Intracellular pH Sensors: Design Principles and Functional Significance

Changes in intracellular pH regulate many cell behaviors, including proliferation, migration, and transformation. However, our understanding of how physiological changes in pH affect protein conformations and macromolecular assemblies is limited. We present design principles, current modeling predictions, and examples of pH sensors or proteins that have activities or ligand-binding affinities that are regulated by changes in intracellular pH.

Changes in intracellular pH (pHi) regulate a number of normal and pathological cell processes. Increases in pHi are permissive for growth factor-induced cell proliferation (22, 40, 80), cell cycle progression (82, 106), and differentiation (12, 107, 112) and are necessary for haptotokinetic migration (21, 44, 47, 85, 96) and ameboid chemotaxis (78, 86, 94, 108). Additionally, increased cytosolic pH is a hallmark of transformed cells from different tissue origins and genetic backgrounds, making it a common characteristic of distinct cancers (16, 36) and possibly a common critical driving force for tumor progression (81, 85). A decrease in cytosolic pH promotes caspase-dependent and -independent apoptosis (46, 59, 119), and in response to some apoptotic signals precedes mitochondrial dysfunction (52). Because cytosolic pH homeostasis is tightly regulated (11), dramatic differences in cell behavior are driven by relatively small changes in pH. The increased pHi in transformed cells is only 0.3–0.4 pH units greater than in normal cells, which is generally maintained at ~7.2, and apoptosis is triggered at 0.3–0.4 pH units lower than normal.

Despite established effects of small changes in pH, on diverse cell functions, our understanding of how these changes affect proteins and macromolecular assemblies driving specific cell processes is limited. The objective of this review is to present design principles, current modeling predictions, and examples of what we term “pH sensors,” or proteins that have activities or ligand-binding affinities that are sensitive to small, physiologically relevant changes in pH. Although changes in solvent pH affect the ionization state of all weak acids and bases, and all cellular proteins contain amino acids with titratable groups, only select proteins appear to be bonaﬁde pH sensors. New insights from protein structures and biomolecular simulations (64) are beginning to reveal the structural basis for tight coupling between protonation state and protein conformation. Exquisitely pH-sensitive conformational switches have been shown to regulate activity of ion (reviewed in Ref. 98) and water (102) channels, affinity of proteins for binding actin ﬁlaments (14, 33, 79, 110), and activity of viral (15, 41) and bacterial (62, 89) proteins controlling host cell entry. Nearly 50% of enzymes contain sensitive histidine residues within their active sites [93], and the structural mechanisms for how physiologically relevant changes in pH regulate the enzymatic activity of the bacterial type III adenyl cyclase (100), β-secretase (32), and glycineamide ribonucleotide transformylase (GART) (67) have been examined.

Regulation by pH Sensors and Signaling Modes

One advantage of using protons for signaling and regulation as opposed to posttranslational modifications or cofactors is the potential for extremely rapid temporal responses. Protons can diffuse through water exceptionally quickly (“Grotthuss diffusion,” made possible in part by the quantum mechanical tunneling ability of protons), possibly facilitated by short-range motion in proteins (58). The variation of pH among subcellular locales makes possible spatial regulation as well. Protons also present an elegant means of regulating protein activity and interactions. A proton is an exceptionally small subatomic particle that can induce a reversible chemical change resulting in substantial electrostatic perturbation, which can drive changes in protein structure, dynamics, and interactions.

As a regulator of protein function, protons can be used in multiple signaling modes. The Bohr effect of pH-driven changes in the afﬁnity of hemoglobin for oxygen binding is the classic example of a single-site, proton-induced allosteric regulation. The effect of pH is mediated by a His-Asp salt bridge (His146 and Asp94 in human hemoglobin), which is disrupted when His146 is deprotonated at increased pH. However, electrostatic interactions, particularly due to chloride binding, also affect proton exchange at His146 (reviewed in Ref. 53). Cooperativity involving binding of ligands or electrostatic coupling of multiple proton binding sites play pivotal roles in pH sensing (28, 95, 115). An example of thermodynamic linkage is the HIV-1 protease, which is required for the formation of mature virions.

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HIV-1 protease undergoes a shift in the pKa of a catalytic aspartyl dyad when bound to inhibitors (105), and deprotonation of the dyad at increased pH dramatically increases the affinity of HIV-1 protease for cationic inhibitors (39). Also, the mammalian prion protein PrpC contains unique octapeptide repeats (PHGGGWGQ) in which histidines function as pH sensors. At neutral and basic pH, deprotonated His in the octapeptide repeats trigger aggregation and increased affinity for Cu²⁺ binding to the repeat domains (FIGURE 1) (63, 120). However, Cu²⁺ binding generates a gain of pH sensitivity for aggregation. The protonation state of coordinating side chains also affects positive cooperativity, as seen with Ca²⁺ binding by the C2 domain (57), a membrane targeting module in phospholipase A2, protein kinase C, and synaptotagmin. Another signaling mode that is becoming increasingly apparent is the role of protons as coincidence regulators with other posttranslational modifications, including phosphorylation or binding of phosphatidylinositol 4,5-bisphosphate (PIP₂). PIP₂ binding could localize a pH sensor to a specific membrane domain where its activity or ligand binding affinity is regulated by localized membrane H⁺ fluxes. Coincidence regulation with phosphorylation state is seen for cofilin, an actin depolymerizing and severing protein that requires independent events of Ser3 dephosphorylation and His133 protonation for binding actin filaments (79).

At the cellular level, physiological changes in pHi act as a signaling feedback mechanism controlling intracellular cation and osmotic homeostasis. The activity of several plasma membrane transport proteins, including channels, exchangers, and pumps, are pH sensitive. Although mechanisms of pH sensing by transport proteins have primarily been inferred from mutagenesis studies, some models have been proposed based on structural data and molecular simulations. In NhaA, a Na-H exchanger in Escherichia coli, its crystal structure (38) and multiconformation continuum electrostatics calculations (75) predict pH sensing is achieved by four clusters of electrostatically coupled amino acids with shifted pKa near the funnel, which regulate long-range conformational changes affecting a distinct H⁺ exchange site. Structural data and molecular dynamics simulations have also been used to predict pH gating by the plant aquaporin channel SoPIP2;1 (102). Proton sensing is predicted to be achieved by a single histidine residue, His193, in the second cytoplasmic loop (D loop). Rotation of the protonated His193 side chain forms a salt bridge with Asp28 that anchors a folded D loop underneath the molecule and occludes the pore. His193 is strictly conserved in plant aquaporins and is critical for pH-sensitive gating by the Arabidopsis aquaporin PIP2;2 (103). Hence, channel gating by intracellular protons may be a conserved mechanism in plant aquaporins to coordinate water transport under anoxic stress. In contrast, several mammalian aquaporins are gated by extracellular protons, analogous to acid-sensing Ca²⁺ and K⁺ channels.

Intracellular pH gating by the renal inwardly rectifying K⁺ channel Kir1.1 (ROMK), which closes in response to pHᵢ < 7.0, acts as a feedback mechanism coupling K⁺ retention during metabolic acidosis. Kir1.1 (ROMK) gating is exquisitely sensitive to changes in pHi as small as 0.2 pH units. The pH sensor, which is structurally distinct from the physical gate for

<table>
<thead>
<tr>
<th>Protein</th>
<th>E. coli glycaminide ribonucleotide transformylase⁽¹⁾</th>
<th>Human glycaminide ribonucleotide transformylase⁽²⁾</th>
<th>Influenza virus hemagglutinin⁽³⁾</th>
<th>Neonatal Fc receptor⁽⁴⁾</th>
<th>Mammalian PrP octapeptides repeats⁽⁵⁾</th>
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<tr>
<td>Structures</td>
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<td><img src="image2.png" alt="Human glycaminide ribonucleotide transformylase" /></td>
<td><img src="image3.png" alt="Influenza virus hemagglutinin" /></td>
<td><img src="image4.png" alt="Neonatal Fc receptor" /></td>
<td><img src="image5.png" alt="Mammalian PrP octapeptides repeats" /></td>
</tr>
<tr>
<td>pH's at which structural data is available</td>
<td>3.5 (blue), 7.5 (purple)</td>
<td>4.2 (blue), 8.2 (purple)</td>
<td>5.0 (right), 7.0 (left)</td>
<td>6.5 (shown); 8.0</td>
<td>4.5 – 7.8</td>
</tr>
<tr>
<td>Aspect of function affected by pH</td>
<td>Catalytic activity</td>
<td>Substrate binding</td>
<td>Membrane fusion</td>
<td>Protein-protein interaction</td>
<td>Aggregation</td>
</tr>
<tr>
<td>Structural mechanism</td>
<td>Local order-disorder transition, residues 110–131 (green) near active site</td>
<td>Conformational change in substrate binding loop, residues 8–14 (wireframe)</td>
<td>Major rearrangement of tertiary structure (residues 40 (blue) to 153 (red) of one monomer shown)</td>
<td>Little structural change; protonation states of His in interfaces modulate interaction strength (His 250 and His 251 shown, with Glu89)</td>
<td>Order-disorder transition</td>
</tr>
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FIGURE 1. Structural mechanisms underlying pH regulation of protein function
Five examples are presented where X-ray crystal or NMR structures have been obtained at two or more values of pH. These pH values sometimes lie outside the range of physiological pH, due to experimental constraints. (1), Ref. 97; (2), Ref. 122; (3), Ref. 15; (4), Ref. 111; (5), Ref. 120.
channel closure, is thought to include a triad of a lysine residue, Lys80, in the first transmembrane domain, and spatially close Arg41 and Arg311 in the NH$_2$- and COOH-terminal cytoplasmic domains, respectively (reviewed in Ref. 90). Lys80 has a downshifted pK$_a$, and its protonation is predicted to induce a conformational change resulting in pore closure. However, Lys80 is not conserved in some pH-sensitive Kir1.1 orthologs, and molecular dynamics simulations predict it is not solvent accessible (84), hence its critical role in pH-gating of ROMK channels is controversial. Of clinical significance are naturally occurring mutations in Kir1.1 that shift pH gating in patients with hyperprostaglandin E syndrome (referred to as Bartter Syndrome), a hereditary renal tubulopathy associated with excessive salt loss.

**Structural Biology of pH Regulation**

In some cases, it has been possible to obtain multiple X-ray crystal structures of a protein at varying pH, providing atomic level details of how protonation can drive structural changes in systems such as bovine beta-lactoglobulin (83), insulin (24, 35), ribonuclease A (8), the response regulator DivK (34), glycaminide ribonucleotide transformylase (97, 122), ovotransferrin (23), carbonic anhydrase II (71), the neonatal Fc receptor (111), gamma-chymotrypsin (26), myoglobin (117), and influenza hemagglutinin (15) (FIGURE 1).

Nuclear magnetic resonance (NMR) is arguably the most powerful method for studying pH-driven changes in protein structure and dynamics. NMR can provide not only an atomic structure of a protein in solution but also information about conformational heterogeneity and dynamics occurring on a variety of time scales. Because the experiments are performed in solution, pH can be modulated easily. Finally, the protonation and deprotonation of titratable groups can be monitored directly by the proton signals; in contrast, protons can only be observed in X-ray crystallography experiments at very high resolution.

On the other hand, one major limitation of NMR is that interpretation of the spectra becomes more difficult as the size of the protein increases, and in practice most NMR studies have been limited to proteins of relatively modest (<150 amino acids, monomeric) size. NMR studies of pH-sensitive proteins or protein fragments in this size range have provided some of the most detailed information on the structural basis of pH regulation. As discussed above, cofilin is a pH-sensitive actin regulatory protein. Pope et al. (79) obtained the structure of human cofilin by NMR and monitored conformational changes as the pH was varied between 6 and 8. NMR methods have also been used to study the pH-dependent behavior of mammalian prion protein (120), low-density lipoprotein receptor (6, 7), receptor-associated protein (49), and villin headpiece (33, 99).

**Computational Tools and Theoretical Insights**

The pK$_a$ of a protonatable group is defined as the pH at which it is 50% protonated. The titration of any single protonatable group is governed by the Henderson-Hasselbach equation, which can be written as $<\alpha> = (10^{pK_a - pH})/(1 + 10^{pK_a - pH})$ where $<\alpha>$ is the fractional protonation of the titratable group with a given pK$_a$. This equation describes a sigmoidal titration curve. Titrating groups found in the standard 20 amino acids are shown in FIGURE 2.

There are two primary challenges in studying the titration behavior of proteins in solution. The first is that the pK$_a$ values of titratable groups vary depending on their environment in the protein. That is, the pK$_a$ of, e.g., a Glu side chain can vary by several units depending on where in the protein it is found. The side chain of free glutamate in solution is ~4, too low to be relevant to almost all biological environments. In a
protein, however, the pKa can vary widely. The two key parameters are the electrostatic potential and solvent accessibility. If a Glu is found in a region of positive electrostatic potential (e.g., near Lys or Arg side chains), the negatively charged form is stabilized, which has the effect of lowering the pKa. Conversely, negative electrostatic potential increases the pKa. Favorable interactions between the side chain and water also help to stabilize the charged form, and so burying a Glu in the interior of a protein can shift the pKa upward. Similar arguments apply to all of the titratable side chains.

The second major challenge in understanding pH titrations of proteins is that they generally contain multiple titratable groups. If these groups are widely separated (~25 Å), then they may titrate independently. However, when two or more titrating groups are spatially close, and particularly when they have similar pKa values, more complicated titration behavior can result. Put simply, the protonation state of each titrating group can affect the pKa (and hence the most probable protonation state) of all of the other titrating groups due to either the direct electrostatic interactions discussed above or conformational changes driven by the changes in protonation state. These interactions between titrating sites can lead to complex, nonsigmoidal titration curves. This also implies that interpreting the effects of mutating titratable residues (e.g., to Ala) can be complicated, because the elimination of a titratable group can impact the titration of other nearby groups. That is, any amino acid substitution that eliminates or introduces a charged group can perturb the pKa of nearby titratable groups, and this effect should be considered when interpreting the results of mutagenesis in studies of pH-dependent properties of proteins. Ullmann and coworkers have published an elegant mathematical formalism for understanding the complex titration behavior of multiple interacting groups (45, 76).

A number of computer programs have been developed to predict the titration behavior of proteins given their structure. One of the challenges in making such predictions is the treatment of water, and for computational efficiency most widely used methods treat water using continuum electrostatics rather than as discrete solvent molecules. The first generation of such programs predicted the titration behavior based on the assumption that the protein remained in a single static conformation. In many cases, such methods could correctly predict qualitative behaviors, such as the direction of pKa shifts for individual side chains. To improve the quantitative accuracy, much effort has been directed toward the development of methods that explicitly treat proteins as conformationally flexible (3, 10, 54, 92, 109, 118, 123). Some of these methods only treat conformational changes in protein side chains, keeping the backbone fixed (30, 113). A new generation of programs, which are referred to as constant-pH molecular dynamics (27, 42, 51, 55, 64, 65) and include versions of the widely used AMBER and CHARMM packages, relaxes this restriction and treats the entire protein as flexible, moving under Newton’s equations of motion. The protonation states are allowed to vary during the simulation in a way that is thermodynamically consistent with the specified pH. These programs hold the promise of providing a detailed understanding of how pH can regulate protein activity. The major limitation, as with any molecular dynamics methods, is that the fundamental time step in the simulation is ~1 fs, which means that it is difficult to study pH-driven conformational changes that occur on time scales longer than a few microseconds.

Computational studies using these methods have helped to develop models for the pH-dependent behavior of human prion protein (48), GART (66, 67), and HIV-1 protease (105). Overall, however, there has been relatively little computational work to elucidate the atomic-level mechanisms of pH regulation. The increasing sophistication of the computational tools suggests that it should be possible to do so, in conjunction with experiments that test the computational predictions.

A Closer Look at the Role of pH Sensors in Selective pH-Dependent Processes

We examine in more detail pH sensors regulating two processes: actin filament dynamics in cell migration and viral and bacterial pathogenesis. These examples demonstrate that multiple pH sensors can act collectively to control a specific cell response. They also exemplify distinct modes of regulation, including single-site allosteric modulation, cooperativity, and coincidence. Moreover, they include proteins regulated by changes in cytosolic pH of 0.5 or less pH units (for actin filament dynamics) as compared with proteins regulated by larger pH gradients across the endosome or phagosome membrane (viral and bacterial pH sensors regulating host cell entry and virulence).

pH sensors regulating actin filament dynamics in cell migration

An increase in pH, is an evolutionarily conserved but poorly understood mechanism necessary for directed cell migration. Early work on the fertilization of sea urchin eggs (5), the acrosomal reaction in *Echinoderm* sperm cells (101), and the motility of *Ascaris* sperm cells (43) indicated that transient increases in pH are necessary for cytoskeletal assemblies driving polarity and movement. Increased pH also is necessary for polarity and directed migration of mammalian fibroblasts (21, 96) and epithelial cells (44, 47, 85) and for chemotaxis of neutrophils (94), leukocytes (86), and *Dictyostelium* cells (78, 108). Regulation of cell motility by changes in pH is a mechanism to temporally and
changes in pHi. Cofilin severing activity is necessary to actin filaments with dependence on physiological destrin, and twinfilin, bind, depolymerize and sever including cofilin, actin-depolymerizing factor (ADF), respectively, are candidate pH sensors.

Cofilin. Most members of the cofilin superfamily, including cofilin, actin-depolymerizing factor (ADF), destrin, and twinfilin, bind, depolymerize and sever actin filaments with dependence on physiological changes in pH. Cofilin severing activity is necessary to generate a rapid (~1 min) increase in actin free barbed (+) ends (69), which promotes the assembly of new actin filaments that drive membrane protrusion at the leading edge and cell migration. Functional (9, 37, 121) and structural (13, 79) data suggest that mammalian cofilin is a coincidence detector requiring two independent activation steps: dephosphorylation of Ser3 by the phosphatases Slingshot (73) or chronophin (31), and deprotonation of His133. Cofilin severs actin filaments by changing the filament helical twist (61), which requires binding actin at two sites: an NH2-terminal G site and a COOH-terminal F site. NMR analysis and molecular dynamics simulations of human cofilin (79) suggest that the F site is retained in a conformation with decreased affinity for actin by a solvent-exposed salt bridge between a protonated His133 imidazole ring and a carboxylate group in Asp98. Although the pKa of His133 has not been determined, functional data indicate that the pH-sensitive switch to activate cofilin occurs between pH 6.9 and 7.2, which suggests a slightly upshifted pKa. With increasing pH and breakage of the salt bridge, significant chemical shift perturbations are seen for a number of nearby residues (79). However, pH-dependent conformational changes in the F site are more subtle compared with phosphorylation-dependent changes in the G site. Computational modeling that we performed using constant-pH molecular dynamics simulations also indicates that conformational changes between pH 6.5 and 8.0 are minor and largely confined to the immediate vicinity of His133 (FIGURE 3). His133 is located among the residues that define the F site, and hence changing the fractional protonation of this titrating residue can directly modulate actin binding without driving large conformational changes.

Increased pH as a coincidence activator of cofilin is an elegant means to generate rapid bursts of localized actin assembly. Dephosphorylated cofilin is highly localized to the submembranous region of protrusions or lamellipodia (19) where it could be “primed” for activation by increased H+ efflux at the plasma membrane. However, cofilin isoforms and related proteins have evolved differences in pH sensitivity. Of the three mammalian cofilins, ADF has a significantly higher activity with increasing pH. Actophorin, the amoebae cofilin, lacks a COOH-terminal His, and its activity is not pH-sensitive (56), and the cofilin-related yeast twinfilin has pH-dependent severing activity, but activity increases with decreasing pH (68). These variations can be exploited in future studies to understand the dynamics of sensitive pH-dependent conformational changes.

Villin. Villin, a member of the gelsolin superfamily, bundles actin filaments to maintain epithelial brush border organization and to promote cell migration. The actin-binding “headpiece” domain at the COOH terminus of villin is a conserved module in more than 20 other proteins and has pH-dependent affinity for binding actin filaments. As determined by circular dichroism spectra, NMR, and 1H/2H amide exchange (33, 99, 110), a pH-sensitive switch induces folding of the NH2-terminal His. NMR analysis and molecular dynamics simulations of human villin (79) suggest that the NH2 terminus of villin headpiece functions as a physiological pH sensor. His41, the only histidine in chicken villin headpiece (HP67), has a downshifted pKa. With a positively charged His imidazole ring at lower pH, hydrophobic packing is destabilized, and the NH2 terminus is unfolded (99, 110). Whether headpiece functions as a physiological pH sensor is uncertain because the NH2 terminus is fully folded at pH 6.5. However, pH-dependent conformational changes and actin binding have been determined only for isolated headpiece, and it is unknown whether a shift in pH sensitivity occurs when headpiece is part of a larger structure.
Talin. Dynamic turnover of cell-substrate or focal adhesions at the cell front is necessary for efficient haptokinetic cell movement and is attenuated at pH < 7.2 (21). The stability of focal adhesions is in part regulated by the focal adhesion-associated protein talin (18), which binds integrins and F-actin to tether the actin cytoskeleton to integrin adhesion complexes. Two distinct pH-dependent processes could disrupt the integrin-talin-actin linkage to permit focal adhesion turnover with small increases in pH. One is cleavage of the mid-region rod domain of talin by the cysteine protease calpain (29), which is activated with increased pH (114). Another is pH-sensitive actin binding by the COOH terminal I/LWEQ domain of talin. Increasing pH > 7.0 lowers the affinity for actin binding by the I/LWEQ domain (50, 88), which might disrupt the talin-actin linkage and allow focal adhesion turnover. In contrast, high-affinity binding of actin by talin at pH < 7.0 would act like a brake to stabilize the linkage and inhibit turnover. Although the structure of the COOH-terminal domain of talin has not been determined, we constructed a homology model of the I/LWEQ domain (FIGURE 4) based on the solved structure of the corresponding domain in huntingtin-interacting protein (Hip)1R. Computational pKa prediction [MCCE (Multi-Conformation Continuum Electrostatics)] (2) using this model revealed that five amino acids have pKa near the physiological range. All five are clustered at one end of the helix-bundled I/LWEQ domain; only one is a His, and the rest are Glu/Asp with predicted upshifted pKa. How or whether this cluster acts as a pH sensor to modulate the affinity for actin binding remains to be determined. The I/LWEQ domain is a conserved actin-binding module (60) also present in Sla2, a yeast protein necessary for cytoskeletal polarity, and the metazoan Sla2 homologs Hip1, the polyglutamine-containing protein that binds huntingtin and is associated with Huntington disease, and Hip1R, which does not bind huntingtin but binds clathrin to regulate endosome and Golgi trafficking.

Viral and bacterial pH sensors in host cell entry and virulence

Most viral and bacterial pH sensors are activated at low pH, which likely is an adaptation to take advantage of the host uptake system (17). In influenza A, two of the three integral membrane proteins necessary for viral uptake, hemagglutinin (HA) and the M2 channel, are pH sensors. At neutral pH, HA monomers are apolar, containing an NH2-terminal hydrophobic “fusion peptide” packed antiparallel to a core α-helical stem domain and a COOH-terminal transmembrane anchor (FIGURE 1). The acidic environment of the host cell endosome triggers a fusogenic conformation of HA, which X-ray crystallography reveals includes movement of the NH2 terminus of >100 Å to align at the top of the stem domain. (FIGURE 1) (15). After fusion, activation of M2, a pH-gated H+ channel, is necessary to acidify the virion as a prerequisite for uncoating. At neutral pH, the channel is closed, and data from NMR analysis (72), UV resonance Raman spectroscopy (74), and molecular dynamics simulations (41) suggest that, when His37 is uncharged, the bulky indole side chain of Trp41 is oriented to occlude the channel pore. His37 has a predicted downshifted pKa of 5.6, and with the acidic pH in the endosome charged His37 allows rotation of Trp41 for channel opening and H+ efflux into the virion. Kass and Arkin (41) predict that the gating model for M2 may be applicable to a number of pH-sensitive K+ channels.

The low endosomal or phagosomal pH also activates a number of pore-forming bacterial toxins. The
biological challenge for these toxins is reversible conversion from a soluble monomeric protein to a transmembrane multimeric channel. Alpha-barrel-forming toxins, such as colicin (70) and diphteria toxin (17), are predicted to form pores by using helical hairpins. They share no significant sequence homology but have remarkable structural homology and a conserved pH-dependent conformational switch. Soluble forms contain layers of buried α-helices at neutral pH that unfold at low pH to a coiled, insertion-competent structure. In contrast, β-barrel-forming toxins assemble as homo-oligomers to form channels and do not share a common pH-activated mechanism. Listeriolysin O, a cytolysin, is active at pH < 6 but unfolded at neutral pH because a β-hairpin containing a triad of acidic residues (Asp208, Glu247, Asp326) is destabilized (89). Alpha-hemolysin of Staphylococcus auerus self assembles at cell membranes and channel conductance increases at pH 6 compared with pH 7. Molecular dynamics simulations suggest that protonation of seven solvent-exposed His144 residues in the oligomer increases channel conductance (1).

Clinical Perspectives

The pH sensors regulated with normal pH homeostasis likely also play roles in pathologies associated with deregulated pH, as seen in transformed or apoptotic cells. Understanding pH-sensitive regulation at the atomic level facilitates the rational design of therapeutics, as recently described in targeting the pH-sensitive HIV-1 protease (4). Gain or loss of pH sensor function can drive pathologies or be engineered to resist pathologies. In some patients with pediatric adrenal cortical carcinoma (ACC), a naturally occurring mutation in the tumor suppressor protein p53 results in a gain of pH sensor function (25). In wild-type p53, Arg337 and Asp352 from different monomers form a salt bridge across helix interfaces that stabilizes tetramer formation necessary for DNA binding and tumor suppressor function. Because side chains in these residues are protonated between pH 5 and 9 (FIGURE 2), the salt bridge and p53 function are relatively pH insensitive. However, in ACC, an Arg337His substitution results in a salt bridge that is sensitive to physiological changes in pH. With increased pH, perhaps during development, deprotonation of His337 disrupts the salt bridge, which destabilizes tetramer assembly. Engineering predictable pH-switches is a relatively unexplored but feasible therapeutic approach, as described recently for a histidine switch in troponin designed to protect against myocardial ischemia (20).

Questions Remaining to be Answered

Clearly, much remains to be learned about the role of pH in regulating protein function and cellular processes, and especially about the link between the two. We conclude by posing some speculative questions that we hope may be answered by future work.

1) What are the principles of ultrasensitive pH switches in proteins? In the cytosol, pH varies only within a small range. In vitro, proteins like phosphofructokinase have striking changes in activity over very small ranges of pH (a few tenths of a pH unit) (104). Most proteins, however, do not show ultra-sensitivity to pH, despite the presence of multiple titrating groups in most cases, and from a theoretical perspective ultrasensitive pH sensing is not well understood. It seems clear that multiple strongly interacting titrating groups must function together to form an ultrasensitive pH switch. Conformational changes in response to protonation state changes likely also play a role. Finally, the activity or binding properties of a protein may depend conditionally on the protonation states of multiple titrating groups, which can lead to more sensitive pH switching. Beyond these simple principles, however, little is understood. The ultimate test would be to design ultrasensitive pH switching into a protein de novo.

2) Do cells use protons to dynamically regulate protein function in the cytosol? The role of pH in regulating protein activity in acidic subcellular compartments such as endosomes is relatively well understood. The dynamic pH changes in the cytosol are smaller, but increasing evidence, reviewed above, suggests that these changes have important cellular consequences, including for cell migration, tumor progression, and apoptosis. However, our understanding of the detailed molecular mechanisms underlying this pH regulation remains fragmentary.

3) Do protons act cooperatively with other signaling mechanisms, such as posttranslational phosphorylation? At a structural level, the addition of a negatively charged phosphate can perturb the pKa of titrating groups; thus it is conceivable that phosphorylation (or dephosphorylation) could be required for pH-sensitive behavior of a particular protein. Even more speculatively, the phosphate group itself has a pKa of ~6.5, roughly the same as histidine, and so the effects of phosphorylation could be pH sensitive (116). Conversely, structural changes induced by pH could promote phosphorylation, e.g., by making the site of phosphorylation more accessible to a kinase.

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References


