellular proton transport (Zhang *et al.* 1992*a*).

Changes in the distribution of V-ATPase within the intercalated cell also participate in the physiological regulation of hydrogen ion secretion. This property was studied using a model of chronic acid administration in rats (Bastani *et al.* 1991). Rats given ammonium chloride in their drinking water developed an acidified blood pH (metabolic acidosis) within a day, and their urine pH dropped from 6.5 to 5.8. Over a period of 2 weeks of chronic acid administration, the kidney showed physiological adaptive changes, with hydrogen ion secretion increasing until the animals were restored back to their normal acid–base status. The role of the renal V-ATPase in this adaptational response was examined. No changes in the quantity of V-ATPase protein or in steady-state levels of mRNA for the enzyme were found at any of the five different time points studied over the 2 week period of acid administration. Anti-V-ATPase immunocytochemistry was then used in the kidney to examine whether a change in the distribution of the enzyme occurred with acid loading. The percentage of intercalated cells with plasma membrane staining was counted as a means for measuring changes in the distribution of the V-ATPase. In control rats or rats subjected to chronic administration of bicarbonate, V-ATPase in intercalated cells of the collecting tubule from kidney medulla was distributed predominantly in intercellular vesicles in the cytoplasm. In contrast, in the rats subjected to chronic acid administration over 2 weeks, V-ATPase in the majority of medullary intercalated cells was detected mostly in the plasma membrane. There was a time-dependent increase in recruitment of V-ATPase from the cytoplasm to the plasma membrane over the 2 week period (Bastani *et al.* 1991). Changes in intercalated cells in the cortical collecting duct were more complex because of the multitude of different morphological types of V-ATPase staining. Nevertheless, an increase in polarization to the apical membrane was also observed in the cortical intercalated cells. These results suggest that the renal adaptational response to increased acid intake does not entail a change in the overall quantity of the V-ATPase, but rather a redistribution of vesicles with V-ATPase from an intercellular pool to the plasma membrane. Similar changes occur in other models for renal adaptation, such as loss of functioning renal mass (B. Bastani and S. Gluck, unpublished results). These results do not exclude a role for changes in the kinetic properties of the renal V-ATPase; the potential involvement of the activator and inhibitor in the adaptational response is currently under investigation.

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