Basolateral carbonic anhydrase IV in the proximal tubule is a glycosylphosphatidylinositol-anchored protein

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Carbonic anhydrase (CA) IV facilitates HCO_3 reabsorption in the renal proximal tubule by catalyzing the reversible hydration of CO_2. CAIV is tethered to cell membranes via a glycosylphosphatidylinositol (GPI) lipid anchor. As there is basolateral as well as apical CAIV staining in proximal tubule, the molecular identity of basolateral CAIV was examined. Biotinylation of confluent monolayers of rat inner medullary collecting duct cells stably transfected with rabbit CAIV showed apical and basolateral CAIV, and in the cell transfectants expressing high levels of CAIV, a transmembrane form was targeted to the basolateral membrane. Basolateral expression of CAIV (~46 kDa) was confirmed in normal kidney tissue by Western blotting of vesicle fractions enriched for basolateral membranes by Percoll density fractionation. We examined the mode of membrane linkage of basolaterally expressed CAIV in the kidney cortex. CAIV detected in basolateral or apical membrane vesicles exhibited similar molecular size by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis following deglycosylation, and was equally sensitive to phosphatidylinositol-specific phospholipase C digestion, indicating that CAIV is expressed on the basolateral membrane as a GPI-anchored protein. Half of the hydratase activity of basolateral vesicles was resistant to SDS denaturation, compatible with being CAIV. Thus, GPI-anchored CAIV resides in the basolateral membrane of proximal tubule epithelia where it may facilitate HCO_3 reabsorption via association with kNBC1.

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Carbonic anhydrases (CAs) facilitate renal acidification in the kidney by catalyzing the hydration of CO_2 or the reverse reaction, the dehydration of bicarbonate.[1] The majority of the CA activity in the kidney (~95%) is comprised of cytosolic CAII.[1] CAIV is one of three membrane-associated CA isoforms,[2] the others being CAXII[3–7] and in rodent species, CAXIV,[8,9] that account for the remaining CA activity in the kidney. In humans, rabbits, and rodent species CAIV is expressed in the proximal tubule (S2 > S1 ≫ S3) and in distal nephron segments including outer and inner medullary collecting ducts (IMCDs) and α-intercalated cells of the cortical collecting duct.[1] A major role for CAIV in urinary acidification has been demonstrated in studies where inhibition of luminal CA activity eliminates nearly all net bicarbonate reabsorption in the proximal tubule[10] and outer medullary collecting duct from the inner stripe,[11] and in CAII-deficient patients and mice where inhibition of CA activity diminishes renal acid secretion.[12,13]

CAIV is unique among CA isoforms, because it is tethered to the plasma membrane via a glycosylphosphatidylinositol lipid anchor (GPI-anchor).[14,15] Most GPI-anchored proteins are targeted to the apical membrane of polarized epithelial cells;[16] nevertheless, CAIV immunoreactivity detected with different CAIV antibodies has been observed on the basolateral membranes of proximal tubule segments in human, rabbit and rodent species.[2,17,18] Studies from our laboratory have functionally demonstrated in the proximal tubule the existence of a basolateral CA activity that facilitates fluid and bicarbonate absorption.[19] These studies suggested that either a transmembrane form is expressed basolaterally or perhaps that GPI-anchored CAIV is additionally targeted to the basolateral membrane. However, with the recent description of CAXII as a major CA isoform that is expressed on the basolateral membranes of acidifying nephron segments,[4] the molecular identity of the basolaterally expressed CA detected by anti-CAIV antibodies required investigation.

In this study, we characterized the polarity and the molecular form of CAIV immunoreactivity in rabbit kidney cortex and in transfected renal IMCD epithelial cells in culture. Whereas in IMCD cells overexpressing CAIV, a transmembrane form is preferentially expressed on the

basolateral membrane, we found that CAIV is normally expressed on both the apical and basolateral membranes of proximal tubule epithelial cells as a GPI-anchored protein.

RESULTS

Trafficking to the basolateral membrane is an intrinsic property of the CAIV molecule

CAIV is tethered to cell membranes via a GPI anchor, and typically GPI-anchored proteins are targeted to the apical membrane of polarized epithelial cells. However, as shown in Figure 1, immunohistochemical staining of kidney cortex with antibodies to CAIV consistently showed basolateral staining of proximal tubules in addition to apical labeling.

The following experiments were designed to determine whether trafficking to the basolateral membrane of renal epithelial cells is an intrinsic property of the CAIV molecule, as well as to ascertain the molecular form of CAIV that is expressed basolaterally. In initial studies the polarity of CAIV expression in an *in vitro* culture model of polarized renal epithelial cells was examined. IMCD cells were selected for these studies owing to their ability to form confluent monolayers with high transepithelial resistance. Confluent monolayers of IMCD cells stably transfected with a full-length CAIV exhibiting transepithelial resistance in the range of 900–1500 Ωcm^2 were subjected to apical and basolateral surface biotinylation. Biotinylated proteins were precipitated with streptavidin (SAv)-agarose and levels of CAIV and apically (GP135) or basolaterally (Na^+,K^+-ATPase) expressed proteins were determined by Western blotting. The results of three independent biotinylation experiments are shown in Figure 2a, where GP135 was precipitated predominantly from cells biotinylated on the apical surface and Na^+,K^+-ATPase was exclusively precipitated from cells biotinylated on the basolateral side, demonstrating the specificity of apical versus basolateral surface biotinylation. CAIV, on the other hand, was equally precipitated from cells biotinylated on either the apical or basolateral side. These results demonstrated that CAIV was sorted to both apical and basolateral membranes of polarized renal epithelial cells.

Because GPI-anchor attachment results in apical sorting of many proteins in polarized epithelial cells, we examined whether expression of a transmembrane form of CAIV explained basolateral expression of CAIV in transfected IMCD cells. CAIV was subcloned into the pcDNA 3.1 vector (see Figure 2b), so that CAIV expressed as a transmembrane protein would contain a 6XHis epitope-tag contiguous with

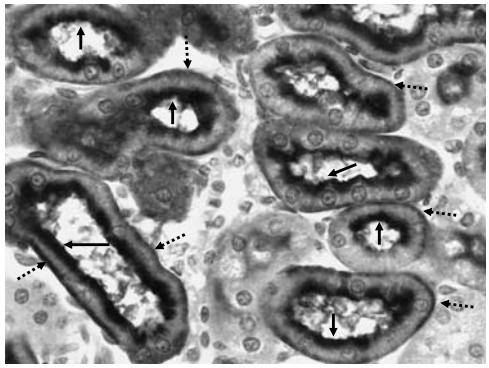

Figure 1 | CAIV immunoreactivity is observed on the apical and basolateral membrane of proximal tubules. Paraffin-embedded sections of rabbit kidney were stained with a goat anti-rabbit CAIV antibody (YDQR). The slide was developed with an HRP-conjugated anti-goat antibody utilizing the DAB substrate and counterstained with hematoxylin. Solid arrows indicate apical staining, whereas dashed arrows point to basolateral staining. Original magnification $\times 100$.

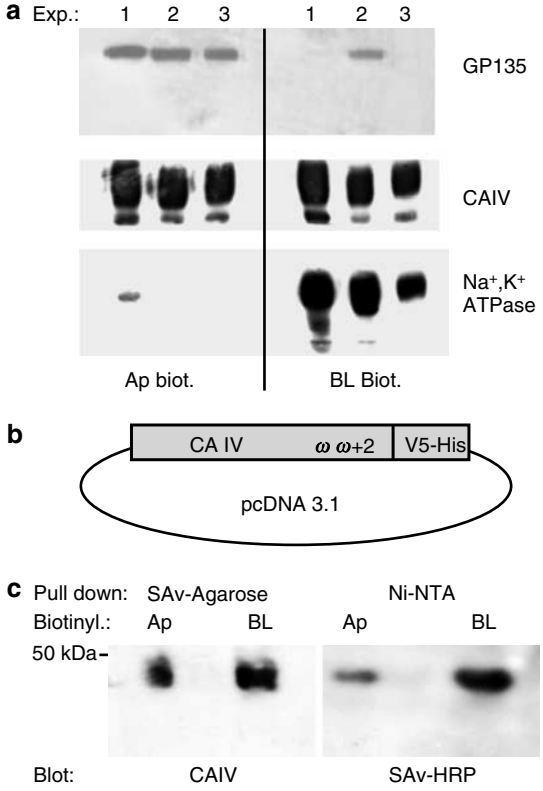

Figure 2 | CAIV is expressed on the apical and basolateral membranes of IMCD transfectants. (**a**) Confluent monolayers of IMCD cells stably transfected with rabbit CAIV were grown on Transwell® permeable supports and surface biotinylated on either the apical or basolateral side. Biotinylated proteins were precipitated from cell lysates with SAv-agarose. Levels of SAv-precipitated apical (GP135), basolateral (Na^+,K^+-ATPase) proteins, and CAIV were determined by Western blotting. Results of three independent surface biotinylation experiments are shown. (**b**) Rabbit CAIV was subcloned into the pcDNA 3.1 vector so that CAIV expressed a transmembrane protein containing the V5-6X His epitope tags. Omega (ω) and ($\omega + 2$) denote the serine residue at which GPI-anchor attachment occurs and the amino-acid residue that plays a key role in the efficiency of lipid anchor linkage, respectively. (**c**) Confluent monolayers of IMCD cells stably transfected with rabbit CAIV-pcDNA 3.1 construct were surface biotinylated on the apical or basolateral side. Biotinylated proteins were precipitated from cell lysates with SAv-agarose or V5-His-tagged proteins with Ni-NTA-agarose. Levels of transfected CAIV precipitated by Ni-NTA-agarose were determined by blotting with SAv-HRP, whereas GPI-linked CAIV precipitated with SAv-agarose was determined by blotting with anti-rabbit CAIV (KDNV). Results presented in (**c**) are representative of two independent experiments.