Modeling Charge Interactions and Redox Properties in DsbA*

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Accurate prediction of charge interactions in macromolecules presents a significant challenge for computational biology. A model for the low Cys30 pKa and oxidizing power of DsbA (Gane, P. J., Freedman, R. B., and Warwicker, J. (1995) J. Mol. Biol. 249, 376–387) has been investigated experimentally (Hennecke, J., Spleiss, C., and Glockshuber, R. (1997) J. Biol. Chem. 272, 189–195), with substitutions for Glu37 and Glu38 and with residues 38–40 removed. Measured changes in Cys30 pKa and redox potential were relatively small and reported to be in contrast to model predictions. It is now shown, particularly with calculations of wild-type-mutant differences for a range of salt concentrations, that the data are consistent with the model and support the key finding that a number of different factors contribute to the oxidizing power of DsbA, so that any particular one need not necessarily be large. A feature of the model is a low protein dielectric, and higher values (which are becoming popular in predictions of pH dependence) are inconsistent with both the difference data and the wild-type Cys30 pKa.

DsbA from *Escherichia coli* is a periplasmic protein of 189 amino acids that catalyzes the introduction of disulfide bonds (1, 2). The oxidizing power of DsbA derives from the low Cys30 pKa of about 3.5 (3, 4), exhibiting a ΔpKa with respect to normal of around −5. DsbA is a valuable link to theory because models must account for the low Cys30 pKa and thereby provide a molecular basis for oxidizing power and physiological function. Continuum electrostatics has become the common method for calculating macromolecular pKa, (5), but it is not yet consistently accurate, with discussion revolving around the choice of macromolecular relative dielectric, εr (6–8). Variation in εr reflects the difficulty of reproducing microscopic solvation effects in a continuum model, particularly where an ionizable group is buried in the macromolecule, so that part of the high relative dielectric (εr) solvation shell is swapped for the lower εp environment (see Fig. 1).

A continuum electrostatic model has been presented for the redox potential difference between *E. coli* DsbA and *E. coli* thioredoxin (9), using structural homology and differencing to circumvent changes in charge burden (see Fig. 1). The low thiolate pKa and oxidizing power of DsbA were suggested to arise from several sources including His32 and Gln97 side chains and summed peptide dipole contributions. Residues Glu37 and Glu38 were each predicted, if deprotonated, to move the Cys30 pKa by about 0.4 in the more reducing direction. The P34H mutation in thioredoxin supports the model implication for DsbA His32 (10), Redox potential and Cys30 pKa measurements have been reported for DsbA mutations Glu37 and Glu38 and for the deletion mutant Δ38–40, which relates to assessment of the peptide dipoles contribution (11). In contrast to the reported disparity with the predictions (11), the current article will demonstrate that data for Δ38–40 are in line with the model. The deleted region is just one part of an implicated section of backbone, and its individual effect, although visible, is not large. The effects of Glu37 and Glu38 depend on their protonation states, and it will be shown that mutant measurements (11) and the atomic structure (12) are consistent with an elevated pKa for one of these residues.

Whereas lower εr gives a reasonable match to experiment in the Cys30 thiolate difference calculations, it generates large discrepancies in unmodified full pKa calculations. A recently introduced modification (8), empirically accounting for changes in water entropy upon charge burial, is shown to perform qualitatively well when applied across the ionizable groups of DsbA.

**EXPERIMENTAL PROCEDURES**

Reduced DsbA has been modeled previously (9) from the crystallographic structure of the oxidized molecule at 2 Å resolution (12) by breaking the Cys30–Cys32 disulfide bond and torsioning Cys30 to maintain van der Waals contact with Cys73. The modeled reduced configuration is similar to that of the homologous Cys30 in the nmr structures of reduced *E. coli* and human thioredoxins (13, 14). In the absence of experimentally determined atomic structures for the DsbA mutants, E37Q and E38Q were assumed to be isosteric with WT, and the deletion mutant Δ38–40 could be made with a C37–C39 link between WT residues 37 and 41, accompanied by only minor conformational rearrangement upon regularization of this region in the program QUANTA, with the CHARMM force field (Molecular Simulations Inc., Waltham, MA). Neither the WT nor Δ38–40 proteins were subjected to extensive energy minimization, so that the structures would remain close to experiment and also match each other away from the deletion site. Calculated differences between WT and the mutants are based on the assumption of minimal structural alteration, which is consistent with stereochemical and hydrogen bonding considerations. The efficacy of such conformational modeling can be assessed when the mutant and reduced WT structures are determined experimentally.

Charge interactions were calculated with FD solutions to the Poisson-Boltzmann equation (15, 16), implemented in the program FDCALC, using εr = 80 and εp ranging from 4 to 80. Calculations of pKa differences between DsbA WT and mutants (see Fig. 1) used reported ionizable charge assignment (9) for groups other than Cys30. In addition, calculations of the electrostatic free energy, differentiated between reduced and oxidized forms and between WT and Δ38–40 mutant, ΔΔG = (ΔGWT,RED − ΔGWT,OX) − (ΔG MUT,RED − ΔG MUT,OX), are compared with ratios of redox equilibrium measurements (11), −RT ln(KWT,OX/KWT,RED), from 0 to 1 M added NaCl, with T = 298.15 K. It is assumed that the structural changes associated with both disulfide bond (Cys30–Cys32) breakage and mutation are confined to separate localized regions and that these localized effects will cancel between WT and mutant (for disulfide bond breakage) and between oxidized and reduced (for the mutation). The calculated ΔΔG value is therefore the remaining long range (electrostatic) interaction between the mutation and the Cys30 thiolate. Ratios of K Quinn at each added NaCl concentration will remove any ionic strength dependence of the glutathione redox potential. Ionic

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1 The abbreviations used are: WT, wild type; FD, finite difference.
FIG. 1. Full $pK_a$ calculations and charge burial within a macromolecule. The $pK_a$ of a schematically drawn cysteine in the WT protein ($pK_a^{WT}$, top left) is derived from the $pK_a$ of the free amino acid ($pK_a^{AA}$, top right) and the electrostatic energy difference between ionization in WT and free amino acid ($\Delta G^{WT}\AA = \Delta G^{WT} - \Delta G^{AA}$), $pK_a^{WT} = pK_a^{AA} + \Delta \alpha \Delta G^{WT AA} = (1/2.303)\Delta \alpha \Delta G^{WT AA}$, where $\Delta \alpha$ is the universal gas constant and $T$ the absolute temperature. The terms $\Delta G^{WT}$ and $\Delta G^{AA}$ contain components from the Born (self) energy (22) and from charge-charge interactions (17). It is assumed that electrostatic contributions associated with neutral cysteine are negligible, focusing on the thiolate interactions in protein and free amino acid. Cysteine $pK_a$ in a mutant ($pK_a^{MUT}$) is also derived from differencing with the free amino acid (lower half of the figure). Significant errors in full $pK_a$ calculations may be associated with modeling charge burial (8), denoted by first hydration (hyd) shell occlusion between free amino acid and protein in this figure, because the Born energy is highly dependent on the difference between $\epsilon_s$ and $\epsilon_e$. Models with low $\epsilon_s$ tend to overestimate the cost of charge burial (6). The figure indicates how differencing between WT and mutant (or related) proteins with structurally similar thiolate environments, $\Delta \alpha \Delta G^{WT MUT} = pK_a^{WT} - pK_a^{MUT}$, circumvents the hydration shell changes that arise from comparison with the free amino acid (9, 23). Suggested mechanisms for full $pK_a$ calculations that reduce the cost of charge burial include the use of relatively high $\epsilon_s$ (6) and the use of lower $\epsilon_e$ together with an empirical estimate of the favorable entropic contribution associated with water liberation from the first hydration shell (8).

strength dependence was incorporated into the FD computations without a Stern layer.

Full $pK_a$ calculations (Fig. 1) used a statistical treatment of interacting ionizable groups (17), extended with a Monte Carlo method for computations with large numbers of such groups (18). This method used 10,000 Monte Carlo steps and a modification that allows for multiple site transitions for pairs that are coupled by an interaction equivalent to more than 2 $pK_a$ units (15). Partial charges (24) and ionizable group (free amino acid) $pK_a$ (6) were allocated. Ionizable residues included were Asp, Glu, His, Arg, Lys, Cys, and the amino-terminal group, whereas the carboxyl-terminal residue (189) is missing from the coordinates, and tyrosines have been omitted from the $pK_a$ calculations. Modification to account empirically for solvent entropic change upon amino acid transfer to protein is $\Delta \epsilon_s = \Delta V E_s$, where $\Delta V$ is the fractional change in first hydration shell volume (calculated from the FD grids) and $E_s$ is a free energy contribution associated with water ordering for a complete first hydration shell (8). Fitting to a range of experimental $pK_a$s, with $\epsilon_s = 4$, gives values of $E_s$ that correspond to about 6 ordered water molecules in the hydration shell of a single charge center group and about 2 for a double charge center group (8). Although there may be further detailed variation between ionizable group types within the charge center groupings, these values are starting points for overall estimates of pH titration curves. Some calculations were made using a Debye-Hückel model with a uniform dielectric, $\epsilon_{\infty} = 50$, in place of the FD procedure. The higher dielectric and the neglect of counterion exclusion from the protein interior in this method (19) reduces the size of electrostatic interactions.

RESULTS AND DISCUSSION

Difference Calculations between WT and Mutants E37Q, E38Q, and E37Q/E38Q—The $pK_a$s of these residues in WT

DsbA are currently unknown, and full $pK_a$ calculations are not sufficiently reliable to provide detailed estimates, largely due to the charge burial term. The more reliable charge-charge estimates yield interactions of about 2 kJ/mol between a deprotonated Glu37 or Glu38 in the Cys30 thiolate. In the WT structure at pH 6.5 (12), Glu37 and Glu38 carboxylates approach within 3 Å (Fig. 2), strongly suggesting that they share a proton and that one of the $pK_a$s will be elevated above neutral pH, so that just one carboxylate to thiolate interaction should be counted for comparison to redox equilibrium measurements at pH 7. The calculated WT to double mutant difference would be this single interaction, approximating no conformational change and limited glutamine side chain charge effects. With regard to the single mutants, Glu38 lies toward the protein exterior and Glu37 facing the protein interior (Fig. 2), so that Glu37 is likely to be buried within the E38Q mutant whereas Glu38 will be solvent-exposed in the E37Q mutant. These considerations would be consistent with deprotonation of Glu38 in the E37Q mutant but neutralization of Glu37 in the E38Q mutant. The calculated $\Delta \Delta G$s, WT to (E37Q, E38Q, E37Q/E38Q) mutants, for this hypothesis with the $\epsilon_s = 4$ model are (0, −2, −2) kJ/mol compared with measured values of (0.6, −2.0, −1.5) in 10 mM sodium phosphate (11). This reasonable agreement, constructed upon a plausible hypothesis for Glu38 in the E37Q mutant but neutralization of Glu37 in the E38Q mutant, demonstrates both the requirement for more accurate full $pK_a$s calculations and the success of the published model (9) in suggesting a route toward engineering more oxidizing DsbA molecules at neutral pH. Comparisons at the acidic pH of the Cys30 $pK_a$s are omitted for this set of mutants because it is likely that both Glu37 and Glu38 will be protonated in this pH region.

Difference Calculations and Salt Dependence for Redox Equilibria of WT versus Δ38–40 Mutant—The deleted residues 38–40 are within a larger polypeptide region (Fig. 2), which in total is predicted to contribute to thiolate stabilization in the low $\epsilon_s$ model. It is possible to make the Glu37–His41 link without substantial disruption to the rest of the protein. Following the discussion in the previous section, Glu37 becomes solvent-exposed in the modeled deletion mutant so that, by analogy with WT Glu38, it is likely to be deprotonated at the neutral pH of $K_w$ measurements. In difference calculations between WT and Δ38–40 it is assumed that the thiolate interactions to the

![Image](http://www.jbc.org/Downloadedfrom)
modeled single negative charge of WT Glu37/Glu38 and the modeled single negative charge of Glu37 in Δ38–40 cancel out. Other ionizable groups are set at normal neutral pH values except for His32 and Glu24, which are in the vicinity of the active site and may have pKa values around neutral pH (9). These ionizations were set to +0.5 and −0.5, respectively. For the WT versus Δ38–40 mutant calculations, both His38 and Glu39 contributions almost cancel on differencing.

Calculated Cys30 thiolate contributions to ΔΔG are compared with experimental values derived from Keq ratios (11) for WT versus Δ38–40 over a range of added NaCl concentrations (Table I). The approximations in the calculations (modeled Δ38–40 conformation, ionizable charge assignment, and Keq contribution from Cys30 thiolate alone included) combined with the small ΔΔG values (thermal energy or less) suggest that qualitative rather than quantitative comparisons should be made. It can be seen that the εp = 4 model is by far the closest to experiment, indicating that charge-charge interactions through a low protein dielectric are important in DsbA and that higher εp values underestimate these interactions. Within the εp = 4 model, the listing of total and non-ionizable interactions shows the importance of partial charge stabilization of the Cys30 thiolate. With the modeled Δ38–40 mutant structurally homologous to WT DsbA, these partial charge interactions can be attributed to WT residues 38–40. The measured effects (11) are therefore consistent with earlier predictions, which estimated interaction energy between the Cys30 thiolate and cumulative peptide dipoles over residues 25–43 at about −20 kJ/mol (9). The measured upward Cys30 pKa shift of 0.5 for the WT to Δ38–40 mutation (11) compares with a value of 0.3 by εp = 4 calculation, demonstrating that these relatively small shifts are roughly in line with the prediction that extensive charge interactions in DsbA play a significant role in generating the low Cys30 pKa and oxidizing power. Residues 38–40 of *E. coli* DsbA are missing in *Vibrio cholerae* DsbA, but a proline causes the same overall kink in the protein backbone (20). The higher thiolate pKa for *V. cholerae* compared with *E. coli* DsbA (21) is consistent with the results for the *E. coli* DsbA Δ38–40 mutant, but more detailed assessment must await full difference calculations between the two WT proteins.

Measurements of folding stabilities for oxidized and reduced *E. coli* DsbA WT and mutants in guanidinium chloride (11) have not been used for comparison because the key determinant of stability in these experiments is the folding transition cooperativity (rather than the transition midpoint) from 1.5–2.5 M guanidinium chloride. Matching computations would therefore be required to account for relatively small differences in ionic strength variation at these high denaturing salt concentrations, which is beyond the scope of current methods. In regard to discussions of the link between redox potential, Cys30 pKa, and reduced/oxidized protein stability, the current calculations are consistent with such a link, with qualitative agreement between the low εp model for Cys30 interactions and Keq measurements. The remaining discrepancy, such as underestimation of experimental values with εp = 4, could signal the breakdown of the various assumptions and/or modeling insufficiency. For example, choice of εp within the lower range (typically 2–4) remains an open question, and εp < 4 would yield higher calculated values. The large difference in scale between calculated values of ΔΔG (Table I) for εp = 4 and higher εp models is due to the largely through-protein nature of the (38–40)/thiolate interactions, suggesting that measurements with Δ38–40 provide a sensitive test of protein dielectric modeling. Single-site mutations that are predicted to yield >0.5 pKa shift relative to WT Cys30 while preserving active site stereochemistry are removal of the His32 ionizable group or removal of the Gln97 side chain amide (9).

![Figure 3](https://www.jbc.org/)

**TABLE I**

| Experimental/ calculated | 0 M NaCl | 0.2 M NaCl | 0.5 M NaCl | 1.0 M NaCl |
|--------------------------|----------|------------|------------|------------|
| **Experimental**         |          |            |            |            |
| (4,80)                   | 1.21 (1.46) | 1.13 (1.40) | 1.12 (1.40) | 1.09 (1.43) |
| (20,80)                  | 0.47 (0.51) | 0.41 (0.45) | 0.38 (0.43) | 0.37 (0.43) |
| (80,80)                  | 0.21 (0.21) | 0.21 (0.21) | 0.19 (0.19) | 0.17 (0.17) |
| εp = 50                  | 0.32 (0.32) | 0.10 (0.10) | 0.05 (0.05) | 0.00 (0.00) |

**Fig. 3.** Ranges of calculated ΔpKₐ for ionizable groups in DsbA WT, according to different models. Each panel plots the number of calculated ΔpKₐ values for intervals over the range from 5 ΔpKₐ units stabilizing (left side) to 5 units destabilizing (right side). Each panel records the experimental (3, 11) and calculated Cys30 ΔpKₐ for comparison and is labeled with the relevant calculation model.
calculations for DsbA WT, thereby introducing the charge burial term (Fig. 1). Extensive studies of computed versus measured pKₐ values in a range of proteins have revealed two important factors. There exists a subset of amino acids with large pKₐ shifts (often linked to function) and a much larger set with small pKₐ shifts tending toward protein stabilization (6). When εₚ is varied to give the best match to experiment, the result tends toward a higher value (e.g., εₚ = 20), which yields the larger set of small pKₐ shifts (6). The Cys³⁰ thiolate of DsbA is an excellent example of the subset of large pKₐ shifts. Although pH titrations of the remaining ionizable groups of DsbA have not been measured, it is appropriate to make a qualitative study of the effect of εₚ variation on the overall form of the pH dependence and to ask whether any of the available models are capable of generating a large and stabilizing ΔpKₐ for Cys³⁰ together with an overall set of small ΔpKₐs that tend toward protein stabilization. Also included is the εₚ = 4 model with the suggested empirical modification to account for hydration entropy change upon charge burial in full pKₐ calculations (8).

Distributions of ΔpKₐ are shown for the various computational models (Fig. 3). The Eₛ parameter in the modification for single charge center groups has been adjusted to match experimental pKₐ values for the DsbA Cys³⁰ and thioredoxin Cys³² thiolates (8), so that reproduction of this match for the DsbA Cys³⁰ pKₐ in the modified εₚ = 4 model is expected. However, application of the Eₛ modification is much more than a device for fit to experiment, because it represents a key part of solvation energetics (solvation entropy), and the derived values fall within the range of measured ionic hydration numbers. It is important to ask whether other models can generate the same agreement for Cys³⁰ and also to analyze the overall distribution of ΔpKₐ values. Modification of the higher εₚ models, with a term accounting for the favorable hydration entropy contribution on charge burial, would have a much smaller effect than with a lower εₚ model, because the magnitude of the modification must not exceed that of the unfavorable Born term to avoid a model that favors general charge group burial. In other words, the higher εₚ models cannot escape from a general underestimation of electrostatic interactions that leaves the Cys³⁰ ΔpKₐ close to zero and in contrast with experiment.

With regard to the overall distribution of ΔpKₐ values, the unmodified εₚ = 4 model shows a large spread, with the extension toward significant destabilization that is characteristic of charge burial in such a model. Application of the modification gives a range of ΔpKₐs tending toward the moderate overall stabilization that is the basis of success in the higher εₚ and εₑ = 50 models (Fig. 3). One of the largest ΔpKₐ shifts upon modification of the εₚ = 4 model is for Cys³⁰, arising from the significant thiolate burial within DsbA. Whereas the modified εₚ = 4 model can target both the overall ΔpKₐ distribution and specific large values, such as that of Cys³⁰, the cost of reducing all charge interactions in the higher εₚ and εₑ = 50 models is likely to be the omission of those larger ΔpKₐs that may be of functional interest. The presence of large calculated ΔpKₐ values other than that of Cys³⁰ in this qualitative application of the modified εₚ = 4 model does not necessarily indicate model breakdown, because they include residues which by various indications may have significantly altered pKₐ values, such as Glu²⁴, Cys³² (3), and Glu₉/Glu₈.

This article shows that a low εₚ continuum electrostatic model is consistent with pKₐ shifts and redox equilibrium measurements for DsbA WT versus mutants (11), reinforcing its value in understanding oxidizing power in this protein family. In the discussion of methods for full pKₐ calculations, DsbA provides a valuable diversion. It is not well characterized in terms of general pKₐ measurements, but the large and functionally significant Cys³⁰ ΔpKₐ provides a crucial test that higher εₚ models fail. Because the modified εₚ = 4 model recovers a reasonable (moderately stabilizing) ΔpKₐ profile as well as the Cys³⁰ ΔpKₐ potential clearly exists for detailed model development against proteins with well characterized pH-dependent properties.

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