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Packing defects functionalize soluble proteins

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ABSTRACT

This work explores the participation of protein packing defects, the so-called *dehydrons*, in biochemical events. We delineate the enabling role of dehydrons as activators of nucleophilic groups. This activation results from the induction of chemical basicity in interfacial water molecules, promoting deprotonation of adjacent nucleophiles. Through multiple steering molecular dynamics with pulling along the proton-displacement coordinate, we show that nucleophilic groups are functionally enabled by nearby dehydrons that promote proton transference. The computations are validated against experimentally determined pKa decreases at functional sites and biochemical probes of deregulated catalytic activity arising from dehydron-generating mutations.

1. Introduction

Dehydrons are packing defects in soluble proteins known to promote protein associations [1] and have been conjectured to induce chemical reactivity in interfacial water [2]. Dehydrons are solvent-exposed backbone hydrogen bonds and, in large concentration on the protein surface, can cause structural disruption by enabling backbone hydration. Thus, dehydrons introduce structure-destabilizing nanoscale cavities on the protein surface. These cavities create interfacial tension since backbone-solvating water under nanoscale confinement is deprived of hydrogen-bonding possibilities [3]. The interfacial tension caused by dehydrons is released upon protein associations that in effect displace the restricted interfacial water, turning dehydrons into promoters of protein interactions [3]. It has been further conjectured that water molecules enveloping dehydrons, thereby restricted in hydrogen-bond coordination, may act effectively as proton acceptors [2]. This chemical behavior appears to arise from a non-Debye polarization-induced negative charge arising as nanoscale confinement hampers the alignment of water dipoles with the electric field [2].

In this work we unravel the mechanism of functional enablement promoted by dehydrons in their role as inducers of chemical basicity of the aqueous interface. To that effect, we investigate the chemical event of proton transference prompted by water molecules at dehydron interfaces. Specifically, we compute the shifts towards lower values in pKa [4] of groups functionalized through dehydron-promoted deprotonation. To assess this activity we consider chemically active dehydrons in the proximity of the functional site, i.e. those for which the water oxygen in the dehydron cavity is within 6 Å of the α-carbon of the functional residue. The method of choice to investigate the chemical event of dehydron-induced proton transference is multiple steering molecular dynamics computation [5]. The nucleophilic group and nearby proton-receptive water molecule at the dehydron interface are treated within a quantum mechanical (QM) scheme while the rest of the molecule and explicit solvent are treated using a classical molecular mechanics (MM) package, in accord with a QM–MM hybrid approach [6,7]. The results are validated against experimentally determined pKa shifts [4] and functional studies of constitutively active mutant enzymes [8] whose aberrant deregulation is shown to arise from the creation of dehydrons not present in the wild type.

The study cases are selected so that the dehydron-promoted lowering of pKa value is significant and cannot be properly captured by current estimators of pKa shift. The latter are typically based on an empirical evaluation of pairwise interactions within a protein environment that favors a particular ionization state. Such estimators do not incorporate the unique electrostatic effects of structural or confined interfacial water molecules [2] into the empirical environmental field [4].

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2. Materials and methods

To determine the functional stimulation caused by dehydrons, we compute the pKa decrease for nucleophilic side-chain groups with dehydrons in their proximity relative to the free residue in solution. As usual, the pKa shift, ΔpKa, estimates the difference in free energy increment, $\Delta\Delta G/RT = [\Delta G(p) - \Delta G(w)]/RT$, of the proton abstraction process in the protein environment (ΔG(p)) relative to the bulk aqueous environment (ΔG(w)). The results are contrasted against experimental data on ΔpKa. The free energy computation follows the multiple steering ansatz [5], where the molecular dynamics (MD) trajectories are generated by treating classically all groups except for those implicated in the chemical step of proton transference. The latter are treated in the quantum-mechanics (QM) density functional theory (DFT) setting [6]. The QM treatment is thus restricted to the side chain of the catalytic residues containing the weak-acid pro-nucleophilic group and to the reactive dehydron-associated water molecule. The reactive water molecule is defined as having its oxygen within 2.5 Å of the transitional proton that is initially covalently attached to a heavy atom (O, S or N) in the pro-nucleophilic group. The latter is generically denoted AH (or AH^+) and the neighboring distance cutoff is set so that the covalent bond A–H (or $[A-H]^+$ if protonation bestows charge) turns into hydrogen bond in the deprotonated state $A^-\cdots H(H_2O)^+$ (or $A\cdots H(H_2O)^+$) that results as the proton is transferred to the nearby dehydron-functionalized water molecule.

We denote by X the proton transference coordinate indicating the distance of the proton to the heavy atom initially covalently attached to it in the weakly acidic pro-nucleophile. Thus, $X(t = 0) = X_0$ is the bond length corresponding to covalent bonding to the heavy atom in the nucleophile and $X–X_0$ measures departure from covalent bond length. We denote by $\boldsymbol{R}$ the structural-coordinate vector for protein chain and water. In accord with the Jarzynski identity [5], $\Delta G(p)/RT = -\log\langle\exp[-W(X, \boldsymbol{R}(X))/RT]\rangle$, where the average $\langle\exp[-W(X, \boldsymbol{R}(X)]\rangle$ (W = computed work) extends over all trajectories $\boldsymbol{R}(X(t))$ with structural conformations steered by the pulling $X = X_0 \rightarrow X = X_0 + v(t_f)$ at constant speed v along the harmonic proton-transference linear coordinate. The pathway ensemble $\{\boldsymbol{R}(X(t))\}$, is generated by choosing initial conformations $\boldsymbol{R_0} = \boldsymbol{R}(X_0)$ within an isothermal/isobaric equilibrated ensemble (T = 298 K). This ensemble realizes the condition $X = X_0$ and is generated by a set of 20 classical thermalization trajectories, each lasting 1 ns, with the PDB-reported structure fixed at the initial condition. The trajectory multiplicity arising from X-pulling is provided by the conformational dispersion in the initial ensemble $\{\boldsymbol{R_0} = \boldsymbol{R}(X_0)\}$, with each initial conformation responding differently to the X-pulling.

The QM region is treated using flexible basis sets of linear combinations of finite atomic orbitals in a real space grid optimized to N-scaling. The basis functions enable the matching of the radial wave function to the core region described by pseudopotentials by using pseudoatomic orbitals (PAOs) [9]. Split valence bases are generated by combining numerical Gaussian orbitals with the minimal basis described. The nuclei and core electrons are represented by norm-conserving pseudopotentials to avoid the computation of core states, a procedure that smoothens out the valence charge density in accord with grid requirements. Within the non-local pseudopotential approximation, a Kohn–Sham Hamiltonian is adopted incorporating the Hartree and exchange–correlation potentials, and a pseudopotential with additive contributions to account for local effects, long-range interactions and operation on valence electrons [10]. Calculations are performed on contracted Gaussian basis sets of double-zeta valence polarized (DZVP) quality (pseudoatomic orbital energy shift = 30 meV, grid cutoff = 135 Ry) [11].

The MM region is treated as detailed in [3,12], where torsional degrees of freedom of backbone and side chains are coarse grained modulo basins of attraction in the potential energy surface in accord with Ramachandran (energetically allowed) regions in local conformation space. Interfacial water dipoles confined to dehydron cavities are subject to a torque resulting from the hindrance to alignment with the electrostatic field [3]. To equilibrate the PDB-reported structures with the solvent, we generated MD trajectories driven by the coarse-grained stochastic process, incorporating the potential energy associated with solvent orientation steering as the reversible work needed to align polarization-induced dipoles due to interfacial water confinement with the Debye electrostatic field [12]. To cover relevant timescales (~10 ns), the dynamics are entrained by the coarser "protodynamics", where the backbone (ϕ, ψ) dihedral torsions are specified "modulo basins of attraction" in the potential energy surface. Coarse moves are defined as transitions between basins of attraction (R-basins) in the Ramachandran torsional (ϕ, ψ)-map for each residue. Thus, each residue is assigned an R-basin after a coarse move, and the coarse state of the chain becomes a conformational ensemble, with each conformation generated by selecting individual (ϕ, ψ)-coordinates within the assigned R-basins [3].

The hybrid Hamiltonian incorporated includes QM-MM coupling comprised of three contributions: (1) electrostatic interaction between electrons and classical charges, (2) electrostatic interactions between nuclei in the QM subsystem and the classical point charges, and (3) a Lennard–Jones 6–12 potential to account for the van der Waals interactions between the atoms in MM and QM regions constructed using the force-field parametrization of Wang et al. [13]. The forces on the QM nuclei are obtained by taking the gradient with respect to atomic positions, and include derivation of the QM–MM coupling energy. The implementation details and description of the package adopted to conduct QM/MM hybrid computations is presented in the Supplementary Data.

3. Results

The quasi-equilibrated work plots harvested in the free-energy computation associated with the dehydron-induced proton transfer event do not portend and are not required to reproduce the actual kinetics, which occurs on much faster timescales. The work performed by the system to reach the point $X = X_0 + vt$ along the proton transference coordinate is shown in Fig. 1a for the imidazole >NH group in His149 of *xylanase* (PDB.1XNB) for ten realizations, $\boldsymbol{R_0} = \boldsymbol{R}(X_0)$ of the initial condition $X = X_0$ with harmonic force constant 48 kJ/mol. The thin lines indicate the work performed on the system at each point $X = X_0 + vt$ along the X-pulling steering trajectory with v = 0.2 Å/ns and t_f = 6 ns. The thick lines correspond to slower pulling at v = 0.1 Å/ns with t_f = 12 ns. The work histories for proton transference from imidazole in a free His amino acid in bulk water are shown in Fig. 1b. The dehydronic environment of His149 in xylanase structure is displayed in Fig. 1c. The His149 residue is required to be deprotonated in its structure-stabilizing function exerted by hydrogen bonding Ser130, internal water and by engaging in a putative aromatic–aromatic interaction with Tyr105 [14]. The case illustrated represents a dramatic pKa shift, with ΔpKa < –3.8, from the pKa value ~6.1 for free protonated imidazole in bulk water to <2.3 in the xylanase environment [4]. The dehydron-functionalized water molecule around vicinal dehydron Ser100-Gly103 in the initial conformation $\boldsymbol{R_0} = \boldsymbol{R}(X_0)$ shown in Fig. 1d serves as acceptor of the imidazole proton in His149, with the associated chemical event schematized in Fig. 1e. Other conformations use the environment around the other vicinal dehydron Thr145-Asn148 for proton acceptance. A computed value of ΔpKa = –3.77 is obtained for 20 trajectories