A 45-kDa protein antigenically related to band 3 is selectively expressed in kidney mitochondria

(anion exchange/renal acidification)

LYNDA S. OSTEDGAARD*, MICHAEL L. JENNINGS†, LAWRENCE P. KARNISKI*, AND VICTOR L. SCHUSTER*‡§

*Department of Medicine, University of Iowa, Iowa City, IA 52242; †Department of Physiology and Biophysics, University of Texas, Galveston, TX 77550; and ‡Department of Medicine, Albert Einstein College of Medicine, Bronx, NY 10461

ABSTRACT   Anion exchange similar to that catalyzed by erythrocyte band 3 occurs across many nonerythroid cell membranes. To identify anion-exchange proteins structurally related to band 3, we immunoblotted rabbit kidney medullary membrane fractions with anti-band 3 antibodies. Immunoblots using antibodies to the cytoplasmic domain of band 3 revealed cross-reactive proteins in the plasma membrane fraction only. In contrast, two monoclonal antibodies against band 3 membrane domain labeled a 45-kDa protein; further immunoblotting and immunogold studies of membrane fractions and kidney sections using one of the anti-membrane domain antibodies showed that labeling was strongest in mitochondria of H^+-secreting collecting duct cells. Tissue-to-tissue expression of the 45-kDa mitochondrial protein was variable: kidney medulla > heart > kidney cortex >> liver. We conclude that a 45-kDa protein with immunological cross-reactivity to the erythrocyte band 3 membrane domain is expressed in mitochondria in a highly cell-specific fashion and speculate that the protein may play a role in mitochondrial anion transport.

Erythrocyte band 3 catalyzes the electroneutral exchange of HCO_3 for Cl (1). The primary sequence of band 3 (2–7) correlates well with the two functional domains: the cytoplasmic domain anchors band 3 to cytoskeletal proteins, and the membrane domain catalyzes anion transport (1).

Many nonerythroid cells also exhibit anion exchange (8). In an attempt to characterize these nonerythroid anion exchangers, several laboratories have performed ultrastructural immunolabeling with domain-specific anti-band 3 antibodies. Antibodies to the cytoplasmic domain were reported to label basolateral membranes of H^+-secreting renal collecting duct cells—i.e., "α" or "a" intercalated cells (9, 10). In contrast, antibodies to a synthetic peptide representing the C-terminal 12 amino acids of murine erythrocyte band 3 membrane domain have been reported to label the Golgi membranes of a variety of cells (11).

We have used antibodies directed at the cytoplasmic and membrane domains of human and rabbit erythrocyte band 3 to probe fractionated kidney membranes. We find that two monoclonal antibodies, directed against human and rabbit band 3 membrane domains, detect a 45-kDa protein that is expressed preferentially in the mitochondria of H^+-secreting cells of medullary collecting duct.

METHODS

Antibody Probes. A polyclonal antibody to the 42-kDa cytoplasmic domain of human erythrocyte band 3, originally raised by P. Low (12), was kindly provided by R. Walder (Univ. of Iowa). A monoclonal antibody (IVF12) against human band 3 membrane domain had been previously gen-

erated and characterized (13). A monoclonal antibody (IIH7) to rabbit membrane domain was made by identical methods (13) except that the rabbit protein was not glutaraldehyde cross-linked.

Membrane Isolation. Erythrocyte ghosts were prepared without protease inhibitors (14). Kidneys from New Zealand White rabbits were perfused with cold phosphate-buffered saline (PBS) plus 0.1 mM phenylmethylsulfonyl fluoride (PMSF) until free of erythrocytes. Outer medulla was homogenized in homogenization buffer (HB) (250 mM sucrose/1 mM EDTA/5 mM Tris·HCl/0.5 mM benzamidine hydrochloride/0.1 mM 1,10-phenanthroline/0.5 μg of leupeptin per ml/0.7 μg of pepstatin A per ml/0.1 mM PMSF, pH 7.4). Membranes were isolated at 4°C by a modification of the Scalera method (15). Homogenate (H) was centrifuged twice at 2500 × g for 20 min. The combined supernatants (S1) were centrifuged at 20,500 × g for 20 min, resulting in a second supernatant (S2) and pellet (P2). P2 consisted of two layers, a fluffy "top" pellet (P2T) and a brown "bottom" pellet (P2B). P2B was resuspended in HB and centrifuged at 20,500 × g for 20 min. The top of the resulting pellet was combined with the original P2T, homogenized in HB, and centrifuged on an 18% Percoll gradient at 30,000 × g for 10 min. The Percoll gradient yielded fractions T1–T3, which were diluted 1:10 in HB and centrifuged twice at 100,000 × g for 1 hr at 4°C to remove Percoll. S2 was further centrifuged at either 30,000 × g or 100,000 × g for 1 hr to obtain a third (microsomal) pellet (P3). For transmission electron microscopic examination, membrane fractions were fixed in 2.5% glutaraldehyde, treated with osmium, and embedded in Spurrs (Fullam, Latham, NY).

Enzyme Markers. Protein was determined by a modified Lowry method (16). Other enzyme activities determined were galactosyltransferase (17), alkaline phosphatase and acid phosphatase (18), succinic dehydrogenase (19, 20), cytochrome c oxidase (21), and Na,K-ATPase (22).

SDS/PAGE, Immunoblotting, and Ultrastructural Immunocytochemistry. Membrane fractions were separated on SDS/6–18% polyacrylamide gel (23), transferred to nitrocellulose, and immunoblotted with anti-band 3 antibodies (24). For qualitative immunoblotting, the secondary antibody was a peroxidase-linked IgG visualized with 4-chloro-1-naphthol. For quantitation, the secondary antibody was ^{125}I-coupled anti-mouse IgG; autoradiographic band density was determined densitometrically. Membrane fractions and blocks of kidney medulla were fixed in periodate/lysine/paraformaldehyde (25) and embedded in L. R. White resin (26). Sections (100 nm) were blocked with normal goat serum in PBS with 0.8% bovine serum albumin and 0.1% gelatin and were incubated sequentially with primary and secondary antibody.

Table 1.   Enrichments of enzyme activities and binding of monoclonal antibody IVF12

Sample	Acid P	Alk P	Na,K-ATPase	Gal T	SDH	Cyt *c* Ox	IVF12
H	1.00	1.00	1.00	1.00	1.00	1.00	1.00
P2T	2.08	4.73	2.25	3.77	1.03	0.89	1.90
	±0.20	±1.54	±0.26	±0.37	±0.21	±0.20	±0.35
T1	2.15	1.20	3.25	4.83	0.69	0.52	0.57
	±0.22	±0.21	±0.31	±0.13	±0.19	±0.13	±0.38
T3	1.50	0.78	0.42	0.50	1.32	2.52	10.85
	±0.37	±0.28	±0.10	±0.10	±0.15	±0.43	±2.25
P2B	2.80	1.53	0.60	0.70	2.15	1.63	8.17
	±0.56	±0.29	±0.17	±0.13	±0.39	±0.23	±2.64
P3	2.15	2.95	3.40	5.50	0.14	0.30	0.00
	±0.05	±0.15	±0.10	±1.30	±0.11	±0.10	±0.00
	n = 4	*n* = 4	*n* = 4	*n* = 3	*n* = 6	*n* = 5	*n* = 3

Acid P, acid phosphatase; Alk P, alkaline phosphatase; Gal T, galactosyltransferase; SDH, succinic dehydrogenase; Cyt *c* Ox, cytochrome *c* oxidase; IVF12, binding of monoclonal antibody IVF12. Values for homogenate (H) are set at 1; values of other fractions are expressed relative to H (±SEM). Assays were performed in duplicate using equal amounts of protein for each fraction.

Other Tissues. Rabbit heart was perfused with PBS/PMSF and membrane fractions were isolated as described above. Mitochondria from perfused rabbit liver were isolated by the method of Samuelson *et al.* (27). Rabbit kidney cortex was prepared according to Karniski and Aronson (28). Mouse kidneys were perfused with PBS/PMSF via the aorta, and membranes were prepared as for rabbit medulla except that whole mouse kidneys were used and the Percoll gradients were omitted.

RESULTS

Kidney Medulla Membrane Fractionation. We first confirmed that the fractionation method separated rabbit kidney medullary membranes. Enrichment of enzymatic markers (Table 1) is expressed as the ratio of the activity in a given fraction relative to that in the homogenate. The upper pellet of the 20,500 × *g* centrifugation (P2T), the top band from the Percoll separation (T1), and the high-speed microsomal pellet (P3) were enriched in plasma membrane markers (alkaline phosphatase and Na,K-ATPase) and Golgi (galactosyltransferase) but contained diminished activity of the mitochondrial markers (succinic dehydrogenase and cytochrome *c* oxidase). In contrast, the bottom pellet of the 20,500 × *g* centrifugation (P2B) and the bottom band from the Percoll gradient (T3) were both enriched in the mitochondrial markers and depleted of the plasma membrane and Golgi markers. We also examined the fractions by transmission electron microscopy (Fig. 1). Fraction T1 consisted of vesicles of heterogeneous size bounded by a single membrane (Fig. 1*A*).

Electron micrographs of fraction P2T were similar (data not shown). In contrast, fractions P2B and the Percoll band T3 contained almost exclusively mitochondria (Fig. 1 *B* and *C*, respectively).

Specificity of Anti-Band 3 Antibodies. Fig. 2 shows the results of immunoblotting rabbit erythrocyte ghosts with a monoclonal antibody to human band 3 membrane domain, antibody IVF12, and with a polyclonal antibody to human band 3 cytoplasmic domain, antibody anti-hCD. IVF12 recognized intact rabbit erythrocyte band 3 at 95 kDa and the membrane domain at 52 kDa (lane 1). Lane 2 shows the reaction of anti-hCD with intact rabbit band 3 at 95 kDa and with a cluster of cytoplasmic domain fragments at 42 kDa. Thus, IVF12 and anti-hCD recognize the rabbit equivalents of the human band 3 membrane and cytoplasmic domains, respectively.

Immunoblots of Kidney Medulla Membrane Fractions. We used these domain-specific antibodies to probe the kidney membrane fractions. Fig. 3*A* shows immunoblots in which the same membrane fractions were probed with different antibodies. Antibody to the cytoplasmic domain revealed bands at 45 and 75–80 kDa in the microsomal pellet P3 (lane 1). These bands were not seen when preimmune serum was used instead of anti-hCD (data not shown). No bands were seen with anti-hCD in the mitochondrial fraction P2B (lane 2).

In contrast, as shown in Fig. 3*B*, the monoclonal antibody IVF12 to human band 3 membrane domain detected a single immunoreactive band at 45 kDa in the mitochondrial membrane fractions (strongest immunoreactivity in pellet P2B; lane 4). Preimmune serum gave no 45-kDa band in P2B (data
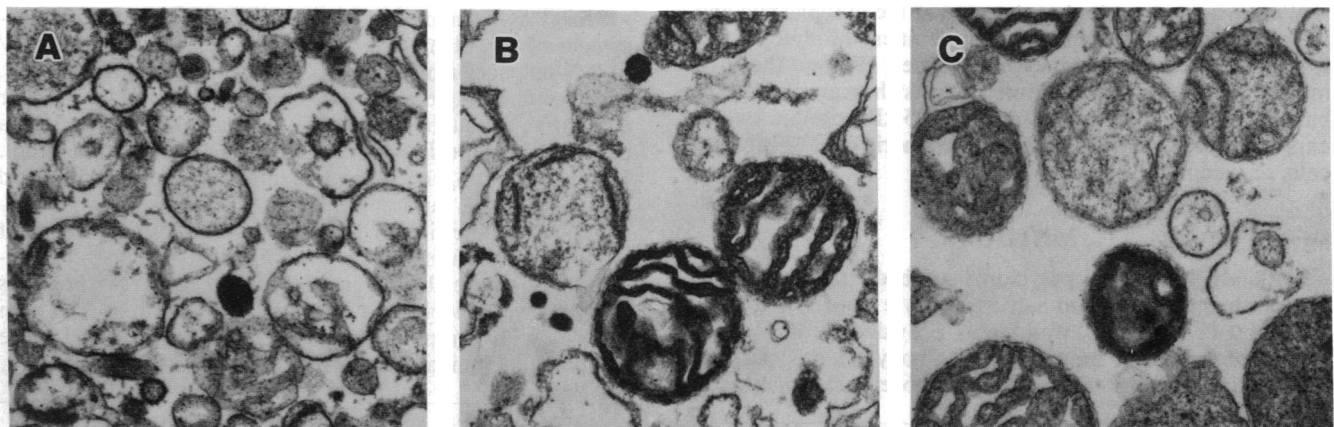

FIG. 1.   Transmission electron micrographs of membrane fractions from rabbit kidney medulla. (*A*) Fraction enriched in plasma membrane markers (T1). (*B* and *C*) Fractions enriched in mitochondrial membrane markers (P2B in *B* and T3 in *C*). (*A*, ×24,800; *B*, ×20,800; *C*, ×19,200.)
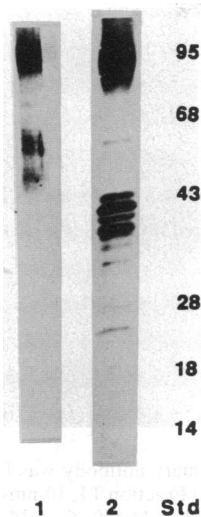
FIG. 2. Immunoblots of rabbit erythrocyte ghosts (25–50 μg of protein per lane). Lanes: 1, primary antibody is IVF12 (1:5000); 2, primary antibody is anti-hCD (1:500). Numbers on right are kDa.

not shown). Weaker immunoreactivity with IVF12 was also evident in P2T (lane 1). After separating P2T on Percoll, fraction T1, which was enriched in plasma membrane markers, had minimal reactivity (lane 2). In contrast, T3, enriched in mitochondria, showed substantial immunoreactivity (lane 3). The high-speed microsomal pellet P3 did not react with the IVF12 antibody (lane 5). Densitometric quantitation of binding of IVF12 to the various membrane fractions is given in Table 1.

It is unlikely that this 45-kDa band results from proteolysis of the 95-kDa erythrocyte band 3 protein. Removal of erythrocytes by perfusion was adequate based on light-level immunocytochemistry. Also, varying the perfusion caused the amount of 95-kDa protein attributable to contaminating erythrocytes, but not the amount of the 45-kDa protein, to change accordingly (data not shown).

We also immunoblotted rabbit erythrocyte ghosts and kidney membranes with a second monoclonal antibody, IIH7, raised to rabbit erythrocyte band 3 membrane domain. IIH7 reacted only weakly at 95 kDa with human erythrocyte band 3 (data not shown). However, IIH7 strongly labeled a 45-kDa band in rabbit kidney membrane fraction P2T (Fig. 4, lane 1). IIH7 also strongly labeled a band at ≈52 kDa and weakly labeled a 95-kDa band in rabbit erythrocyte ghosts

(lane 2). Thus, two separate monoclonal antibodies, raised to the membrane domains of rabbit and human band 3, identify a 45-kDa protein in rabbit kidney medullary membranes.

Immunocytochemistry. We localized binding of antibody IVF12 in fractionated kidney medulla membranes and tissue sections by using ultrastructural immunocytochemistry (similar studies with monoclonal antibody IIH7 were unsuccessful). In the two fractions enriched in mitochondria (P2B and T3), labeling was present over mitochondria (Fig. 5 *A* and *B*). We saw no labeling upon omitting primary antibody or with preimmune serum instead of IVF12 (data not shown). The minimal labeling observed in plasma membrane fraction T1 was confined to the occasional contaminating mitochondrion (Fig. 5*C*, arrow).

Light-level microscopy immunocytochemistry of kidney medulla using IVF12 gave a broad, basal pattern of staining in a subpopulation of collecting duct cells (Fig. 6*A*). Other studies from our laboratory have shown that these cells are α intercalated cells (29). Fig. 6 *B* and *C* shows kidney sections labeled with IVF12 and immunogold. The gold particles were distributed primarily over mitochondria (Fig. 6*C*); however, low-density labeling of the basal plasma membrane was occasionally noted (Fig. 6*B*). Fig. 6*D* shows that the mitochondrial distribution is basal in these cells, offering a likely explanation for the broad basal pattern of staining seen at the light microscope level.

We quantitated the total number of gold particles, the total number of mitochondria, and the number of gold particles associated with mitochondria. For six mitochondria-rich (α intercalated) cells incubated with IVF12, there was a positive correlation between the number of mitochondria and the number of gold particles ($r = 0.72$; $P < 0.01$). We found no such correlation for intercalated cells incubated with preimmune mouse serum instead of IVF12 or for nonintercalated cells incubated with IVF12.

The relatively low density of mitochondrial labeling (≈1 gold particle per mitochondrion) appears to result from low antigen abundance. We compared the labeling density of human erythrocytes (26) to that of rabbit α-cell mitochondria by using the same immunogold methods and antibody IVF12. Human erythrocytes had a labeling density of ≈1.7 gold particles per μm of erythrocyte membrane length. For rabbit mitochondrial membranes, the lower limit estimate of antigenic density was ≈1.7 × 10^{-3} gold particles per μm of membrane length (cristae membrane included in the calculation).

Other Mitochondria-Rich Tissues. We examined whether the 45-kDa protein is also expressed in the mitochondria of other tissues that are relatively rich in mitochondria. Although we saw a faint band in the mitochondrial fraction from kidney cortex, it was substantially less dense than that found in the medullary fraction (data not shown). As kidney cortical mitochondria are largely of proximal tubule rather than collecting duct origin (30), this result suggests selective expression of this protein in only a subset of kidney mito-
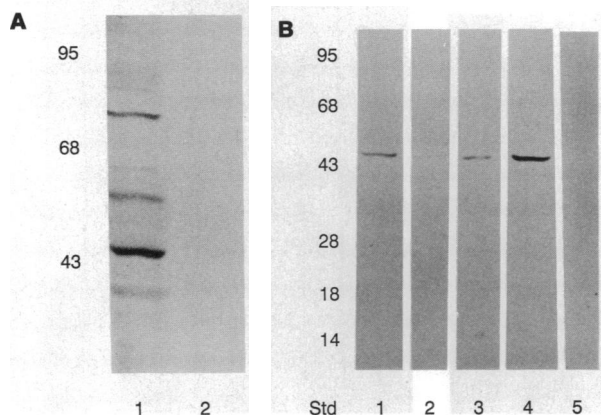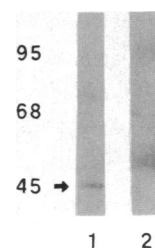

FIG. 3. Immunoblots of rabbit kidney membrane fractions (100 μg of protein per lane) using anti-band 3 antibodies. (*A*) Membranes were blotted with anti-hCD. Lanes: 1, fraction P3; 2, fraction P2B. (*B*) Membranes were blotted with IVF12. Lanes: 1, P2T; 2, T1; 3, T3; 4, P2B; 5, P3. Numbers on left are kDa.

FIG. 4. Immunoblots using monoclonal antibody IIH7 against rabbit erythrocyte membrane domain. Lanes: 1, rabbit kidney membrane fraction P2T (100 μg); 2, rabbit erythrocyte ghosts (50 μg). Numbers on left are kDa.
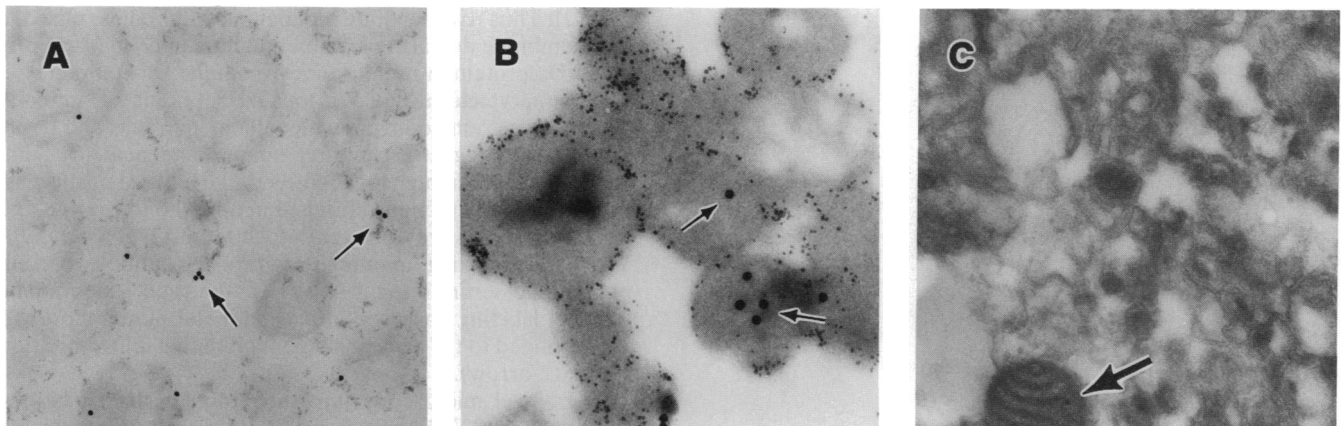
FIG. 5.   Ultrastructural immunocytochemical labeling of isolated membrane fractions. The primary antibody was IVF12. (*A*) Fraction T3, 10-nm-diameter gold particles (arrows). (*B*) Fraction T3, 30-nm-diameter gold particles (arrows). (*C*) Fraction T1, 10-nm-diameter gold particles. In *C*, note the single gold particle over the one mitochondrion in the field (arrow). (*A*, ×18,400; *B*, ×34,560; *C*, ×38,000.)

chondria. Fig. 7*A* shows an immunoblot of crude mitochondrial fractions from rabbit liver and heart. Whereas liver mitochondria were devoid of immunoreactivity (Fig. 7*A*, lane 1), heart mitochondria showed an immunoreactive band at 45 kDa (lane 2).

Finally, we examined a species other than rabbit. When blotted against IVF12, mouse erythrocytes had the expected 95- to 100-kDa band (data not shown). The kidney plasma membrane (P2T) fraction from mouse kidneys perfused free of erythrocytes showed a faint reaction similar to that of rabbit medullary plasma membranes prior to Percoll separation (Fig. 7*B*, lane 1). The mitochondrial fraction had the same strongly reactive 43- to 45-kDa band previously seen in the rabbit (lane 2).

DISCUSSION

In the present study, we have identified a 45-kDa protein in rabbit kidney medullary mitochondria that is immunologically similar to the membrane domain of human and rabbit erythrocyte band 3. The finding of a band 3-like protein in mitochondria is important because it suggests the presence of a previously unrecognized class of mitochondrial anion transporters, and because it expands the family of known band 3-like proteins.

In the course of these studies, we adapted for the rabbit kidney medulla a method of cell membrane fractionation originally developed for the rat kidney cortex (15). By characterizing the membrane fractions both ultrastructurally and with enzymatic markers, we documented that the method adequately separates plasma membranes from mitochondrial membranes. Data from the rat have shown that the basolateral membranes of intercalated cells have no enrichment for Na,K-ATPase (31), raising the possibility that this enzyme is an inadequate marker for these membranes. However, in the rabbit outer medullary collecting duct, especially in the inner stripe, Na,K-ATPase is equally distributed across all cell types (32). Thus, we are probably correct in concluding that the basolateral membranes of α-intercalated cells comigrated with the Na,K-ATPase activity in our isolation procedure. Also, to the extent that binding of antibodies to the band 3 cytoplasmic domain is a marker for α-intercalated cell basolateral membranes (9, 10), we can conclude from our immunoblotting data that the P3 fraction isolated here is enriched in α-cell basolateral membranes (Fig. 3*A*).

Immunocytochemically, several laboratories have reported band 3 immunoreactivity at the basal region of medullary collecting duct intercalated cells in rat, rabbit, and human kidneys (9, 10, 29, 33–35). In two of these studies, localization at the ultrastructural level, using antibodies directed at the cytoplasmic domain of band 3, revealed
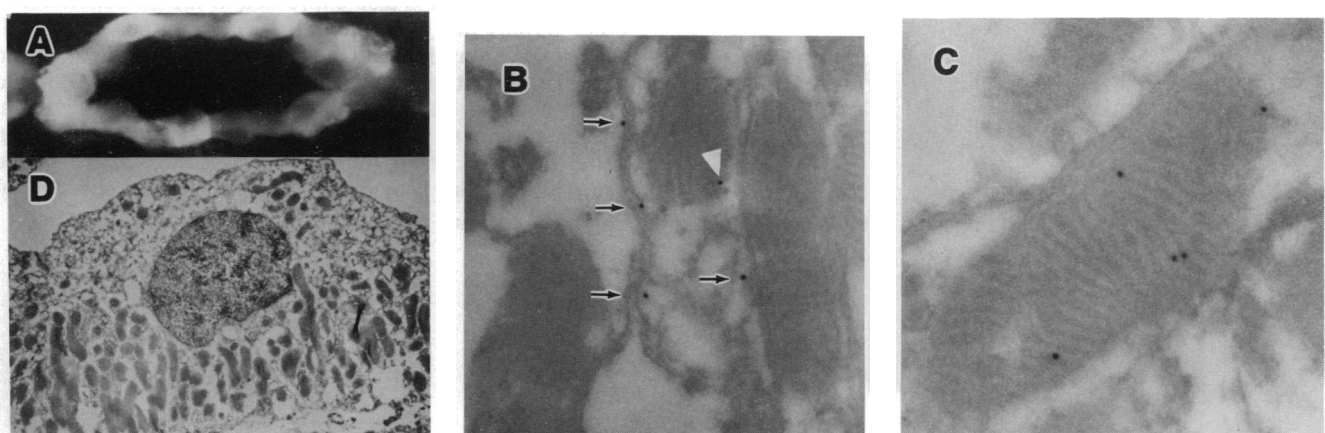

FIG. 6.   (*A*) Light microscope level immunocytochemical labeling of rabbit medulla with antibody IVF12. The α-intercalated cells show a broad, basal staining pattern (10-μm section). (*B*) Ultrastructural immunocytochemical labeling as in *A*. Two infoldings of the basolateral membrane demonstrate gold labeling (arrows). One mitochondrion is also labeled (arrowhead). (*C*) Mitochondrial labeling with monoclonal antibody IVF12. (*D*) A medullary collecting duct cell with basally distributed mitochondria (compare mitochondrial distribution to the light microscope staining pattern in *A*). (*A*, ×1000; *B*, ×30,240; *C*, ×60,000; *D*, ×4800.)

Cell Biology: Ostedgaard *et al.*

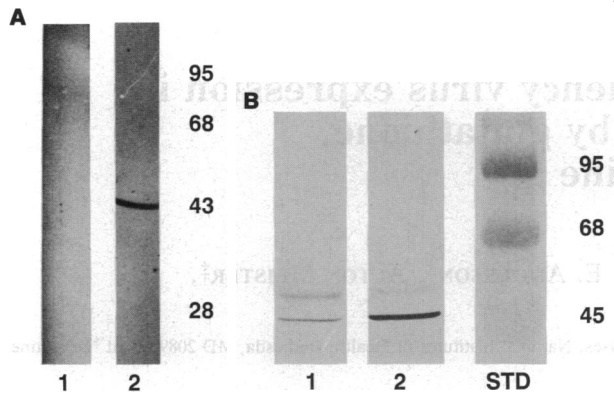*Proc. Natl. Acad. Sci. USA 88 (1991)*   985

FIG. 7. Immunoblots of membranes with monoclonal antibody IVF12. (*A*) Rabbit mitochondrial membranes. Lanes: 1, liver; 2, heart. (*B*) Murine membranes. Lanes: 1, kidney plasma membrane fraction P2T; 2, kidney mitochondrial fraction P2B; 3, size standards (kDa).

labeling restricted to the basolateral plasma membrane (9, 10). In the present study, two monoclonal antibodies against erythrocyte band 3 membrane domain of either human or rabbit recognized a 45-kDa protein in rabbit kidney membranes. Extensive study with one of these antibodies showed that it labeled mitochondrial fractions on immunoblots and immunocytochemically labeled mitochondria in isolated fractions as well as in sections of medullary tissues. Although we occasionally observed labeling of basolateral plasma membranes in tissue sections (Fig. 6*B*), results that are consistent with the known presence of a band 3-like protein in the basolateral membrane of α-intercalated cells (9, 10), the predominant finding of the present studies is that the epitope recognized by antibody IVF12 is most abundant in α-cell mitochondria. In support of these data, we have also found that this antibody binds to a 45-kDa protein in the mitochondria, but not the plasma membranes, of human oncocytomas (36), tumors that are extremely rich in mitochondria and are thought to arise from α-intercalated cells (37).

At 45 kDa, the present mitochondrial protein differs in size from intact erythrocyte band 3 (95 kDa), from the "kidney band 3" protein encoded by the most-abundant band 3-like mRNA in rat and mouse kidney (80–95 kDa) (38, 39), and from other reported proteins that are immunologically similar to band 3 (11, 33, 34, 40, 41). The 45-kDa protein is unlikely to be a proteolytic cleavage product of either erythrocyte band 3 or the larger kidney band 3. Monoclonal antibody IVF12 does not recognize the 43- to 45-kDa cytoplasmic domain of either rabbit or human erythrocyte ghosts, and the present 45-kDa protein is consistently detected in the mitochondrial fraction regardless of the degree of removal of contaminating erythrocytes. Although our anti-hCD antibody recognized an ≈80-kDa protein in the plasma membrane fraction (Fig. 3*A*, lane 1), proteolysis of such a protein seems unlikely to produce a 45-kDa peptide that fractionates with the mitochondria.

The function of the mitochondrial band 3-like protein is unknown. Antigenic cross-reactivity between band 3 and the present protein does not necessarily imply a functional relationship. However, anion exchangers as well as a conductive anion pathway have been described in liver, heart, and kidney mitochondria (42–44). None of the anion transporters purified or cloned to date is 45 kDa, making it likely that the present protein represents a previously unreported entity. A characteristic feature of known mitochondrial anion transporters is the highly tissue-specific manner in which they are expressed. Similarly, the present mitochondrial protein is expressed only in certain mitochondria (kidney medulla >

heart > kidney cortex >> liver). Future studies will need to directly determine the function and substrate specificity of the 45-kDa protein identified here.

1. Jennings, M. L. (1989) *Annu. Rev. Biophys. Biophysical Chem.* **18**, 397–430.
2. Kopito, R. R. & Lodish, H. F. (1985) *Nature (London)* **316**, 234–238.
3. Demuth, D. R., Showe, L. C., Ballantine, M., Palumbo, A., Fraser, P. J., Cioe, L., Rovera, G. & Curtis, P. J. (1986) *EMBO J.* **5**, 1205–1214.
4. Cox, J. V. & Lazarides, E. (1988) *Mol. Cell. Biol.* **8**, 1327–1335.
5. Kim, H.-R. C., Yew, N. S., Ansorge, W., Voss, H., Schwanger, C., Vennstrom, B., Zenke, M. & Engel, J. D. (1988) *Mol. Cell. Biol.* **8**, 4416–4424.
6. Tanner, M. J. A., Martin, P. G. & High, S. (1988) *Biochem. J.* **256**, 703–712.
7. Lux, S. E., John, K. M., Kopito, R. R. & Lodish, H. F. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9089–9093.
8. Lowe, A. G. & Lambert, A. (1983) *Biochim. Biophys. Acta* **694**, 353–374.
9. Verlander, J. W., Madsen, K. M., Low, P. S., Allen, D. P. & Tisher, C. C. (1988) *Am. J. Physiol.* **255**, F115–F125.
10. Drenckhahn, D. & Merte, C. (1987) *Eur. J. Cell Biol.* **45**, 107–115.
11. Kellokumpu, S., Neff, L., Jämsä-Kellokumpu, S., Kopito, R. & Baron, R. (1988) *Science* **242**, 1308–1311.
12. Low, P. S., Westfall, M. A., Allen, D. P. & Appell, K. C. (1984) *J. Biol. Chem.* **259**, 13070–13076.
13. Jennings, M. L., Anderson, M. P. & Monoghan, R. (1986) *J. Biol. Chem.* **261**, 9002–9010.
14. Dodge, J. T., Mitchell, C. & Hanahan, D. J. (1963) *Arch. Biochem. Biophys.* **100**, 119–130.
15. Scalera, V., Huang, Y.-K. & Hildman, B. (1981) *Membr. Biochem.* **4**, 49–61.
16. Peterson, G. S. (1977) *Anal. Biochem.* **83**, 346–356.
17. Bergeron, J. J. M., Ehrenreich, P., Siekevitz, P. & Palade, G. E. (1973) *J. Cell Biol.* **9**, 73–88.
18. Engstrom, L. (1961) *Biochim. Biophys. Acta* **52**, 36–48.
19. Butcher, R. G. (1970) *Exp. Cell Res.* **60**, 54–58.
20. Pennington, R. J. (1961) *Biochem. J.* **80**, 649–654.
21. Wharton, D. C. & Tzagaloff, A. (1965) *Biochim. Biophys. Acta* **96**, 245–250.
22. Forbush, B., III (1983) *Anal. Biochem.* **128**, 159–163.
23. Laemmli, U. K. (1974) *Nature (London)* **227**, 680–685.
24. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
25. McLean, I. W. & Nakane, P. F. (1974) *J. Histochem. Cytochem.* **22**, 1077–1083.
26. Timms, B. G. (1986) *Am. J. Anat.* **175**, 267–275.
27. Samuelson, A. C., Stockert, R. J., Novikoff, A. B., Novikoff, P. M., Saez, J. C., Spray, D. C. & Wolkoff, A. W. (1988) *Am. J. Physiol.* **254**, 829–838.
28. Karniski, L. P. & Aronson, P. S. (1987) *Am. J. Physiol.* **253**, F513–F521.
29. Schuster, V. L., Bonsib, S. M. & Jennings, M. L. (1986) *Am. J. Physiol.* **251**, C347–C355.
30. Simpson, D. P. (1988) *Am. J. Physiol.* **254**, F863–F870.
31. Kashgarian, M., Biemesderfer, D., Caplan, M. & Forbush, B, III (1985) *Kidney Int.* **28**, 899–913.
32. Ridderstrale, Y., Kashgarian, M., Koeppen, B. M., Giebisch, G., Stetson, D. L., Ardito, T. & Stanton, B. (1988) *Kidney Int.* **34**, 655–670.
33. Drenckhahn, D., Schluter, K., Allen, D. P. & Bennett, V. (1985) *Science* **230**, 1287–1289.
34. Wagner, S., Vogel, R., Lietze, R. & Drenckhahn, D. (1987) *Am. J. Physiol.* **253**, F213–F221.
35. Alper, S. L., Natale, J., Gluck, S., Lodish, H. F. & Brown, D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5429–5433.
36. Bonsib, S. M., Williams, R. D., Ostedgaard, L. S., Li, L., Severson, C. D. & Schuster, V. L. (1990) *Kidney Int.* **37**, 224 (abstr.).
37. Störkel, S., Pannen, B., Thoenes, W., Steart, P. V., Wagner, S. & Drenckhahn, D. (1988) *Virchows Arch. B* **56**, 185–189.
38. Brosius, F. C., III, Alper, S. L., Garcia, A. M. & Lodish, H. F. (1989) *J. Biol. Chem.* **264**, 7784–7787.
39. Kudrycki, K. E. & Shull, G. E. (1989) *J. Biol. Chem.* **264**, 8185–8192.
40. Thomas, H. A., Machen, T. E., Smolka, A., Baron, R. & Kopito, R. R. (1989) *Am. J. Physiol.* **257**, C537–C544.
41. Kopito, R. R., Lee, B. S., Simmons, D. M., Lindsey, A. E., Morgans, C. W. & Schneider, K. (1989) *Cell* **59**, 927–937.
42. Lanoue, K. F. & Schoolwerth, A. C. (1984) in *Bioenergetics*, ed. Ernster, L. (Elsevier, Amsterdam), pp. 221–268.
43. Aquila, H., Link, T. A. & Klingenberg, M. (1987) *FEBS Lett.* **212**, 1–9.
44. Beavis, A. D. & Powers, M. F. (1989) *J. Biol. Chem.* **264**, 17148–17155.