A Possible CO₂ Conducting and Concentrating Mechanism in Plant Stomata SLAC1 Channel

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Abstract

**Background:** The plant SLAC1 is a slow anion channel in the membrane of stomatal guard cells, which controls the turgor pressure in the aperture-defining guard cells, thereby regulating the exchange of water vapour and photosynthetic gases in response to environmental signals such as drought, high levels of carbon dioxide, and bacterial invasion. Recent study demonstrated that bicarbonate is a small-molecule activator of SLAC1. Higher CO₂ and HCO₃⁻ concentration activates S-type anion channel currents in wild-type Arabidopsis guard cells. Based on the SLAC1 structure a theoretical model is derived to illustrate the activation of bicarbonate to SLAC1 channel. Meanwhile a possible CO₂ conducting and concentrating mechanism of the SLAC1 is proposed.

**Methodology:** The homology structure of Arabidopsis thaliana SLAC1 (AtSLAC1) provides the structural basis for study of the conducting and concentrating mechanism of carbon dioxide in SLAC1 channels. The pKₐ values of ionizable amino acid side chains in AtSLAC1 are calculated using software PROPKA3.0, and the concentration of CO₂ and anion HCO₃⁻ are computed based on the chemical equilibrium theory.

**Conclusions:** The AtSLAC1 is modeled as a five-region channel with different pH values. The top and bottom layers of channel are the alkaline residue-dominated regions, and in the middle of channel there is the acidic region surrounding acidic residues His332. The CO₂ concentration is enhanced around 10⁴ times by the pH difference between these regions, and CO₂ is stored in the hydrophobic region, which is a CO₂ pool. The pH driven CO₂ conduction from outside to inside balances the back electromotive force and maintain the influx of anions (e.g. Cl⁻ and NO₃⁻) from inside to outside. SLAC1 may be a pathway providing CO₂ for photosynthesis in the guard cells.

Introduction

In biology, a stoma is a tiny pore, found in the epidermal tissues of leaves and stems, which is used for gas exchange. The pore is bordered by a pair of kidney-shaped parenchyma cells known as guard cells, which are responsible for regulating the pore aperture of the opening [1]. Ambient carbon dioxide enters the plant leaves through these stomatal pores, where it is used in photosynthesis. Oxygen produced by photosynthesis in the spongy layer cells (parenchyma cells with pectin) of the leaf interior exits through these same openings. In plant respiration the oxygen enters the plant through the stomata, too. Also, water vapor is released into the atmosphere through these pores in a process called transpiration [2,3].

The plant SLAC1 is a slow anion channel in the membrane of stomatal guard cell, which controls the turgor pressure in the aperture-defining guard cells of plant stomata [4–8], thereby regulating the exchange of water vapour and photosynthetic gases in response to environmental signals such as drought, high levels of carbon dioxide, and bacterial invasion [5,6]. Studies proved that SLAC1 is activated by phosphorylation from the OST1 kinase [9,10]. OST1 activity is negatively regulated by the ABI1 phosphatase [11], which is in turn inhibited by the stomatal ABA receptors PYR and RCAR [12] when in the ternary hormone-receptor-phosphatase complex [13,14]. Thereby, ABA stimulates SLAC1 channel activity. Resulting Cl⁻ efflux through SLAC1 causes membrane depolarization, which activates outward rectifying K⁺ channels, leading to KCl and water efflux to reduce turgor further and cause stomatal closure.

Recent study demonstrated that bicarbonate is a small-molecule activator of SLAC1 [15–17]. Elevated intercellular concentration of HCO₃⁻ with low concentration of CO₂ and H⁺ activated S-type anion channel, whereas low [HCO₃⁻] at higher [CO₂] and [H⁺] did not [15]. Thereby the bicarbonate activates the SLAC1 anion channels. However, the molecular mechanisms that underlie the SLAC1 activation and stomatal CO₂ signalling have remained relatively obscure. Some logical questions arise from these new findings. How does the concentration of HCO₃⁻ and CO₂ activate...
the SLAC1 to maintain the influx of anions and adjust the pressure in guard cells of stomata? Is there any connection between influx of anions (Cl\(^-\) and NO\(_3\)\(^-\)) and the concentration of HCO\(_3\)\(^-\) and CO\(_2\) in SLAC1 channel?

Recently an atomic-resolution crystal structure of the TehA from *Haemophilus influenzae* at 1.20 Å resolution was solved by Chen et al. [18,19] with the PDB codes 3M71, 3M72, 3M73 and 3M7L [http://www.rcsb.org/pdb/], notably HiTehA (*Haemophilus influenzae* TehA) [19]. Then a homology model of *Arabidopsis thaliana* SLAC1 (AtSLAC1) was developed by Chen et al. [18], which is substantially similar to the bacterial homologues. This milestone work provided the structural basis for solving the questions. In this study we perform a theoretical analysis for the activation mechanism of bicarbonate based on the protein structure of AtSLAC1 [18] using physicochemical calculation tools.

**Results**

The crystal structure of the HiTehA is a trimer consisting of three tightly associated subunits. Each protomer of HiTehA and AtSLAC1 has ten transmembrane helices. The fold of SLAC1 protomer is novel: tandemly repeated helical hairpins are arranged in two layers with quasi-five-fold symmetry. Fig. 1 shows the alignment of AtSLAC1 model structure and its template HiTehA. The backbones of two structures overlap very nicely. The extracellular inter-helix loops are short (1–5 residues), whereas the intracellular inter-helix connections are longer (Fig. 1 A). The top (outside the membrane) and the bottom (inside the membrane) of the SLAC1 channel are filled by water molecules. In Fig. 1 the residue Phe262 (colored in yellow) is in the center of stomatal channel, which is the gate of the channel. The ten helices of the two layers in SLAC1 channel are connected by flexible loops. It is anticipated that the ‘triple-barrel’ structure of the AtSLAC1 channel makes the diameter of the channel is adjusted by pressure change in the guard cells.

![Figure 1](https://example.com/figure1.png)

**Amino acid composition of AtSLAC1**

The amino acid composition and distribution in HiTehA and AtSLAC1 are shown in Fig. 2, where the acidic residues are colored in pink, alkaline residues in blue, polar residues in light blue, and hydrophobic residues in light green. The channel gate 262Phe (in HiTehA) and 462Phe (in AtSLAC1) are shown in yellow. The acidic and alkaline residues are shown in space filling render. Most alkaline residues (blue) and acidic residues (pink) concentrate locate in the top layer and bottom layer of the channel. The hydrophobic residues (light green) are in the middle, the transmembrane part of the channel.

The values of acidic ionizing constant (pK\(_a\)) of residues are essential for the CO\(_2\) conducting and concentrating. The classification of 20 natural amino acids is listed in Table 1. In Table 1 the pK\(_a\) values of amino acid side chains are only the model values [21]. The effective pK\(_a\) values of residues in the protein may be very different from the model values because of the special protein environment. The pK\(_a\) values of ionizable residues in AtSLAC1 are calculated using software PROPKA3.0 [21–23] and listed in Table 2.

In Table 2 there are 44 alkaline residues and 16 acidic residues. Most acidic and alkaline residues are located in the top and the bottom of AtSLAC1 channel. The amino acid distribution in the top layer of AtSLAC1 channel is shown in Fig. 3 A and B.
layer there are five acidic residues (His219, His293, Asp351, Asp412, and Glu464) and 14 alkaline residues (Lys211, Arg289, Lys290, Tyr291, Lys347, Tyr408, Cys414, Arg416, Cys418, Lys61, Tyr462, Tyr469, and Arg472). In the bottom layer, as shown in Fig. 3 C and D, there are 7 acidic residues (Glu252, Glu257, His260, Glu380, Glu385, His387, and His496) and 20 alkaline residues (Cys192, Tyr243, Lys246, Cys247, Tyr250, Lys255, Arg256, Tyr258, Arg263, Lys310, Lys320, Arg321, Arg322, Cys324, Lys325, Tyr373, Arg375, Lys384, Tyr390, and Lys440). In both top layer and bottom layer the alkaline residues exceed the acidic residues much more. The alkaline condition in the top and bottom layers is in favor of CO2 absorption and storage.

CO2 conducting mechanism

Fig. 4 shows the cartoon model of the AtSLAC1 stomatal channel, which is used to illustrated the conducting mechanism of carbon dioxide. The SLAC1 channel is divided into five regions. The first region is the top layer of SLAC1 channel, which is modeled as an alkaline aqueous solution, because it is dominated by alkaline residues and filled by water molecules. Below the first region there is a water pool, the second region in the channel, which is surrounded by polar residues and filled by water molecules. In Fig. 4 the third region is also filled by water molecules, surrounding the acidic residue His332 (space filling render in dark red), which has the second lowest pKa value (pK_a = 3.65) in Table 2. The fourth region is a hydrophobic region, formed mainly by hydrophobic residues, which is an empty cavity. The fifth region is the bottom layer of the channel, which is formed by alkaline and acidic residues and filled by water molecules. The fifth region is an alkaline solution, because where are much more alkaline residues (blue) than the acidic residues (pink). However, in the bottom layer there are one or two acidic exits of CO2 formed by the acidic residues.

The carbon dioxide conducting mechanism can be illustrated based on the cartoon model of SLAC1 channel in Fig. 4. The CO2 conductance is a six-step procedure. The CO2 is first absorbed from the atmosphere into the alkaline solution in the region 1, forming hydrogen carbonate ion HCO_3^- . In the second step the HCO_3^-

Figure 2. Amino acid distributions in Arabidopsis thaliana SLAC1 (AtSLAC1) and in Haemophilus influenzae TehA (HTehA). (A) Amino acid distributions of AtSLAC1. The acidic residues are shown in blue space filling render, and the acidic residues in pink space filling render. The polar residues are in light blue line drawing, and the hydrophobic residues are in light green line drawing. (B) Amino acid distributions of HTehA. The acidic residues (pink) and the alkaline residues (blue) are concentrated in top and bottom layers, and the hydrophobic residues (light green) are arranged in the middle of the channel. (C) The transmembrane model of AtSLAC1. The top and bottom layers are filled by water molecules. In the channel there are two water-rich regions, in which the water molecules are shown in space filling drawing. The dark gray ball indicates the hydrophobic region, where is an empty cavity.

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migrates to the aqueous solution in the region 2. Then in the third step the ion HCO$_3^-$ enters the acidic region 3 centered by His332 (pKa = 3.65), where it dissociates to CO$_2$ in the acidic condition. In the fourth step the saturated CO$_2$ in the acidic region 3 comes to the hydrophobic region 4, which is a carbon dioxide storage pool. In the fifth step, from the carbon dioxide pool the CO$_2$ dissolves in the alkaline solution in the region 5, forming hydrogen carbonate ion HCO$_3^-$.

Finally in the sixth step, the ion HCO$_3^-$ dissociates to CO$_2$ in the acidic exit of the region 5, and comes to the cell plasma through the acidic exit. The step 1, transfer of CO$_2$ from atmosphere to region 1, is a gas-solution equilibrium process. The step 2 of HCO$_3^-$ migration from region 1 to region 2 is caused by concentration gradient. The steps 4 and 5 are also the gas-solution equilibrium process. The reversible conversion of CO$_2$ to HCO$_3^-$ is driven by pH differences between different regions. It is much faster than the conversion in uniform solution with constant pH value.

**CO$_2$ concentrating mechanism**

The CO$_2$ conductance from atmosphere to cell plasma through SLAC1 channel enhances the CO$_2$ concentration remarkably. Assuming in atmosphere the concentration of carbon dioxide is [CO$_2$]$_{air}$ and the pH value in alkaline solution of the region 1 is pH = 9.0, the concentration of hydrogen carbonate ion HCO$_3^-$ is calculated as follows.

CO$_2$(g) + H$_2$O(l) $\rightleftharpoons$ HCO$_3^-$ + H + (aq), $K_a = 4.60 \times 10^{-7}$ mol/L

$$\frac{[\text{HCO}_3^-][\text{H}^+]}{[\text{CO}_2]_{\text{air}}} = K_a$$

$$[\text{HCO}_3^-] = \frac{[\text{CO}_2]_{\text{air}}}{[\text{H}^+]}, K_a = 10^9 [\text{CO}_2]_{\text{air}}, K_a$$

### Table 1. Chemical properties and classification of 20 natural amino acids.

| Amino acid | pK$_a$ | Classification |
|------------|--------|----------------|
| Asp (D)    | 3.93   | Acidic         |
| Glu (E)    | 4.37   | Acidic         |
| His (H)    | 6.50   | Acidic         |
| Arg (R)    | 12.50  | Alkaline       |
| Lys (K)    | 10.50  | Alkaline       |
| Tyr (Y)    | 10.00  | Alkaline       |
| Cys (C)    | 9.00   | Alkaline       |
| Ser (S)    | -      | Polar          |
| Thr (T)    | -      | Polar          |
| Asn (N)    | -      | Polar          |
| Gin (Q)    | -      | Polar          |
| Trp (W)    | -      | Polar          |
| Gly (G)    | -      | Hydrophobic    |
| Ala (A)    | -      | Hydrophobic    |
| Val (V)    | -      | Hydrophobic    |
| Leu (L)    | -      | Hydrophobic    |
| Ile (I)    | -      | Hydrophobic    |
| Met (M)    | -      | Hydrophobic    |
| Phe (F)    | -      | Hydrophobic    |
| Pro (P)    | -      | Hydrophobic    |

*The referent pK$_a$ values are from references [18–20].

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### Table 2. The pK$_a$ values of ionizable residues in AtSLAC1.

| Acidic Residues | Alkaline Residues |
|-----------------|-------------------|
|                | No.   | A.A. | pK$_a$ | No.   | A.A. | pK$_a$ |
| 351             | ASP   | 3.93 |      | 192   | CYS   | 10.68 |
| 412             | ASP   | 2.41 |      | 196   | CYS   | 12.10 |
| 252             | GLU   | 4.37 |      | 247   | CYS   | 9.78  |
| 257             | GLU   | 5.79 |      | 274   | CYS   | 11.49 |
| 308             | GLU   | 4.48 |      | 298   | CYS   | 9.47  |
| 352             | GLU   | 4.66 |      | 324   | CYS   | 8.64  |
| 380             | GLU   | 4.58 |      | 414   | CYS   | 9.61  |
| 385             | GLU   | 4.35 |      | 418   | CYS   | 10.18 |
| 464             | GLU   | 7.03 |      | 487   | CYS   | 9.37  |
| 219             | HIS   | 6.73 |      | 243   | TYR   | 13.83 |
| 260             | HIS   | 4.28 |      | 250   | TYR   | 10.12 |
| 293             | HIS   | 6.02 |      | 258   | TYR   | 11.73 |
| 332             | HIS   | 3.65 |      | 291   | TYR   | 10.79 |
| 364             | HIS   | 5.67 |      | 304   | TYR   | 11.06 |
| 387             | HIS   | 4.52 |      | 312   | TYR   | 10.31 |
| 496             | HIS   | 5.98 |      | 365   | TYR   | 13.99 |
| 373             | TYR   | 10.23 |     | 390   | TYR   | 12.32 |
| 408             | TYR   | 10.38 |     | 426   | TYR   | 10.56 |
| 448             | TYR   | 11.41 |     | 462   | TYR   | 13.40 |
| 469             | TYR   | 10.04 |     | 211   | LYS   | 10.07 |
| 246             | LYS   | 9.86  |     | 255   | LYS   | 10.56 |
| 290             | LYS   | 10.03 |     | 310   | LYS   | 9.20  |
| 320             | LYS   | 9.64  |     | 325   | LYS   | 10.10 |
| 347             | LYS   | 10.05 |     | 355   | LYS   | 9.73  |
| 384             | LYS   | 9.93  |     | 440   | LYS   | 10.44 |
| 461             | LYS   | 9.12  |     | 256   | ARG   | 12.55 |
| 263             | ARG   | 12.14 |     | 289   | ARG   | 12.36 |
| 321             | ARG   | 12.15 |     | 322   | ARG   | 12.57 |
| 375             | ARG   | 11.25 |     | 416   | ARG   | 9.90  |
| 432             | ARG   | 12.41 |     | 472   | ARG   | 12.39 |

Table 1. Chemical properties and classification of 20 natural amino acids.

Table 2. The pK$_a$ values of ionizable residues in AtSLAC1.
In the acidic region 3 surrounding His332, the hydrogen carbonate ion \( \text{HCO}_3^- \) dissociates to \( \text{CO}_2 \). This is just the reverse reaction of the above equation, \( K_b = K_a^{-1} \). Assuming in the acidic region 3 the pH value is 5.0, the concentration \([\text{CO}_2]_3\) in region 3 is calculated as follows.

\[
\text{HCO}_3^{(aq)} + \text{H}^+ + \text{CO}_2^{(g)} + \text{H}_2\text{O}(1), \quad K_b = K_a^{-1}
\]

\[
\frac{[\text{CO}_2]_3}{[\text{HCO}_3^-][\text{H}^+]} = K_b
\]

\[
\frac{[\text{CO}_2]_3}{[\text{HCO}_3^-][\text{H}^+]} = K_b
\]

Therefore, the \( \text{CO}_2 \) concentration in the region 3 of SLAC1 channel is \( 10^5 \) times higher than the concentration in the atmosphere.

The above calculation for \( \text{CO}_2 \) concentrating mechanism is not rigorous because of the following two problems. The first problem is that the calculation uses the assumed pH values in the alkaline solutions (pH = 9) and in the acidic regions (pH = 5). The second problem is that the chemical equilibrium equation holds for macro system, however, the SLAC1 channel is not a macro system. Although the above calculation is not rigorous, it still can be used to illustrate the \( \text{CO}_2 \) concentrating in the SLAC1 channel qualitatively. If the alkaline residues and acidic residues in the first, third, and fifth regions are treated as the donors and receptors of \( \text{H}^+ \), \( \text{OH}^- \), and \( \text{HCO}_3^- \), we can get the same qualitative results.
If the concentration (partial pressure) of CO$_2$ in atmosphere is [CO$_2$]$_{air} = 0.0003$ atm, according to the above calculations, the CO$_2$ concentration in the hydrophobic region 4 (CO$_2$ pool) is

$$\frac{1}{2} \frac{[CO_2]}{[C_{138}]}_{pool} \approx 10^4 \frac{[CO_2]}{[C_{138}]}_{air} \approx 3.0 \text{ atm}$$

The very high concentration of CO$_2$ in AtSLAC1 channel may be overestimated. However, the carbon dioxide concentration in plant SLAC1 channel must be higher than that in the atmosphere.

Activation mechanism of SLAC1

Recent study revealed that the bicarbonate is a small-molecule activator of SLAC1 [15]. The activation mechanism of HCO$_3^-$ to AtSLAC1 can be illustrated based on the CO$_2$ conduction and concentration model of AtSLAC1 proposed in this study. The Arabidopsis thaliana SLAC1 was identified as a slow anion channel [18]. Study shows that electrostatic features of the pore coupled with electrophysiological characteristics indicate that selectivity among different anions is largely a function of the energetic cost of ion dehydration [18]. The relative anion permeability sequence of SLAC1 is $I^- > NO_3^- > Br^- > Cl^-$ [18, 24, 25]. The SLAC1 channel transports anions (Cl$^-$ and NO$_3^-$) from inside of guard cell to outside across the membrane. The one-way conduct will make the electrostatic potential inside the guard cell is higher than the outside. The back electromotive force will stop the conduction. On the other hand, the conduction of anion HCO$_3^-$ through SLAC1 channel is from outside to inside driven by pH difference. The one-way conduct will make the electrostatic potential inside the guard cell is higher than the outside. The back electromotive force will stop the conduction. On the other hand, the conduction of anion HCO$_3^-$ through SLAC1 channel is from outside to inside driven by pH difference. The difference between anion HCO$_3^-$ and other anions (I$^-$, NO$_3^-$, Br$^-$, and Cl$^-$) is that the anion HCO$_3^-$ is pH sensitive, which has higher concentration in alkaline solution, and dissociates to CO$_2$ in acidic solution. Therefore, pH value has strong modulation ability to anion HCO$_3^-$ than to other anions. The conduction of CO$_2$ (in HCO$_3^-$ form) in SLAC1 channel from outside to inside is a necessary condition to balance the back electromotive force and maintain the influx of other anions (Cl$^-$, NO$_3^-$, I$^-$ and Br$^-$) from inside to outside. In this way the bicarbonate plays the role of activator for SLAC1 channel.

Discussion

Usually the reversible conversion of between CO$_2$ and HCO$_3^-$ is a very slow process without the catalysis by carbonic anhydrases. This is the phenomena of the conversion between CO$_2$ and HCO$_3^-$ in a uniform solution with constant pH value. The proposed model of SLAC1 channel consists of several regions with different pH values. This is only possible in a micro channel. Just the different pH values elevate the concentration of CO$_2$, and make the conversion between CO$_2$ and HCO$_3^-$ much faster than in uniform macro solution. This is like the case when a drop of hydrochloric acid is put in NaHCO$_3$ solution, the CO$_2$ escapes out quickly.

The function of CO$_2$ conduction and concentration of SLAC1 channel is highly interesting, because it implies a possible pathway of CO$_2$ supply in plant. As we known the stomatal aperture is the regular pathway of CO$_2$ supplying to cells in leaves for photosynthetic reactions. However, the pathway of CO$_2$ influx to the guard cells self is unclear. The proposed mechanism of CO$_2$ conduction and concentration indicates that the SLAC1 channel may be a possible pathway providing CO$_2$ for photosynthesis in guard cells. The high concentration of CO$_2$ (or HCO$_3^-$) in the plant SLAC1 channel not necessarily means the high concentration of CO$_2$ (or HCO$_3^-$) in guard cells, because the transfer of...
carbon dioxide in the cell plasma of guard cells to the enzyme RuBisCo needs the help of enzyme CA (carbonic anhydrase) [26–31]. However, the CO2 concentrating in SLAC1 channel may be a mechanism dealing with the instant fluctuation of carbon dioxide in environment.

The possible function of CO2 conduction and concentration in SLAC1 channel is supported by the water-channel protein aquaporin [32–35]. The role of aquaporin in CO2 diffusion in higher plants was first examined by Terashima and Ono [36]. A significant decrease of g (internal CO2 conductance) was detected in the presence of HgCl2, an inhibitor of most aquaporins, which is the evidence indicating involvement of aquaporins in CO2 diffusion across the plasma membrane [36]. Then the role of aquaporin in CO2 diffusion inside plant leaves was further confirmed by Hanba et al. [37].

In the cartoon model of AtSLAC1 channel (Fig. 4), the top region and bottom region are modeled as the alkaline solutions. However, the two regions are best to be described as the alkaline buffer solutions, because of the alternately distribution of alkaline residues and acidic residues. The alkaline residue-dominated buffer solution not only can maintain the constantly higher pH value, but also can accommodate more CO2 (or HCO3−). In the AtSLAC1 model the acidic His332 in the region 3 plays an important role, by which the CO2 concentration in the hydrophobic region (CO2 pool) is enhanced greatly. Actually, histidine can play the role of both proton donor and acceptor. The transfer of HCO3− to CO2 in the His323 may be the speed-control step in the slow anion channel.

Carbon dioxide is a key reactant in plant photosynthesis. The continuing rise in of green house gas CO2 in atmosphere is predicted to have diverse and dramatic effects on the productivity of agriculture, plant ecosystems, and global climate [38–40]. The CO2 conducting mechanism and concentrating mechanism in plant SLAC1 channel, derived in this study based on the structure of AtSLAC1, may provide useful insight into this important research topic.

**Materials and Methods**

**Amino acid classification and pKa calculation**

The plant SLAC1 anion channel has a novel amino acid composition, and its unique mechanism for CO2 conductance can be illustrated using the physicochemical properties of amino acids. The properties of 20 natural amino acids and the pKa values of side chains are listed in Table 1. The 20 amino acids are classified into four types: acidic, alkaline, polar, and hydrophobic. In this study the acidic residues includes Asp, Glu and His, and the alkaline residues are Arg, Lys, Tyr, and Cys [21–23]. Five amino acids (Ser, Thr, Asn, Gln, and Trp) are classified as the polar residues. The remaining 8 amino acids are hydrophobic residues. In Table 1 the pKa values of ionizable amino acid side chains are model values [29], which may be very different from the effective pKa values in protein environment.

The effective pKa values of ionizable amino acids in HiTchA and AtSLAC1 are calculated using software PROPKA3.0 [21-23].

\[
pKa_{(pro)} = pKa_{(mod)} + 1 \frac{AG}{2.303kT}
\]

In the above calculation equation the AG is the free energy change of a residue side chain from exposed environment to the protein fold environment. When a protein folds, the titratable amino acids in the protein are transferred from a solution-like environment to an environment determined by the 3-D structure of the protein. In the unfolded protein the titratable side chain of amino acid typically exposes to water. When the protein folds the side chain could be buried deeply in the protein interior with no exposure to solvent. Furthermore, in the folded protein the side chain may be closer to other titratable groups in the protein and will also interact with permanent charges (e.g., ions) and dipoles in the protein. All of these effects alter the pKa, value of the amino acid side chain. The pKa calculation methods generally calculate the effect of the protein environment on the model pKa value of an amino acid side chain.

**Calculation of pH value in CO2 solution**

The CO2 conducting and concentrating mechanism of plant SLAC1 channel relate with the unique physicochemical properties of carbon dioxide. When carbon dioxide dissolves in water, it exists in equilibrium with carbonic acid.

\[
{\text{CO2} + \text{H2O} \rightarrow \text{HCO3}^- + \text{H}^+}
\]

Carbonic acid is diprotic having two protons, which may dissociate from the parent molecule. Thus there are two dissociation constants.

\[
\text{HCO3}^- + \text{H}^+ \rightarrow \text{H}_2\text{CO3}
\]

The first one is the dissociation into the hydrogen carbonate ion HCO3−, and the second is the dissociation of the bicarbonate ion into the carbonate ion CO32−. However, in aqueous solution carbonic acid only exists in equilibrium with carbon dioxide, and the concentration of H2CO3 is much lower than the dissolved CO2 concentration. Since it is not possible to distinguish between H2CO3 and dissolved CO2 by conventional methods, the dissolving and ionizing equation of CO2 in aqueous solution may be rewritten as follows,

\[
\text{CO2} + \text{H2O} \rightarrow \text{HCO3}^- + \text{H}^+ \quad \text{Ka} = 4.60 \times 10^{-7}
\]

Whereas this Ka is quoted as the dissociation constant of carbonic acid, and it might better be referred to as the acidity constant of dissolved carbon dioxide, as it is particularly useful for calculating the pH of CO2-containing solutions. In the alkaline solution, the higher pH value ([lower concentration [H+]]) is favor in CO2 dissolution [higher concentration [HCO3−]]. On the other hand, lower pH value ([higher concentration [H+]]) makes the HCO3− to dissociate to CO2.

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**Author Contributions**

Conceived and designed the experiments: QSD RBH. Performed the experiments: QSD CHW. Analyzed the data: QSD CHW. Contributed reagents/materials/analysis tools: QSD XWF. Wrote the paper: QSD RBH.
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