Acute pH-dependent Regulation of AE2-mediated Anion Exchange Involves Discrete Local Surfaces of the NH2-terminal Cytoplasmic Domain

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We have previously defined in the NH2-terminal cytoplasmic domain of the mouse AE2/SLC4A2 anion exchanger a critical role for the highly conserved amino acids (aa) 336–347 in determining wild-type pH sensitivity of anion transport. We have now engineered hexa-Ala ((A)6) and individual amino acid substitutions to investigate the importance to pH-dependent regulation of AE2 activity of the larger surrounding region of aa 312–578. 4,4'-Disothiocyanostilbene-2,2'-disulfonic acid (DIDS)-sensitive 36Cl– efflux from AE2-expressing Xenopus oocytes was monitored during changes in pHi or pHo in HEPES-buffered and in 5% CO2/HCO3–-buffered conditions. Wild-type AE2-mediated 36Cl– efflux was profoundly inhibited at low pHo, with a pHo50 value = 6.75 ± 0.05 and was stimulated up to 10-fold by intracellular alkalinization. Individual mutation of several amino acid residues at non-contiguous sites preceding or following the conserved sequence aa 336–347 attenuated pHi and/or pHo sensitivity of 36Cl– efflux. The largest attenuation of pH sensitivity occurred with the AE2 mutant (A)357–362. This effect was phenocopied by AE2 H360E, suggesting a crucial role for His360. Homology modeling of the three-dimensional structure of the AE2 NH2-terminal cytoplasmic domain (based on the structure of the corresponding region of human AE1) predicts that those residues shown by mutagenesis to be functionally important define at least one localized surface region necessary for regulation of AE2 activity by pH.

The on-line version of this article (available at http://www.jbc.org) contains Table I and Fig. S1.

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1 The abbreviations used are: aa, amino acid(s); DIDS, 4,4'-disothiocyanostilbene-2,2'-disulfonic acid; MES, 4-morpholine-ethanesulfonic acid.
erved residues aa 336–347 play a similar role in pH-dependent regulation of the AE3 polypeptide and are likely to be important in other SLC4 transporters, as well (20).

In the present work we extend our mutagenic analysis to the AE2 NH2-terminal cytoplasmic domain residues that precede and follow the highly conserved aa 336–347 sequence, as guided by our earlier sequential deletion studies (18, 20). We demonstrate that several non-contiguous regions of the AE2 NH2-terminal cytoplasmic domain contain conserved amino acids whose mutation alters the regulation of anion exchange activity by pH, and/or by pHi. Homology modeling suggests that a substantial subset of these residues contributes to at least one pH-sensitive surface of this domain. A preliminary report of this work has been published (21).

MATERIALS AND METHODS

Reagents—Na36Cl was purchased from ICN (Irvine, CA). Other chemical reagents were of analytical grade and obtained from Sigma, Calbiochem, or Fluka. Restriction enzymes and T4 DNA ligase were from New England Biolabs (Beverly, MA), Taq DNA polymerase and dNTPs were from Promega (Madison, WI) or Invitrogen.

Solutions—ND-96 medium consisted of (in mM): 96 NaCl, 2 KCl, 1.8 CaCl2, 1 MgCl2, 5 HEPES, and 2.5 sodium pyruvate, pH 7.40. Flux media lacked sodium pyruvate. pH values of 7.0, 8.0, and 8.5 in room air flux media were achieved with 5 mM HEPES, 5 mM MES was used for room air flux media of pH values 5.0 and 6.0. In Cl−-free solutions, NaCl was replaced isomotically with 96 mm sodium isethionate, and equimolar potassium, calcium, and magnesium gluconate substituted for the weak acid salt, sodium butyrate, was used as an equimolar substitution in the experiments in Cl−-free solutions of pH 5.0 and 6.0.

To vary pH within the physiological range, NaHCO3 was replaced isosmotically with 96 mm sodium isethionate, and equimolar potassium was added to the media. The pH of CO2/HCO3− buffer media of pH values 5.0, 6.0, 7.0, 8.0, and 8.5, then to a solution at pH 8.5 in the presence of DIDS (13, 19). Rate constants measured at each pH value for wild-type AE2 and for the tested AE2 mutants in each individual experiment were fit (Sigmoid Plot) to the following first-order logistic sigmoid equation,

\[
v = \left( V_{\text{max}} \times 10^{-K} \right) / \left( 10^K + 10^r \right) + d
\]

where \( v \) = AE2-mediated Cl− efflux rate constant, \( V_{\text{max}} \) = the maximum AE2-mediated Cl− efflux rate constant, \( x = \text{pH} \) at which the rate constant was measured, \( K = \text{pH}_{50} \), the pH at which \( v \) is half-maximal, \( r \) is the y axis intercept. Any pH value at 20 cpm (peak 30 cpm). Within each experiment, water-injected and AE2 RNA-injected oocytes from the same frog were subjected to parallel measurements. On each experimental day, AE2 Cl− efflux activity of the tested mutant AE2 polypeptides was compared at pH 7.4 to wild-type AE2 activity (“basal activity”). Each AE2 mutant was tested in oocytes from at least two frogs, and most in oocytes from three frogs. These data are summarized in Supplemental Materials Table I.

Measurement of \( pH \)-Dependence of AE2 Efflux—Individual oocytes maintained in Cl−-free solution at pH 7.4 were exposed sequentially to Cl−-containing ND-96 at pH 5.0, 6.0, 7.0, 8.0, and 8.5, then to a solution at pH 8.5 in the presence of DIDS (13, 19). Rate constants measured at each pH value for wild-type AE2 and for the tested AE2 mutants in each individual experiment were fit (Sigmoid Plot) to the following first-order logistic sigmoid equation.

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**RESULTS**

**Roles of Candidate SH3 Binding Domain and Histidine Residues within Amino Acids 312–317 for AE2 Regulation by pH$_i$ and pH$_f$**—We reported previously that the region encompassing amino acids 310–347 within the NH$_t$-terminal cytoplasmic domain of the murine AE2 anion exchanger is required for normal regulation of AE2-mediated Cl$^-$/H$^+$ transport by pH$_i$ and pH$_f$ (19, 20). As shown in Fig. 1A, aa 312–317 includes two prolines that comprise a potential SH3 domain binding site, as well as two potentially titratable histidine residues. We mutated each residue individually to Ala and assessed the effects on Cl$^-$/Cl$^-$ exchange of varying pH$_i$ or pH$_f$. Fig. 1B indicates that most mutants exhibited $^{36}$Cl$^-$ efflux activity at pH 7.4 comparable with that of wild-type AE2. Fig. 1C profiles $^{36}$Cl$^-$ efflux activity (normalized as described under “Materials and Methods”) as a function of pH$_f$ for wild-type AE2, the two AE2 mutants P313A and H317A, and the double mutant H314A/H317A. The pH$_f$ value at which the rate constant for wild-type, AE2-mediated $^{36}$Cl$^-$ efflux was half-maximal (pH$_f^{50}$) at 6.75 ± 0.05 (n = 24; Fig 1, C and E). The single mutant P313A and the double mutant H313A/H317A each exhibited a pH$_f$ dependence indistinguishable from that of wild-type AE2, whereas the pH$_f^{50}$ value of the AE2 mutant H317A was shifted to a more acidic pH value of 6.20 ± 0.13 (n = 11, p < 0.05). Fig. 1E summarizes, for all mutants tested, the pH$_f^{50}$ values measured from data like that shown in Fig. 1C. Although mutagenesis of either His$^{314}$ or His$^{317}$ led to an acid-shift in pH$_f^{50}$ value, the double mutant H314A/H317A exhibited wild-type regulation by pH$_f$.

We next investigated the effect of changing pH$_i$ at a constant pH$_f$, upon activity of the same set of AE2 mutants. Bath addition and removal of the weak acid, butyrate, respectively, decreases and increases oocyte pH$_i$ (19). Fig. 1D shows a representative efflux trace in which $^{36}$Cl$^-$ efflux via wild-type AE2, and via the mutants P313A, H317A, and H314A/H317A, was reduced at low pH$_i$ (butyrate addition) and subsequently stimulated when pH$_i$ was elevated (by butyrate removal). Fig. 1F summarizes similar experiments for all mutants tested, results are expressed as the $^{36}$Cl$^-$ efflux rate constant at low pH$_i$ (in the presence of 40 mM butyrate) normalized to that measured at high pH$_i$ (following extracellular butyrate removal, see “Materials and Methods” and Supplemental Materials Fig. S1). The data do not suggest involvement of the proline and histidine residues in pH$_i$-dependent regulation of AE2 activity, because their mutation to alanine did not prevent reduction of $^{36}$Cl$^-$ efflux at low pH$_i$. Note that while the AE2 mutants, H314A and H317A, were altered in their sensitivity to pH$_i$, their sensitivity to pH$_f$ was unchanged (Fig. 1F). The data also indicate that pH$_i$-dependent regulation of AE2 does not require binding of a SH3 domain-containing protein at this site, because the proline mutants exhibited normal sensitivity to both pH$_i$ and pH$_f$.

**Individual Mutations within AE2 aa 318–323 Identify Residues Important for Regulation of Cl$^-$/H$^+$ Transport by pH$_i$ and pH$_f$**—Our previous study using hexa-Ala bloc substitutions implicated aa 318–323 as important for the regulation of AE2 activity by pH (20). However, the AE2 mutant (A)$_6$318–323 expressed too low an activity at pH$_i$ 7.4 to allow study of inhibition by extracellular acidification. Therefore, we re-evaluated this region by alanine scan of the individual residues (Fig. 2A). Fig. 2B shows that all mutants studied retained $^{36}$Cl$^-$ efflux activity at pH$_i$ 7.4, sufficient for an analysis of pH$_i$ sensitivity. Fig. 2C shows that the AE2 mutant, E318A (open circles), exhibited a pH$_i$ dependence similar to that of wild-type AE2 (filled circles), except for its incomplete inhibition at pH$_i$ 5.0. In contrast, the AE2 mutant F320A (filled squares) and the double mutant E318A/E322G (open squares) displayed acid-shifted pH$_i^{50}$ values of 5.83 ± 0.12 (n = 10, p < 0.05) and 6.20 ± 0.13 (n = 7, p < 0.05), respectively. Because both of these mutants also exhibited incomplete inhibition at pH$_i$ 5.0, these pH$_i^{50}$ values represent maximal estimates.

Fig. 2E summarizes the pH$_i$ sensitivity of all the point mutants, plotted as pH$_i^{50}$ values derived from results like those illustrated in Fig. 2C. Two individual mutations (F320A and L323A) and the (inadvertently derived) double mutation E318A/E322G led to significant shifts in pH$_i^{50}$ values relative to that of wild-type AE2. Alanine substitution of other residues did not significantly shift pH$_i^{50}$ values. Mutation of the hydrophilic phenylalanine (Phe$^{322}$) to the similarly sized but more hydrophilic tyrosine (as in the corresponding position of mAE1) also acid-shifted the pH$_i^{50}$ value, but to a lesser degree. Note that, although individual mutations of glutamate 318 or 322 to alanine had no effect on the pH$_i^{50}$ value, there was an acid-shift for the double mutant.

We next tested the pH$_i$ sensitivity of the same AE2 mutants. Fig 2D shows a representative experiment in which $^{36}$Cl$^-$ efflux via wild-type AE2 and the mutant E318A was inhibited at low pH$_i$ (in the presence of butyrate) and subsequently stimulated by an elevation of pH$_i$ (induced by extracellular butyrate removal). In contrast, mutants F320A and E318A/E322G both showed a significantly reduced inhibition at low pH$_i$ (and therefore a reduced stimulation upon elevation of pH$_i$). Fig. 2F summarizes the pH$_i$ sensitivity of all the point mutants tested here in comparison with wild-type AE2. The mutants F320A, F320Y, and L323A, and the double mutants E318A/E322G and E318V/E322A each displayed a decrease in $^{36}$Cl$^-$ efflux activity at low pH$_i$ (in the presence of 40 mM butyrate) that was significantly lower than wild-type. This loss of inhibition by acid pH$_i$ was also manifest in AE2 F320A-mediated Cl$^-$/HCO$_3^-$ exchange (not shown). These data therefore identify multiple amino acid residues whose mutation alters wild-type regulation of AE2 activity by both pH$_i$ and pH$_f$. The next segment of AE2, aa 324–335, was not further examined because the AE2 mutants A$_{324}$–329 and A$_{330}$–335 exhibited wild-type regulation by both pH$_i$ and pH$_f$ (20).

**The Effect of Individual Mutations within AE2 aa 348–355 on Regulation by pH$_f$**—We determined previously that the adjacent, strongly conserved AE2 region encompassing aa 336–347 was critical for wild-type sensitivity to pH$_i$ and pH$_f$ (20). However, the AE2 region aa 348–355 immediately adjacent to the conserved critical region of aa 336–347 is only partially conserved across the SLC4 gene family. This adjacent region includes several glutamic acid residues. Prompted by our earlier identification of Glu$^{346}$ and Glu$^{347}$ as important in regulation of AE2 by pH$_i$, we subjected AE2 residues 348–355 individually to alanine scan mutagenesis. Each of these AE2 mutants displayed near wild-type $^{36}$Cl$^-$ efflux activity at pH$_i$ 7.4, and retained wild-type regulation by pH$_i$ (Supplemental Materials Table I).

**Mutagenesis of AE2 Residues 356–362 Identifies a Conserved Histidine Residue Important for pH$_f$ and pH$_i$ Sensitivity**—The high degree of conservation of AE2 Trp$^{356}$ among SLC4 polypeptides extends through the adjacent region encompassing aa 357–362. Therefore, we proceeded to test the role of this region by hexa-Ala bloc substitution, and by individual Ala substitution of Trp$^{356}$ and the candidate pH-sensitive His$^{360}$ residue (Fig. 3A). All mutants were highly active at pH 7.4 (Fig. 3B). The pH$_i$ versus $^{36}$Cl$^-$ efflux activity profiles of W356A, (A)$_{357}$–362, and H360E were each acid-shifted when compared with that of wild-type AE2 (Fig. 3C). Whereas the pH$_i^{50}$ value of W356A was acid-shifted to a moderate degree, the pH$_i^{50}$ value of (A)$_{357}$–362 was acid-shifted by –1 pH unit. As
summarized in Fig. 3, this shift was partially phenocopied by the individual mutant H360E, suggesting a role for His360 in the regulation of AE2 by pHo.

We next compared pHo sensitivity of AE2 mutants W356A, P313A, P316A, P313/316A, H314A, H317A, and H314/317A with wild-type AE2 (Fig. 3D). In contrast to wild-type AE2, which exhibits a low rate constant at
acid pH, and is subsequently stimulated by raising pH, each of these mutants had robust $^{36}\text{Cl}^{-}$ efflux activity at low pH, and were minimally responsive to subsequent elevation of pH.

These data are the first to define a histidine residue within the NH$_2$-terminal cytoplasmic domain whose mutation alters AE2 regulation by both pH$_o$ and pH$_i$.
Mutations within AE2 Region aa 391–408 Define Residues Important for Regulation of Cl	extsuperscript– Transport by pH

Our previous study of NH	extsubscript{2}-terminal deletion constructs identified AE2 aa 391–510 as important for regulation by pH (19). Subsequent deletion analysis narrowed this area to aa 391–410 (data not shown). As an initial step toward identification of individual amino acid residues required for this regulation, we tested the functional properties of three hexa-Ala bloc substitutions spanning this region and then tested individual substitution mutants (Fig. 4A). As shown in Fig. 4B, only (A)	extsubscript{6397–402} mutant lacked activity at pH 7.4 sufficient to evaluate its regulation by pH. (Fig. 4C). Fig. 4C summarizes pH sensitivity of the active mutants. The (A)	extsubscript{6391–396} mutant exhibited a pH	extsubscript{50} value of 6.95 ± 0.09 (n = 6, p > 0.05), similar to that of wild-type AE2, 6.71 ± 0.09 (n = 16), whereas the (A)	extsubscript{6357–362} mutant displayed a significantly acid-shifted pH	extsubscript{50} value of 6.05 ± 0.19 (n = 10, p < 0.05). Thus aa 403–408 appear important for pH	extsubscript{i} dependent regulation of AE2. In contrast, both AE2 mutants (A)	extsubscript{6391–396} and (A)	extsubscript{6000–396} exhibited wild-type pH	extsubscript{i} sensitivity (p < 0.05) (Fig. 4D). The AE2 (A)	extsubscript{6397–402} mutant appeared to be insensitive to regulation by pH (Fig. 4D), but its low basal activity (Fig. 4B) mandates caution in interpreting this potentially interesting result.

Amino acids with fixed-charge side chains play important roles in pH-dependent AE2 regulation by aa 336–347 (20). We therefore tested possible regulatory contributions from AE2 residues Glu	extsuperscript{346}, Asp	extsuperscript{360}, and Lys	extsuperscript{365} by studying function of the individual Ala substitution mutants. All mutants displayed robust activity at pH	extsubscript{i} 7.4 (Fig. 4B), and wild-type pH	extsubscript{50} values (Fig. 4C), and wild-type or near wild-type sensitivity to pH (Fig. 4D). Overall, the experiments highlight aa 403–408 as important for pH	extsubscript{i}-dependent but not pH	extsubscript{i}-independent regulation of AE2. However, the individual Ala substitutions studied did not suffice to alter AE2 regulation by pH.

Role of a Predicted Side Chain Interaction in the Regulation of AE2-mediated Cl	extsuperscript– Transport by pH

Wild-type AE2, H360E, and W356A did not suffice to alter AE2 regulation by pH. However, the individual Ala substitutions studied did not suffice to alter AE2 regulation by pH. The (A)	extsubscript{6391–396} mutant exhibited a robust activity at pH	extsubscript{i} 6.05. In contrast, the double mutant E346H/H360E failed to rescue the altered phenotype. We have shown previously that single amino acid substitutions within the highly conserved region aa 336–347 that alter pH	extsubscript{i} dependence were sufficient to alter pH	extsubscript{i} sensitivity. Therefore, we investigated the functional importance of this putative intra-monomeric interaction by creation of the paired mutations E346H, H360E, and the double mutant. Fig. 5B shows that all mutants displayed sufficient activity to allow analysis of 36Cl	extsuperscript– efflux during changes of pH	extsubscript{r} and pH	extsubscript{i}. The pH	extsubscript{50} values of mutants E346H and H360E were each acid-shifted compared with wild-type AE2 (Fig. 5D). However, this altered phenotype was not rescued in the AE2 double mutant E346H/H360E, as shown in Fig. 5, C and D.

The AE2 single and double mutants were also assessed for altered regulation by pH, (Fig. 5E). Whereas wild-type AE2 exhibited 20% 36Cl	extsuperscript– efflux activity at low pH, (in the presence of butyrate), AE2 mutants E346H and H360E each retained ~80% efflux activity. Again, however, the double mutant E346H/H360E failed to rescue the altered phenotype. We have shown previously that single amino acid substitutions within the highly conserved region aa 336–347 that alter pH	extsubscript{i} sensitivity of Cl	extsuperscript–/Cl	extsuperscript– exchange similarly alter the pH sensitivity of Cl	extsuperscript–/HCO	extsubscript{3} exchange (20). We therefore examined pH sensitivity of Cl	extsuperscript–/HCO	extsubscript{3} exchange mediated by AE2 H360E and the double mutant E346H/H360E (Fig. 5F). The 36Cl	extsuperscript– efflux activity of both mutants in the presence of butyrate was significantly higher than for wild-type AE2. Thus, the presence of HCO	extsubscript{3} did not allow the double mutant to rescue the altered pH	extsubscript{i} sensitivity of the two individual mutants. The combined data fail to support a functional role for Glu	extsuperscript{346}/His	extsuperscript{360} interaction in determining the pH sensitivity of AE2. The data do, however, confirm that AE2 structure-function relationships derived from measurements of pH	extsubscript{r} sensitivity of Cl	extsuperscript–/Cl	extsuperscript– exchange in Xenopus oocytes apply equally to the pH	extsubscript{i} sensitivity of AE2-mediated Cl	extsuperscript–/HCO	extsubscript{3} exchange.
Role in AE2 Regulation of a Residue Predicted in AE1 to Control pH-dependent Conformational Change—Mutation of the conserved hAE1 NH2-terminal cytoplasmic domain amino acid residues Glu291 to Gln, Arg, or Leu abolishes the pH-dependent change in Stokes radius normally observed with both natural and recombinant AE1 cytoplasmic domain dimers. This phenotype most likely arises by alteration of the hinge angle between the large globular domain and the dimerization arm (25), as revealed by the crystal structure of the hAE1 NH2-terminal cytoplasmic domain oligomer (10). We hypothesized that a similar pH-dependent movement around a homologous hinge region might contribute to pH-dependent regulation of AE2-mediated anion exchange. We therefore tested the effect of the corresponding AE2 mutation D578K on pH-sensitive anion exchange. AE2 D578K had wild-type activity at pH 7.4 (Fig. 6B), and exhibited a modestly acid-shifted pH<sub>50</sub> value of 6.46 ± 0.07 (n = 16, Fig. 6D). In addition, AE2 D578K exhibited only minimal sensitivity to pH<sub>i</sub>, retaining nearly 60% activity in the presence of butyrate (Fig. 6E). Thus, a mutation in AE2 which, in the corresponding region of AE1 changes the pH-dependent dimer structure, is also involved in the pH sensitivity of the 36Cl<sup>-</sup> transport.

Because the carboxylate of AE2 D578 is modeled to reside 7 Å from the ε-amino group of the pH-sensitive Lys344 (Fig. 6A), we tested the hypothesis that a mid-range electrostatic interaction between Asp578 and Lys344 might contribute to basal AE2 function or to its regulation by pH. The AE2 mutation K344D exhibited a substantial acid shift in pH<sub>50</sub> with a value of 5.86 ± 0.14 (n = 13) (p < 0.05 compared with the wild-type value of 6.75 ± 0.10, n = 14) (Fig. 6D). However, the double mutant D578K/K344D, with its acid-shifted pH<sub>50</sub> value of 5.91 ± 0.06 (n = 11), failed to rescue wild-type pH<sub>i</sub> sensitivity (Fig. 6, C and D).

A role for the putative Asp578/Lys344 interaction was also tested in the regulation of AE2 by pH<sub>i</sub> (Fig. 6E). Whereas AE2 D578K displayed a moderately reduced sensitivity to pH<sub>i</sub> (60% activity in presence of 40 mM butyrate, compared with wild-type AE2 (∼20% activity, n = 17), the K344D mutant was completely insensitive to pH<sub>i</sub>. The double mutant K344D/D578K was similarly insensitive to pH<sub>i</sub> not only in the assay of Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange (Fig. 6F), but also in the assay of Cl<sup>-</sup>/H<sup>+</sup> exchange (Fig. 6F). Thus, the absence of a phenotypic second site reversion in the pH regulation profiles of AE2 D578K/K344E fails to support a role for electrostatic interaction between these two nearby residues in the regulation of AE2 by pH.

Modeling the Non-contiguous Amino Acids Required for pH<sub>i</sub> Regulation of AE2 within the Putative Structure of AE2 aa 317–623—We have modeled the mouse AE2 NH2-terminal cytoplasmic domain (Fig. 7) based upon the crystal structure of the hAE1 NH2-terminal cytoplasmic domain oligomer (10). Although the structured hAE1 aa 55–356 correspond to mAE2 aa 317–605, the DeepView program yielded a model that omit-

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Footnotes:
2 J. Zhou and P. S. Low, personal communication.
previously studied aa 336–347, a contiguous surface. The pH-sensitive Asp578 is immediately adjacent to this surface. In contrast, the less intensively studied pH-sensitive regions of aa 403–408 (in pink) and 397–402 (out of view at lower right) are located on the other side of this model of part of the AE2 NH2-terminal cytoplasmic domain (Fig. 7B). The sequence alignment of Fig. 7C reveals strong conservation of pH-sensitive AE2 residues in the closely related Na+-independent Cl-/HCO3- exchanger, AE3, and throughout the SLC4 gene family, including Na+-dependent bicarbonate transporters not known to transport chloride. Although Na+-HCO3- cotransport in cardiomyocytes is regulated by pH independently of HCO3- concentration (26), acute regulation by pH of individual recombinant Na+-dependent HCO3- transporters has yet to be reported. The conservation among NBC cotransporters of amino acid residues implicated in regulation of AE2 and AE3 activities by pH strongly suggests similarity in mechanism of regulation.

**Fig. 5.** Role of a predicted intra-monomeric interaction in regulation of AE2-mediated Cl− transport by pH. A, structure of AE2 aa 317–363 as modeled on the template of the crystal structure of the AE1 NH2-terminal cytoplasmic domain (see “Materials and Methods”). The predicted side chain proximity of the functionally important AE2 residues Glu346 and His360 suggested a possible contribution of their electrostatic interaction to regulation of AE2 by pH. B, 36Cl− efflux rate constants for (n) oocytes measured at pH 7.4 in oocytes expressing wild-type AE2 or the indicated mutants (mean ± S.E.). C, regulation by pH of normalized 36Cl− efflux from oocytes expressing wild-type AE2 (filled circles) and AE2 E346H/H360E (filled squares). Values are mean ± S.E. D, pH_{50} values exhibited by wild-type AE2 and the indicated mutants. E, normalized rate constant (± S.E.) of 36Cl− efflux in the presence of 40 mM butyrate in HEPES-buffered medium, from (n) oocytes expressing wild-type or mutant AE2. F, normalized rate constant (± S.E.) of 36Cl− efflux in the presence of 40 mM butyrate in Cl−-free HCO3−-buffered medium, from (n) oocytes expressing wild-type or mutant AE2. Asterisks (*) indicate p < 0.05 compared with wild-type AE2.
DISCUSSION

The current work expands considerably the range of mutations in the AE2 NH$_2$-terminal cytoplasmic domain known to alter pH$_{i}$ and pH$_{o}$ sensitivity of anion transport. The importance of this domain in conferring pH sensitivity has already been documented, with particular emphasis on aa 336–347, the most highly conserved portion of the domain among bicarbonate transporters in the SLC4 superfamily (19, 20). Less well conserved NH$_2$-terminal cytoplasmic regions have also been implicated, including aa 318–323 (20). Additional residues influencing pH sensitivity reside in the AE2 transmembrane domain (13, 19).

Our present work highlights NH$_2$-terminal cytoplasmic domain residues that influence pH sensitivity, but which are non-contiguous with the highly conserved aa 336–347 region. Mutation of these residues decreases pH sensitivity of AE2-

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**Fig. 6. Role of residues with predicted importance in pH-sensitive conformational sensing in the regulation of AE2-mediated Cl$^-$/H$^+$ transport by pH.**

A. Structure of AE2 aa 317–610 as modeled on the template of the crystal structure of the AE1 NH$_2$-terminal cytoplasmic domain (see "Materials and Methods"). The predicted proximity of functionally important Lys$^{344}$ and to Asp$^{578}$ (predicted to be of conformational importance) suggested a possible contribution of their electrostatic interaction to regulation of AE2 by pH. 36Cl$^-$ efflux rate constants for (n) oocytes measured at pH$_{o}$ 7.4 in oocytes expressing wild-type or mutant AE2 polypeptides (mean ± S.E.). C. Regulation by pH$_{o}$ of normalized 36Cl$^-$ efflux from oocytes expressing wild-type AE2 (filled circles) and AE2 K344D/D578K (filled squares). Values are mean ± S.E. D, pH$_{50}$ values of wild-type and mutant AE2 polypeptides. E, normalized 36Cl$^-$ efflux rate constant (±S.E.) in the presence of 40 mM butyrate in HEPES-buffered medium from (n) oocytes expressing wild-type AE2 or the indicated mutants. F, normalized 36Cl$^-$ efflux rate constant (±S.E.) in the presence of 40 mM butyrate in Cl$^-$-free HCO$_3$-buffered medium from (n) oocytes expressing wild-type or mutant AE2 polypeptides. Asterisks (*) indicate $p < 0.05$ compared with wild-type AE2.
mediated \( \text{Cl}^- \) transport. It is notable, therefore, that homology modeling based on the structure of the corresponding region of human AE1 (Zhang et al. (10), see “Materials and Methods”). The structural model (above) and the linear schematic (below) each indicate residues that when mutated alter AE2 regulation by \( \text{pH}_i \), \( \text{pH}_o \), or by both \( \text{pH}_i \) and \( \text{pH}_o \). In the case of acid/base transporters, mutation of two juxtamembrane His residues of the COOH-terminal intracellular cytoplasmic domain associated with AE2 regulation by \( \text{pH}_o \) and \( \text{pH}_i \) shifts its \( \text{pH}_i \) sensitivity (30). This result suggests that His residue(s) may also be important for pH sensing by the AE2 protein. In the present work, individual Ala substitutions of AE2 His314 or His317 each acid-shifted the \( \text{pH}_o \) sensitivity but were without effect on the \( \text{pH}_i \) sensitivity. Protonation of these His residues, however, is unlikely to account for \( \text{pH}_o \) sensitivity, as the double mutant H314A/H317A had no effect (Fig. 1). This pattern contrasts with the consequences of His mutagenesis in the NH2-terminal and COOH-terminal cytoplasmic tails of the K+ channel Kir1.1. In that protein, although two His residues of the NH2-terminal tail and one of the 6 His residues in the COOH-terminal tail are dispensable for maintenance of pH

**Role of Charged and Titratable Amino Acid Residues in AE2 Regulation by pH**

**Histidine**—Exofacial His protonation controls the pH-dependent activity of many ion channel polypeptides (27–29).
sensitivity, individual mutation of four of the COOH-terminal tail His residues attenuates pH sensitivity, and mutation of all four residues nearly completely abolishes it (27).

In contrast to the complex effects of His314 and His317 mutations in AE2, the single H360E mutation exerted a dramatic effect on both pHi and pHo sensitivity. His360 resides within the more highly conserved stretch of aa 357–362. Its mutation replicated the effects of the A6−357–362 hexa-Ala bloc substitution in acid shifting the pHi activity curve of AE2, and in nearly completely abolishing the regulation of transport by pHi (Fig. 3). This is the largest attenuation of pH sensitivity yet observed for an individual mutation in the AE2 NH2-terminal cytoplasmic domain. His360 may thus form part of the “pH modulator” site of AE2. Homology modeling predicts surface exposure of His360 on this portion of AE2. The functional consequences of the H360E mutation may reflect a predicted side chain rotation and displacement that changes the shape as well as the electrostatics of the local protein surface.

Glutamate—In addition to histidine, mutation of other NH2-terminal charged residues can influence the pH sensitivity of AE2. Most notable among these are the two glutamate residues Glu346 and Glu347 (within the highly conserved region, aa 336–347) that have previously been shown to be important for conferring pH sensitivity (20). We now report that although other individual glutamate AE2 mutants, namely E318A and E322A, exhibited essentially wild-type pH sensitivity, the double mutant exhibited an acid-shifted pHi dependence and a loss of pHo sensitivity (Fig. 2). Individually mutating other glutamate residues to alanine (Glu350, Glu351, Glu352, and Glu354) was without effect (Supplemental Materials Table I), despite the fact that these residues are close to the important Trp356 highlighted in Fig. 7 (see also Ref. 20). Similarly, the individual mutant E399A did not replicate the altered pH regulation of the AE2 bloc mutant A6−397–402 (Fig. 4). In summary, of 10 glutamate residues mutated so far in the NH2-terminal region, no more than four seem to play a role in setting the pH sensitivity of the transporter.

Aspartate and Lysine—We had hypothesized that charged residues Asp405 and Lys408 might be responsible for the acid-shifted pHi(50) value of AE2 hexa-Ala bloc substitution mutant A6−403–408 as their side chains are predicted to face outward (see Fig. 7). However, individual mutation of each residue of alanine was again without effect on regulation of AE2 by pHi or pHo suggesting that neither residue acting individually mediates pHi sensitivity of AE2. It remains to be investigated whether the double charge mutation or individual mutation of one of the uncharged residues in this region might contribute to pHi regulation of AE2.

Mutation of human AE1 residue Glu291 abolishes pH-dependent changes in Stokes radius of the soluble recombinant AE1 NH2-terminal cytoplasmic domain dimer.2 In view of this, we tested the possibility that mutation of the corresponding AE2 residue, Asp578, might modulate or abolish pH sensitivity of AE2-mediated Cl− transport. Indeed, AE2 D578K exhibited a significant acid shift in pHi(50) value, along with significant reduction in pHi sensitivity (Fig. 6). Thus, our results may reflect a similar pH-dependent shape change in AE2 that is associated with a regulatory change in anion transport.

Tests of Charge-Pair Interaction within AE2—Because AE2 D578K exhibited altered pH sensitivity, we examined the modeled AE2 structure for residues that might interact with Asp578. Nearest neighbor search analysis (DeepView; see “Materials and Methods”) estimated a distance of ~7 Å between Asp578 and Lys345, whose mutation we earlier showed to alter pH sensitivity of AE2 (Fig. 6A). This distance is within the 8–9-Å range of stabilizing electrostatic interactions detected in a survey of many protein structures (23). We therefore hypothesized that a charge reversal experiment with the AE2 double mutant D578K/K344D might rescue the wild-type pH sensitivity lost in the individual mutants, but this was not the case (Fig. 6, D–F).

A nearest neighbor search was also performed for His360, whose mutation produced such a large alteration in the pH dependence of AE2 (see above). This revealed within a distance of 4 Å the residue Glu346 that, when changed to Ala, selectively altered regulation by pHi (20). The individual mutations H360E and E346H indeed each altered regulation of AE2 by both pHi and pHo (Fig. 5, C–F), in CO2/HCO3−-buffered as well as in HEPES-buffered conditions. Again, however, the AE2 double mutant E346H/H360E failed to restore wild-type regulation by pH. Thus, initial analysis of two possible intramolecular electrostatic side chain interactions fails to provide functional evidence for their importance, perhaps reflecting a structural rather than a regulatory role for the interactions. Alternatively, it might reflect inaccuracies in the AE2 structure predicted by homology modeling, such that other residues near His360 and Asp578 might be more appropriate targets for mutagenesis.

Role of Non-charged Residues in AE2 Regulation by pHi

Proline—Although charged, proton-transportable residues are attractive candidates for a pH sensor of the transporter it is clear that, for AE2, non-charged residues must also play a role (20). The proline-rich AE2 sequence PRARPRPPKPHEVF (aa 306–320) meets “moderate stringency” criteria as a potential binding site for SH3 domains of cortactin or phosphatidylinositol 3-kinase p85 subunit (scansite.mit.edu), and so proline residues may conceivably play a role in the biochemical regulation of AE2 activity. Although the modestly conserved AE2 aa 312–317 do not correspond to a structured region of the crystallized AE1 cytoplasmic domain (10), proline mutagenesis was used to test for possible involvement of an SH3-binding protein in the pH-dependent regulation of anion transport. However, neither basal activity nor pH-regulated activity of AE2 was altered by individual or tandem Ala substitution of Pro312 and Pro316. In contrast, the two proline-rich regions of the NHE2 Na+/H+ exchanger are known to bind in vitro to a range of SH3 domains, although NHE1, NHE3, and NHE4 lack this property (31). Mutation of both proline-rich regions in NHE2 is reported to alter the polarity of sorting of the transport protein in epithelial cells, although it does not alter the kinetic regulation of ion transport (31). In contrast, AE2 interactions with SH3 domain-containing proteins have yet to be reported, and the phosphatidylinositol 3-kinase inhibitor wortmannin is without effect on AE2-mediated rates of [36Cl]− efflux at pH7.4 (24). Overall there is, to date, no compelling evidence for a role of the NH2-terminal cytoplasmic domain prolines or SH3 domain-binding proteins in the regulation of AE2 by pH.

Other Hydrophobic and Aromatic Residues—In the present work, individual mutation of AE2 aa 318–323 has identified the conserved residue Phe320 as important in AE2 regulation by pH. Mutation of Phe320 to Ala, or to the corresponding mouse AE1 residue Tyr, acid-shifted the pHo versus pHi activity curve of AE2, and Phe320 exposed at the surface (see Fig. 7). Both substitutions of Phe320 are likely to alter the size of the hydrophobic surface patch to which the phenyl side chain of Phe320 is normally predicted to contribute, a feature that may lead to the
observed reduction in pH sensitivity. The predicted surface location of Phe320 suggests that its phenol group may interact with either the COOH-terminal transmembrane domain or directly with the lipid bilayer.

Within aa 348–356 immediately adjacent to the highly conserved AE2 aa 336–347, only the mutation W356A substantially decreased AE2 pH sensitivity (Fig. 3). This Trp residue is also highly conserved within the SLC4 gene family (Fig. 7). AE2 homology modeling predicts partial surface exposure of the Trp356 indole, where it is surrounded within a putative 5-Å radius by multiple glutamates and arginines (see “Materials and Methods”). The W356A substitution predicts a large reduction in the hydrophobic patch area, accompanied by rearrangement of surrounding charged residues. It is therefore of interest that, in the membrane-spanning gramicidin channel, tryptophan side chains near the lipid-water boundary are oriented away from the aqueous ion pore and serve to tune the proton conductance of the channel (32).

**Larger Scale Structural Considerations in the Regulation of AE2 by pH**

The involvement of NH2-terminal cytoplasmic domain amino acids in the regulation of AE2 by pH is more readily explained than their involvement in regulation by pH. In the latter case, direct protonation by extracellular H+ is not a possibility. Mutations of AE2 NH2-terminal cytoplasmic domain residues might alter the pH sensitivity of AE2 by transmission of a local conformational change across the bilayer to residues accessible to extracellular solvent. This would represent a type of static “inside-out signaling,” akin to that exhibited by extracellular matrix receptors. Some of the pH regulation-associated residues may be important in maintaining the structural integrity of the NH2-terminal cytoplasmic domain. This could be achieved through intra-monomeric or inter-monomeric interactions. For example, a conformational change accompanying a mutation might indirectly alter the conformation of the AE2 substrate binding pocket, or the conformation of a portion of the transmembrane domain required for anion translocation, thereby altering the pH sensitivity of the transporter. A mutation may also alter the pH of other critical residues whose protonation/deprotonation controls a local or larger scale conformational change within the NH2-terminal cytoplasmic domain. In addition, the integrated control of anion transport by pH is likely to depend on interactions between the surface of the NH2-terminal cytoplasmic domain and the cytoplasmic vestibule of the anion translocation pathway. Alternatively there may be interaction between the NH2-terminal cytoplasmic surface and transmembrane domain structures that remotely stabilize the transport pathway.

**Non-contiguous NH2-terminal Cytoplasmic Domain Amino Acids Cluster to Form a Surface That Contributes to AE2 Regulation by pH**

The finding that multiple, non-contiguous regions of the NH2-terminal cytoplasmic domain of AE2 are involved in defining the pHi and pHo sensitivity of AE2 has similarities with the regulation by pH of potassium channels (33, 34) and the Na+/H+ exchanger NHE1 (35, 36). In those proteins, non-contiguous amino acids were proposed to be involved in pH gating, or to constitute part of the “pH sensor.” In the present work, many of the residues that influence the pH sensitivity of AE2 are predicted to form a single, localized surface on the cytoplasmic domain. This surface cluster of 12 residues includes 7 residues with charged side chains. The relatively high charge density of this area may be affected by local changes of pH. Thus, this surface may modulate the sensing of pH by the transmembrane anion-translocating portion of the AE2 protein.

**Conservation of Amino Acid Residues Important in pH Regulation of AE2**

As shown in Fig. 7 many of the residues newly identified as important for normal regulation of AE2-mediated Cl− transport by pHi and pHo are conserved among other SLC4 bicarbonate transporters. The degree of conservation of a region or of individual residues may reflect the functional importance to pH-dependent regulation of related transporters. For example, the AE3 glutamate residues that correspond to the functionally important Glu146 and Glu147 of AE2 are also important for the pH-dependent modulation of AE3 (20). Similarly, the cytoplasmic subdomains found to be important in the pH dependence of NHE1 activity are comparably conserved in other sodium/hydrogen exchangers (35).

In summary, the results of the present study suggest the presence of a localized pH-sensitive surface on the AE2 NH2-terminal cytoplasmic domain that contributes to the pHi and pHo dependence of AE2-mediated anion transport. This highlights the complexity of the pH sensing apparatus. In addition to structures within the NH2-terminal cytoplasmic domain, identification of other pH sensing structures within the COOH-terminal transmembrane domain, as well as the anion transport sites themselves, will be required to elucidate more completely the molecular mechanism of AE2 regulation by pH. Experiments toward this end are underway.

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**REFERENCES**

1. Vaughan-Jones, R. D. (1979) J. Physiol. [Lond.] 295, 111–137.
2. Vaughan-Jones, R. D. (1986) J. Physiol. [Lond.] 379, 497–508.
3. Alper, S. L. (1994) Cell. Physiol. Biochem. 4, 265–281.
4. Alper, S. L., Darman, R. B., Chernova, M. N., and Dahl, N. K. (2002) J. Nephrol. 15, S41–S53.
5. Bevensee, M. O., Alper, S. L., Aronson, P. S., and Boron, W. F. (2000) in The Kidney: Physiology and Pathophysiology (Seldin, D.W., and Giebisch, G., eds) Third Ed., pp. 391–442. Lippincott, Williams and Wilkins, Philadelphia.
6. Vince, J. W., and Reithmeier, R. A. (2000) Biochemistry 39, 5527–5533.
7. Sterling, D., Reithmeier, R. A., and Casey, J. R. (2002) J. Biol. Chem. 276, 47886–47894.
8. Grinstein, S., Ship, S., and Rothstein, A. (1978) Biochim. Biophys. Acta 507, 294–304.
9. Kopito, R. R., Lee, B. S., Simmons, D. M., Lindsey, A. E., Morgans, C. W., and Schneider, K. (1989) Cell 59, 927–937.
10. Zhang, D., Kiyatkin, A., Bolin, J. T., and Low, P. S. (2000) Blood 96, 2925–2933.
11. Funder, J., and Wieth, J. O. (1976) Am J. Physiol. 230, C1295–C1307.
12. Zhang, Y., Chernova, M. N., Stuart-Tilley, A. K., Jiang, L., and Alper, S. L. (1996) J. Biol. Chem. 271, 5741–5749.
13. Jennings, M. L. (1992) in The Kidney: Physiology and Pathophysiology (Seldin, D.W., and Giebisch, G., eds) Second Ed., pp. 113–145. Raven Press, New York.
14. Reiner, C., Votava, L., Gary, N., and Reiner, M. (1986) J. Biol. Chem. 261, 11117–11125.
15. Leem, C.-H., Lapid-Gossmann, D., and Vaughan-Jones, R. D. (1999) J. Biol. Chem. 274, 158–180.
16. Lee, B. S., Gunn, R. B., and Kopito, R. R. (1991) J. Biol. Chem. 266, 11448–11454.
17. Jiang, L., Stuart-Tilley, A. K., Parkash, J., and Alper, S. L. (1994) Am. J. Physiol. 267, C845–C856.
18. Stewart, A. K., Chernova, M. N., Kunes, Y. Z., and Alper, S. L. (2001) Am. J. Physiol. 281, C1344–C1354.
19. Stewart, A. K., Chernova, M. N., Shmukler, B. E., Wilhelm, S., and Alper, S. L. (2002) J. Gen. Physiol. 120, 707–722.
20. Stewart, A. K., Chernova, M. N., Kerr, N., Alper, S. L., and Vaughan-Jones, R. D. (2003) J. Am. Soc. Nephrol. 14, 304a.
21. Chernova, M. N., Humphreys, B. D., Robinson, D. H., Stuart-Tilley, A. K., Garcia, A. M., Brosius, F. C., and Alper, S. L. (1997) Biochim. Biophys. Acta 1329, 111–123.
22. Kumar, S., and Nussinov, R. (2002) Biochim. Biophys. Acta 1585, 1595–1612.
23. Chernova, M. N., Stewart, A. K., Jiang, L., Friedman, D. J., Kunes, Y. Z., and Alper, S. L. (2003) Am. J. Physiol. 284, C1235–C1246.
24. Chang, S. H., and Low, P. S. (2003) J. Biol. Chem. 278, 6879–6884.
25. Chen, P. F., Dilworth, E., Swietach, P., Goddard, R. D., and Vaughan-Jones, R. D.
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27. Chanhevalap, S., Yang, Z., Cui, N., Qu, Z., Zhu, G., Liu, C., Giwa, L. R., Abdulkadir, L., and Jiang, C. (2000) J. Biol. Chem. 275, 7811–7817
28. Morton, M., O’Connell, A. D., Sivaprasadarao, A., and Hunter, M. (2003) Pflugers Arch. 445, 577–583
29. Weeks, D., Gushansky, G., Scott, D. R., and Sachs, G. (2004) J. Biol. Chem. 279, 9944–9950
30. Cha, B., Oh, S., Shanmugaratnam, J., Donowitz, M., and Yun, C. C. (2003) J. Membr. Biol. 191, 49–58
31. Chow, C., Woodside, M., Demaurex, N., Yu, F. H., Plant, P., Rotin, D., Grinstein, S., and Orlowski, J. (1999) J. Biol. Chem. 274, 10481–10488
32. Gowen, J. A., Markham, J. C., Morrison, S. E., Cross, T. A., Busath, D. D., Mapes, E. J., and Schumaker, M. F. (2002) Biophys. J. 83, 890–898
33. Schulte U., Hahn, H., Konrad, M., Jeck, N., Deret, C., Wild, K., Weidemann, S., Ruppersberg, J. P., Fakler, B., and Ludwig J. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 15298–15303
34. Flagg, T. P., Yoo, D., Sciortino, C. M., Tate, M., Romero, M. F., and Welling, P. A. (2002) J. Physiol. 544, 351–362
35. Ikeda, T., Schmitt, B., Pouyssegur, J., Wakabayashi, S., and Shigekawa, M. (1997) J. Biochem. (Tokyo) 121, 295–303
36. Wakabayashi, S., Hisamitsu, T., Pang, T., and Shigekawa, M. (2003) J. Biol. Chem. 278, 11828–11835
37. Parker, M. D., Ourmuzdi, E. P., and Tanner, M. J. (2001) Biochim. Biophys. Acta 1538, 1109–1116
Acute pH-dependent Regulation of AE2-mediated Anion Exchange Involves Discrete Local Surfaces of the NH$_2$-terminal Cytoplasmic Domain

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