Sodium-Dependent Chloride Transport in Basolateral Membrane Vesicles Isolated from Rabbit Proximal Tubule†

Pei-Yuan Chen‡ and A. S. Verkman*

Division of Nephrology, Cardiovascular Research Institute, University of California, San Francisco, California 94143

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ABSTRACT: The mechanisms for Cl transport across basolateral membrane vesicles (BLMV) isolated from rabbit renal cortex were examined by using the Cl-sensitive fluorescent indicator 6-methoxy-*N*-(3-sulfopropyl)quinolinium (SPQ). The transporters studied included Cl/base exchange, Cl/base/Na cotransport, K/Cl cotransport, and Cl conductance. Initial rates of chloride influx (J_{Cl}) were determined from the measured time course of SPQ fluorescence in BLMV following inwardly directed gradients of Cl and gradients of other ions and/or pH. For a 50 mM inwardly directed Cl gradient in BLMV which were voltage and pH clamped (7.0) using K/valinomycin and nigericin, J_{Cl} was 0.80 ± 0.14 nmol s^{-1} (mg of vesicle protein)$^{-1}$ (mean ± SD, n = 8 separate preparations). In the absence of Na and CO_2/HCO_3 in voltage-clamped BLMV, J_{Cl} increased 56% ± 5% in response to a 1.9 pH unit inwardly directed H gradient; the increase was further enhanced by 40% ± 3% in the presence of CO_2/HCO_3 and inhibited 30% ± 8% by 100 μM dihydro-4,4′-diisothiocyanostilbene-2,2′-disulfonic acid. Na gradients did not increase J_{Cl} in the absence of CO_2/HCO_3; however, an outwardly directed Na gradient in the presence of CO_2/HCO_3 increased J_{Cl} by 31% ± 8% with a Na K_D of 7 ± 2 mM. These results indicate the presence of Cl/OH and Cl/HCO_3 exchange, and Cl/HCO_3 exchange trans-stimulated by Na. There was no significant effect of K gradients in the presence or absence of valinomycin, suggesting lack of significant K/Cl cotransport and Cl conductance under experimental conditions. The Na-dependent Cl/HCO_3 exchanger may provide an important route for Cl exit across the proximal tubule basolateral membrane.

Approximately 50% of transepithelial Cl resorption in the mammalian proximal tubule occurs by a transcellular route in which Cl moves serially across the apical (brush border) and basolateral membranes (Alpern et al., 1985; Baum & Berry, 1984; Cassola et al., 1983). Despite good understanding of transport mechanisms for Na, K, and H across proximal tubule cell membranes, the mechanisms for Cl transport remain controversial. On the apical membrane, net NaCl transport has been postulated to occur by parallel Na/H and Cl/base or Cl/formate exchange with rapid recycling of formic acid (Karniski & Aronson, 1985). At the basolateral membrane, there is considerable controversy about the Cl exit pathways both in vesicle and in intact tubule studies. Cl has been suggested to exit by Cl conductance, Cl/anion exchange, K/Cl cotransport, and some form of Na/Cl/HCO_3-coupled transport (Boron & Boulpaep, 1983; Eveloff & Warnock, 1987; Grassl & Aronson, 1986; Grassl et al., 1987; Dobbins, 1985; Rector, 1983).

We recently developed a method to examine Cl transport mechanisms based on quenching of the Cl-sensitive fluorescent indicator 6-methoxy-*N*-(3-sulfopropyl)quinolinium (SPQ) (Illsley & Verkman, 1987). SPQ can be loaded into membrane vesicles by incubation and washing without membrane toxicity. SPQ fluorescence then reports the instantaneous intravesicular Cl concentration with high sensitivity, without interference by other physiological ions, ionophores, or pH. The SPQ method has been validated by comparison of fluorescence with ^{36}Cl uptake results in several systems including red cells (Illsley & Verkman, 1987), human placental microvillus vesicles (Illsley et al., 1988), and rabbit renal brush border vesicles (Chen et al., 1988). Compared to ^{36}Cl uptake measurements in membrane vesicles, the SPQ fluorescence method is more rapid, has better sensitivity and time resolution, and requires a smaller sample size. In membrane vesicle experiments, it is possible to examine accurately the early time course of Cl transport in response to ion or pH gradients and to induced diffusion potentials, at times well before initial gradients have collapsed.

This new method has been applied to examine the mechanisms of Cl transport in proximal tubule basolateral membrane vesicles (BLMV) isolated from rabbit renal cortex. Rates of Cl flux in nanomoles per second per milligram of protein were measured in response to a simultaneous gradient of Cl and Na, H, or K. We find evidence to support the presence of stilbene-inhibitable Cl/OH(HCO_3) exchange and Na/Cl/HCO_3-coupled transport which requires bicarbonate but not potassium. We find no K/Cl cotransport or Cl conductance in these membranes.

Experimental Procedures

Materials. SPQ was synthesized as described previously (Wolfbeis & Urbano, 1982). Diphenylamine-2-carboxylate (DPC) was purchased from ICN Biomedicals, Inc. (Plainview, NY), and was added from a 100 mM stock solution in ethanol. Dihydro-4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (H_2DIDS) was purchased from Molecular Probes (Junction City, OR) and prepared freshly as a 5 mM stock solution in buffer and protected from light exposure. All other chemicals were obtained from Sigma (St. Louis, MO). Valinomycin,

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* Address correspondence to this author.

‡ Present address: Department of Internal Medicine, Taipei Medical College, Taiwan, Republic of China.

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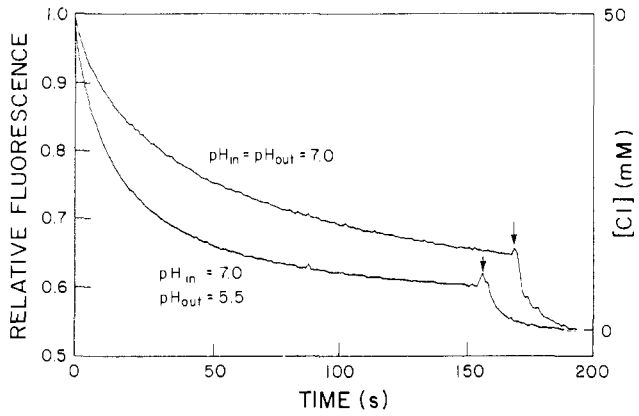

FIGURE 1: Basolateral membrane Cl influx driven by a pH gradient. BLMV (voltage clamped to 0 mV) at a concentration of 10 μg of membrane protein/mL containing SPQ were subjected to a 50 mM inwardly directed Cl gradient in the presence and absence of a 1.5 pH unit inwardly directed H gradient. The time course of relative SPQ fluorescence is shown on the ordinate. The arrows indicate addition of 10 μL of 1% Triton X-100 to the cuvette.

nigericin, monensin, and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) were added from 25 mg/mL stock solutions in ethanol. All solutions used were filtered through 0.22-μm Millipore filters prior to experiments to remove dust particles which scatter light.

Membrane Preparation. Basolateral membrane vesicles (BLMV) were prepared as described previously (Ives et al., 1983; Verkman & Ives, 1986). Briefly, the renal cortex was dissected from the kidneys of 2–3-kg female New Zealand white rabbits and homogenized at 4 °C in 250 mM sucrose, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid–tris(hydroxymethyl)aminomethane (Hepes–Tris), and 5 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA)–Tris, pH 7.0, with a Sorvall omni-mixer. Crude membrane was isolated by differential centrifugation and loaded onto a linear 35–48% sucrose gradient at pH 7. After equilibrium centrifugation for 16 h, fractions were collected and assayed for maltase and (Na/K)-ATPase activities. The fractions with highest (Na/K)-ATPase and lowest maltase activities were pooled. BLMV were enriched in (Na/K)-ATPase specific activity >15-fold and in maltase activity <0.3-fold over the crude homogenate. BLMV were deenriched in intracellular membranes 5–1.2-fold as judged from the activities of acid phosphatase (marker of lysosomes), thiamin pyrophosphatase (Golgi), NADPH–cytochrome-*c* reductase (endoplasmic reticulum), and succinate–cytochrome-c reductase (mitochondria) (Ives et al., 1983). BLMV were incubated in the desired buffers for 24 h at 4 °C, centrifuged, and stored at 4 °C until the time of the experiment (<48 h).

Vesicles were loaded with SPQ by incubation of BLMV (10 mg of protein/mL) with 10 mM SPQ at 4 °C in the dark for 18–24 h. Extravesicular SPQ was removed by three washes in >25 volumes of buffer not containing SPQ. BLMV were homogenized, resuspended at a concentration of 10 mg of protein/mL, and maintained at 4 °C until the time of the experiment (<4 h). For experiments performed in the presence or absence of CO_2/HCO_3, BLMV and solutions were bubbled with 5% CO_2/95% N_2 or 100% N_2, respectively, for 2 h. For some experiments, valinomycin and/or nigericin (25 μg/mg of vesicle protein), and H_2DIDS or DPC (100 μM), were added to the vesicles 1 h prior to experiments.

Fluorescence Measurements. The time course of SPQ fluorescence was measured by using an SLM 4800 fluorometer (Urbana, IL) interfaced to an IBM PC/XT computer. Temperature was controlled with a Haake A80 refrigerated water bath. Unless otherwise noted, all experiments were performed at 23 °C. Fluorescence was excited at 350 nm (8-nm bandpass) and measured by using Schott Glass KV408 and GG420 cut-on filters passing light above 420 nm. Acrylic cuvettes (Sarstadt, West Germany) were used for all experiments.

Cl influx experiments were initiated by adding BLMV (20–50 μg of protein) in 150 mM (NMG/K/Na) gluconate and appropriate buffer [Hepes–Tris or 2-(*N*-morpholino)-ethanesulfonic acid (MES)–Tris] to 2 mL of isotonic, isoosmotic buffer[1] with specified ion or pH gradients. Solutions were stirred continuously by a magnetic stirrer. Fluorescence intensities were averaged over 1-s time intervals by the on-line computer, displayed graphically, and stored on disk for analysis. At the completion of each experiment, 10 μL of the detergent Triton X-100 (1%) was added to expose all SPQ to the solution Cl concentration (Illsley & Verkman, 1987; Chen et al., 1988). Cl flux (J_{Cl}) in nanomoles per second per milligram of protein was calculated from the initial rate of change of SPQ fluorescence, the SPQ fluorescence vs [Cl] calibration curve, and the BLMV glucose space (4 μL/mg of protein) as described in detail previously (Illsley & Verkman, 1987). All measurements were performed in quadruplicate using at least three separate BLMV preparations unless otherwise indicated. Data in tables are reported as J_{Cl} values in sets of experiments performed on a single typical BLMV preparation. For statistical comparisons, data in the text are generally reported in paired studies, as the ratio of J_{Cl} measured under "test" conditions (inward Cl gradient + test ion or pH gradient) to J_{Cl} measured under "control" conditions (inward Cl gradient). The number of separate BLMV preparations used for pair comparisons is given in parentheses.

RESULTS

BLMV were voltage clamped with K/valinomycin and pH clamped with nigericin to eliminate diffusion potentials and changes in intravesicular pH. In eight sets of experiments, each set performed in quadruplicate on a separate BLMV preparation, J_{Cl} for a 50 mM inwardly directed Cl gradient at pH 7.0 was 0.80 ± 0.14 nmol s^{-1} (mg of protein)$^{-1}$ [mean ± SD (n = 8)]. In paired experiments, J_{Cl} was inhibited 34% ± 11% (n = 5) by the stilbene inhibitor H_2DIDS (100 μM) but not significantly by the chloride channel blocker DPC (DiStefano et al., 1985) [100 μM, 2% ± 3% inhibition (n = 3)]. A typical experimental curve is shown in Figure 1. There is a slow time course of decreasing fluorescence corresponding to Cl influx and intravesicular SPQ quenching. Addition of

[1] Because SPQ fluorescence rather than ^{36}Cl radioactivity is used as an indicator of chloride transport, it is important to recognize and eliminate the several possible effects of vesicle volume changes on SPQ fluorescence. If intra- and extravesicular osmolarities are not matched, or if solute reflection coefficients differ from unity, there will be rapid osmotic water movement (<200 ms; Verkman & Ives, 1986), causing a change in SPQ concentration and in vesicle volume. The change in SPQ concentration will not affect the signal because SPQ self-quenching does not occur under experimental conditions (Illsley & Verkman, 1987). The change in vesicle volume will alter ion gradients and apparent times for Cl entry. Several control experiments were performed to demonstrate that the results were not affected by vesicle volume changes. Stopped-flow light-scattering measurements under experimental conditions showed no measurable change in vesicle volume within the first 1000 ms after mixing. The time courses of SPQ fluorescence performed under "volume-clamp" conditions, in which 600 mM sucrose was present in the intra- and extravesicular solutions, were the same as those measured with the usual buffer osmolarity. Lastly, SPQ fluorescence was independent of time following exposure of vesicles to pH and Na gradients in the absence of Cl.

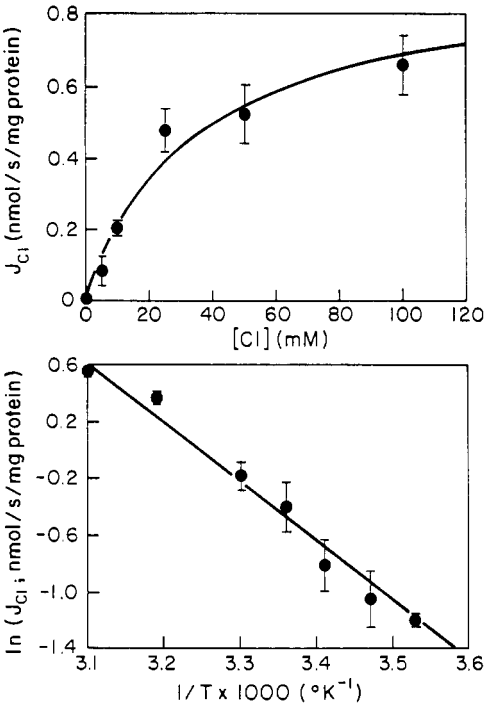

FIGURE 2: Characteristics of basolateral membrane Cl/OH exchange. (Top) BLMV at pH 7.0 were subjected to 5–100 mM inwardly directed Cl gradients and a 1.5 pH unit inwardly directly H gradient. The ordinate represents the magnitude of H_2DIDS-sensitive Cl influx measured from the difference in J_{Cl} in the absence and presence of 100 μM H_2DIDS. Each data point is the mean ± SD of measurements performed in quadruplicate using two separate BLMV preparations. Data are fitted to a single-site saturable binding model with K_D = 34 ± 8 mM. (Bottom) Arrhenius plot for BLMV Cl/OH exchange. J_{Cl} was determined for a 50 mM inwardly directed Cl gradient and a 1.5 pH unit inwardly directed H gradient at several temperatures between 10 and 50 °C. Each point is the mean ± SD of quadruplicate determinations of J_{Cl} using two separate BLMV preparations. The fitted activation energy was 11 ± 2 kcal/mol.

increasing valinomycin (50–100 μg/mg of vesicle protein) resulted in no further increase in J_{Cl} for these experiments and those involving pH and Na gradients (data not shown), demonstrating adequacy of the voltage clamp at 25 μg of valinomycin/mg of vesicle protein.

Cl/Base Exchange. Effects of pH and Na gradients, in the presence and absence of CO_2/HCO_3, were studied to examine the characteristics of BLMV Cl/base exchange. In voltage-clamped BLMV in the absence of Na and CO_2/HCO_3, a 1.5 pH unit inwardly directed H gradient increased J_{Cl} by 40% ± 4% (n = 3) (see Figure 1). The increase was inhibited 30% ± 3% (n = 3) by 100 μM H_2DIDS.[2] These results indicate the presence of Cl/OH exchange in BLMV.

Cl/OH exchange in the absence of Na and CO_2/HCO_3 was examined further from the dependences of J_{Cl} on external pH, [Cl], temperature, and voltage. In voltage-clamped BLMV (pH_{in} 7.0), J_{Cl} was (in nanomoles per second per milligram of protein, quadruplicate determinations in one preparation) 0.64 ± 0.02 (pH_{out} 7.0), 0.80 ± 0.12 (pH_{out} 6.5), 1.21 ± 0.10 (pH_{out} 6.0), 1.54 ± 0.08 (pH_{out} 5.5), and 1.99 ± 0.08 (pH_{out} 5.0). For a 1.5 pH unit inwardly directed H gradient (pH_{in} 7.0; pH_{out} 5.5), the H_2DIDS-sensitive component of J_{Cl} (Figure 2, top panel) was saturable with Cl, K_D = 34 ± 8 mM. In temperature-dependence studies (Figure 2, bottom panel), a single activation energy of 11 ± 2 kcal/mol was measured. In the absence of a pH gradient (pH 7.0), a 60-mV internal-positive K/valinomycin diffusion potential ($[K]_{in}$ = 5 mM; $[K]_{out}$ = 50 mM) did not increase J_{Cl} significantly [−6% ± 7% (n = 3)] over J_{Cl} measured with a 0-mV potential ($[K]_{in}$ = $[K]_{out}$ = 50 mM). In the presence of a 1.5 pH unit inwardly directed H gradient, a 60-mV internal-positive K/valinomycin diffusion potential also did not increase J_{Cl} [3% ± 5% (n = 3)]. These result suggest that Cl/OH exchange is neutral and probably has 1:1 stochiometry.

To determine whether Cl/HCO_3 exchange occurs, J_{Cl} was measured for a 0 and 1.9 pH unit inwardly directed H gradient in the absence and presence of CO_2/HCO_3 (Table I). The presence of CO_2/HCO_3 results in a significant increase in J_{Cl} both with and without a pH gradient. In paired studies comparing J_{Cl} in the presence and absence of a pH gradient, J_{Cl} increased by 56% ± 5% (n = 3) in the absence of CO_2/HCO_3. In the presence of a pH gradient, addition of CO_2/HCO_3 resulted in a 40% ± 3% (n = 3) increase in J_{Cl}; 100 μM H_2DIDS inhibited J_{Cl} for a 1.9 pH unit inwardly directed H gradient by 30% ± 8% (n = 3).

Na-Dependent Cl/Base Exchange. The presence of a Na-dependent Cl transport process in BLMV was examined from the effect of Na gradients on J_{Cl} in the presence and absence of CO_2/HCO_3 (Table II). Experiments were carried out with a 1.9 pH unit inwardly directed H gradient. In the absence of CO_2/HCO_3, there was no effect of inwardly or outwardly directed Na gradients. In the presence of CO_2/HCO_3, a 25 mM outwardly directed Na gradient increased J_{Cl} by 31% ± 8% (n = 5); there was a small decrease in J_{Cl} [−15% ± 5% (n = 3)] by a 25 mM inwardly directed Na gradient. The Na effect was not due to a Na diffusion potential because BLMV were voltage clamped with K/valinomycin. In addition, an outwardly directed Na gradient would induce an internal negative diffusion potential which would decrease J_{Cl} if parallel Na and Cl conductances were present.

Table I: Effect of CO_2/HCO_3 on Cl Influx[a]

	pH_{in}/pH_{out}		
		7.44/5.50	
	7.44/7.44	−H_2DIDS	+H_2DIDS
−CO_2/HCO_3	0.85 ± 0.10	1.33 ± 0.15	0.94 ± 0.11
+CO_2/HCO_3	1.21 ± 0.08	1.86 ± 0.16	1.30 ± 0.17

[a] BLMV (voltage clamped with K/valinomycin) with internal pH 7.44 were suspended in buffer containing 50 mM Cl at pH 7.44 or 5.50 in the presence and absence of CO_2/HCO_3 as described under Experimental Procedures. H_2DIDS was present at 100 μM concentration when indicated (+H_2DIDS). Each value is the mean ± SD for J_{Cl} (nanomoles per second per milligram of protein) measured in quadruplicate in one set of experiments. Results of paired studies performed using several separate BLMV preparations are given in the text.

Table II: Effect of Outward Na Gradient and K on Cl Influx[a]

	−Na	+Na
−CO_2/HCO_3	1.33 ± 0.15	1.32 ± 0.16
+CO_2/HCO_3	1.86 ± 0.11	2.42 ± 0.18

[a] BLMV (voltage clamped with K/valinomycin) at pH 7.44 in the presence and absence of CO_2/HCO_3 were subjected to a 50 mM inwardly directed Cl gradient and a 1.9 pH unit inwardly directed Na gradient. Each value is the mean ± SD for J_{Cl} (nanomoles per second per milligram of protein) measured in quadruplicate in one set of experiments. Results of paired studies performed using several separate BLMV preparations are given in the text.

[2] There are several reasons why a relatively small percentage of H_2DIDS inhibition was measured. Because of the interference of H_2DIDS with the SPQ fluorescence signal at [H_2DIDS] >100 μM, experiments were performed at that concentration. Because it is likely that the apparent K_I for H_2DIDS in the presence of 50 mM Cl is >25–50 μM, 100 μM H_2DIDS would only give partial inhibition of J_{Cl}. In addition, there may be other Cl pathways in the membrane vesicles (that may not be present in the intact tissue) that are nonspecific (e.g., H/Cl cotransport) which would not be modified by specific, high-affinity inhibitors.

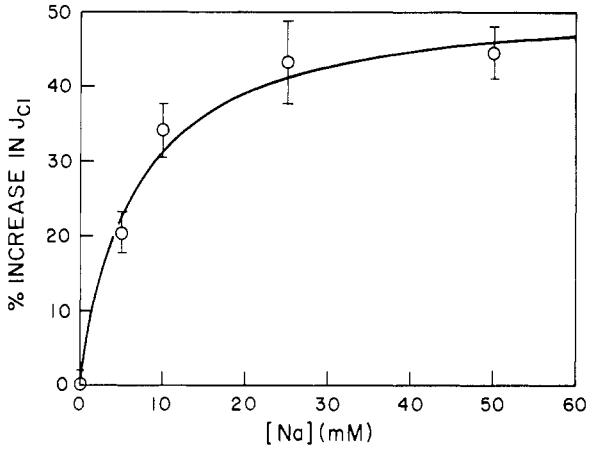

FIGURE 3: Dependence of Cl influx on outward Na gradient. BLMV at pH 7.44 (voltage clamped to 0 mV) were subjected to a 50 mM inwardly directed Cl gradient and a 1.9 pH unit inwardly directed H gradient in the presence of CO_2/HCO_3 and a variable outwardly directed Na gradient. The ordinate is expressed as the percentage increase in J_{Cl} in the presence of intravesicular Na over that measured in the absence of a Na gradient. Each point is the mean ± SD of quadruplicate determinations of J_{Cl} in two separate BLMV preparations. Data were fitted to a single-site, saturable binding model with K_D = 7 ± 2 mM.

Table III: Effect of Voltage and K Gradient on Cl Influx[a]

	$[K_{in}]/[K_{out}]$	
	50/50	5/50
+valinomycin	0.82 ± 0.07	0.79 ± 0.05
−valinomycin	0.80 ± 0.05	0.79 ± 0.04

[a] BLMV at pH 7.0 were subjected to a 50 mM inwardly directed Cl gradient in the presence and absence of valinomycin, and of a K gradient. Each value is the mean ± SD for J_{Cl} (nanomoles per second per milligram of protein) measured in quadruplicate in one set of experiments. Results of paired studies performed using several BLMV preparations are given in the text.

The Na effect cannot be due to electrogenic $Na/3HCO_3$ cotransport (Akiba et al., 1986; Grassl & Aronson, 1986; Yoshitomi et al., 1985) because an outwardly directed Na gradient would decrease intravesicular $[HCO_3]$, resulting in a decreased driving force for Cl/HCO_3 exchange, and thus a decrease in J_{Cl}. These data indicate the presence of a CO_2/HCO_3-dependent, Cl influx trans-stimulated by Na gradients.

The $Na/HCO_3/Cl$ transport system was further characterized by studying the dependence of J_{Cl} on intravesicular [Na] (Figure 3). J_{Cl} was saturable with a Na K_D of 7 ± 2 mM. To examine whether the Na effect required K, the effect of an outwardly directed Na gradient on J_{Cl} with K replaced by NMG and in the presence of CO_2/HCO_3 was determined. A 25 mM outwardly directed Na gradient increased J_{Cl} by 30% ± 8% (n = 3) in the absence of K, indicating that the $Na/HCO_3/Cl$ transport system does not require K. Again, an increase in J_{Cl} would not be observed in the presence of parallel Na and Cl conductances.

K/Cl Cotransport and Cl Conductance. The presence of Cl conductance was studied from the effects of K gradients on J_{Cl} in the presence and absence of valinomycin (Table III). With equal [K] on both sides of the membrane (50 mM), addition of valinomycin did not affect J_{Cl} significantly [2% ± 3% change (n = 3)], indicating that the relative BLMV Cl to K conductance ratio is very low. In the presence of a 60-mV internal positive membrane potential, J_{Cl} was not increased significantly over that measured in the presence of a 0-mV membrane potential [2% ± 1% (n = 3)]. Similarly, there was no effect of a 60-mV potential on J_{Cl} in the presence of 2 mM Mg on both sides of the membrane (not shown). Therefore, voltage-driven chloride influx is minimal in BLMV.

The presence of K/Cl cotransport was examined by voltage clamping BLMV with 50 mM Na and the ionophores monensin and CCCP. In these experiments, the internal K concentration was 0 mM, so that it would be possible to observe K/Cl cotransport that is saturable at low K. Under these conditions, an inwardly directed K gradient might drive inward Cl transport by K/Cl cotransport but not by Cl conductance. BLMV (voltage and pH clamped with 25 μg/mL monensin and CCCP) containing 50 mM sodium gluconate and 50 mM NMG gluconate, pH 7.0, were added to an external solution containing 50 mM NaCl and 50 mM NMG gluconate, pH 7.0 (control), or 50 mM NaCl and 50 mM potassium gluconate, pH 7.0 (K gradient). Control J_{Cl} [0.64 ± 0.11 nmol s^{-1} mg^{-1} (n = 6)] was not different from J_{Cl} [0.63 ± 0.09 nmol s^{-1} mg^{-1} (n = 6)] in the presence of a K gradient. To ensure that the pH clamp was adequate, J_{Cl} in the presence of a 1.5 pH unit inwardly directly H gradient [0.69 ± 0.12 nmol s^{-1} mg^{-1} (n = 6)] was not different from the control or K gradient rates, indicating that the pH clamp was effective. These results indicate that K/Cl cotransport is absent in BLMV under the experimental conditions.

Discussion

Several possible modes of BLMV Cl transport were examined: $Cl/OH(HCO_3)$ exchange and Na-coupled HCO_3/Cl transport; K/Cl cotransport; and Cl conductance. Initial rates of Cl influx into BLMV were measured by using SPQ, a Cl-sensitive, entrapped fluorescent indicator. The application of SPQ for measurement of membrane transport was described first by Illsley and Verkman (1987) and has been applied to measure Cl transport in human red cell ghost membranes (Illsley & Verkman, 1987), apical membrane vesicles isolated from renal proximal tubule (Chen et al., 1988), human placenta (Illsley et al., 1988), and bovine trachea (Fong et al., 1987), and intact cells of the isolated perfused rabbit proximal tubule (Krapf et al., 1988). The mechanisms of Cl transport in BLMV were examined from the measured rates of Cl influx in response to concurrent gradients of Cl and other ions or pH, and from the effects of transport inhibitors on Cl influx.

The data presented demonstrate that BLMV contain a voltage-insensitive Cl/OH exchanger inhibited by the stilbene anion-exchange inhibitor H_2DIDS. The exchange rate was enhanced in the presence of CO_2/HCO_3, indicating that both OH and HCO_3 can exchange with Cl. In contrast, CO_2/HCO_3 did not enhance the rate of Cl/OH exchange in renal brush border membrane vesicles under the same experimental conditions (Chen et al., 1988). While a Na gradient parallel to the Cl gradient did not enhance BLMV Cl/HCO_3 exchange, a Na gradient antiparallel to the Cl gradient enhanced Cl/HCO_3 exchange with a Na K_D of 7 mM. This effect required CO_2/HCO_3 but did not require K. The data presented do not support the presence of K/Cl or Na/K/Cl cotransport and Cl conductance.

The rate of the neutral, H_2DIDS-sensitive Cl/OH exchanger increased with increasing pH gradient size and was saturable in Cl (K_D = 34 mM). It had an apparent single activation energy of 11 kcal/mol. Similar Cl/OH exchangers have been reported in epithelial brush border membranes from rat (Cassano et al., 1984), rabbit (Chen et al., 1988), dog (Guggino et al., 1983), and *Necturus* (Seifter & Aronson, 1984) kidneys and rat (Cassano et al., 1984) and rabbit (Knickelbein et al., 1985) intestines, and in basolateral membranes from the rabbit (Grassl et al., 1987) and rat kidney

(Grassl et al., 1985). In contrast to the Cl/OH exchanger present in the rabbit brush border membrane, the BLMV exchanger can exchange HCO_3 with Cl. In the isolated perfused rabbit proximal straight tubule using Cl-sensitive microelectrodes, Sasaki et al. (1987) reported a Cl-related HCO_3 exit mechanism which accounted for only a small portion of the total HCO_3 exit.

We find that an outwardly directed Na gradient increases BLMV Cl influx only in the presence of CO_2/HCO_3. The effect was seen both in voltage-clamped BLMV and in the absence of K. These findings are not consistent with the presence of the furosemide-sensitive Na/K/2Cl cotransporter, parallel Na and Cl conductances, and $Na/3HCO_3$ cotransport which might function to carry both HCO_3 and Cl. In addition, the $Na/3HCO_3$ cotransporter has been reported not to carry Cl (Grassl & Aronson, 1986; Jentsch et al., 1985; Sasaki & Berry, 1984). These data suggest that the rabbit proximal tubule basolateral membrane contains a $Na-HCO_3/Cl$ exchanger.

A $Na-HCO_3/Cl$ transport system was first described in the snail neuron (Thomas, 1977), barnacle muscle (Boron et al., 1981), and squid giant axon (Boron & Russell, 1983) and subsequently characterized in the *Amphiuma* intestinal mucosa (White, 1986) and in cultured bovine corneal endothelium (Jentsch et al., 1985). With the use of Cl and pH-sensitive microelectrodes, this transporter was described in the basolateral membrane of *Necturus* proximal tubule (Guggino et al., 1983). The $Na-HCO_3/Cl$ exchanger appears to be inhibited by stilbenes, is electroneutral, and may exchange one Na and two HCO_3, or one Na and one CO_3, for one Cl, or one Na and one HCO_3 for one H and one Cl (Boron & Boulpaep, 1983; Boron & Russell, 1983; Guggino et al., 1982; Jentsch et al., 1985); however, the exact mechanism of this transport system has been difficult to define. Recently, Grassl et al. (1987) used ^{22}Na uptake methods to show that basolateral membrane vesicles from rat kidney cortex contained a $Na-HCO_3$ for Cl exchanger; however, the saturability characteristics and stoichiometry of this system were not defined.

The presence of K/Cl cotransport in proximal tubule basolateral membranes has been an unresolved question. In experiments using ^{36}Cl uptake in BLMV, Grassl et al. (1987) found no K/Cl cotransport, while Eveloff and Warnock (1987) found evidence for K/Cl cotransport. In the present studies, we find no evidence for K gradient stimulated chloride influx both in the absence and in the presence of valinomycin, and with a Na/monensin/CCCP voltage clamp, ruling out both K/Cl cotransport and voltage-driven Cl conductance. In the intact proximal tubule, there have been no experimental studies to address whether K/Cl cotransport is present; however, there is good evidence that basolateral Cl conductance is minimal or absent (Burckhardt et al., 1984). In a mathematical model of the proximal tubule reported by Verkman and Alpern (1987), a neutral basolateral K exit pathway was required; however, the nature of the pathway was not specified.

In the intact proximal tubule, approximately 50% of Cl resorption (lumen to capillary) is transcellular. On the basis of studies in brush border membranes and in the intact proximal tubule, Cl probably enters the cell across the brush border membrane by Cl/OH and Cl/formate exchangers, operating in parallel to Na/H antiport. On the basolateral membrane, several modes of Cl exit have been suggested as plausible mechanisms including Cl conductance, K/Cl cotransport, and Na-coupled $Cl/OH(HCO_3)$ exchange. The electrochemical driving forces in the intact proximal tubule for Cl conductance, K/Cl cotransport, and $Na-HCO_3/Cl$ exchange favor Cl exit across the basolateral membrane. For the $Na-HCO_3/Cl$ exchanger, Na and HCO_3 will move from capillary to cell, a direction opposite to the necessary physiological resorption of Na and HCO_3, requiring exit of Na and HCO_3 by the $Na/3HCO_3$ cotransport and the (3Na/2K)-ATPase. The electrochemical driving force for Cl/OH exchange favors Cl entry across the basolateral membrane and thus cannot account for Cl exit. Further experiments and mathematical modeling in the intact proximal tubule will be required to evaluate fully the data from vesicle studies and to define conclusively the mechanisms of basolateral Cl exit.

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Poly(aspartic acid)-Dependent Fusion of Liposomes Bearing the Quaternary Ammonium Detergent [[[(1,1,3,3-Tetramethylbutyl)cresoxy]ethoxy]ethyl]dimethylbenzylammonium Hydroxide

Michael Beigel,[‡] Mordechai Keren-Zur,[‡] Yehudit Laster,[§] and Abraham Loyter*[,§]
International Genetic Sciences Partnership, P.O. Box 4309, 91042 Jerusalem, Israel, and Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel
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ABSTRACT: Addition of the quaternary ammonium detergent [[[(1,1,3,3-tetramethylbutyl)cresoxy]ethoxy]ethyl]dimethylbenzylammonium hydroxide (DEBDA[OH]) and the fluorescent probes *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine and *N*-(lissamine rhodamine B sulfonyl)phosphatidylethanolamine (*N*-NBD-PE and *N*-Rh-PE, respectively) to liposomes composed of phosphatidylcholine (PC) and cholesterol (chol) resulted in the formation of fluorescently labeled liposomes bearing DEBDA[OH]. Incubation of the anionic polymer poly(aspartic acid) (PASP) with such liposomes resulted in strong agglutination, indicating an association between the negatively charged PASP and the positively charged liposome-associated DEBDA[OH]. Addition of PASP to a mixture of fluorescently labeled and nonlabeled liposomes, both carrying DEBDA[OH], resulted in a significant increase in the extent of fluorescence, namely, fluorescence dequenching. The degree of the fluorescence dequenching was dependent upon the ratio between the nonfluorescent and the fluorescent liposomes, upon the temperature of incubation, and upon the amount of DEBDA[OH] which was associated with the liposomes. Electron microscopic observations revealed that large liposomes were formed upon incubation of liposomes bearing DEBDA[OH] with PASP. The results of the present work strongly indicate that the fluorescence dequenching observed is due to a process of PASP-induced liposome-liposome fusion.

Membrane fusion is an important event in many biological processes such as endocytosis, exocytosis, fertilization, and infection of cells by animal viruses (Poste & Nicolson, 1978). The complexity of biological membranes and the difficulty in studying and analyzing their isolated components have promoted the use of model systems for the elucidation of the molecular mechanism of membrane fusion (Düzgünes, 1985).

The most widely used model system has been fusion between liposomes composed of negatively charged phospholipids such as phosphatidylserine (PS)[1] (Papahadjopoulos et al., 1975; Düzgünes et al., 1981) and cardiolipin (Wilschut et al., 1982). Fusion of such liposomes can be promoted by either divalent metals such as Ca^{2+} (Wilschut et al., 1980) or by polycations such as polylysine (Gad et al., 1985; Uster & Deamer, 1985) and polyhistidine (Wang & Huang, 1984). Increasing the molar content of phosphatidylcholine (PC) is inhibitory, while addition of phosphatidylethanolamine (PE) increases the fusion competence of phospholipid vesicles (Uster & Deamer, 1985).

Fusion between liposomes can be quantitatively followed through the use of fluorescently labeled probes and energy-transfer methods (Struck et al., 1981). With these methods,

* Author to whom correspondence should be addressed.
[‡] International Genetic Sciences Partnership.
[§] The Hebrew University of Jerusalem.

[1] Abbreviations: chol, cholesterol; DEBDA[Cl], [[[(1,1,3,3-tetramethylbutyl)cresoxy]ethoxy]ethyl]dimethylbenzylammonium chloride; DEBDA[OH], [[[(1,1,3,3-tetramethylbutyl)cresoxy]ethoxy]ethyl]dimethylbenzylammonium hydroxide; DQ, fluorescence dequenching; HTC, hepatoma tissue culture cells; *N*-NBD-PE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine; *N*-Rh-PE, *N*-(lissamine rhodamine B sulfonyl)phosphatidylethanolamine; PASP, poly(aspartic acid); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; REV, reverse-phase evaporated vesicles.

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