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Backbone conformational dependence of peptide acidity

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ABSTRACT

Electrostatic interactions at the protein surface yield over a billion-fold range of amide hydrogen exchange rates. This range is equivalent to the maximal degree of attenuation in exchange rates that have been shown to occur for amides buried within the protein interior. Continuum dielectric analysis of Ala-Ala, Ala-Gly, Gly-Ala and trans-Pro-Ala peptide conformer acidities predicts that the relative orientation of the two neighboring peptide groups can account for a million-fold variation in hydroxide-catalyzed hydrogen exchange rates. As in previous protein studies, an internal dielectric value of 3 was found to be applicable to simple model peptides, presumably reflecting the short lifetime of the peptide anion intermediate. Despite the million-fold range in conformer acidities, the small differences in the experimental exchange rates for these peptides are accurately predicted. Ala-Ala conformers with an extended N-terminal residue and the C-terminal residue in the α conformation are predicted to account for over 60% of the overall hydrogen exchange reaction, despite constituting only 12% of the protein coil population.

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1. Introduction

Protein amide hydrogen exchange is commonly interpreted in terms of a conformational protection factor. The ratio of the observed amide exchange rate k_{ex} to the exchange rate for the corresponding local sequence in a model peptide k_{pep} is used to predict the equilibrium constant for the conformational transition by which a structurally buried amide becomes transiently exposed to the solvent phase (i.e., $\Delta G = -RT\ln(k_{ex}/k_{pep})$) [1]. Central to this analysis is the assumption that exposure to solvent results in amide exchange rates that are equal to those for simple model peptides. However, hydrogen exchange measurements for amides that are exposed to solvent in the protein X-ray structure can dramatically conflict with this peptide normalization assumption. The amide hydrogens of *Pyrococcus furiosus* rubredoxin that are solvent-exposed in the 1.1 Å X-ray structure [2] exhibit a billion-fold range of hydroxide-catalyzed exchange rates [3]. In particular, the well exposed (3 Å^2) Val 38 amide hydrogen exchanges at a rate that is nearly 10^7-fold slower than that of the corresponding Trp-Val model peptide [4]. Conversely, His 38 in the active site of the **a** domain of the human protein disulfide isomerase exchanges at a rate 400-fold faster than the corresponding model peptide value [5]. Application of the standard protection factor analysis to these two static solvent-exposed amides yields a 13 kcal/mol range of apparent conformational stabilities. This range is at least as large as the maximal global stability of any protein predicted from hydrogen exchange measurements which has been independently verified by either calorimetric or spectroscopic methods [6–8]. Given that these static solvent-exposed amides need not require any conformational transition for hydrogen exchange to occur, it is of interest to explore in detail how the remainder of the protein structure can give rise to such a large range of exchange rates.

Amides are known to react with the hydroxide ion as Eigen [9] normal acids, so that the exchange rate of an amide is equal to the product of the diffusion-limited rate and the fraction of forward-reacting encounters $K_c/(K_c+1)$, where K_c is the equilibrium constant for the transfer of a proton from the amide to an hydroxide ion [10,11]. In turn, electrostatic interactions modulate the acidity of an amide by altering the difference in solvation free energy between the neutral and anionic peptide states [12,13]. In the earlier rubredoxin study [3], Poisson–Boltzmann continuum electrostatic calculations were carried out on the deprotonated peptide form of each static solvent-exposed amide, so as to predict the free energy of proton transfer between these sites. With one exception, the predicted acidities for all of these amides agreed with the observed hydrogen exchange rate constants to within a rmsd of 6 [3].

To analyze the electrostatic contributions to the rate of hydrogen exchange that arise solely from the adjacent peptide units, dipeptide sequences containing only alanine, glycine and proline residues within a large set of high resolution X-ray structures were identified. Continuum dielectric calculations were then carried out on implicitly solvated models of the corresponding N-acetyl-X-X-N-methylamide conformers. By operationally turning off the remainder of the interactions that result from the protein native structure, the contribution to amide acidity that arises from the orientation of the adjacent peptide units can be assessed.

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The Protein Coil Library of Rose and coworkers [14] was used to identify target peptide conformers from among the 17,422 protein segments in their library that had been selected from X-ray structures having resolution limits of 1.6 Å and *R* values of 0.25 or better. As for coil libraries in general, the helical and sheet segments of the protein backbones were removed from this Protein Coil Library to yield a set of peptide conformers that are commonly believed to offer the most robust representation presently available for the conformational distribution characteristic of the unstructured protein denatured state and of its constituent peptide components.

Such a conformationally filtered set offers an independent method for assessing the reliability of the proposed peptide conformer electrostatic calculations. In experimental measurements of model peptides, substitution of alanine on either side of the exchange site yields less than a 3-fold change in the amide exchange rate for most sidechain types [4]. However, evidence is presented here which indicates that the orientation of the adjacent peptide units gives rise to a million-fold range in amide acidities for individual conformations that are observed in high resolution protein structures. Yet, despite this wide range of acidities for individual peptide conformers, averaging over the full coil library distribution faithfully predicts the much smaller differences in exchange rates that are observed among simple model peptides.

2. Computational methods

2.1. Continuum dielectric calculations

The 17,422 protein segments selected from X-ray structures having at least 1.6 Å resolution and *R* values of 0.25 or better in the Protein Coil Library of Rose and coworkers [14] were screened for Ala-Ala, Ala-Gly, Gly-Ala, Gly-Gly and trans-Pro-Ala peptides, after the terminal residues of each segment had been excluded so as to further reduce residual conformational bias that could arise from the presence of adjacent regular secondary structure [15]. The Reduce program [16] was used to add hydrogens to the heavy atoms in the backbone segment that extends between the C^{α} atoms on either side of the selected dipeptide, yielding N-acetyl-X-X-N-methylamides. Nonlinear Poisson–Boltzmann calculations were carried out using the DelPhi algorithm [17], with CHARMM22 atomic charge and atomic radius parameters [18]. Except where stated otherwise, the excess charge of the amide anion was localized on the nitrogen atom. This selection reflects the superior performance that was observed for this charge distribution in the present study as well as in previous studies of both model peptide [12] and protein [3] hydrogen exchange. A 0.25 Å grid spacing was used with a 40% filling factor and an ionic radius of 2.0 Å. The external dielectric value was set to 78.5 and an ionic strength of 0.15 M was used, so as to mimic the conditions of our earlier rubredoxin study [3].

2.2. N-methylacetamide modeling

A population of structures for the reference compound N-methylacetamide was generated so as to provide consistency with electrostatic calculations on peptide conformations obtained from the Protein Coil Library. The central peptide unit for each of the Gly-Gly peptides was converted to N-methylacetamide by transformation of the first nitrogen and the last carbonyl carbon to hydrogen atoms. DelPhi calculations were carried out on a lattice grid containing each of these Gly-Gly derived N-methylacetamide molecules with a second N-methylacetamide molecule of fixed geometry. All atoms from each of the two molecules were separated by at least 8 Å. At this distance the variation in the differential electrostatic solvation free energies predicted for the two amide anions was found to be less than 0.1 kT. Assuming an internal dielectric constant value of 3, 68% of the Gly-Gly derived N-methylacetamide molecules had predicted amide pK values within 0.1 units of the mean electrostatic solvation free energy ($\Delta pK = \Delta kT/\ln$ [10]). Averaging over this subpopulation of Gly-Gly derived structures was used to determine an N-methylacetamide reference molecule with C^{α}–C, C–O, C–N and N–C^{α} bond lengths of 1.52, 1.23, 1.33 and 1.45 Å, respectively, and C^{α}–C–O, C^{α}–C–N and C–N–C^{α} bond angles of 120.6°, 116.3° and 121.0°, respectively. These values closely correspond to those for the small molecule X-ray structure [19].

2.3. Crystallographic dipeptide conformer calculations

Electrostatic calculations on the Ala-Ala, Ala-Gly, Gly-Ala and trans-Pro-Ala peptides were carried out similarly with N-methylacetamide added to the box for internal referencing of the differential electrostatic potential. For each peptide conformer, three calculations were carried out: the peptide anion in the presence of the neutral N-methylacetamide; the neutral peptide in the presence of the N-methylacetamide anion; and the neutral peptide in the presence of the neutral N-methylacetamide which serves as the common reference state. In this approach, the coordinates of the reference molecule are not superimposed upon those of the ionizing site of the target molecule in the lattice grid. As a result, incomplete cancellation of the electrostatic self-energy terms can result. To assess the extent of incomplete cancellation, the difference in electrostatic solvation free energy predicted between the neutral and anionic N-methylacetamide molecules for each of the 679 Ala-Ala conformer calculations was found to vary with an rmsd of 0.083 kT, indicating an average grid error of 0.036 pH units in the relative pK values.

3. Results and discussion

In our earlier study of the static solvent-exposed amides in *Pf* rubredoxin [3], the electrostatic solvation free energies of the individual peptide anions were calculated as a function of the force field electrostatic parameter set (CHARMM22 [18], AMBER parm99 [20] and PARSE [21]), the protein internal dielectric constant, and the distribution of the excess negative charge between the amide nitrogen and the carbonyl oxygen of the ionizing peptide linkage. An optimal correlation between the second order rate constants of hydroxide-catalyzed exchange and the predicted differences in the amide pK values was obtained from the use of an internal dielectric value of 3 with the CHARMM22 electrostatic parameter set applied to the peptide anion charge localized on the amide nitrogen. The poorer correlation that was obtained when the excess negative charge of the peptide anion was divided equally between the amide nitrogen and the carbonyl oxygen agrees with the results that were previously reported in continuum dielectric modeling of hydrogen exchange in simple peptides [12].

3.1. Prediction of peptide conformer acidity

In the present study, the optimal electrostatic parameters obtained in our earlier *Pf* rubredoxin study were applied to a set of N-acetyl-Ala-Ala-N-methylamide peptide conformers which were implicitly solvated at 0.15 M ionic strength. A total of 679 Ala-Ala conformations were identified in 17,422 protein segments in the Protein Coil Library [14]. The DelPhi [17] program was used to calculate the electrostatic potential of the central peptide anion for each terminally blocked Ala-Ala conformer, relative to an N-methylacetamide anion reference.

When the CHARMM22 electrostatic parameters were applied to the coil library-derived Ala-Ala peptides with the internal dielectric set to 3, the conformer acidities were found to span a range of 6 pH units (Fig. 1a). A similar range of conformer acidities is predicted for the Ala-Gly peptides (Fig. 1b) as well as for the other coil library peptides considered here. Hence, within the range of conformations observed in native protein structures, the local backbone conformation of the adjacent peptide groups is predicted to give rise to more

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