

Negatively-Charged Residues and Hydrogen Bonds Tune the Ligand Histidine pK_a -Values of Rieske Iron-Sulfur Proteins

Supporting Information

Astrid R. Klungen and G. Matthias Ullmann

The microscopic pK -values in Figure 1 relate to the microscopic equilibrium constants K as $pK = -\lg K$. From the law of mass action, the microscopic equilibrium constants are defined as $K = \frac{[\text{deprotonated}][\text{H}^+]}{[\text{protonated}]}$, where squared brackets denote the chemical activities of the respective species. For brevity, the different microstates will be denoted slightly deviating from Figure 1, for example with $^{\text{ox}}141\text{H}, 161\text{H}$ (oxidized and doubly protonated) and $^{\text{redH}}141, 161$ (reduced at the histidine-coordinated iron and doubly deprotonated).

For the oxidized state of the cluster, four different protonation microstates have to be considered when calculating macroscopic equilibrium constants. Following the law of mass action, the macroscopic equilibrium constants for the oxidized state can be obtained as

$$\begin{aligned} K_{\text{mac}}^{\text{ox1}} &= \frac{[^{\text{ox}}141, 161\text{H}][\text{H}^+] + [^{\text{ox}}141\text{H}, 161][\text{H}^+]}{[^{\text{ox}}141\text{H}, 161\text{H}]} \\ &= K_{11}^{\text{ox}} + K_{21}^{\text{ox}}, \end{aligned} \tag{1}$$

and

$$\begin{aligned} K_{\text{mac}}^{\text{ox2}} &= \frac{[^{\text{ox}}141, 161][\text{H}^+]}{[^{\text{ox}}141, 161\text{H}] + [^{\text{ox}}141\text{H}, 161]} \\ &= \frac{[^{\text{ox}}141, 161][\text{H}^+] \cdot [^{\text{ox}}141, 161][\text{H}^+]}{[^{\text{ox}}141, 161\text{H}] \cdot [^{\text{ox}}141, 161][\text{H}^+] + [^{\text{ox}}141\text{H}, 161] \cdot [^{\text{ox}}141, 161][\text{H}^+]} \end{aligned}$$

$$\begin{aligned}
&= \frac{[\text{ox}141,161][\text{H}^+][\text{ox}141,161][\text{H}^+]}{[\text{ox}141,161\text{H}][\text{ox}141\text{H},161]} \\
&= \frac{[\text{ox}141,161][\text{H}^+]}{[\text{ox}141\text{H},161]} + \frac{[\text{ox}141,161][\text{H}^+]}{[\text{ox}141,161\text{H}]} \\
&= \frac{K_{12}^{\text{ox}} \cdot K_{22}^{\text{ox}}}{K_{22}^{\text{ox}} + K_{12}^{\text{ox}}}.
\end{aligned} \tag{2}$$

In the calculation of macroscopic equilibrium constants for the reduced cluster, a total of eight microstates has to be considered. The macroscopic equilibrium constants of the reduced cluster are calculated as

$$\begin{aligned}
K_{\text{mac}}^{\text{red1}} &= \frac{[\text{redH}141,161\text{H}][\text{H}^+] + [\text{redH}141\text{H},161][\text{H}^+] + [\text{redC}141,161\text{H}][\text{H}^+] + [\text{redC}141\text{H},161][\text{H}^+]}{[\text{redH}141\text{H},161\text{H}] + [\text{redC}141\text{H},161\text{H}]} \\
&= \frac{\frac{[\text{redH}141,161\text{H}][\text{H}^+]}{[\text{redH}141\text{H},161\text{H}]} + \frac{[\text{redH}141\text{H},161][\text{H}^+]}{[\text{redH}141\text{H},161\text{H}]} + \frac{[\text{redC}141\text{H},161\text{H}]}{[\text{redH}141\text{H},161\text{H}]} \cdot \left(\frac{[\text{redC}141,161\text{H}][\text{H}^+]}{[\text{redC}141\text{H},161\text{H}]} + \frac{[\text{redC}141\text{H},161][\text{H}^+]}{[\text{redC}141\text{H},161\text{H}]} \right)}{1 + \frac{[\text{redC}141\text{H},161\text{H}]}{[\text{redH}141\text{H},161\text{H}]}} \\
&= \frac{K_{11}^{\text{redH}} + K_{21}^{\text{redH}} + K_1^{\text{R}} (K_{11}^{\text{redC}} + K_{21}^{\text{redC}})}{1 + K_1^{\text{R}}}
\end{aligned} \tag{3}$$

with $K_1^{\text{R}} = \frac{[\text{redC}141\text{H},161\text{H}]}{[\text{redH}141\text{H},161\text{H}]}$, and

$$\begin{aligned}
K_{\text{mac}}^{\text{red2}} &= \frac{[\text{redH}141,161][\text{H}^+] + [\text{redC}141,161][\text{H}^+]}{[\text{redH}141,161\text{H}] + [\text{redH}141\text{H},161] + [\text{redC}141,161\text{H}] + [\text{redC}141\text{H},161]} \\
&= \frac{[\text{redH}141,161]^2[\text{H}^+]^2 + [\text{redC}141,161][\text{redH}141,161][\text{H}^+]^2}{[\text{redH}141,161\text{H}][\text{redH}141,161][\text{H}^+] + [\text{redH}141\text{H},161][\text{redH}141,161][\text{H}^+] + [\text{redC}141,161\text{H}][\text{redH}141,161][\text{H}^+] + [\text{redC}141\text{H},161][\text{redH}141,161][\text{H}^+]} \\
&= \frac{\frac{[\text{redH}141,161]^2[\text{H}^+]^2}{[\text{redH}141,161\text{H}][\text{redH}141\text{H},161]} + \frac{[\text{redC}141,161][\text{redH}141,161][\text{H}^+]^2}{[\text{redH}141,161\text{H}][\text{redH}141\text{H},161]}}{\frac{[\text{redH}141,161][\text{H}^+]}{[\text{redH}141\text{H},161]} + \frac{[\text{redH}141,161][\text{H}^+]}{[\text{redH}141,161\text{H}]} + \frac{[\text{redH}141,161][\text{H}^+]}{[\text{redH}141,161\text{H}][\text{redH}141\text{H},161]} \cdot ([\text{redC}141,161\text{H}] + [\text{redC}141\text{H},161])} \\
&= \frac{K_{12}^{\text{redH}} \cdot K_{22}^{\text{redH}} \cdot \frac{[\text{redC}141,161][\text{H}^+]}{[\text{redC}141,161\text{H}][\text{redC}141\text{H},161]} + \frac{[\text{redH}141,161][\text{H}^+]}{[\text{redH}141,161\text{H}][\text{redH}141\text{H},161]} \cdot \frac{[\text{redC}141,161]^2[\text{H}^+]^2}{[\text{redC}141,161\text{H}][\text{redC}141\text{H},161]}}{\left(K_{22}^{\text{redH}} + K_{12}^{\text{redH}} \right) \cdot \frac{[\text{redC}141,161][\text{H}^+]}{[\text{redC}141\text{H},161][\text{redC}141,161\text{H}]} + \frac{[\text{redH}141,161][\text{H}^+]}{[\text{redH}141,161\text{H}][\text{redH}141\text{H},161]} \cdot \left(K_{22}^{\text{redC}} + K_{12}^{\text{redC}} \right)}
\end{aligned}$$

$$\begin{aligned}
&= \frac{K_{12}^{\text{redH}} \cdot K_{22}^{\text{redH}} \cdot \frac{K_{12}^{\text{redC}} \cdot K_{22}^{\text{redC}}}{[\text{redC141,161}][\text{H}^+]} + \frac{K_{12}^{\text{redH}} \cdot K_{22}^{\text{redH}}}{[\text{redH141,161}][\text{H}^+]} \cdot K_{12}^{\text{redC}} \cdot K_{22}^{\text{redC}}}{\left(K_{22}^{\text{redH}} + K_{12}^{\text{redH}}\right) \cdot \frac{K_{12}^{\text{redC}} \cdot K_{22}^{\text{redC}}}{[\text{redC141,161}][\text{H}^+]} + \frac{K_{12}^{\text{redH}} \cdot K_{22}^{\text{redH}}}{[\text{redH141,161}][\text{H}^+]} \cdot \left(K_{22}^{\text{redC}} + K_{12}^{\text{redC}}\right)} \\
&= \frac{\left(1 + \frac{[\text{redC141,161}]}{[\text{redH141,161}]}\right) \cdot K_{12}^{\text{redH}} \cdot K_{22}^{\text{redH}} \cdot K_{12}^{\text{redC}} \cdot K_{22}^{\text{redC}}}{\left(K_{22}^{\text{redH}} + K_{12}^{\text{redH}}\right) \cdot K_{12}^{\text{redC}} \cdot K_{22}^{\text{redC}} + \frac{[\text{redC141,161}]}{[\text{redH141,161}]} \cdot K_{12}^{\text{redH}} \cdot K_{22}^{\text{redH}} \cdot \left(K_{22}^{\text{redC}} + K_{12}^{\text{redC}}\right)} \\
&= \frac{\left(1 + K_2^{\text{R}}\right) \cdot K_{12}^{\text{redH}} \cdot K_{22}^{\text{redH}} \cdot K_{12}^{\text{redC}} \cdot K_{22}^{\text{redC}}}{\left(K_{22}^{\text{redH}} + K_{12}^{\text{redH}}\right) \cdot K_{12}^{\text{redC}} \cdot K_{22}^{\text{redC}} + K_2^{\text{R}} \cdot K_{12}^{\text{redH}} \cdot K_{22}^{\text{redH}} \cdot \left(K_{22}^{\text{redC}} + K_{12}^{\text{redC}}\right)} \tag{4}
\end{aligned}$$

with $K_2^{\text{R}} = \frac{[\text{redC141,161}]}{[\text{redH141,161}]}$.

With $\Delta G_{\text{reduction}} = RT \cdot \ln K_{\text{reduction}} = -F \cdot E$ and accordingly $K_{\text{red}} = \frac{[\text{reduced}]}{[\text{oxidized}][\text{e}^-]} = \exp\left(-\frac{F}{RT} \cdot E\right)$, where R is the universal gas constant, T the temperature, F the Faraday constant and E the reduction potential, K_1^{R} in equation 3 can be written as

$$\begin{aligned}
K_1^{\text{R}} &= \frac{[\text{redC141H, 161H}]}{[\text{redH141H, 161H}]} \\
&= \frac{[\text{redC141H, 161H}]}{[\text{ox141H, 161H}][\text{e}^-]} \cdot \frac{[\text{ox141H, 161H}][\text{e}^-]}{[\text{redH141H, 161H}]} \\
&= \frac{\exp\left(-\frac{F}{RT} \cdot E_{\text{Cys}}^{\text{prot}}\right)}{\exp\left(-\frac{F}{RT} \cdot E_{\text{His}}^{\text{prot}}\right)} \\
&= \exp\left(-\frac{F}{RT} \left(E_{\text{Cys}}^{\text{prot}} - E_{\text{His}}^{\text{prot}}\right)\right),
\end{aligned}$$

with the meaning of $E_{\text{Cys}}^{\text{prot}}$ and $E_{\text{His}}^{\text{prot}}$ as shown in Figure 1. Analogously, K_2^{R} in equation 4 can be written as

$$\begin{aligned}
K_2^{\text{R}} &= \frac{[\text{redC141, 161}]}{[\text{redH141, 161}]} \\
&= \exp\left(-\frac{F}{RT} \left(E_{\text{Cys}}^{\text{deprot}} - E_{\text{His}}^{\text{deprot}}\right)\right).
\end{aligned}$$

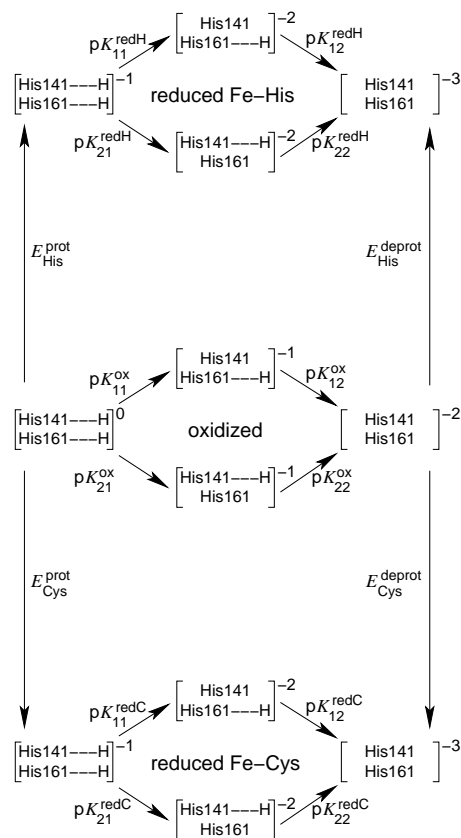


Figure 1: Twelve possible microstates of the Rieske cluster and their interconversion. Numbering of ligand histidines corresponds to the amino acid sequence of the Rieske protein from bovine mitochondrial cytochrome bc_1 . The electron can formally reduce either the histidine-coordinated iron (reduced Fe-) or the cysteine-coordinated iron (reduced Fe-Cys). For each of the three different redox states there are four protonation states: both histidines can be protonated, His141 protonated and His161 deprotonated, His161 protonated and His141 deprotonated, or both histidines deprotonated. The net charges of the cluster (iron-sulfur core with ligand side-chains) are given.