

Bestrophin Cl^- channels are highly permeable to HCO_3^-

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Qu Z, Hartzell HC. Bestrophin Cl^- channels are highly permeable to HCO_3^- . *Am J Physiol Cell Physiol* 294: C1371–C1377, 2008. First published April 9, 2008; doi:10.1152/ajpcell.00398.2007.—Bestrophin-1 (Best1) is a Cl^- channel that is linked to various retinopathies in both humans and dogs. Dysfunction of the Best1 Cl^- channel has been proposed to cause retinopathy because of altered Cl^- transport across the retinal pigment epithelium (RPE). In addition to Cl^- , many Cl^- channels also transport HCO_3^- . Because HCO_3^- is physiologically important in pH regulation and in fluid and ion transport across the RPE, we measured the permeability and conductance of bestrophins to HCO_3^- relative to Cl^- . Four human bestrophin homologs (hBest1, hBest2, hBest3, and hBest4) and mouse Best2 (mBest2) were expressed in HEK cells, and the relative HCO_3^- permeability ($P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$) and conductance ($G_{\text{HCO}_3^-}/G_{\text{Cl}^-}$) were determined. $P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$ was calculated from the change in reversal potential (E_{rev}) produced by replacing extracellular Cl^- with HCO_3^- . hBest1 was highly permeable to HCO_3^- ($P_{\text{HCO}_3^-}/P_{\text{Cl}^-} = \sim 0.44$). hBest2, hBest4, and mBest2 had an even higher relative HCO_3^- permeability ($P_{\text{HCO}_3^-}/P_{\text{Cl}^-} = 0.6\text{--}0.7$). All four bestrophins had HCO_3^- conductances that were nearly the same as Cl^- ($G_{\text{HCO}_3^-}/G_{\text{Cl}^-} = 0.9\text{--}1.1$). Extracellular Na^+ did not affect the permeation of hBest1 to HCO_3^- . At physiological HCO_3^- concentration, HCO_3^- was also highly conductive. The hBest1 disease-causing mutations Y85H, R92C, and W93C abolished both Cl^- and HCO_3^- currents equally. The V78C mutation changed $P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$ and $G_{\text{HCO}_3^-}/G_{\text{Cl}^-}$ of mBest2 channels. These results raise the possibility that disease-causing mutations in hBest1 produce disease by altering HCO_3^- homeostasis as well as Cl^- transport in the retina.

bicarbonate transport; pH; retinal pigment epithelium; retinopathy

BESTROPHINS ARE A NEWLY IDENTIFIED family of membrane proteins that exhibit Cl^- channel activity and also function as regulators of voltage-gated Ca^{2+} channels (for review, see Ref. 10). Human bestrophin 1 (hBest1) was first identified as the gene responsible for Best vitelliform macular dystrophy (Best disease) (24, 28), but Best1 mutations have subsequently been shown to be associated with several other retinopathies in humans and dogs (10). The Best1 gene product is located in the basolateral membrane of the retinal pigment epithelium (RPE) (2, 21). It has been proposed that Best1-associated retinopathies are caused by Cl^- channel dysfunction because hBest1 mutations characteristically reduce electrooculogram (EOG) light peak, which reflects a basolateral Cl^- conductance in the RPE (7, 10), and because many disease-causing mutations alter the Cl^- channel function of hBest1 (40, 46, 47). However, an alternative view holds that hBest1 is not a Cl^- channel and that its ability to regulate voltage-gated Ca^{2+} channels is responsible for the disease (10, 22, 23, 35).

HCO_3^- is an important physiological anion that is involved in several physiological processes including pH regulation (4). Transmembrane movement of HCO_3^- is mediated by specific HCO_3^- transporters in many tissues including RPE (4, 7). Photoreceptors have a very high metabolic rate that produces large quantities of CO_2 and HCO_3^- (41). HCO_3^- is removed from the retina by transepithelial transport by the RPE (7), which is mediated at least partly by an electrogenic Na^+ - 2HCO_3^- cotransporter in the apical membrane of the RPE (11, 15, 16, 18) and a $\text{Cl}^-/\text{HCO}_3^-$ exchanger in the basolateral membrane (7).

Although many Cl^- channels are permeable to HCO_3^- , it is not known whether ion channels in RPE may also participate in HCO_3^- homeostasis. Anion channels such as CFTR, ClC , CaCC, and ligand-gated anion channels are permeable to HCO_3^- anions, but the HCO_3^- permeability is usually <25% of the Cl^- permeability (20, 31, 36, 43). As a potential anion channel in the basolateral membrane of the RPE, hBest1 could possibly be involved in movement of HCO_3^- from inside the RPE to the choroid (30, 39). In this study, we examined the permeability of bestrophins to HCO_3^- in transfected HEK293 cells. We found that bestrophins have a surprisingly high permeability and conductance to HCO_3^- anions. This conclusion suggests that disease-causing mutations in hBest1 may result in defective transport of both Cl^- and HCO_3^- . The loss of normal HCO_3^- transport by RPE may contribute to development of Best disease.

MATERIALS AND METHODS

Generation of mutations in bestrophins and heterologous expression. hBest1, hBest2, hBest3, and hBest4 cDNAs in pRK5 vectors were generously provided by Jeremy Nathans (John Hopkins University). Mouse (m)Best2 cDNA in pCMV-SPORT6 vector was purchased from the American Type Culture Collection (ATCC) (IMAGE clone ID: 4989959). Site-specific mutations in hBest1 and mBest2 were made with a PCR-based site-directed mutagenesis kit (Quick-change; Stratagene) as described previously (30). Bestrophins or hBest1 and mBest2 mutants were cotransfected into HEK293 cells (ATCC) with FuGene-6 transfection reagent (Roche) with pEGFP (Invitrogen) to identify transfected cells. Bestrophin wild-type or mutant cDNA (0.1–1.0 μg) was used to transfect one 35-mm culture dish. One day after transfection, cells were trypsinized and replated on glass coverslips for electrophysiological recording. Single transfected cells were used for patch-clamp experiments within 3 days after transfection.

Electrophysiology. Recordings were performed with the whole cell recording configuration of voltage patch clamp (26). Patch pipettes were made with borosilicate glass (Sutter Instrument), pulled by a Sutter P-2000 puller (Sutter Instrument), and fire polished. Patch pipettes had resistances of 2–3.5 M Ω (see below). The bath was

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grounded via a 3 M KCl⁻ agarose bridge connected to a Ag/AgCl⁻ reference electrode. Changes of chamber solutions were performed by perfusing a 1-ml chamber at a speed of ~4 ml/min. The chamber was covered, and 5% (for 30 mM HCO₃⁻ solutions) or 30% (for 140 mM HCO₃⁻ solutions) CO₂ in O₂ was blown between the cover and the chamber solution surface to keep the pH and PCO₂ constant. To produce a current-voltage (*I-V*) curve in response to changed extracellular anions, it was important to obtain data relatively quickly before intracellular anion concentrations changed significantly. To this end, 200-ms voltage ramps from -100 to +100 mV with a 10-s start-to-start interval were used instead of voltage steps. Because the currents are time independent, voltage ramps provide a reliable *I-V* relationship. Holding potential was 0 mV. Data were acquired by an Axopatch 200A amplifier controlled by Clampex 8.1 via a Digidata 1322A data acquisition system (Axon Instruments). Experiments were conducted at room temperature (22–24°C). Liquid junction potentials were calculated by using Clampex 8.1 to correct reversal potential (E_{rev}) of various ionic conditions. The standard pipette solution (high intracellular Ca²⁺ solution) contained (in mM) 146 CsCl, 2 MgCl₂, 5 Ca²⁺-EGTA, 8 HEPES, and 10 sucrose, pH 7.3, adjusted with *N*-methyl-D-glucamine (NMDG). The calculated Ca²⁺ concentration in the internal solution was 4.5 μM (30). The standard extracellular solution (150 mM Cl⁻ solution) contained (in mM) 140 NaCl, 4 KCl, 2 CaCl₂, 1 MgCl₂, 10 glucose, and 10 HEPES, pH 7.3 with NaOH. This combination of solutions set E_{rev} for Cl⁻ currents to zero, while cation currents carried by Na⁺ or Cs⁺ had very positive or negative E_{rev} , respectively. To change extracellular anions from Cl⁻ to HCO₃⁻, Cl⁻ was replaced on an equimolar basis with HCO₃⁻ (140 mM

HCO₃⁻/10 mM Cl⁻ solution). Solution osmolarity was 303–306 mosM for both intra- and extracellular solutions (Micro Osmometer model 3300, Advanced Instrument). Small differences in osmolarity were adjusted by addition of sucrose or NMDG-gluconate. In some cases, extracellular Cl⁻ was replaced on an equiosmolar basis with SO₄²⁻ (100 mM Na₂SO₄, 1 mM CaCl₂, 10 mM HEPES, pH 7.3), which is relatively impermeant through bestrophin Cl⁻ channels, to verify that the current was carried by Cl⁻ or HCO₃⁻. To maintain pH, 140 mM HCO₃⁻ solutions were bubbled with 30% CO₂ and 30 mM HCO₃⁻ solutions were bubbled with 5% CO₂. In addition, both solutions contained 10 mM HEPES, which also helped maintain the pH to some degree. Because it is difficult to maintain the pH of HCO₃⁻-buffered solutions, we monitored the pH at the level of the bath and found that pH was maintained in the range of 7.3–7.5. hBest1 currents were unaffected by this variation in pH. The NMDG⁺-HCO₃⁻ solution was prepared by bubbling NMDG⁺ solution with 100% CO₂ to pH 7.4.

Analysis of data. For the calculations and graphical presentation, we used OriginPro 7.0 software (Microcal). Data analyzed with Student's *t*-test are presented as means ± SE. HCO₃⁻ permeability relative to Cl⁻ ($P_{HCO_3^-}/P_{Cl^-}$) was determined by measuring the shift in E_{rev} on change of the bath solution from one containing 150 mM Cl⁻ to another containing 140 mM HCO₃⁻/10 mM Cl⁻ (31). The permeability ratio was estimated with the Goldman-Hodgkin-Katz equation: $P_{HCO_3^-}/P_{Cl^-} = \frac{[Cl^-]_i/[HCO_3^-]_o \exp(\Delta E_{rev}F/RT)}{[Cl^-]_o/[HCO_3^-]_i}$, where ΔE_{rev} is the difference between the reversal potential obtained with the HCO₃⁻ anion on one side of the cell and that observed with symmetrical Cl⁻ on both sides; [Cl⁻] and [HCO₃⁻] are Cl⁻ and

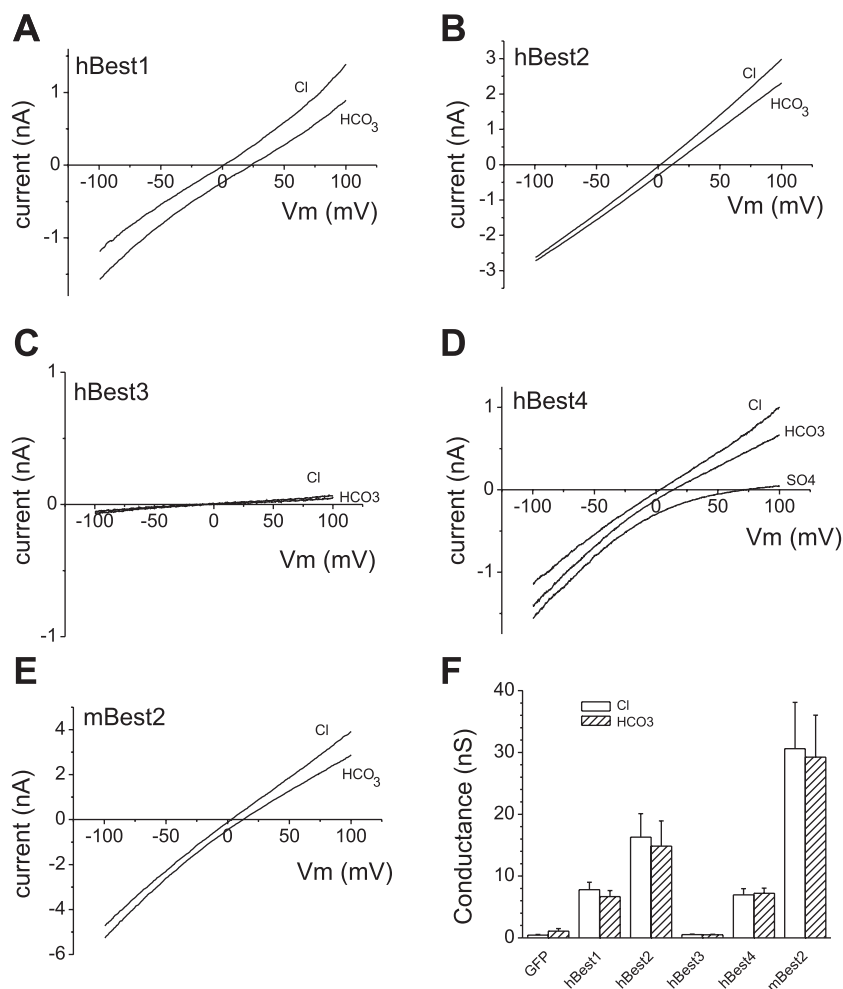


Fig. 1. Bestrophins are permeable to HCO₃⁻. Human (h)Best1 (A), hBest2 (B), hBest3 (C), hBest4 (D), or mouse (m)Best2 (E) cDNA in mammalian expression was cotransfected into HEK293 cells along with green fluorescent protein (GFP) to identify the transfected cells. The green cells were selected for whole cell voltage-clamp recording. Cells were stimulated by ramp voltages, and Cl⁻ currents were recorded with pipette solution containing high Ca²⁺ (see MATERIALS AND METHODS). For measurement of bestrophin permeabilities to HCO₃⁻ relative to Cl⁻, 140 mM NaCl in bath solution was replaced with 140 mM NaHCO₃ (see MATERIALS AND METHODS), which was bubbled with 30% CO₂ to maintain pH. A–E show representative cells ($n = 5–11$). SO₄²⁻ solution was used to check the presence of leak current in D. V_m , membrane potential. F: average values of slope conductances of bestrophin-expressed currents. hBest3+GFP- or GFP only-transfected cells showed negligible conductance to both Cl⁻ and HCO₃⁻.

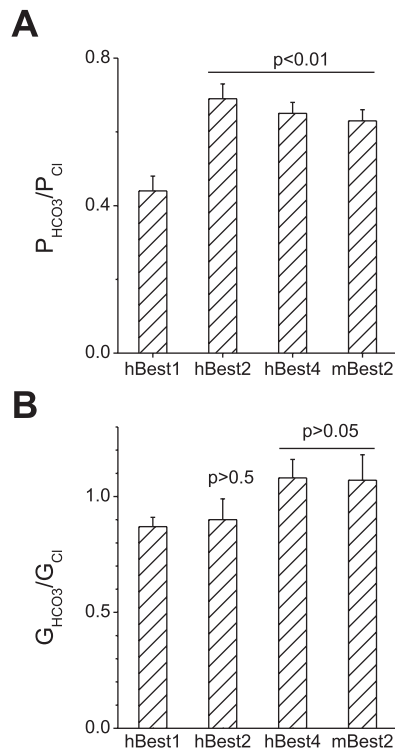


Fig. 2. Relative permeability and conductance of bestrophins to HCO₃⁻. **A**: relative permeabilities were determined by measuring the shift in reversal potential (E_{rev}) produced by switching from 140 mM Cl⁻ to 140 mM HCO₃⁻ and using the Goldman-Hodgkin-Katz equation to calculate relative permeability ($P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$; see MATERIALS AND METHODS). **B**: relative conductance ($G_{\text{HCO}_3^-}/G_{\text{Cl}^-}$) was measured as the slope of the current-voltage (I - V) curve at ± 25 mV around E_{rev} ($n = 5$ – 11). Statistical comparisons between hBest1 and other bestrophins are shown in **A** and **B**.

HCO₃⁻ concentrations; and subscripts i and o indicate intracellular and extracellular, respectively. F , R , and T are Faraday constant, temperature, and gas constant, respectively. HCO₃⁻ conductances relative to Cl⁻ ($G_{\text{HCO}_3^-}/G_{\text{Cl}^-}$) were obtained from the measurement of the slope of the I - V relationship between -25 and $+25$ mV from E_{rev} .

RESULTS

Bestrophins are highly permeable to HCO₃⁻ anions. Five bestrophins (hBest1, hBest2, hBest3, hBest4, and mBest2) that had previously been well characterized in heterologous cell systems (30, 32, 33, 39, 40) were selected for this study (Fig. 1). hBest3 exhibited tiny anion currents under the standard voltage protocol [as previously reported (29)] (Fig. 1C);

the other four bestrophins were highly conductive and permeable to HCO₃⁻. The slope conductance with HCO₃⁻ in the bath was very similar to the conductance with Cl⁻ in the bath, showing that Cl⁻ and HCO₃⁻ were transported equally well by bestrophins (Fig. 1F and Fig. 2B). E_{rev} was shifted to the right when 140 mM Cl⁻ in the bath was replaced with HCO₃⁻. This is consistent with HCO₃⁻ having a lower permeability than Cl⁻, but the shift was relatively small. $P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$ calculated from the shift in E_{rev} by the Goldman-Hodgkin-Katz equation was 0.44 ± 0.4 for hBest1, 0.69 ± 0.4 for hBest2, 0.65 ± 0.03 for hBest4, and 0.63 ± 0.3 for mBest2 (Fig. 2A). These values are remarkably high compared with other Cl⁻ channels. Most Cl⁻ channels have $P_{\text{HCO}_3^-}/P_{\text{Cl}^-} < 0.25$. HEK293 cells transfected with green fluorescent protein (GFP) only had negligibly small Cl⁻ or HCO₃⁻ conductances (Fig. 1F), indicating that Cl⁻ and HCO₃⁻ anions were conducted by transfected bestrophins.

Na does not affect hBest1 permeability to HCO₃⁻. Although we believe that HCO₃⁻ permeability is mediated by hBest1, another possibility is that hBest1 regulates some kind of electrogenic transporter, such as the slc4 Na⁺-HCO₃⁻ transporter family, that is responsible for the current. To determine whether the permeation of HCO₃⁻ was dependent on Na⁺, we examined the HCO₃⁻ current in a Na⁺-free solution consisting of NMDG⁺-HCO₃⁻. When the bath solution was changed to NMDG⁺-HCO₃⁻, the E_{rev} shifted toward the right about the same amount as with NaHCO₃ (Fig. 3A), indicating that the presence of Na⁺ did not exert a significant effect on HCO₃⁻ permeation. The calculated $P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$ and $G_{\text{HCO}_3^-}/G_{\text{Cl}^-}$ were statistically the same for NaHCO₃ and NMDG⁺-HCO₃⁻ (Fig. 3, B and C). The result supports the conclusion that hBest1 is permeable to HCO₃⁻ anions.

Effects of pH. There are two concerns about using HCO₃⁻ solutions. The first concern is maintenance of extracellular pH, because pH buffering depends on equilibration between the gaseous environment and the bathing solution. To be sure that pH was well controlled, we monitored extracellular pH at the bath and found that it was maintained within a range of 7.3–7.5. To determine whether changes in extracellular pH in this range might affect the hBest1 channel, we compared hBest1 currents recorded with HEPES buffer at pH 7.3 and *N*-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS) buffer at pH 8.1 (Fig. 4A). Within this range, the E_{rev} of hBest1 current was unaffected by extracellular pH and the current amplitude was reduced only $10.6\% \pm 2.6\%$ ($n = 5$). The second concern is that on switching from HEPES-buffered solution to HCO₃⁻-CO₂ solution, one might expect the cytosol-

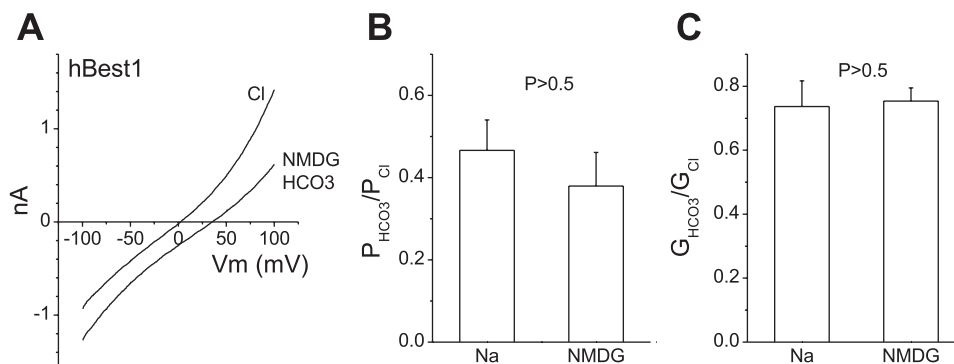
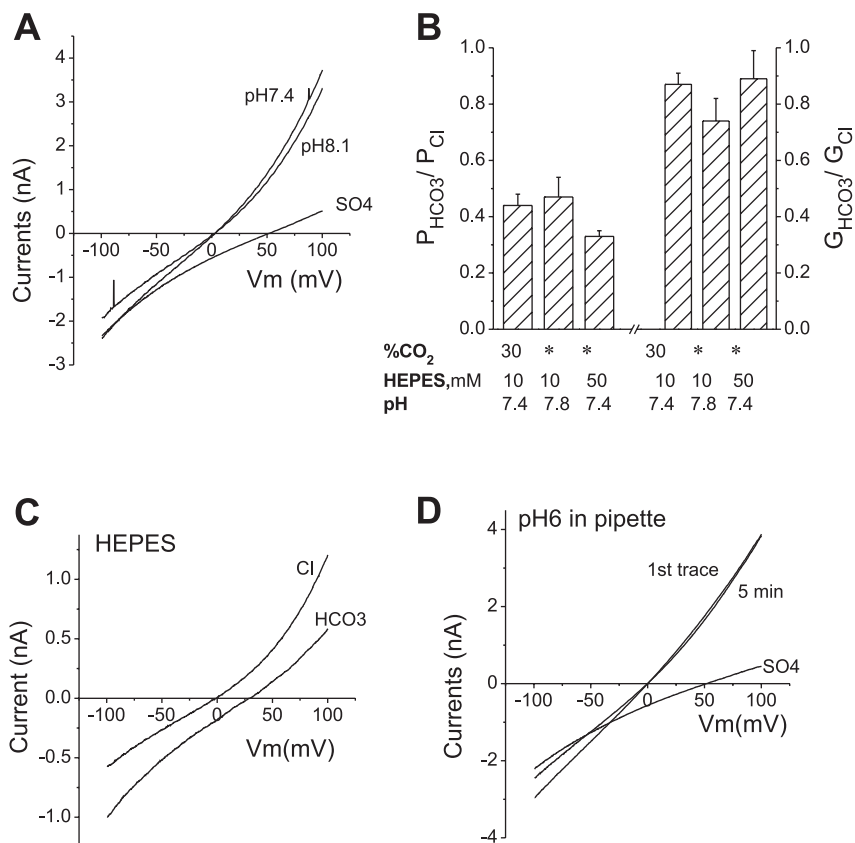


Fig. 3. Na⁺ did not affect HCO₃⁻ permeation across hBest1 channels. Whole cell recordings were performed as in Fig. 1 except that Na⁺ was replaced with NMDG⁺; 140 mM NMDG⁺-Cl⁻ was replaced with 140 mM NMDG⁺-HCO₃⁻ (see MATERIALS AND METHODS) and E_{rev} measured. **A**: representative I - V relationships of an hBest1-expressing HEK cell bathed in NMDG⁺-Cl⁻ or NMDG⁺-HCO₃⁻. **B** and **C**: average relative permeabilities and conductances of hBest1 with Na⁺ or NMDG⁺ as cation. There were no significant differences between the 2 cations ($P > 0.5$, $n = 4$ – 5).

Fig. 4. Effects of pH on hBest1-expressed currents. **A**: representative *I-V* curve from an hBest1-expressing cell bathed in normal extracellular solution (HEPES, pH 7.3) and an extracellular solution of pH 8.1 (TAPS). Raising extracellular pH from 7.3 to 8.1 had no significant effect on hBest1 currents. Replacement of extracellular Cl⁻ with SO₄²⁻ largely blocked outward currents. **B**: comparison of relative permeability and conductance of HCO₃⁻ under various conditions as indicated. The 30% CO₂/10 HEPES/pH 7.4 bars are the same data as in Fig. 1. There are no significant differences among the 3 groups ($P > 0.1$, $n = 5-11$). *, Ambient. **C**: effect of buffering the solutions with 50 mM HEPES. All solutions, both internal and external, contained 50 mM HEPES. **D**: representative *I-V* curves from hBest1-expressing cells with internal pH of 6.0. The pH 6.0 internal solution was buffered with MES; The result is typical of 3 cells.

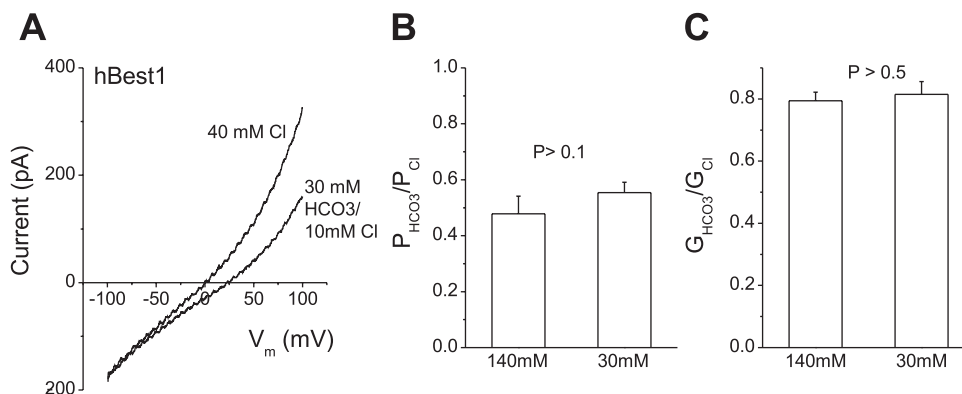


lic solution to acidify transiently as CO₂ diffuses into the cell more rapidly than HCO₃⁻. To minimize the change in intracellular pH, we measured $P_{HCO_3^-}/P_{Cl^-}$ and $G_{HCO_3^-}/G_{Cl^-}$ with 140 mM HCO₃⁻-10 mM HEPES solution (pH 7.8) that was not gassed with CO₂ (Fig. 4B). $P_{HCO_3^-}/P_{Cl^-}$ and $G_{HCO_3^-}/G_{Cl^-}$ measured under these conditions were statistically the same as for solutions that were bubbled with CO₂ (Fig. 4B). We also performed experiments with 50 mM HEPES buffer at pH 7.4 in both the internal and external solutions to better control pH. $P_{HCO_3^-}/P_{Cl^-}$ and $G_{HCO_3^-}/G_{Cl^-}$ were the same as in experiments with 10 mM HEPES (Fig. 4, B and C). Finally, to assess whether any uncontrolled intracellular pH change might affect hBest1 current, we observed changes of the currents recorded with MES-buffered solutions at pH 6.0. Current amplitudes did not change during 5-min recording with intracellular pH = 6.0, and the E_{rev} were not affected (Fig. 4D). These control exper-

iments rule out possible changes in pH as contributing significantly to the outcome or interpretation of the experiments.

HCO₃⁻ permeability of hBest1 at physiological concentrations. The concentrations of HCO₃⁻ used above were nonphysiological. [HCO₃⁻] in RPE cells is ~23 mM (7). To observe how HCO₃⁻ anions permeate at physiological concentrations, we compared hBest1 currents generated in the presence of symmetrical 125 mM Cl⁻ to those obtained with 25 mM HCO₃⁻ added to the 125 mM Cl⁻ solution. Addition of 25 mM HCO₃⁻ increased the anion current amplitude at +100 mV by ~16% ($n = 7$). However, changes in E_{rev} were negligible. To measure a shift in E_{rev} reliably, we compared currents in 40 mM Cl⁻ to those in 30 mM HCO₃⁻/10 mM Cl⁻. Osmolarity and ionic strength were kept constant by the addition of 120 mM NMDG-gluconate. The currents in 30 mM Cl⁻ were smaller than in 150 mM Cl⁻, as expected for a channel with a

Fig. 5. Permeability of hBest1 in low HCO₃⁻ concentrations. Internal [Cl⁻] was 40 mM. External solution contained 40 mM NaCl or 30 mM NaHCO₃ solution (with other components supplied as in 140 mM Cl⁻ solution; see MATERIALS AND METHODS). **A**: current traces of a representative cell recorded with low permeant anion concentrations on both sides of the cell. **B** and **C**: relative HCO₃⁻ permeabilities (B) and conductances (C) for high (140 mM) and low (30 mM) HCO₃⁻ concentrations ($n = 4-8$).



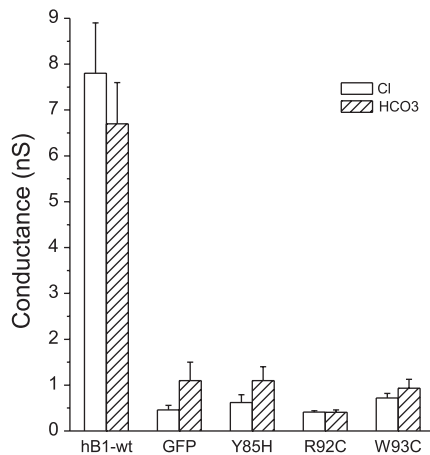


Fig. 6. Loss of both Cl⁻ and HCO₃⁻ conduction through hBest1 due to disease-causing mutations. Mutant cDNAs were transfected into HEK cells for whole cell current recording as described in Fig. 1. Average values of slope conductances were calculated as described in MATERIALS AND METHODS ($n = 5-10$). hB1-wt, hBest1 wild type.

low Cl⁻ affinity (Fig. 5A). E_{rev} was shifted to the right when Cl⁻ was replaced with HCO₃⁻ (Fig. 5A). The calculated permeability and conductance ratios were virtually identical to the values obtained with 140 mM HCO₃⁻ (Fig. 5, B and C).

hBest1 disease-causing mutants do not conduct Cl⁻ or HCO₃⁻. We hypothesized that Cl⁻ and HCO₃⁻ may permeate hBest1 through the same pore. To test this hypothesis, we selected three disease-causing mutations (Y85H, R92C, and W93C) that occur in transmembrane domain 2 and are thought to disrupt the channel pore structure (30, 40). These mutations have previously been shown to be nonfunctional as Cl⁻ chan-

nels. All three mutations lost their ability to conduct both Cl⁻ and HCO₃⁻ (Fig. 6). The anion conductances that remained were similar to those in cells that were transfected with GFP alone. These data provide additional evidence that hBest1 encodes the channel responsible for the HCO₃⁻ conductance and also shows that it is likely that Cl⁻ and HCO₃⁻ share the conduction pathway.

V78C mutation changes permeability of mBest2 to HCO₃⁻. In a study of the mBest2 pore structure, we found that the V78C mutation showed the highest selectivity between anionic and cationic sulfhydryl reagents (33). For this reason, we thought that this mutation might differ from wild type in relative Cl⁻ and HCO₃⁻ permeability. We found that the mutation did alter the relative permeability and conductance of mBest2 to HCO₃⁻ (Fig. 7). Both $G_{HCO_3^-}/G_{Cl^-}$ and $P_{HCO_3^-}/P_{Cl^-}$ for the V78C mutation were less than wild type. This is consistent with the fact that HCO₃⁻ has a negative charge that is distributed over two oxygen atoms. Introducing a mutation at the site that exhibits high charge selectivity may result in lower HCO₃⁻ permeability compared with Cl⁻.

DISCUSSION

Anion channels such as CFTR, ClC, CaCC, and ligand-gated anion channels are permeable to HCO₃⁻, although their permeabilities to HCO₃⁻ are lower than Cl⁻ (20, 31, 36, 43). In this study we found that several bestrophin Cl⁻ channels were also permeant to HCO₃⁻. Interestingly, the conductance for HCO₃⁻ was very similar to Cl⁻ under a variety of conditions, suggesting that under physiological conditions a significant amount of the hBest1 current may be carried by HCO₃⁻.

Like bestrophin, CFTR also transports HCO₃⁻. However, with CFTR, the mechanisms are complicated. From single-

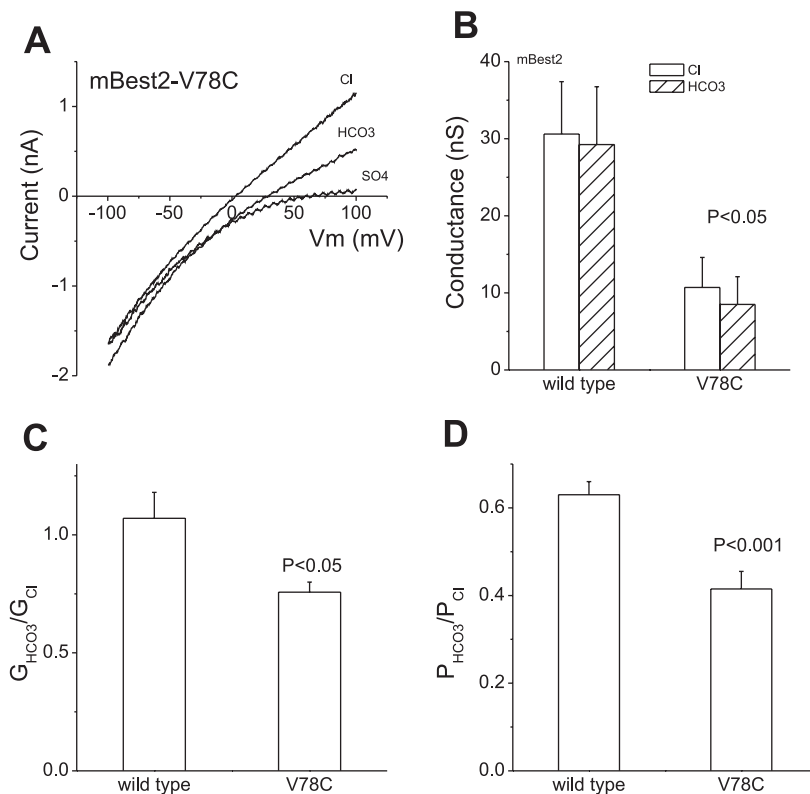


Fig. 7. V78C mutation changes the permeability of mBest2 to HCO₃⁻. As described in Fig. 1, the V78C mutant cDNA was transfected into HEK cells for whole cell current recordings. A: representative current traces. B–D: V78C mutation significantly attenuated slope conductances (B), $G_{HCO_3^-}/G_{Cl^-}$ (C), and $P_{HCO_3^-}/P_{Cl^-}$ (D) compared with mBest2 wild type ($n = 5-11$). Note that in B, slope conductances for both wild type and V78C between Cl⁻ and HCO₃⁻ are not significantly different ($P > 0.5$).

channel analysis, it is clear that CFTR itself can transport HCO₃⁻ (20, 27). Estimates of CFTR $P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$ range from 0.1 to 0.4. The highest end of this range is slightly less than the value we have obtained for bestrophins. However, in addition to transporting HCO₃⁻ itself, CFTR regulates HCO₃⁻ secretion via electrogenic Cl⁻/HCO₃⁻ exchangers of the SLC26 family (13, 14, 38). It appears that the bulk of HCO₃⁻ secretion is mediated by SLC26 transporters, because HCO₃⁻ transport by CFTR is very small under conditions of physiological Cl⁻ concentration (37, 44). There are several reasons to believe that with bestrophin channels HCO₃⁻ is conducted by the same pore that conducts Cl⁻. First, the hBest1 mutations we tested affect Cl⁻ and HCO₃⁻ conductance similarly. However, it is possible that a wider sampling of mutations may reveal a dissociation of HCO₃⁻ and Cl⁻ transport. In the case of CFTR, certain mutations like G551S affect HCO₃⁻ conductance without changing Cl⁻ conductance significantly. Second, both Cl⁻ and HCO₃⁻ conductances require intracellular Ca²⁺ in order for the conductance to be turned on. This suggests that the Cl⁻ and the HCO₃⁻ conductance pathways are gated in a similar manner. Also, the bestrophin currents are quite large and do not exhibit significant rectification or time dependence. These properties are incompatible with either electroneutral or electrogenic exchangers. Although the possibility exists that the HCO₃⁻ conductance in the bestrophin-transfected cells may be due to upregulation of other anion channels or exchangers by bestrophin, we think this is unlikely given that mutations affect Cl⁻ and HCO₃⁻ conductance similarly. Single-channel analysis would be useful in helping to answer this question.

Possible role of HCO₃⁻ in Best disease. The mechanisms underlying Best disease are not known and are presently controversial (10). There are two hypotheses, which may not be mutually exclusive. One hypothesis is that hBest1 is a Cl⁻ channel and that dysfunction of the Cl⁻ channel disrupts the interaction between photoreceptors and RPE somehow resulting in the accumulation of lipofuscin in the subretinal space and RPE cells (8, 39, 46, 47). The other hypothesis is that hBest1 is a regulator of voltage-gated Ca²⁺ channels (22, 23, 35). Our finding that hBest1 is highly permeable to HCO₃⁻ adds another dimension to the problem. If hBest1 is also capable of transporting HCO₃⁻, it is possible that abnormal HCO₃⁻ transport contributes to the disease (9).

HCO₃⁻ is an important anion in retinal physiology. Retina is one of the most metabolically active tissues in the body and produces large amounts of CO₂ (41, 42). Because photoreceptor function is inhibited by low pH (17), it is essential that CO₂ be removed from the subretinal space. The mechanisms by which pH is controlled in the retina are incompletely understood. Several HCO₃⁻ transport pathways have been shown to exist in the RPE (11, 12, 15). HCO₃⁻ is moved from the subretinal space into the RPE cells by an apical Na⁺-dependent HCO₃⁻ transporter (7). Because the Na⁺-HCO₃⁻ transporter NBC1 (SLCA4) has been localized to the apical membrane of the RPE (3), NBC1 is one candidate for the apical transporter. HCO₃⁻ leaves the RPE into the choriocapillaris by a Cl⁻/HCO₃⁻ exchanger whose molecular identity remains unknown. This basolateral transporter could possibly be a member of the SLC4 or SLC26 families (1, 25, 34). There is physiological evidence supporting both electroneutral and electrogenic exchange (5, 6, 12, 19), and different species may use different transporters. The concentration of HCO₃⁻ in the choriocapil-

laris is maintained at a low level because a functional complex of carbonic anhydrase 4 and NBC1 in the choriocapillaris ensures transport of HCO₃⁻ into the blood. Recently, it was found that mutations in carbonic anhydrase 4 impair pH regulation and cause retinal photoreceptor degeneration (45). Although carbonic anhydrase 4 is expressed in the choriocapillaris, this result emphasizes the importance of removal of HCO₃⁻ and CO₂ from the retina.

The question arises as to whether the basolateral efflux of HCO₃⁻ may also occur through anion channels. One advantage of exchangers is that they can harness the downhill electrochemical movement of one ion to drive the uphill movement of another. The intracellular concentration of HCO₃⁻ in RPE cells has been estimated to be 17–23 mM (7, 19). If the extracellular HCO₃⁻ concentration on the basolateral side is maintained low by HCO₃⁻ transport into the blood by the NBC1-carbonic anhydrase 4 complex in the choriocapillaris, the electrochemical driving force will strongly favor HCO₃⁻ efflux from the RPE. If this reasoning is correct, there is no need for an exchanger mechanism to drive HCO₃⁻ efflux. Actually, depending on the RPE membrane potential and Cl⁻ equilibrium potential, the Cl⁻ driving force could attenuate HCO₃⁻ efflux through a Cl⁻/HCO₃⁻ exchanger rather than promoting it. Thus a role for channel-mediated HCO₃⁻ efflux should be considered. Because hBest1 is localized on the basolateral membrane (21) and has a high HCO₃⁻ permeability, hBest1 would be a reasonable candidate for a HCO₃⁻ channel in this membrane. Cellular HCO₃⁻ plays several fundamental roles in cells: metabolism, regulation of pH, and regulation of cell volume (4). Therefore, disturbance of HCO₃⁻ transport in RPE by hBest1 mutations could cause Best disease by multiple mechanisms.

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