Distinct Histidine Residues Control the Acid-induced Activation and Inhibition of the Cloned K<sub>ATP</sub> Channel*  

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The modulation of K<sub>ATP</sub> channels during acidosis has an impact on vascular tone, myocardial rhythmicity, insulin secretion, and neuronal excitability. Previous studies have shown that the cloned Kir6.2 is activated with mild acidification but inhibited with high acidity. The activation relies on His-175, whereas the molecular basis for the inhibition remains unclear. To elucidate whether the His-175 is indeed the protonation site and what other structures are responsible for the pH-induced inhibition, we performed these studies. Our data showed that the His-175 is the only proton sensor whose protonation is required for the channel activation by acidic pH. In contrast, the channel inhibition at extremely low pH depended on several other histidine residues including His-186, His-193, and His-216. Thus, proton has both stimulatory and inhibitory effects on the Kir6.2 channels, which attribute to two sets of histidine residues in the C terminus.

ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) channels couple the intermediary metabolism to cellular activity and play important roles in numerous cellular functions such as insulin secretion, neuronal excitability, vascular tones, and muscle contractility (1–6). The K<sub>ATP</sub> channels consist of the pore-forming Kir6 and sulfonylurea receptor (SUR) subunits. Without SUR, the Kir6.2 alone can express functional channels with essential ATP sensitivity when the last 26 or 36 amino acids are deleted (i.e., Kir6.2ΔC26 or Kir6.2ΔC36) (7). The K<sub>ATP</sub> channels are modulated by multiple cytosolic factors, likely through allosteric mechanisms. Their hallmark feature is the sensitivity to intracellular ATP that inhibits channel activity. ADP and phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) are another two regulators with opposite effects to ATP (8–10). In the presence of PIP<sub>2</sub>, the IC<sub>50</sub> of ATP concentration increases by at least 100-fold, allowing these channels to be activated at near-physiological ATP levels (11–12). Similar to ATP, ADP, and PIP<sub>2</sub>, proton is a potent K<sub>ATP</sub> regulator in a number of tissues (13–21). Some studies have suggested that proton rather than ATP or ADP is the ultimate signal reflecting the metabolic status in the muscle cells (9).

The pH sensitivity is more complex in K<sub>ATP</sub> than in all other Kir channels. In excised patches, the K<sub>ATP</sub> first undergoes activation with mild acidosis and then is strongly inhibited with further acidification (15, 16, 20). The activation is a reversible process relying on inherent properties of the channel protein, whereas the inhibition manifests itself when the activation is removed and shows rather poor reversibility (20). We have shown previously that His-175 is crucial for the pH-dependent channel activation (20). This histidine residue is conserved in Kir6 but not seen in any other Kir channels. Therefore, detailed studies of such a critical residue may lead to a discovery of molecular intervention to K<sub>ATP</sub> channels by manipulating this histidine residue. Although histidine is the ultimate proton sensor according to its side-chain pK<sub>a</sub> value, it can be involved in channel gating via other mechanisms. Thus, the demonstration of the real proton sensor becomes crucial in the understanding of the pH-dependent K<sub>ATP</sub> activation. To determine whether titration of the side-chain amine group of His-175 indeed occurs at acidic pH, we performed systematic mutagenesis experiments on the His-175 using whole-cell voltage clamp and single-channel recordings. We reasoned that if the channel activation requires protonation of the His-175, a replacement of it with a positively charged residue should lead to an enhancement of the base-line currents. On the other hand, its substitution with a neutral or negative residue should have no effect or even suppress the base-line currents if the channel expression in the plasma membranes remains unchanged. Furthermore, mutations to any other amino acids should abolish the acid-induced channel activation, because of their titratability in an environment that favors the histidine titration at pH 6–7.

The pH-dependent channel inhibition is another important characteristic of the K<sub>ATP</sub>, which is shown in excised patches at low pH. Since such an inhibition is also pH-dependent, it should have its own pH-sensing mechanisms in the Kir6.2 protein, which may depend on histidine residues as well. To understand the proton-sensing mechanisms for the Kir6.2 activation and inhibition, we performed these experiments in which all histidine residues in the Kir6.2 protein were studied either alone or in combination with other histidines. Our results indicate that there are two sets of histidine residues in the Kir6.2 channels critical for the pH-dependent activation and inhibition, respectively.

MATERIALS AND METHODS  
Oocytes from *Xenopus laevis* were used in the present studies. Frogs were anesthetized by bathing them in 6.3% 3-aminobenzoic acid ethyl ester. A few lobes of ovaries were removed after a small abdominal incision (~5 mm). The surgical incision was then closed, and the frogs were allowed to recover from the anesthesia. *Xenopus* oocytes were treated with 2 mg/ml collagenase (type I, Sigma) in the OR2 solution (in mM): NaCl 82, KCl 2, MgCl<sub>2</sub> 1, and HEPES 5, pH 7.4, for 90 min at room temperature. After 3 washes (10 min each) of the oocytes with the OR2

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‡ The abbreviations used are: SUR, sulfonylurea receptor; PIP<sub>2</sub>, phosphatidylinositol 4,5-bisphosphate; wt, wild type.
solution, cDNAs (25–50 ng in 50 nl of water) were injected into the oocytes. The oocytes were then incubated at 18 °C in the ND-96 solution containing (in mM) NaCl 96, KCl 2, MgCl₂ 1, CaCl₂ 1.8, HEPES 5, and sodium pyruvate 2.5 with 100 mg/ml filter geneticin added, pH 7.4.

Mouse Kir6.2 (GenBank™ accession number D50581) cDNA was genetically provided by Dr. S. Seino and subcloned into a eukaryotic expression vector (pcDNA3.1, Invitrogen Inc., Carlsbad, CA). Site-specific mutations were produced using a site-directed mutagenesis kit (Strategene, La Jolla, CA). The correct mutations were confirmed with DNA sequencing.

Whole-cell currents were studied on the oocytes 2–4 days after injection. Electrophysiological voltage clamp was performed using an amplifier (Geneclamp 500, Axon Instruments Inc., Foster City, CA) at room temperature (−24 °C). The extracellular solution contained (in mM): KCl 90, MgCl₂ 3, and HEPES 5, pH 7.4. The HEPES buffer was chosen because of its buffering range and membrane impermeability, as shown in our previous studies (20, 23).

Experiments were performed in a semi-closed recording chamber (BSC-HT, Medical System, Greenvalle, NY), in which oocytes were placed on a supporting nylon mesh, and the perfusion solution bathed both the top and bottom surface of the oocytes. The perfusate and the superfusion gas entered the chamber from two inlets at one end and flowed out at the other end. There was a 3 × 15-mm gap on the top cover of the chamber, which served as the gas outlet and an access to the oocytes for recording microelectrodes. At base line, the chamber was ventilated with atmospheric air. Exposure of the oocytes to CO₂ was carried out by switching a perfusate that had been bubbled for at least 30 min with a gas mixture containing CO₂ at various concentrations balanced with 21% O₂ and N₂ and superfused with the same gas (23–25). The high dissolved CO₂ resulted in a detectable change in intra- or extracellular pH values as fast as 10 s in these oocytes.

Macroscopic and single-channel currents were recorded in excised patches at room temperature (−24 °C) as described previously (24, 25). In brief, the oocyte vitelline membranes were mechanically removed after exposing to hypertonic solution (400 mM) for 5 min. Recordings were performed on the stripped oocytes using the same solution applied to bath and recording pipettes. The solution contained (in mM) KCl 10, potassium fluoride 150, NaCl 96, MgCl₂ 1, CaCl₂ 1.8, HEPES 5, and sodium pyruvate 2.5 with 100 mg/ml filter geneticin added, pH 7.4. A parallel perfusion system was used to deliver low pH perfusates at a rate of ~1 ml/min with no dead space (24, 25). Macroscopic and single-channel currents were analyzed using the pClamp 6 software as detailed previously (24, 25).

Data were further filtered (1 kHz) with a Gaussian filter. This filtering causes events shorter than 200 ms to be ignored. No correction was made for the missed events. Single-channel conductance was measured as a slope conductance with at least two voltage points. The open-state probability (P₀) was calculated by first measuring the time, tᵢ, spent at current levels corresponding to j = 0, 1, 2, −N channels open (24, 25). The P₀ was then obtained as P₀ = (Σj = 0, 1, 2, −N tᵢj/N, where N is the number of channels active in the patch, and T is the duration of recordings. P₀ values were calculated from stretches of data having a total duration of 20–60 s. The current amplitude was described using Gaussian distributions, and the difference between two adjacent fitted peaks was taken as unitary current amplitude.

The charge density of the side chain of an amino acid was calculated using the classical Henderson-Hasselbach equation pH = pKᵢ + log ([base]/[acid]). The ratio of [base]/[acid] was calculated using the side-chain pKᵢ value of a titratable amino acid in free base at different pH levels and was used as an index for the ratio of the protonated versus non-protonated state of the amino acid. The pH-current relationship was described using a sum of a double Hill equation: y = m₀/(1 + (pKᵢ/pKᵢ)²) + m₁/(1 + (pKᵢ/pH)²) − m, where pKᵢ is the midpoint channel activation; h₁ is the Hill coefficient for channel activation; pKᵢ is the midpoint channel inhibition; h₂ is the Hill coefficient for channel inhibition; and m = 1.2 (assuming 80% peak activation is reach before rundown starts). Data are presented as means ± S.E. Student’s t or analysis of variance test was used. Differences of CO₂ and pH effects before versus during exposures were considered to be statistically significant if p ≤ 0.05.

RESULTS

Biphasic Responses of Kir6.2 to Acidic pH—Whole-cell currents were studied in the two-electrode voltage clamp mode using an extracellular solution containing 90 mM K⁺. Depolarizing and hyperpolarizing command pulses were given to the cell in a range from −160 (−120 mV in some cells) to 100 mV with a 20-mV increment at a holding potential of 0 mV. Under such a condition, the inward rectifying currents were observed 2–4 days after cDNA injection. Consistent with our previous observations, currents produced by Kir6.2 and SUR1 or a single Kir6.2AC36 were equally stimulated when the cell was exposed to 15% CO₂ (Fig. 1A). Therefore, to simplify the studies, the Kir6.2AC36 was used. This effect resulted from intracellular acidification and was independent of ATP, as it was seen in the excised patch in the absence of ATP (20). In excised patches, the channel displayed a rapid inhibition at extremely acidic pH (20), which showed a poor reversibility and appeared similar to channel rundown (Fig. 1B).

His-175 as the Proton Sensor for the Acid-induced Activation—As we reported previously (20), mutation of His-175 to lysine (a residue found in several other Kir channels, H175K) or alanine (H175A) completely eliminated the acid-induced channel activation. Instead, these mutants were inhibited during hypercapnia in whole-cell recordings (Fig. 2A) and by acidic pH in excised patches (Fig. 1B). To determine how this histidine residue is involved in pH-dependent Kir6.2 activation, systematic mutation analysis was carried out. If certain side-chain properties of this residue such as the size, charged state, or hydrophobicity but not the titratability were critical for the proton sensitivity, a substitution of the histidine with another amino acid with similar side-chain properties would retain, to certain degree, the pH-dependent activation. To test this hypothesis, the His-175 was mutated to positively charged (H175K and H175R), neutral polar (H175N and H175C), neutral non-polar (H175A), or negatively charged (H175E and H175D) amino acids. When these mutants were tested, we found that the pH-dependent activation was totally lost in all these mutants (Fig. 2B).

Among these substitutes are arginine and alanine. Arginine...
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The channel activation depends on His-175. A, site-specific mutation of His-175 to lysine eliminates the channel activation during hypercapnia. Instead, the mutant channel is slightly inhibited. B, effects of systematic mutations of His-175 on the $K_{ATP}$ sensitivity. The His-175 was systematically mutated to various amino acids and then the mutant channels were studied with CO$_2$. None of the mutants shows activation during hypercapnia. Several His-175 mutants were rescued by introducing an additional mutation of Cys-166 to serine shown with *. They also lose the CO$_2$-induced activation. Note that BL control indicates base-line channel activity before CO$_2$ exposure.

is highly hydrophilic and capable of forming hydrogen bonds with adjacent residues, whereas alanine is not. Arginine but not alanine has a large side chain and is positively charged at physiological pH. Despite these distinct properties, replacement of the His-175 with arginine affected the CO$_2$ sensitivity almost identically to that with alanine.

We noticed that the amplitude of base-line currents before CO$_2$ exposure were closely associated with the charge characteristics of the amino acid at this location. Mutations to positively charged amino acids yielded channels with large base-line currents (H175K, 8.1 ± 1.1 μA, n = 8; H175R, 9.2 ± 1.1 μA, n = 5); mutations to neutral amino acid gave rise to moderate currents (H175A, 2.4 ± 0.4 μA, n = 11) similar to that of the wt Kir6.2ΔC36 (H175H, 2.1 ± 0.3 μA, n = 12); and no detectable current was seen when His-175 was mutated to negatively charged residues (H175D and H175E). The relationship of base-line currents with charge density was calculated according to the Henderson-Hasselbalch equation (see “Materials and Methods”). When the base-line currents were plotted against the charge density, a strong correlation was revealed with $r > 0.99$ and $p < 0.001$ (Fig. 3), indicating that the current amplitude is a function of the positive charge at this location.

There are two possible explanations for the difference in the base-line currents among these mutants. First, mutations to acidic or neutral amino acids are nonfunctional, owing to either misfolding, defective endoplasmic reticulum trafficking, or poor membrane insertion of the channel proteins. Second, the substitution with negative or neutral residues may lead most of expressed channels to the closed state, whereas mutations to the positive ones favor channel openings. If the mutant channels are expressed in the plasma membranes and stay in the closed state, their membrane expression can be demonstrated by activating these channels. Because the $K_{ATP}$ channels are normally inhibited by ATP, lowering the ATP sensitivity can enhance the open-state probability, increasing the likelihood to see their expression. Therefore, another mutation on Cys-166 was introduced, as the C166S mutant has been reported previously to stabilize the channel to the open conformation (21, 26). By adding the C166S mutation, several His-175 mutants (H175D, H175C, H175E, and H175Q) that failed to show channel activity were rescued (Table I). All of them were inhibited during CO$_2$ exposure, even though the side-chain properties of the amino acids vary greatly (Table I). These results therefore suggest that the limited currents seen with negative charge or neutrality at locus 175 are likely to be due to the inadequate channel openings rather than the lack of functional expression.

To understand further the relationship of the open-state probability ($P_o$) with the number of expressed channels, we studied single-channel currents in excised inside-out patches. The H175K had higher base-line activity ($P_o = 0.123 ± 0.016$, $n = 4$, $p = 0.001$) than the wt channel ($P_o = 0.021 ± 0.040$, $n = 5$) and H175A mutant ($P_o = 0.029 ± 0.006$, $n = 5$) (Fig. 4A). Based on the relationship $G = gNP_o$ (where $G$ is conductance of the macroscopic currents; $g$ is single-channel conductance, and $N$ is number of functional channels), the number of functional channels expressed in the plasma membranes can be estimated. We thereby measured base-line macroscopic currents and the single-channel conductance under the identical conditions. The base-line macroscopic currents were 26.1 ± 2.3 pA ($n = 12$) in the Kir6.2ΔC36, 30.3 ± 4.4 pA ($n = 7$) in the H175A, and 93.6 ± 17.4 pA ($n = 8$) in the H175K. The single-channel conductance was 74.1 ± 1.0 pS ($n = 15$) in the Kir6.2ΔC36, 74.0 ± 2.0 pS ($n = 4$) in the H175A, and 67.0 ± 1.3 pS ($n = 5$) in the H175K. We found that the large base-line H175K currents were due to a higher $P_o$ instead of a greater expression of functional channels in the plasma membranes, as the $G/P_o$ ratio, or the $N$ value, for the H175K is not greater than those for H175A and Kir6.2ΔC36 (Fig. 4B).

The whole-cell base-line currents of the H175K were also about 3–4 times larger than those of wt Kir6.2ΔC36 and H175A (Table I). The change in the base-line currents was not caused by a decrease in ATP sensitivity either, since H175K and H175A had a similar ATP sensitivity as wt Kir6.2ΔC36 (20). These results thus indicate that protonation of the His-175 occurs at acidic pH leading to the channel activation.

The H175K mutant showed high channel activity at pH 7.4–9.0. The channel activity decreased when the pH levels became higher or lower. The pK value for channel activation was pH 10.5 (Fig. 5A), suggesting that the lysine residue is also protonated at near its pK value for free-base amino acids in the microenvironment of this residue.

Fig. 3. The relationship of the current amplitude with the charge density at residue 175. There is a strong correlation between the base-line channel activity and the charge density of residue 175 when the currents are plotted as a function of the charge density (closed circle). The relationship of currents versus the charge density can be expressed using a linear regression: $y = 6.39x + 2.39$, where $y$ is currents and $x$ is charge density. Statistics shows $r > 0.99$ and $p < 0.001$ for regression.
The PHDsec and Chou-Fasman analyses show that the secondary structure around the His-175 is α-helical. Thus, we studied two residues in the immediate vicinity of the His-175, which are aligned onto the same surface of His-175. Interestingly, when we neutralized Glu-179, the E179Q mutant showed a biphasic response with the maximal channel activity seen at pH 8.0. Further mutation analysis was performed. Mutations of one of several histidines were constructed on the H175D, H175K, or C166S/H175D mutants. The rationale for choosing the C166S/H175D as a template is that this mutant is strongly inhibited by CO₂, raising the resolution of the CO₂/pH-induced channel inhibition (Fig. 6A). Note that the C166S mutant itself is not inhibited by 15% CO₂ (Table I), similar to what we have shown recently (21). Consistent with the CO₂ sensitivity of whole-cell currents, the C166S/H175D mutant was inhibited by intracellular acidosis in inside-out patches with pK = 6.68 (h = 2.1, n = 4, Fig. 6, B and C). Concerning the CO₂/pH sensitivities, the mutant channel behaved just like Kir1.1 and Kir2.3

| Name          | BL current | CO₂ effect | n  |
|---------------|------------|------------|----|
| Kir6.2C36     | 2.1 ± 0.3  | 148.0 ± 14.9 | 12 |
| H175A         | 2.0 ± 0.4  | 28.7 ± 5.9  | 3  |
| H175K         | 8.0 ± 1.0  | 21.4 ± 2.1  | 8  |
| H175R         | 9.2 ± 1.1  | 7.8 ± 2.9   | 5  |
| Multiple histidine mutants |          |            |    |
| *H175D       | 15.8 ± 4.8 | -67.6 ± 6.0 | 5  |
| *H175D/H46A  | 11.7 ± 1.1 | -67.6 ± 2.6 | 4  |
| *H175D/H70/0A| 26.4 ± 5.4 | -76.6 ± 2.6 | 4  |
| *H175D/H186A | 2.4 ± 0.2  | -5.4 ± 3.1  | 4  |
| *H175D/H1933 | 32.7 ± 6.7 | -4.7 ± 2.8  | 5  |
| *H175D/H216Q | 15.7 ± 6.0 | -6.9 ± 4.1  | 4  |
| *H175D/H259A | 10.5 ± 3.4 | -66.7 ± 5.4 | 4  |
| *H175D/H276A | 11.1 ± 2.5 | -67.1 ± 3.3 | 5  |
| *H175D/H277Q/278N | 13.1 ± 4.0 | -54.5 ± 3.6 | 4  |
| H175K         | 8.1 ± 1.1  | -21.4 ± 2.1 | 8  |
| H175K/H186A  | 17.0 ± 2.4 | 6.3 ± 3.4   | 5  |
| H175K/H1933  | 8.0 ± 0.4  | 0.8 ± 7.6   | 7  |
| H175K/H216Q  | 8.4 ± 1.7  | 2.6 ± 7.9   | 4  |
| H175K/H186A/H1913N | 15.5 ± 2.9 | 3.6 ± 0.9   | 5  |
| H175K/H70A   | 15.1 ± 3.2 | -11.9 ± 0.9 | 7  |
| H175K/H259A  | 5.0 ± 1.0  | 18.4 ± 0.7  | 5  |
| H186A        | 2.0 ± 0.7  | 169.1 ± 29.4 | 6 |
| H193N        | 2.0 ± 0.3  | 179.3 ± 29.3 | 4 |
| H216Q        | 2.1 ± 0.4  | 139.7 ± 14.6 | 4 |
| H186A/H193N  | 2.7 ± 0.4  | 190.6 ± 37.3 | 4 |
| H186A/H193N/H216Q | 3.5 ± 0.5  | 213.7 ± 29.1 | 5 |

* The mutants with * were constructed using the C166S mutant as a template. The abbreviations used are: BL, base line; n, number of observations; NF, nonfunctional.
channels where histidines are critical for the acid-induced channel inhibition (23, 27–29). Therefore, we systematically studied all histidine residues in the channel protein. We found that His-193 was a player. The acid-induced inhibition was almost completely removed by mutation of His-193 to glutamine (Fig. 7, A and B). Mutations of His-186 and His-216 had a similar effect, whereas replacements of other histidines did not (Fig. 8 A and Table I). Mutation of these histidine residues did not compromise the channel activation. Indeed, the magnitude of the activation was significantly larger in the H186A/H193N/H216Q triple mutant than in the wt Kir6.2/H193N (Fig. 8, B and C, Table I). Interestingly, mutations of these histidine residues markedly diminished the channel rundown, as shown in the H186A/H193N/H216Q and C166S/H175D/H193N (Fig. 9, A and B). These results thus indicate that another set of histidine residues are involved in the pH-dependent inhibition of Kir6.2 channels.

**DISCUSSION**

Proton is an important regulator of the $K_{ATP}$ channels (30). However, previous studies on the pH sensitivity of the $K_{ATP}$ in several tissues and cells were controversial and even contradictory. Channel response varies from activation (16, 19), no effect (22, 31), to inhibition (13, 18). By using the cloned $K_{ATP}$ channels, we have shown recently that acidic pH has dual effects depending on the pH range. The channels are activated by a moderate decrease in intracellular pH and inhibited at high acidity. Such two-phase effects, which have been observed in insulin-secreting cells and cardiac myocytes (15, 16), may underlie the controversy in the pH sensitivity of the cell-endogenous $K_{ATP}$.

Our previous studies have shown that the channel activation relies on His-175 (20). Mutation of this histidine residue completely eliminates the acid-induced channel activation (20). The molecular basis of the His-175-dependent channel activation was examined in the present study. Our data have shown that protonation of His-175 is essential for the channel activation. Indeed, the magnitude of the activation was significantly larger in the H186A/H193N/H216Q triple mutant than in the wt Kir6.2ΔC36 (Fig. 8, A and B, and Table I). Interestingly, mutations of these histidine residues markedly diminished the channel rundown, as shown in the H186A/H193N/H216Q and C166S/H175D/H193N (Fig. 9, A and B). These results thus indicate that another set of histidine residues are involved in the pH-dependent inhibition of Kir6.2 channels.
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Involvement of other histidine residues in the acid-induced channel inhibition. $A$, the CO$_2$-induced channel inhibition was studied in several histidine mutants constructed on the basis of the C166S/H175D that shows a strong inhibition by CO$_2$ (see Fig. 6A). The channel inhibition is greatly diminished when His-186, His-193, or His-216 is mutated, whereas mutations of other histidine residues have no significant effect. $B$, mutation of these histidines alone or combined does not reduce the channel activation during CO$_2$ (15%) exposure. Indeed, the CO$_2$ sensitivity of the H186A/H193N/H216Q triple mutant is significantly enhanced, which is detailed in C. *, $p < 0.05$; **, $p < 0.001$; dashed line, control level of channel response to 15% CO$_2$.

Fig. 8.

At this stage, the physiological significance for the pH-dependent inhibition of Kir6.2 is still unclear. Since such an inhibition shows very poor recovery and is clearly seen in excised patches but not in whole-cell recordings, it may be related to the channel rundown. Previous studies on the cell-endogenous $K_{ATP}$ have shown that channel rundown can be greatly attenuated when the cytosolic side of patch membranes is exposed to protease (33, 34). Therefore, it is possible that the $K_{ATP}$ channel rundown involves a large scale of movement of intracellular protein domains as demonstrated in the Kir1.1 (32), whereas these histidine residues may play a part in the movement or even possibly initiate the movement.

The $K_{ATP}$ channels are gated by several intracellular regulators including ATP, ADP, Pi, and proton. More complicated are the channel gating mechanisms, as proton itself has biphasic effects on channel activity showing how sophisticated the $K_{ATP}$ gating can be. Although the precise mechanisms for channel gating are still not clear, a better understanding of the $K_{ATP}$ regulation can be achieved with the availability of the information about all its key regulators. Therefore, the demonstration of the molecular basis for the pH-dependent channel activation and inhibition in the present study provides important information in this aspect.

In conclusion, the Kir6.2 channel shows a biphasic response to acidic pH. The channel activation requires a histidine residue (His-175) near the M2 region in the C terminus. The His-175 is very likely to be the proton sensor that couples the pH change to channel activation. The channel inhibition, on the other hand, is related to another three histidine residues, i.e. His-186, His-193, and His-216. Mutations of these residues greatly diminish the pH-dependent inhibition. Thus, proton has both stimulatory and inhibitory effects on the $K_{ATP}$ channels by acting on two distinct sets of histidine residues.

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