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Identifying Residues That Cause pH-Dependent Reduction Potentials

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Supporting Information

ABSTRACT: The pH dependence of the reduction potential $E°$ for a metalloprotein indicates that the protonation state of at least one residue near the redox site changes and may be important for its activity. The responsible residue is usually identified by site-specific mutagenesis, which may be time-consuming. Here, the titration of $E°$ for *Chromatium vinosum* high-potential iron−sulfur protein is predicted to be in good agreement with experiment using density functional theory and Poisson−Boltzmann calculations if only the sole histidine undergoes changes in protonation. The implementation of this approach into CHARMMing, a user-friendly web-based portal, allows users to identify residues in other proteins causing similar pH dependence.

Knowing the protonation state of ionizable residues in the active sites of proteins and enzymes is important in understanding their mechanisms. For instance, the protonation states of histidines near the metal redox site of metalloproteins are often important in identifying proton coupling in electron transfer reactions or transferrable protons in enzymatic mechanisms. Thus, the determination of pK_a values for residues within a protein has been a major goal for computational methods for many decades,[1−6] with varying degrees of success.[7] However, often the real question is simply to identify the residue causing an observed pH dependence as opposed to the actual values of the pK_a.

For metalloproteins, the reduction potential $E°$ can serve as a microscopic probe of the residues that affect the metal redox site, which is often the functionally important site. Thus, the pH dependence of $E°$, which can be measured using electrochemical or spectroscopic methods, indicates that the protonation state of one or more residues is affecting the redox site. However, the residue is usually experimentally identified by site-specific mutagenesis and repetition of the titration, which can be time-consuming especially without an existing expression system and may lead to other unpredicted changes in the protein. A fast, simple computational procedure for identifying the residues that contribute to the pH dependence of $E°$ for a metalloprotein is presented here as an alternative, or at least a prelude, to mutagenesis studies.

The pH dependence of $E°$ for *Chromatium vinosum* high-potential iron−sulfur protein (CvHiPIP) (Figure 1) is a useful test case. It contains a single histidine at residue 42 and exhibits a pH dependence of $E°$ near pH 6−7.[8] This histidine has been identified as the cause because the pH dependence in the wild type (wt) disappears when it is mutated to a glutamine in the H42Q mutant.[9] First, we show how well the computational methods used here reproduce the experimental results from CvHiPIP. Next, we demonstrate how the computational procedure using these methods would be used when the residue responsible for the pH dependence is not known, using CvHiPIP as an example.

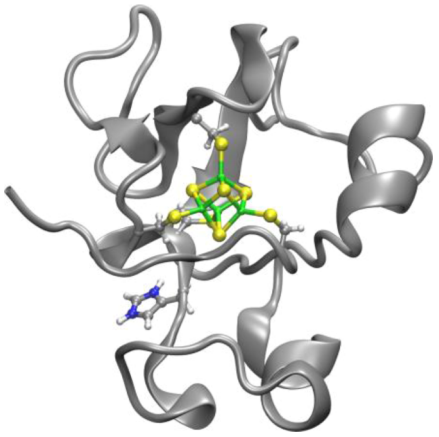

Figure 1. Ribbon drawing of *C. vinosum* high-potential iron−sulfur protein, with the redox site and histidine in ball-and-stick form.

The computational approach used here will be termed DFT +PB, where DFT refers to density functional theory calculations and PB refers to Poisson−Boltzmann continuum electrostatic calculations. The plus sign (+) indicates that the two calculations are performed independently but are linked by using partial charges from the DFT calculations to represent the DFT region in the PB calculation. Recently, we have reported accurate calculations of $E°$ versus the standard hydrogen electrode using the DFT+PB approach for [4Fe-4S] proteins,[10,11] showing that DFT partial charges give an accurate description of the redox site. In our work, the redox site contribution is from carefully benchmarked DFT calculations of redox site analogues in the gas phase[12] and the protein contribution is from PB calculations of the protein in a continuum solvent using crystal structures of the proteins.[10] This approach for calculating $E°$ has been implemented into CHARMMing,[13] a web-based portal for biomolecule calculations. In this implementation, the redox site

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contribution comes from a library of our DFT results while the protein contribution is calculated using APBS[14] for protein coordinates chosen by the user. Because only the PB calculations are performed, each E° calculation takes ~15 min on the server or a workstation. In addition, because dynamical effects are not included, further investigations by molecular dynamics simulations can be set up using other modules in the CHARMMing portal.

First, the pH dependencies of E° for wt and H42Q CvHiPIP were examined to show how well the approach works by comparing its results to the experimental results. The PB calculations used crystal structures of wt [Protein Data Bank (PDB) entry 1CKU] at 1.20 Å resolution[15] and H42Q (PDB entry 1B0Y) at 0.93 Å resolution.[15,16] E° was calculated using the DFT+PB approach for three states of the protein: with all ionizable residues protonated, with all ionizable residues with a pK_a of >4 protonated, and with all ionizable residues with a pK_a of >8 protonated. pK_a values were assumed for the ionizable residues: pK_a = 3.5 for all aspartates and glutamates, pK_a = 6.3 for all histidines, and pK_a > 8 for the rest. Then, the E° values as a function of pH were estimated assuming that the Henderson−Hasselbach equation determines the relative populations of each type of protonation state (see the Supporting Information).

The predicted results using DFT+PB at pH 7 for wt and H42Q are slightly too positive, by 29 and 34 mV, respectively (Table 1). These results are within the predicted range of error

Table 1. Calculated and Experimental[9] E° Values (millivolts) for HiPIP

	E°_{cal}	E°_{exp}
wt at pH 7	384 ± 30	355 ± 2
H42Q at pH 7	391 ± 30	357 ± 2

for DFT+PB because a deviation from experiment of ~30 mV was found for structures of this resolution when other proteins were examined.[10,11] However, these results also show a systematic deviation for the entire titration curve because the same crystal structure is used for each calculation for the entire titration curve. Although the experiment may also give rise to error, the data shown here from experiments performed using cyclic voltammetry gave an E° of 355 mV at pH 7,[9] in good agreement with a previous study following the absorption spectra, which gave an E° of 356 mV at pH 7[8] (more discussion of the error is given in the Supporting Information). Overall, the agreement indicates that PB electrostatics with DFT redox site partial charges gives a good estimate of the protein contribution, especially because the overall E° is the sum of large and opposing sign contributions, and factors such as the dynamics of the protein or changes in the redox site do not appear to be present.

Because the errors are systematic for a given protein, the predicted titration curves for wt and H42Q can be shifted such that they match the respective experimental value at pH 7. These results agree well with the experimental titration data (Figure 2). In particular, the approximately 25 mV increase in E° for wt between pH 8 and 5.5 is in excellent agreement with experiment, while no such increase is seen in E° for H42Q for either calculation or experiment. Moreover, these results also help to confirm that the histidine is responsible. Because the nearest atom of the histidine is 6.5 Å from the nearest atom of the [4Fe-4S] redox site, this indicates that PB electrostatics is

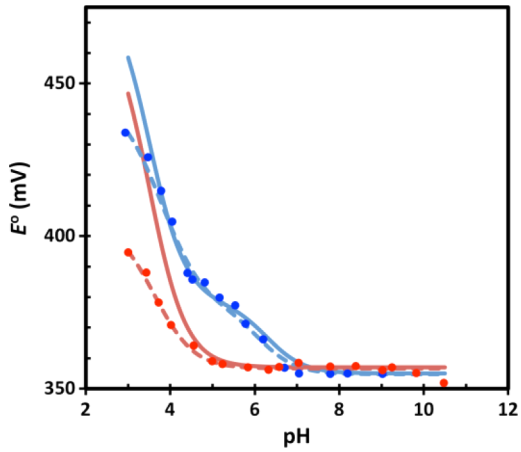

Figure 2. pH dependence of the predicted (—), experimental[9] (●), and best-fit-to-experiment[9] (---) reduction potentials of wt HiPIP (blue) and its H42Q mutant (red).

able to predict even relatively small changes in E° caused by residues that may not be close to the redox site.

In CvHiPIP, the identity of the residue causing the pH dependence is straightforward because there is only one histidine and the site-specific mutagenesis data are consistent. However, generally the responsible residue is not known, and site-specific mutants are not available. In addition, more than one residue may contribute, especially because E° is sensitive to the protonation of even relatively distant residues. Thus, we demonstrate how our computational procedure may be used in such cases, again using CvHiPIP as an example. First, the DFT+PB-calculated E° should be within 50 mV of the experimental E° as in Table 1 to ensure that the approach is appropriate: otherwise, factors such as dynamics of the protein or changes in the redox site may be important. Then, one can focus on the relative E° as in Figure 2. Good agreement between the calculated (blue solid line in Figure 2 or 3) and experimental (blue filled circles in Figure 2 or 3) E° indicates the pH dependence is being reproduced. Next, the residue responsible for the pH dependence can be identified by a procedure similar to experimental site-specific mutagenesis in which the calculation outlined above is repeated but instead of protonating all histidines at pH 6.3, all but one are protonated

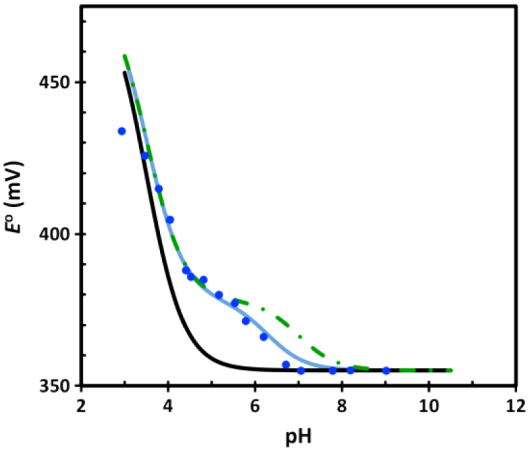

Figure 3. Reduction potentials of wt HiPIP predicted with the pK_a of H42 equal to 6.3 (blue), 4 (black), and 7 (green) and experimental data[9] (blue circles).

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(i.e., the pK_a of that histidine is changed to 3.5). If the curve calculated when the pK_a of a specific histidine is shifted to 3.5 has little or no pH dependence near 6−7 (black line in Figure 3), this histidine would be identified as contributing to the pH dependence.

In addition, the pK_a of the histidine in CvHiPIP should be close to 6.3 because the experimental titration curve (blue circles in Figure 3) is reproduced well by the curve calculated assuming a pK_a of 6.3 for histidine (blue line in Figure 3). However, a deviation of the predicated curve from experiment in the range of pH 6−7 indicates that the histidine has a pK_a shifted from 6.3. In that case, the histidine could be identified computationally and its pK_a determined by adjusting the pK_a for each histidine. For instance, when the pK_a of a particular histidine is set to 7 (green dotted−dashed line in Figure 3), better agreement with experiment would indicate that the pK_a of that histidine is 7, which is of course not the case here.

This procedure has been demonstrated for identifying histidines that cause pH changes but can be used to study other ionizable residues. In addition, only histidines, aspartic acids, and glutamic acids were considered to be ionizable here, but the method can be extended to include other ionizable residues as well.

■ ASSOCIATED CONTENT

Supporting Information

Brief methods, including the relationship between $E°$ and pK_a and pH, and discussion of error with references. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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