Halide Permeation in Wild-Type and Mutant Cystic Fibrosis Transmembrane Conductance Regulator Chloride Channels

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ABSTRACT Permeation of cystic fibrosis transmembrane conductance regulator (CFTR) Cl− channels by halide ions was studied in stably transfected Chinese hamster ovary cells by using the patch clamp technique. In cell-attached patches with a high Cl− pipette solution, the CFTR channel displayed outwardly rectifying currents and had a conductance near the membrane potential of 6.0 pS at 22°C or 8.7 pS at 37°C. The current–voltage relationship became linear when patches were excised into symmetrical, N-tris(hydroxymethyl)methyl-2-aminomethane sulfonate (TES)-buffered solutions. Under these conditions, conductance increased from 7.0 pS at 22°C to 10.9 pS at 37°C. The conductance at 22°C was ~1.0 pS higher when TES and HEPES were omitted from the solution, suggesting weak, voltage-independent block by pH buffers. The relationship between conductance and Cl− activity was hyperbolic and well fitted by a Michaelis-Menten–type function having a $K_m$ of ~38 mM and maximum conductance of 10 pS at 22°C. Dilution potentials measured with NaCl gradients indicated high anion selectivity ($P_{Na}/P_{Cl} = 0.003–0.028$). Biionic reversal potentials measured immediately after exposure of the cytoplasmic side to various test anions indicated $P_I (1.8) > P_B (1.3) > P_Cl (1.0) > P_3 (0.17)$, consistent with a “weak field strength” selectivity site. The same sequence was obtained for external halides, although inward F− flow was not observed. Iodide currents were protocol dependent and became blocked after 1–2 min. This coincided with a large shift in the (extrapolated) reversal potential to values indicating a greatly reduced $I^-/Cl^-$ permeability ratio ($P_I/P_{Cl} < 0.4$). The switch to low $I^-$ permeability was enhanced at potentials that favored $Cl^-$ entry into the pore and was not observed in the R347D mutant, which is thought to lack an anion binding site involved in multi-ion pore behavior. Interactions between $Cl^-$ and $I^-$ ions may influence $I^-$ permeation and be responsible for the wide range of $P_I/P_{Cl}$ ratios that have been reported for the CFTR channel. The low $P_I/P_{Cl}$ ratio usually reported for CFTR only occurred after entry into an altered permeability state and thus may not be comparable with permeability ratios for other anions, which are obtained in the absence of iodide. We propose that CFTR displays a “weak field strength” anion selectivity sequence.

KEY WORDS: iodide permeability • lyotropic sequence

INTRODUCTION

The cystic fibrosis transmembrane conductance regulator (CFTR)1 belongs to the “ATP binding cassette” superfamily of membrane transport proteins. Although its functions are still debated, several lines of evidence suggest that its primary function is that of a tightly regulated $Cl^-$ channel. Heterologous expression of CFTR generates a low conductance $Cl^-$ channel that is indistinguishable from that found in epithelial cells (Gray et al., 1989; Champigny et al., 1990; Tabcharani et al., 1990; Berger et al., 1991; Kartner et al., 1991; Rommens et al., 1991; Tabcharani et al., 1991). Mutations in CFTR alter the ionic selectivity of the expressed whole cell currents (Anderson et al., 1991), single channel conductance (Sheppard et al., 1993; Tabcharani et al., 1993; McDonough et al., 1994) and sensitivity to channel blockers (Lindsell and Hanrahan, 1996a; McDonough et al., 1994). When CFTR is purified to homogeneity and incorporated into bilayers, it forms channels closely resembling those in CFTR-expressing cells (Bear et al., 1992). Mutations in CFTR that cause it to be mislocalized (i.e., $ΔF508$) or unresponsive (i.e., G551D) are associated with severe forms of cystic fibrosis, whereas mutations that only partially reduce CFTR conductance (R347P,H, Sheppard et al., 1993; Tabcharani et al., 1993), open probability (R117H, Sheppard et al., 1993; Becq et al., 1994; intracellular loop IV mutants, Seibert et al., 1996), or processing (A455E, Sheppard et al., 1995) are associated with milder symptoms. Thus, the primary, though perhaps not exclusive, function of CFTR is to mediate $Cl^-$ permeation.

CFTR gating by nucleotides and regulation by phosphorylation have been characterized in some detail but less is known regarding its permeation properties. Most single channel measurements of CFTR selectivity have not been carried out under biionic conditions, although permeability ratios have been estimated using...
cell-attached and excised membrane patches (Gray et al., 1989; Champigny et al., 1990; Kartner et al., 1991; Bajnath et al., 1993), whole cell patches (Cliff and Frizzell, 1990; Anderson et al., 1991; Bear and Reyes, 1992), cell monolayers (Bell and Quinton, 1992; Kottra, 1996), and fused cells (Schröder and Frömter, 1995). The $P_{i}/P_{Cl}$ ratios in these studies ranged between 0 and 2.0, with most groups reporting $\sim$0.4. Low $I^{-}$ permeability has become a diagnostic for identifying CFTR-mediated macroscopic conductance; therefore, it seems important to assess whether the variation in permeability ratios reflects different experimental conditions in the various studies or some intrinsic property of the channel.

In this paper, we describe the halide permeability of single wild-type and mutant CFTR $Cl^{-}$ channels stably expressed in Chinese hamster ovary cells. CFTR was highly anion selective and the initial halide permeability ratios under biionic conditions were consistent with the lyotropic sequence. Interestingly, the relative $I^{-}$ permeability was strongly protocol dependent, with high initial $P_{i}/P_{Cl}$ switching abruptly to a low value similar to the ratios usually reported for macroscopic CFTR currents. This switch to low $I^{-}$ permeability was accelerated by holding the membrane at potentials that favored $Cl^{-}$ entry into the channel, and was not observed in R347D CFTR, a mutant shown previously to lack multilayer behavior in mixtures of $Cl^{-}$ and thiocyanate. Preliminary reports of this work have appeared (Tabcharani and Hanrahan, 1993).

**Methods**

**Cells**

Chinese hamster ovary (CHO) cells expressing wild-type CFTR or the mutant R347D (arginine 347 in the sixth predicted membrane spanning region mutated to aspartate) have been described previously (Tabcharani et al., 1991; Chang et al., 1993; Tabcharani et al., 1993). They were plated in DMEM containing 8% fetal bovine serum and methotrexate (100 µM) on glass coverslips at a density of $\sim 10^5$/cm$^2$ and maintained at 37°C in 5% CO$_2$ for 3–5 d before patch clamp experiments.

**Solutions**

The standard recording solutions in the pipette and bath contained (mM): 150 NaCl, 2 MgCl$_2$, 10 NaFES, pH 7.4. A 100× stock solution of 50 mM dibutyryl cyclic AMP (db-cAMP), 1 mM forskolin, and 1 mM 3-isobutyl-1-methylxanthine (IBMX) was used to raise intracellular cAMP in intact cells during cell-attached recordings. Forskolin, IBMX, db-cAMP, and adenosine triphosphate were from Sigma Chemical Co. (St. Louis, MO). Channel activity was maintained when recording from excised patches using 180 nM protein kinase A catalytic subunit and 1 mM MgATP, as described previously (Tabcharani et al., 1991, 1993). To measure cation permeability, reversal potentials were measured when 110 mM NaCl in the bath or pipette solution was replaced by 220 mM sucrose. Halide permeation was studied under biionic conditions by replacing salts in the pipette or bath solutions with those of the appropriate anion.

**Single Channel Recording**

Cells were transferred to a chamber containing 0.5 ml bath solution. Pipettes were prepared using a conventional puller (PP-83; Narishige Instruments Lab., Tokyo, Japan) and had 4–6 MΩ resistance when filled with 150 mM NaCl solution. The Ag/AgCl electrodes in the patch pipette and bath solutions were protected from $I^{-}$ poisoning when necessary by NaCl agar bridges. The bath agar bridge had the same composition as the pipette solution or the agar bridge in the pipette when one was necessary. Liquid junction potentials were determined using a flowing 3M KCl electrode (Neher, 1992) and reported voltages have been corrected accordingly. Single-channel currents were amplified (Axopatch 1C; Axon Instruments Inc., Foster City, CA), recorded onto video cassette tape by a pulse-coded modulation-type recording adapter (DR384; Neurodata Instrument Co., Delaware Water Gap, PA) and low pass filtered during play back using an 8-pole Bessel-type filter (902 LPF; Frequency Devices Inc., Haverhill, MA). The final recording bandwidth was $\sim$ 3 dB at 230 Hz, and records were sampled at 1 kHz and analyzed using a microcomputer as described previously (Tabcharani et al., 1989). $V_p$ refers to the command voltage applied to the pipette interior with reference to the bath during cell-attached patch recording. By convention, the membrane potential ($V_m$) in excised patches is the potential of the bath solution with reference to that of the pipette.

Current–voltage (i/V) relationships were calculated by a semi-automated procedure in which all-points histograms were computed screen by screen and displayed sideways next to the data so that current amplitudes and peaks could be verified using cursors. Also, the number of data points used in the histogram was adjusted to minimize the effect of any drift so that only those points immediately before and after a transition were included (between 100 ms and 8 s of the record). Histogram peaks were broader when patches contained multiple channels (usually between one and five channels), but this did not affect estimates of unitary amplitudes, which were averages of at least 10 determinations between several levels. Periods of severe baseline drift were not included in the analysis. Open events were measured at each potential and entered into an i/V curve, which was displayed at the end of the run. Reversal potentials were estimated by fitting a polynomial function to the i/V curve. Slope conductance was determined by linear regression over the voltage ranges specified in results. Permeability ratios were calculated using the equation $P_{i}/P_{Cl} = \exp(-E_{rev}/RT)$, where $E_{rev}$ is the reversal potential and the other terms have their usual meanings. The relationship between conductance and symmetrical $Cl^{-}$ activity was fitted using the Michaelis-Menten equation $y = y_{max}/(1 + [K_m/[Cl^{-}]]$), where $y$ is conductance, $y_{max}$ the saturating conductance of the channel, $K_m$ the apparent affinity of the channel for $Cl^{-}$ ions, and ([$Cl^{-}$]) the $Cl^{-}$ activity calculated using the Debye-Hückel theory (Robinson and Stokes, 1970). Single channel experiments were performed at room temperature ($\sim$22°C) unless otherwise indicated.

**Results**

Fig. 1, A and B show cell-attached recordings of CFTR channels at 37 and 22°C. The current–voltage (i/V) relationships were essentially linear at positive applied membrane voltages, but outwardly rectified at hyperpolarizing potentials (Fig. 1 C). Rectification under these cell-attached conditions presumably reflects (a) the low intracellular $Cl^{-}$ activity anticipated from ion-selective microelectrode studies in other cells, and (b) voltage-
dependent fast block of CFTR by large intracellular anions (Linsdell and Hanrahan, 1996b).

When patches were excised and bathed symmetrically with 150 mM NaCl solutions at different holding potentials (Fig. 2 A), single channel conductance was 7.0 ± 0.1 pS. Using different patches to test each Cl⁻ concentration, the i/V relationship remained linear in the range ±80 mV as Cl⁻ activity was varied symmetrically (Fig. 2 B). Activity coefficients for Cl⁻ in 15, 30, 45, 75, 150, and 400 mM NaCl solutions were estimated by the modified Debye-Hückel theory to be 0.93, 0.85, 0.87, 0.79, 0.75, and 0.69, respectively (Robinson and...
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Stokes, 1970). Plotting mean CFTR conductance at each Cl$^-\,$ activity yielded a rectangular hyperbola that was well described by the Michaelis-Menten equation when $K_m = 37.6 \, \text{mM Cl}^-\,$ activity and $G_{\text{max}} = 10.0 \, \text{pS}$ (Fig. 2 C).

The conductances reported here are consistent with previous reports from this laboratory, although values in the literature vary over a considerable range (4–12 pS), presumably due to different experimental conditions. HEPES and some other N-substituted taurine buffers are known to inhibit the conductance of anion channels (e.g., Hanrahan and Tabcharani, 1990); therefore, we compared i/V curves obtained in solutions buffered symmetrically by the zwitterionic buffers TES and HEPES with those obtained when solutions were weakly buffered using the cationic buffer TRIS (1 mM; Fig. 3). TES and HEPES at concentrations typically used in patch clamp experiments (10–40 mM) reduced single channel conductance by $\sim 14\%$. This effect was small compared with the inhibition of the outwardly rectifying anion channel that would occur with the same concentration of HEPES ($\sim 65\%$; Hanrahan and Tabcharani, 1990). TES was chosen as the pH buffer in subsequent studies because it is a stronger buffer at pH 7.4 and has low reactivity compared with TRIS; however, it should be noted that CFTR conductances reported in this paper will be reduced at all potentials. The conductance of the CFTR channel bathed symmetrically with 154 Cl$^-\,$ solutions was increased at 37$^\circ\text{C}$ as reported previously for endogenous CFTR channels on T$_{84}$ cells (Tabcharani et al., 1990), and the i/V relationship remained linear (not shown).

CFTR channels were selective for Cl$^-\,$ over Na$^+\,$ according to dilution potential measurements with a NaCl gradient (not shown). Single channel currents reversed at $-27.8 \pm 0.7 \, \text{mV} \,(n = 4)$ with 150 mM NaCl in the pipette and 40 mM NaCl $+ 220 \, \text{mM sucrose}$ in the bath solution, which yields $P_{\text{Na}}/P_{\text{Cl}} = 0.028$. The zero current potential was $+30.0 \pm 0.9 \, \text{mV} \,(n = 2)$ when the orientation of the salt gradient was reversed, which gave $P_{\text{Na}}/P_{\text{Cl}} = 0.003$. These ratios are too low to be measured accurately but are generally consistent with the values reported previously (0.125, Tabcharani et al., 1990; 0.04–0.07, Tabcharani et al., 1991; 0.01–0.16, Anderson et al., 1991; and 0.2, Bear and Reyes, 1992).

Permeation by external Br$^-\,$ and F$^-\,$ was studied under biionic conditions at 22$^\circ\text{C}$. Bromide currents were clearly observed at positive potentials with 150 mM NaBr solution in the pipette and 150 mM NaCl solution in the bath (Fig. 4 A). The reversal potential was shifted from 0 to $-5.2 \, \text{mV}$ under these conditions, indicating a permeability ratio $P_{\text{Br}}/P_{\text{Cl}} = 1.22$. Nevertheless, the mean slope conductance between $+60$ and $+80 \, \text{mV}$ suggested the ratio $G_{\text{Br}}/G_{\text{Cl}} = 0.48$. External Br$^-\,$ had no apparent effect on Cl$^-\,$ flow from the cytoplasmic side. With NaF solution in the pipette, large negative currents ($\sim 0.2 \, \text{pA}$) carried by outward Cl$^-\,$ flow were observed at 0 mV (Fig. 4 A). These Cl$^-\,$ currents decreased in amplitude as the potential was increased, and became undetectable when it approached $+50 \, \text{mV}$ (<0.05 pA). External F$^-\,$ was not measurably permeant; therefore, a reversal potential could not be determined. Extrapolation of the i/V curve suggests an upper limit for the permeability ratio ($P_{\text{F}}/P_{\text{Cl}} < 0.1$). Ex-
external F\(^-\) also inhibited outward Cl\(^-\) flow at negative potentials (Fig. 4 B).

Fig. 5 A shows single channel currents carried by Br\(^-\) under biionic conditions with Br\(^-\) on the cytoplasmic side. The i/V relationship was outwardly rectified and reversed at +5.8 ± 3.5 mV (Fig. 5 B), indicating P\(_{\text{Br}}\)/P\(_{\text{Cl}}\) = 1.30. Despite the high permeability ratio, slope conductances at negative potentials suggested G\(_{\text{Br}}\)/G\(_{\text{Cl}}\) = 0.48, similar to the ratio obtained with external Br\(^-\)\. The i/V relationship at 37°C with internal Br\(^-\) reversed at +8.3 ± 1.3 mV, indicating P\(_{\text{Br}}\)/P\(_{\text{Cl}}\) = 1.36 (data not shown). Replacing NaCl in the bath with NaF shifted the reversal potential to negative potentials, indicating low F\(^-\) permeability (Fig. 5 B). However, in contrast to the results obtained with external F\(^-\), currents carried by internal F\(^-\) could be observed at appropriate voltages (Fig. 5 B). The i/V relationship determined under these biionic conditions reversed at −46.7 mV, indicating P\(_{\text{F}}\)/P\(_{\text{Cl}}\) = 0.17. Comparing the slope conductances at negative voltages when patches were bathed with symmetrical Cl\(^-\) or with biionic conditions with F\(^-\) in the bath and Cl\(^-\) in the pipette yielded G\(_{\text{F}}\)/G\(_{\text{Cl}}\) ≈ 0.5.

The P\(_{\text{F}}\)/P\(_{\text{Cl}}\) ratios that have been reported for CFTR vary over a wide range. Most whole-cell patch clamp and cell monolayer studies have indicated that the P\(_{\text{F}}\)/P\(_{\text{Cl}}\) of CFTR-mediated conductance is between 0.4 and 0.6.
Indeed, $P_I < P_{Cl}$ has been used as a diagnostic property for distinguishing CFTR-mediated conductance from those mediated by other $Cl^-$ channels, such as the outwardly rectifying anion channel. Nevertheless, several studies of CFTR have described substantially higher $I^-$ permeability (e.g., $P_I/P_{Cl} \approx 1.0$, Gray et al., 1990; 1.1, Kartner et al., 1991; 1.9, Tabcharani et al., 1992; 1.3, Dousmanis and Gadsby, 1994).

We examined $I^-$ permeation under biionic conditions and found that the current–voltage relationship was strongly dependent on the voltage protocol. With NaI solution in the pipette, large currents carried by inward $I^-$ flow were initially observed at positive potentials and could be recorded up to $\sim 2 \text{ min}$ (Fig. 6 A). These iodide currents became smaller as the holding potential was made increasingly negative and reversed polarity near $-20 \text{ mV}$, indicating $P_I/P_{Cl} = 2.1$ (Fig. 6 B). However, when the membrane potential was subsequently repolarized after briefly recording $Cl^-$ currents at negative potentials, the relationship clearly diverged from the initial $i/V$ curve; i.e., negative currents (carried by outward $Cl^-$ flow) were clearly observed at 0 mV, but positive currents (carried by $I^-$) could no longer be recorded (Fig. 6 B; $n = 3$–6 patches). The channel remained in this low $I^-$ permeability state as long as $I^-$ was present, but $Cl^-$ currents reappeared when $I^-$ was washed from the bath. The reappearance of $Cl^-$ currents occurred with a time course that was too fast to be resolved with these methods. Extrapolation of the second $i/V$ curve suggested a reversal potential greater than $+20 \text{ mV}$; i.e., $P_I < P_{Cl}$, although this reversal could not be measured. We refer to the initial, high $I^-$ permeability state as unblocked ($I_{unbl}$), and the low $I^-$ permeability state that develops later as blocked ($I_{bl}$). The switch from $I_{unbl}$ to $I_{bl}$ could not be distinguished during recordings at negative potentials because the amplitudes of negative currents were the same in both states. The switch to low iodide permeability was more rapid at voltages that would drive $Cl^-$ into the pore and was observed in every experiment. Thus, $I^-$ currents were observed at positive potentials for 0.5–2 min before the channel switched to the $I_{bl}$ state. By contrast, $I^-$ currents became blocked within seconds at the same potentials during test pulses from a negative holding potential where $Cl^-$ ions would carry the current. Fig. 7 shows an example of a patch excised into $Cl^-$-containing bath solution exposed to $I^-$ solution in the pipette. Iodide currents disappeared when channels were held at $V_m = +20 \text{ mV}$, although $Cl^-$ currents were observed during steps to negative potentials (data not shown).

Similar hysteresis and block were observed with $I^-$ on the cytoplasmic side (Fig. 8). Iodide currents could be recorded for several minutes with $I^-$ in the bath and $Cl^-$ in the pipette if patches were excised while holding the membrane at negative potentials. When the potential was increased, the resulting $i/V$ relationship was outwardly rectified and reversed near $+16 \text{ mV}$, indicating $P_I/P_{Cl} = 1.8$, but transitions abruptly switched from the unblocked ($I_{unbl}$) state to the “blocked” ($I_{bl}$) state have $\sim 40\%$ lower conductance. Entering $I_{bl}$ coincided with a negative shift in the (extrapolated) reversal potential to at least $-20 \text{ mV}$, consistent with a large decrease in relative iodide permeability to $P_I/P_{Cl} \geq 0.4$ ($n = 6$–10), similar to that reported in many previous studies of macroscopic $I^-$ conductance (e.g., Anderson et al., 1991; Bell and Quinton, 1992). Each point in Fig. 8 is the mean of 3–10 observations; however, points were collected from at least 20 patches because the unblocked state was transient and not all $i/V$ points could be obtained from each patch. It was not possible to

![Figure 6](image-url)
record iodide currents after the switch between $I_{bl}$ and $I_{unbl}$ had occurred; however, the number of CFTR channels observed before and after were always similar. When channels had been allowed to enter the $I_{bl}$ state and the bath solution containing NaI, MgATP, and PKA was completely replaced by NaCl, transitions initially disappeared, consistent with the known dependence of CFTR gating on ATP. Restoring MgATP and PKA to the NaCl solution reactivated $I_{unbl}$ transitions, allowing Cl− currents to be recorded at both positive and negative holding potentials.

The switch from high to low I− permeability was observed whenever I− was present on one side of the membrane, and was enhanced by holding at potentials where the current would be carried by Cl−. Thus, it may depend on the entry of Cl− ions into the pore. The switch was not instantaneous since I− currents were sometimes observed for a few seconds after pulses had been applied to drive Cl− through the channel, as if I− or Cl− were binding at a site in the pore where permeating anions are not required to move in single file, preventing permeation by I− but allowing Cl− to pass through. This behavior was not symmetrical since Cl− flow from the “trans” side was reduced by internal (Fig. 6) but not external (Fig. 8) iodide.

The switch from $I_{bl}$ to $I_{unbl}$ was dependent on ionic strength (Fig. 9; see also Tabcharani et al., 1992). When biionic (NaCl/Nal) experiments were carried out using salts at 400 rather than 150 mM, only the high I− permeability state was observed. With external I− and internal Cl−, the mean reversal potential was $−19.6 ± 0.2$ mV at 400 mM, compared with $−18.1 ± 0.01$ mV at 150 mM in the $I_{unbl}$ state. With internal I−, the corresponding reversal potentials were $17.5 ± 0.9$ mV and $15.5 ± 1.4$ mV, respectively. Both internal and external I− induced outward rectification under biionic conditions (Fig. 9).

Thus, $G_{I} > G_{Cl}$ when I− was present externally, whereas $G_{I} < G_{Cl}$ when I− was present intracellularly. This discrepancy only occurred when I− and Cl− were present on opposite sides of the membrane; the i/V curve with symmetrical I− solutions was linear and yielded a conductance $(5.4 ± 0.15$ pS, $n = 4$) that was slightly lower than with symmetrical Cl− solutions (Fig. 10). The reversal potentials and biionic permeability ratios for wild-type CFTR are summarized in Table I.

**Role of Arg347 in I− Permeation**

Previous studies have suggested that the sixth membrane spanning region of CFTR lines the pore (Anderson et al., 1991; Sheppard et al., 1993; Tabcharani et al., 1993; McDonough et al., 1994; Cheung and Akabas, 1996; Linsdell and Hanrahan, 1996a) and that the arginine at position 347 may form part of an anion binding site. Mutations that remove the positive charge at this site eliminate voltage-dependent block by internal thiocyanate and abolish the anomalous mole fraction effects that are normally observed in mixtures of Cl− and thiocyanate (Tabcharani et al., 1993). To assess the possible role of this residue in I− permeation and block, we studied the I− selectivity of a mutant in which this residue was converted to aspartate (R347D). Fig. 11 compares the i/V relationships obtained when the mutant channel was bathed with symmetrical Cl− or with external Cl− and internal I−. In symmetrical Cl− solutions, the i/V relationship was linear and conductance was reduced by ~60%, as reported previously (Tabcharani et al., 1993). With I− on the cytoplasmic side, the reversal potential was $+1.0$ mV, consistent with an equilibrium permeability ratio $P_{I}/P_{Cl} = 0.96$. Despite this loss of selectivity between I− and Cl−, the mutant resembled the wild-type channel in having low I− conductance and I− inhibition of Cl− flow from the “trans” side. Most significantly, the permeability ratio $P_{I}/P_{Cl}$ was not protocol dependent in the R347D mutant. Iodide currents through R347D could be measured indefinitely after Cl− currents had been allowed to flow for several minutes. The inability of R347D to distinguish between I− and Cl− ions suggests arg347 may contribute to the selectivity filter.

**Discussion**

Comparing the selectivity of whole cell currents generated by wild-type and mutant CFTR helped establish that CFTR forms a Cl− conductive pathway (Anderson et al., 1991). In studies of macroscopic anion conductances, low $P_{I}/P_{Cl}$ ratios in the range 0.4–0.6 have be-
come a hallmark of cAMP-stimulated Cl\(^-\) currents, and have been used to distinguish CFTR-mediated currents from those activated by calcium and cell swelling (Cliff and Frizzell, 1990). Nevertheless, there is confusion at the single channel level and values for P\(_I\)/P\(_{Cl}\) of zero (Champigny et al., 1990; Tabcharani et al., 1992) and 1.0–1.8 have been reported (Gray et al., 1990; Kartner et al., 1991). The present study shows that P\(_I\)/P\(_{Cl}\) is strongly protocol dependent, as if I\(^-\) were binding in the pore when Cl\(^-\) is also present, thereby reducing Cl\(^-\) current in one direction and blocking I\(^-\) flux in the other. This “low iodide permeability” state is not observed when measuring ratios for other anions; therefore, the P\(_I\)/P\(_{Cl}\) ratio for this state may not be comparable with P\(_{Br}\)/P\(_{Cl}\) and P\(_F\)/P\(_{Cl}\) determined in the absence of I\(^-\). We propose that the high initial iodide permeability in the I\(_{unbl}\) state is comparable with ratios for the other anions, and the selectivity sequence for CFTR is P\(_I\) > P\(_{Br}\) > P\(_{Cl}\) > P\(_F\); i.e., the lyotropic series observed in many other anion channels. This would be consistent with the high permeability of CFTR to lyotropic polyatomic anions such as SCN\(^-\) reported previously (Gray et al., 1989; Tabcharani et al., 1993).

**CFTR Conductance**

The conductance of CFTR channels (6 pS at 22°C) recorded on CHO cells at the membrane potential, which was not measured during these experiments, is similar to that reported previously in single channel studies of human pancreatic (Gray et al., 1989), intestinal (Tabcharani et al., 1990; Bear and Reyes, 1992; Bajnath et al., 1993), and airway epithelial cells (Haws et al., 1992) expressing endogenous CFTR, and on a variety of heterologous expression systems (e.g., Bear et al., 1991; Berger et al., 1991; Kartner et al., 1991; Tabcharani et al., 1991; Cliff et al., 1992; Sheppard et al., 1993; Venglarik et al., 1994).

The conductance of recombinant CFTR channels at 37°C in this study is similar to previous measurements on T84 (8.6 pS cell-attached configuration; Tabcharani et al., 1990) and CHO cells (9.6 pS excised; Tabcharani et al., 1991) and on NIH-3T3 fibroblasts (8.5 pS cell-attached, Fischer and Machen, 1994; 10.1 pS excised, Carson et al., 1994). Outward rectification in cell-attached patches was originally attributed to the Cl\(^-\) concentration gradient between the pipette solution and cytoplasm because i/V relations became linear when patches were excised into symmetrical Cl\(^-\) solutions (Gray et al., 1989; Tabcharani et al., 1991). Moreover, when CFTR was studied in SF9 cells infected with a recombinant Baculovirus containing the CFTR gene, both the membrane and reversal potentials for CFTR currents approached 0 mV, and the i/V became linear, suggesting that elevation of cell Cl\(^-\) under these conditions was sufficient to eliminate rectification (Kartner et al., 1991). More recent studies indicate CFTR can be blocked by large anions from the cytoplasmic side in a voltage-dependent manner (Linsdell and Hanrahan, 1996b), thus flickery block by large intracellular anions may contribute to the rectification seen in the cell-attached patches. Commonly used pH buffers such as HEPES and TES caused a voltage-independent decrease in single channel conductance; however, differences in temperature are considered the most likely explanation for the range of conductances reported for CFTR. There may also be some variation in the conductance of CFTR homologues in other species (e.g., Gray et al., 1988; Riordan et al., 1994).

The dependence of conductance on Cl\(^-\) activity was well fitted by the Michaelis-Menten relationship without including a term for surface charge effects (see also...
Linsdell et al., 1997b). Interestingly, the $K_m$ estimated from the fits is similar to the intracellular $\text{Cl}^-$ activity measured previously in secretory epithelia (e.g., Welsh et al., 1982). Thus, like many enzymes, the affinity of CFTR is close to its substrate concentration. A similar series of experiments carried out to assess the effects of mutations in the sixth membrane spanning region (TM6) yielded similar values for $K_m$ and $G_{\text{max}}$ (Linsdell et al., 1997b), and were used when fitting barrier models (Linsdell et al., 1997a).

**Anion–Cation Selectivity**

CFTR was highly selective for $\text{Cl}^-$ over sodium ($P_{\text{Na}}/P_{\text{Cl}^-} < 0.028$). This ratio is somewhat lower than reported previously (Tabcharani et al., 1990; Anderson et al., 1991; Bear, 1992), but is consistent with the observation that CFTR conductance and permeability ratios are not affected when sodium is replaced by $N$-methyl-$d$-glucamine or tetramethylammonium (Gray et al., 1988; Kartenner et al., 1991; Bear, 1992). This insensitivity of $\text{Cl}^-$ conductance to the nature of the cation suggests the permeation mechanism in CFTR differs from that described for a neuronal $\text{Cl}^-$ channel, where cations permeate relatively well and are proposed to form ion pairs with permeating $\text{Cl}^-$ ions in the pore (Franciolini and Non

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**Figure 9.** Comparison of current–voltage relationships obtained under biionic conditions with external or internal iodide solution. A and B show results obtained with external Na and internal NaCl solutions, when pipette and bath solutions were 400 and 150 mM, respectively. C and D show biionic current–voltage relationships obtained with intracellular $I^-$ when solutions were 400 or 150 mM, respectively.

**Table 1**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Reversal potential</th>
<th>Permeability ratio ($P_{\text{Na}}/P_{\text{Cl}^-}$)</th>
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<td>$\text{Cl}^-$</td>
<td>0</td>
<td>1.00</td>
<td>8</td>
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<tr>
<td>$\text{Br}^-$</td>
<td>−5.23 ± 1.04</td>
<td>1.22 ± 0.05</td>
<td>4</td>
</tr>
<tr>
<td>$\text{F}^-$</td>
<td>+5.84 ± 3.53</td>
<td>1.26 ± 0.17</td>
<td>4</td>
</tr>
<tr>
<td>$\text{I}^-$</td>
<td>—</td>
<td>&lt;0.04</td>
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<tr>
<td>$I_{\text{G}(\text{unbl})}$</td>
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<td>2.00 ± 0.11</td>
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<tr>
<td>$I_{\text{G}(\text{bl})}$</td>
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</tr>
<tr>
<td>$I_{\text{G}(400 \text{mM})}$</td>
<td>−19.60 ± 0.20</td>
<td>2.08 ± 0.04</td>
<td>6</td>
</tr>
<tr>
<td>$I_{\text{G}(\text{unbl})}$</td>
<td>+15.50 ± 1.42</td>
<td>1.82 ± 0.10</td>
<td>6</td>
</tr>
<tr>
<td>$I_{\text{G}(34)}$</td>
<td>—</td>
<td>&lt;0.04</td>
<td>10</td>
</tr>
<tr>
<td>$I_{\text{G}(400 \text{mM})}$</td>
<td>+17.50 ± 0.90</td>
<td>1.9 ± 0.06</td>
<td>6</td>
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</tbody>
</table>

*Solution containing $\text{Cl}^-$ at the same concentration as the test anion bathed the opposite side; $\text{unbl}$ refers to the unblocked, high iodide permeability state; $\text{bl}$ is the $I^-$-blocked state; $\text{400}$ refers to biionic conditions with 400-mM salt concentrations bathing both sides.
Br̄ was present on the extracellular or cytoplasmic side. It is similar to the ratio estimated previously in cell-attached patches when pipette Cl− was partially replaced by Br− (PBr/PCl = 1; Gray et al., 1990). The rectifying i/V relations indicate low Br̄ conductance, although the ratio calculated at these potentials (GBr/GCl = 0.48) can be only a first approximation if Br̄ permeation is affected by Cl− on the opposite side (see below). That such "trans" effects occur in CFTR is evident from the inhibition of outward Cl− flow by external F− (Fig. 4), and from the discrepancy between values of Gs/GCl determined using asymmetrical solutions vs. biionic conditions. Low Gbr/Gcl ratios have been reported for single channels under nonbiionic conditions (Gray et al., 1990), but were not observed in whole cell experiments under biionic conditions (Anderson et al., 1991). Although the present results do not explain the high Gbr/Gcl ratios in the latter study, they suggest that the discrepancy was not caused by the use of biionic conditions. The high permeability ratio and low conductance ratio suggest that Br̄ ions are slowed during permeation by binding within the pore.

Fluoride currents and a bionic reversal potential were observed with internal, but not external F−. Anderson et al. (1991) reported Pf/Pcl ratios of 0.11 for the apical membrane of T84 cells, and 0.3 for whole cell currents in transfected NIH 3T3 cells. Dousmanis and Gadsby (1994) reported ratios of 0.2–0.3 for PKA-stimulated whole-cell Cl− current in cardiac myocytes. In attempting to reconcile these data, we note that whole cell measurements are expected to be more susceptible to contamination by parallel pathways for F− movement. On the other hand, single channel recordings are more likely to underestimate permeability when the ion inhibits gating or has very low permeability. Our inability to measure currents carried by external F− may indeed reflect block by external F−, since Cl− currents are also reduced by external F−.

Evidence for Two States Having Different Iodide Permeabilities

Early studies of CFTR channels on pancreatic duct, which involved partial replacement of pipette Cl− with I−, suggested that Pf/Pcl is near 1.0 (Gray et al., 1990). Ratios ≥1 were obtained in early studies of recombinant CFTR (1.2, Kartner et al., 1991; 1.7, Tabcharani et al., 1992) and in several cell types that express CFTR endogenously (1.51, Chan et al., 1992; 1.31, Walsh and Long, 1992; 1.29, Dousmanis and Gadsby, 1994). Nevertheless, most permeability ratios obtained from macroscopic currents have been <1.0 (e.g., 0.4, Cliff and Frizzell, 1990; 0.5–0.6, Anderson et al., 1991; 0.4–0.6, Anderson and Welsh, 1991; 0.5, Haws et al., 1992; 0.6, Bear and Reyes, 1992; 0.9, Bell and Quinton, 1992; 0.9, Overholt et al., 1993). The present results indicate that

Bromide and Fluoride Permeation

The Br−/Cl− permeability ratio for CFTR was high under biionic conditions (1.3) and was identical whether
the I⁻ permeability ratio depends on the protocol used and that both high and low values can be observed in the same CFTR channel. Thus, I⁻ currents were recorded for several minutes when patches were excised and held at potentials that would drive I⁻ through the channel. Under these conditions, the i/V relationship indicated unequivocally that P₁ > P₃Cl. After a variable time interval at negative potentials, the channel switched to a different conductance state in which P₁ < P₃Cl and I⁻ currents disappeared at positive potentials. This state, which we shall call I₃bl, had an extrapolated reversal potential indicative of P₃Cl/PI << 1, although the ratio could not be determined because I⁻ currents were not observed. Impermeability to iodide would agree with some previous single channel studies (Champigny et al., 1990; Cliff et al., 1992). Iodide alone did not induce the I₃bl state, which did not occur when the channel was bathed in symmetrical I⁻ solutions. The conductance in symmetrical I⁻ solutions was 77% of that measured with symmetrical Cl⁻ solution (Fig. 10). Low iodide permeability was not caused by oxidized forms of I⁻ such as I₃⁻ or IO₃⁻ (Läuger et al., 1967; Finkelstein and Cass, 1968), since it was not affected by addition of the reducing agent thiosulfate (20 mM) to the I⁻ solution (data not shown). The switch from P₁ > P₃Cl to P₁ < P₃Cl was enhanced at potentials that would favor Cl⁻ entry into the channel, and once the switch occurred, it was not overcome by reversing the voltage to again drive I⁻ through the channel.

Mutating Arg³⁴⁷ Abolishes the Switch to Low Iodide Permeability

A gross alteration in pore structure (e.g., induced by the simultaneous presence of both Cl⁻ and I⁻) seems unlikely to explain the low I⁻ permeability of I₃bl state, considering that Cl⁻ permeation from the outside was only partially reduced and Cl⁻ permeation from the inside was unaffected. When studied only from the inside after the switch to I₃bl, iodide behaved as if it were an impermeant anion. We do not know the precise mechanism of the switch in permeability, but suggest that Cl⁻ or I⁻ binds in a non–single file region of the pore, preventing I⁻ permeation but allowing Cl⁻ ions to pass through. The notion that the delayed block of I⁻ currents involves Cl⁻ entry and ion–ion interactions within the pore is strengthened by the finding that a mutation eliminates anomalous mole fraction behavior in mixtures of Cl⁻ and thiocyanate also abolished the switch from I₃unbl to I₃bl.

Cl⁻ currents were restored as quickly as measurements could be made after replacing the I⁻ solution bathing one side of the patch with Cl⁻ solution (i.e., within a few seconds; data not shown). Since P₁/P₃Cl for I₃bl and I₃unbl encompass the range of values reported previously, the intermediate ratios that have been obtained in studies of macroscopic currents may have been due to different proportions of channels in each permeability state. The switch to the I₃bl state and the apparent decrease in P₁/P₃Cl were abolished when salt concentrations were increased from 150 to 400 mM NaCl/NaI (Fig. 9). Whether this reflects concentration-dependent multi-ion interactions in the pore (e.g., Friel and Tsien, 1989) remains to be determined.

**CFTR Has a "Weak Field Strength" Selectivity Sequence**

Information on anion selectivity from a large number of biological and nonbiological systems has been used to construct empirical selectivity isotherms (Wright and Diamond, 1977). Based on 81 quantitative anion sequences from various systems, only 5 of 24 possible sequences were observed. Selectivity was modeled by calculating the difference between the free energy of hydration of the anions and electrostatic interactions between each anion and model sites (Eisenman, 1962). Once the equilibrium permeability ratio P₁/P₃Cl was known for a particular system, the ratios for the other two halides could be predicted using the isotherms (Wright and Diamond, 1977). As shown in Fig. 12 A, this holds true for CFTR, but only when P₁/P₃Cl of the I₃unbl state is considered. When CFTR switched to low I⁻ permeability, the “Type I” sequence (P₁ > P₃Cl > P₅) was changed to one that does not resemble any of the known sequences, although it is most consistent with a “moderate field strength” Type III sequence (P₁ > P₃Cl > P₅ > P₃). Whereas low P₁/P₃Cl was only observed in the I₃bl state induced by I⁻, P₅/P₃Cl and P₅/P₃Cl were determined in the complete absence of I⁻ when the channel would presumably be in the I₃unbl state. Thus, the validity of combining permeability ratios obtained in these different states is questionable and we propose that only the high initial P₁/P₃Cl ratio obtained immediately after exposure to iodide can be compared with bionic P₅/P₃Cl and P₅/P₃Cl ratios.

**Arg³⁴⁷ May Contribute to a Weak Field Strength Site for Iodide**

High macroscopic P₁/P₃Cl ratios have been reported previously for CFTR channels in which positively charged residues in the membrane spanning regions were mutated to negatively charged residues (K95E, 1.43; K335E, 1.37; R347E, 0.9; R1030E, 0.81; Anderson et al., 1991). The P₁/P₃Cl ratio obtained for R347D under bionic conditions is intermediate between I₃unbl and I₃bl in the wild-type channel, and is consistent with the value of 0.9 reported previously for R347E (Anderson et al., 1991). This suggests that arg³⁴⁷ may play some role in selecting among anions. Arginine has several properties that favor the binding of lyotropic anions. Studies with the model compound n-propylguanidine indicate that arginine is by far the most hydrophilic amino acid, with a partition coefficient between water and vapor phases
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Using a low value of $\text{PI/PCl}$, they do not fit any of the anion site. On the other hand, when ratios for other anions are plotted reasonably well and indicate a Type I (low field strength) selectivity after mutation of arg347 suggests it might contribute by resonance amongst the carbon and all three nitrogen atoms of the $\delta$-guanido group. The very large solvent-accessible surface area of the arginine side chain ($196 \, \text{Å}^2$) would be expected to further weaken the field strength of this diffuse charge. The resonance of electrons in the $\delta$-guanido group of arginine would favor induction of a cationic dipole. The relative polarizability of halide crystals (Tessman et al., 1953) follows the sequence $\Gamma^- (2.17) > \text{Br}^- (1.41) > \text{Cl}^- (1.0) > \text{F}^- (0.22)$, almost identical to the selectivity sequence for CFTR reported in this paper. Because the side chain and anion are both easily induced to form dipoles, dispersion forces may be important in stabilizing the interaction between the arginine residue and lyotropic anions.

Halide selectivity in CFTR cannot be attributed exclusively to the region around arg347, however, because high $P_I/PCl$ ratios have been reported previously for other pore mutants (K95D and K335E; Anderson et al., 1991), and because R347D retains some preference for Br$^-$ over Cl$^-$ and selects strongly against F$^-$ (our unpublished observations). $P_I/PCl$ was altered by a mutation that abolished the anomalous mole fraction effect (AMFE, R347D); however, these properties are not strictly correlated because K335E also had high $P_I/PCl$ in a previous study (Anderson et al., 1991) and yet displays an AMFE in SCN$^-\text{Cl}^-$ mixtures (Tabcharani et al., 1993).

Lyotropic Selectivity in CFTR

The sequence $\Gamma^- > \text{Br}^- > \text{Cl}^- > \text{F}^-$ is part of the lyotropic series first reported by Hofmeister (1888). It is observed when the effects of anions on protein solubility, anion adsorption to lipid bilayers, and surface potentials at air–water interfaces are characterized (for review of early literature, see Dani et al., 1983). Studies with model compounds indicate that such sequences require both an anion-attracting positive charge or dipole and a neighboring hydrophobic group. The fact that many anion channels have nearly identical halide sequences suggests that selectivity may be determined by a common structural element, such as the cationic dipole of the peptide bond, rather than a specific amino acid side chain or arrangement of amino acids. Binding of lyotropic anions to the amide dipole of the peptide backbone in proteins has been proposed previously (Robinson and Jencks, 1965) and amide dipoles in solution are anion attracting and confer lyotropic anion selectivity (Hamabata and Von Hippel, 1973). Lyotropic selectivity also requires a neighboring hydrophobic region where extensive hydrogen bonding between water molecules causes them to be more structured. Preferential association of lyotropic anions such as I$^-$ at these sites will be solvent driven when it is energetically more favorable for water molecules (which would interact only weakly with I$^-$) to hydrogen bond with this iceberg network on the hydrophobic surface than to associate with the I$^-$ ion. Lyotropic anions bind to lipid bilayers and generate negative surface charge (McLaughlin et al., 1975). If similar binding occurs at

![Figure 12](Image)

**Figure 12.** Evidence that the CFTR Cl$^-$ channel in the $I_{\text{unbl}}$ state has a low field strength selectivity sequence. (A) Selectivity isotherms replotted from Wright and Diamond (1977) with permeability ratios obtained for CFTR denoted by ○. Dashed vertical line at right shows that when ratios for other halides are plotted against the high (initial) $P_I/PCl$ obtained in this study, they follow the isotherms reasonably well and indicate a Type I (low field strength) site. On the other hand, when ratios for other anions are plotted using a low value of $P_I/PCl$, they do not fit any of the anion sequences that have been reported for other biological and nonbiological systems. (B) Relationship between unhydrated diameters of the halides ($F^- < Cl^- < Br^- < I^-$) and their respective permeability ratios (○). Also shown is the relationship between unhydrated diameter and hydration energy ($\Delta H$).
hydrophobic sites in the mouth of the pore, charge
screening might account for the loss of unusual effects
of iodide at 400-mM salt concentrations.

The finding that a large, weakly hydrated halide such as Br− permeates through CFTR more readily than a smaller anion such as F−, which has high hydration en-

nergy (ΔG_H2O; Fig. 12 B), suggests that the pore must be
sufficiently narrow to require at least partial dehydra-
tion of the anion. The following paper estimates the
functional diameter of single CFTR channels using
polyatomic anions of known dimensions.

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