Role of solvation in pressure-induced helix stabilization

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In contrast to the well-known destabilization of globular proteins by high pressure, recent work has shown that pressure stabilizes the formation of isolated α -helices. However, all simulations to date have obtained a qualitatively opposite result within the experimental pressure range. We show that using a protein force field (Amber03w) parametrized in conjunction with an accurate water model (TIP4P/2005) recovers the correct pressure-dependence and an overall stability diagram for helix formation similar to that from experiment; on the other hand, we confirm that using TIP3P water results in a very weak pressure destabilization of helices. By carefully analyzing the contributing factors, we show that this is not merely a consequence of different peptide conformations sampled using TIP3P. Rather, there is a critical role for the solvent itself in determining the dependence of total system volume (peptide and solvent) on helix content. Helical peptide structures exclude a smaller volume to water, relative to non-helical structures with both the water models, but the total system volume for helical conformations is higher than non-helical conformations with TIP3P water at low to intermediate pressures, in contrast to TIP4P/2005 water. Our results further emphasize the importance of using an accurate water model to study protein folding under conditions away from standard temperature and pressure. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4901112>]

I. INTRODUCTION

The dependence of protein folding equilibria on thermodynamic control variables can provide important insights into the fundamental forces which stabilize folded structures.¹ Variation of temperature is the most commonly used approach due to the ease with which this can be achieved. A detailed comparison of the temperature-dependence of protein stability with that of the hydrophobic effect, for example, strongly supports the role of hydrophobic interactions in stabilizing protein structure.^{2,3}

A variable which is less exploited is pressure, due to the greater demands of the experiments required.^{4,5} However, it is well-known that high pressure tends to destabilize protein native structures,^{6,7} indicative of a positive change of reaction volume for folding. A possible resolution of this initially counterintuitive effect⁸ was proposed to be the existence of cavities within the folded protein,^{9,10} with substantial support for this hypothesis coming from experiments on cavity-forming mutants.^{10,11} It was also proposed that high pressures can lead to water penetration of protein's hydrophobic core due to reduced solvent-solute interfacial free energy.¹²

However, the pressure-dependence of the folding equilibria for small, independently folding elements of secondary structure such as hairpins and helices clearly cannot fit the same picture. Since they contain no evident internal cavities or well-defined hydrophobic core, any pressure dependence would have to come from other effects, which might be ob-

sured when studying the overall folding of a globular protein. Recent experimental work on the pressure dependence of the helix-coil equilibrium, using either FTIR spectroscopy^{13,14} or triplet state quenching experiments¹⁵ has in fact found the opposite trend to that for protein folding: namely a negative reaction volume for helix formation, resulting in pressure stabilization of helices for all positive pressures. The origin of this effect is not clear, especially considering the small magnitude of the volume change (≈ 0.2 – 1.0 cm³ mol⁻¹ per residue). Molecular simulation could potentially help to explain the origin of this result; however, simulation studies of the pressure dependence of helix formation have qualitatively contradicted experimental results, finding instead helix destabilization at low to intermediate pressures, only turning over to stabilization at very high pressure.^{16–18}

Here, we investigate the pressure dependence of helix formation for a model 15-residue helix-forming peptide using two different force field combinations: the Amber ff03* protein force field¹⁹ together with explicit TIP3P water²⁰ and the Amber ff03w protein force field²¹ with the TIP4P/2005 water model.²² In agreement with earlier studies with TIP3P water on the effect of pressure on helix formation, we find a positive reaction volume for helix formation at low to intermediate pressures. In contrast, a qualitatively correct result is obtained with TIP4P/2005, i.e., a negative reaction volume for helix formation. This difference, obtained for the same sequence with almost identical protein force fields, suggests a key role for water. There are essentially two ways in which water can be envisaged to influence the reaction volume: (i) it may alter the conformations sampled for a given total number of helical residues, particularly for the non-helical states and

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(ii) different water models may be more or less closely coordinated with helical than with non-helical structures. While there is no doubt that water influences the conformational sampling, we show that in fact the different solvation of helical and non-helical states plays a key role in determining the volume changes, and for both water models opposes the much larger decrease in volume excluded to water associated with helix formation. The result obtained with TIP3P water at low to intermediate pressures is qualitatively inconsistent with experiment, as the total system volume is higher for helical structures than for non-helical structures. Our results highlight the importance of using an accurate water model for capturing biomolecular equilibria at state points away from standard conditions.

II. METHODS

A. Simulation methods

Replica exchange molecular dynamics (REMD) simulations with gromacs 4.0.7 or 4.5.3²³ were used to sample the folding of the blocked peptide Ac-(AAQAA)₃-NH₂ with either (i) the Amber ff03* force field¹⁹ and TIP3P water model²⁰ or (ii) the Amber ff03w force field²¹ and the TIP4P/2005 water model.²² For ff03*, 42 replicas spanning a temperature range from 275 to 496 K were used, and for ff03w, 40 replicas spanning 250 to 452 K. Periodic boundary conditions with a truncated octadron minimum image cell of initial edge 4.5 nm were used. Simulations were maintained at a constant pressure with a Parrinello-Rahman barostat²⁴ and temperature was controlled via Langevin dynamics with a friction coefficient of 0.2 ps⁻¹ (ff03*) or 1.0 ps⁻¹ (ff03w). Note that the difference in friction coefficients will not alter the equilibrium properties considered here, and both values are sufficient to maintain constant temperature. Simulations were run for 100–200 ns per replica. A separate set of REMD runs was performed for each pressure, using 1 bar, 2 kbar, 4 kbar, 8 kbar, and 12 kbar for Amber ff03* and 1 bar, 1 kbar, 4 kbar, and 8 kbar for Amber ff03w.

To determine the change of volume associated with helix formation, initial configurations were obtained by random selection from the 298 K replica of the ff03* simulations. For each number of helical residues (0, 3, 4, ... 15), 20 configurations were chosen with that helix content. From each of these configurations, 10 ns simulations were performed using position restraints to keep the peptide close to its initial structure, with force constants of 1000 kJ mol⁻¹ nm⁻² on each cartesian coordinate, and using both the ff03* and ff03w force fields. Average total system volumes were computed from each of these runs.

B. Helix formation

We define helical states using the backbone Ramachandran angles, in the spirit of the Lifson-Roig theory. We define the helical region of the Ramachandran map as $\phi \in [-100^\circ, -30^\circ]$ and $\psi \in [-67^\circ, -7^\circ]$. A helical segment is defined as three consecutive residues with their backbone torsion angles lying in this range. Therefore, the total number of helical

residues in the blocked 15-residue peptide considered can take on values in {0, 3, 4, 5, ..., 14, 15}. This method gives results which are consistent with other definitions of helix (Supporting Fig. S1).¹⁹

C. Helix-coil models

Simulation data on helix formation were initially described by a Lifson-Roig model,²⁵ with nucleation parameter v and elongation parameter w . These parameters were fitted by maximizing the likelihood of the observed conformations, given the equilibrium probabilities generated from the Lifson-Roig partition function, using a previously described procedure.¹⁹ Since the experimental data had been fitted to the Zimm-Bragg model for helix formation,²⁶ we also converted the fit parameters to the Zimm-Bragg nucleation and elongation parameters σ and s respectively, using the approximate relations:²⁷

$$\begin{aligned}\sigma &\approx \frac{v^2}{(1+v)^4}, \\ s &\approx \frac{w}{1+v}.\end{aligned}\quad (1)$$

D. Thermodynamic model

The dependence of the helix elongation free energy, ΔG_{el} on pressure and temperature was fitted to a thermodynamic model:

$$\begin{aligned}\Delta G_{\text{el}}(P, T) = & \Delta H_0 + \Delta C_P(T - T_0) \\ & - T \Delta S_0 - T \Delta C_P \ln(T/T_0) \\ & + \Delta V(P - P_0) + \frac{\Delta \beta}{2}(P - P_0)^2 \\ & + \Delta \alpha(T - T_0)(P - P_0).\end{aligned}\quad (2)$$

In this expression, ΔH_0 , ΔS_0 , and ΔV_0 are the change of enthalpy, entropy, and volume as a result of helix elongation at reference conditions, taken to be $T_0 = 298$ K and $P_0 = 1$ bar pressure. In addition, a constant change of heat capacity ΔC_P , change of linear expansion coefficient $\Delta \alpha$ and change of compressibility $\Delta \beta$ were assumed. The data were fitted by non-linear least squares, and errors were estimated by Monte Carlo bootstrapping.

III. RESULTS

In order to determine the effects controlling helix stability under pressure, we carefully selected two protein force fields, Amber ff03* and Amber ff03w. These models are almost identical, being originally based on the standard Amber ff03 force field.²⁸ They only differ in that an additional empirically determined Fourier term has been added to the ψ backbone torsion angle in each case, in order to approximately match experimental helix propensities near 300 K.^{19,21} In the case of ff03*, the calibration was done in conjunction with the TIP3P water model, while for ff03w it was done with TIP4P/2005 water (below, it will be assumed when discussing Amber ff03* and ff03w that the TIP3P and TIP4P/2005 water models were used, respectively). This allows us to test

FIG. 1. Pressure-dependence of fraction helix. (a) Amber ff03w, TIP4P/2005 water; (b) Amber ff03*, TIP3P water.

specifically the effects of the water model using closely related protein force fields that have very similar helical populations under standard conditions of temperature and pressure. We study the 15-residue peptide Ac-(AAQAA)₃-NH₂, as a model system which is known to form helix at low temperature,²⁹ and which has been extensively characterized in previous simulations.^{19,21,30}

REMD simulations were performed in order to sample the temperature-dependent helix-coil equilibrium. The average fraction helix at each temperature is shown in Figure 1, as determined using backbone dihedral angles. Very similar results are obtained using the standard DSSP³¹ algorithm, which instead uses backbone hydrogen bonds (see Fig. S1 of the supplementary material³²). As expected both Amber ff03* and ff03w populate 20%–30% helix at 300 K and 1 bar pressure, where they were parametrized against experimentally determined helix populations. For Amber ff03w, all pressures used resulted in a stabilization of the peptide, even at 1 kbar. On the other hand, over a wide range, from 1 bar to 8 kbar, pressure had very little effect on the overall helix propensity for Amber ff03*: a slight decrease in helix fraction at temperatures greater than ~300 K is observed. Only for a pressure of 12 kbar is a significant stabilization obtained. Qualitatively, these results suggest a negative reaction volume for helix formation using Amber ff03w, but a very small or positive reaction volume at low to intermediate pressure using Amber ff03*.

We quantify the effect of pressure on the helix-coil equilibrium using a thermodynamic model. However, helix-formation is not a simple two-state process, and involves a broad spectrum of populated intermediates. Therefore, we first fit a simple Ising-like statistical mechanics model which can faithfully capture the helix-coil transition. The two classic partition functions for helix-coil formation are those by Zimm and Bragg²⁶ and by Lifson and Roig,²⁵ which are approximately equivalent.²⁷ We have determined parameters for both models here. The data were initially fitted to the Lifson-Roig model, using a previously described maximum likelihood method,¹⁹ yielding a nucleation parameter ν and an elongation parameter w . These parameters were then converted into the corresponding parameters for the Zimm-Bragg model, σ and s . Overall, all these parameters show essentially similar trends to the global fraction of helix, but can be more justifiably fitted to a thermodynamic two-state model since they describe the microscopic transitions involving the flipping of individual residues between helical and extended conformations. Below we focus on the Zimm-Bragg model, as this has been used to characterize the experimental data.^{14,15} In particular, the elongation parameter s corresponds to the equilibrium constant for adding a single helical hydrogen bond.

We fitted a thermodynamic model to the elongation free energy, here defined as $\Delta G_{el}(P, T) = -RT \ln s(P, T)$. The model includes changes of enthalpy ΔH_0 , entropy ΔS_0 and reaction volume ΔV_0 under standard conditions, as well as constant differences in heat capacity ΔC_p , isothermal compressibility $\Delta\beta$ and linear thermal expansion coefficient $\Delta\alpha$ to describe the temperature- and pressure-dependence. The same model was fitted to the data for $RT \ln s(P, T)$ reported by Imamura *et al.*, based on FTIR measurements.¹⁴ The fits to the raw data are shown in Fig. S2 of the supplementary material.³² In Fig. 2 we show the stability diagram for each force field and for experiment; the fitted parameters are listed in Table I.

Overall, the stability diagram obtained with the Amber ff03w force field is very similar to experiment, bearing in mind that the experiments were done on a different alanine-based peptide (AK20)—we note that the stability diagrams for the AK16 peptide obtained by Hatch *et al.*¹⁷ with Amber ff03* and TIP3P are qualitatively very similar to those we obtain for Ac-(AAQAA)₃-NH₂ with the same force field and water model. The agreement is particularly good in the range of temperature and pressure probed by the experiments, indicated by the broken lines in Fig. 2. In both cases, increasing pressure clearly stabilizes helical states, as also reflected in the negative reaction volume of $-0.8 \text{ cm}^3 \text{ mol}^{-1}$ for experiment and $-1.5 \text{ cm}^3 \text{ mol}^{-1}$ for Amber ff03w (Table I). It also captures quite well the overall enthalpy and entropy changes for adding a helical residue, as previously noted.²¹ In contrast, the stability diagram for the ff03* force field differs in some important respects. Application of low pressures will have little effect on, or slightly destabilize helical, reflected in the small positive $\Delta V_0 = 0.4 \text{ cm}^3 \text{ mol}^{-1}$ (Table I) for helix formation. Additionally, the changes in enthalpy and entropy are almost half the experimental estimates, indicative of too low a cooperativity of helix melting.¹⁹ For experiment and both force fields, the changes in heat capacity are small. This

FIG. 2. Stability diagrams for helix elongation. The free energy associated with elongating a helix by one residue, ΔG_{el} is obtained from the Zimm-Bragg²⁶ elongation parameter s as $\Delta G_{el} = -RT \ln s$, as a function of temperature and pressure. Shown are the stability diagrams obtained by fitting Eq. (2) to (a) experimental $RT \ln s(P, T)$ obtained from Imamura and Katu¹⁴ for the peptide Ac-AA(AAKAA)₃AAY-NH₂; (b) constant-pressure replica exchange simulations of the peptide Ac-(AAQAA)₃-NH₂ with the ff03w force field²¹ and TIP4P/2005 water model;²² (c) constant-pressure replica exchange simulations of Ac-(AAQAA)₃-NH₂ with the ff03* force field¹⁹ and TIP3P water model.²⁰ Dashed magenta box indicates approximate region covered by experimental data. Energy units are kJ mol⁻¹.

is in contrast to protein folding,³ and may indicate a limited role for the hydrophobic effect in stabilizing helices. For both force fields, the difference in thermal linear expansion coefficients, $\Delta\alpha$, is small and positive, ~ 4 cm³ mol⁻¹ K⁻¹. This implies a change of reaction volume with temperature which will slightly increase the positive reaction volume for Amber ff03*, but is insufficient to change the sign of the negative

TABLE I. Parameters for fits of helix elongation free energy ΔG_{el} to thermodynamic model (Eq. (2)). Numbers in brackets are the error in the last significant figure estimated by bootstrap Monte Carlo.

Parameter	Units	Expt.	ff03w	ff03*
ΔH_0	kJ mol ⁻¹	-5.2(3)	-4.1(1)	-2.9(1)
ΔS_0	J mol ⁻¹ K ⁻¹	-15(1)	-13.4(2)	-9.7(3)
ΔC_p	J mol ⁻¹ K ⁻¹	0(1)	2(1)	-1(1)
ΔV_0	cm ³ mol ⁻¹	-0.8(1)	-1.5(1)	0.4(2)
$\Delta\beta$	$\times 10^{-5}$ cm ³ mol ⁻¹ bar ⁻¹	6(3)	22(2)	-13(2)
$\Delta\alpha$	$\times 10^{-3}$ cm ³ mol ⁻¹ K ⁻¹	-3(2)	4.1(6)	3.4(4)

reaction volume using Amber ff03w. Over the range of temperature where liquid water is stable the effect is too small to qualitatively change the pressure-dependence of the two models.

The differences in enthalpy and entropy between the two solvent models have been explained in terms of the strength of solvent interactions with the peptide chain.²¹ These differences also result in a more expanded unfolded state for ff03w relative to ff03*. How can the differences in reaction volume be understood? The first explanation would be in terms of changes in the peptide free energy surface—i.e., different conformations are preferred by each force field, particularly for non-helical states. This is undoubtedly a contribution, since these free energy surfaces are evidently different. In Fig. 3, we show representative two-dimensional free energy surfaces as a function of the radius of gyration and the number of helical residues. As anticipated, the radius of gyration of the helical states is similar, but the non-helical states are much more collapsed in the case of ff03* compared to ff03w. If one supposes that a more collapsed unfolded state is associated with a smaller volume, then this would be consistent with the observed differences in reaction volume for helix formation for the two force fields.

In order to investigate the above hypothesis, we determined the approximate volume excluded to water by each peptide conformation by using the Connolly volume: this is the volume which is inaccessible to a sphere of radius 0.14 nm. This is the simplest way in which the volume of a configuration can be estimated. The average Connolly volumes for configurations with the same number of helical residues are shown in Fig. 4, for the replica at 298 K. The results clearly show that more helical states have a smaller total volume for both ff03* and ff03w, which is qualitatively consistent with the negative change of volume for helix formation observed experimentally. However, it does not explain why under low pressure conditions the reaction volume for TIP3P may be positive, and the overall changes of 10–15 cm³ mol⁻¹ per helical residue are about an order of magnitude larger than the volume changes estimated from the thermodynamic fits in Table I. It also does not explain the reduction of reaction volume for ff03* with pressure, such that it becomes negative at sufficiently high pressures. Although we find that helical states do have smaller Connolly volume above 4 kbar, in agreement with earlier findings based on the radius of gyration,¹⁸ we also find an even greater reduction in volume for non-helical states so that the

FIG. 3. Free energy surfaces for radius of gyration and number of helical residues. (a) Amber ff03w, (b) Amber ff03*. Magenta line indicates mean radius of gyration for a given number of helical residues.

difference in volume between non-helical and helical states would become more positive with increasing pressure, the opposite trend to that observed for the total reaction volume of the system. Therefore, a simple picture based only on properties of the peptide configurations does not tell the whole story.

Naturally, the surrounding solvent may also play a role in determining the dependence of system volume on peptide conformation, and previous studies have suggested that peptide solvation changes with increasing pressure,^{16,33} and changes in water structure with increasing pressure are known to alter the hydrophobic effect.⁹ We probed for this by randomly drawing configurations from the 298 K replica from the 1 bar Amber ff03* REMD simulation, and then determining for each of these the average system volume using different force fields and system pressures (1 bar and 4 bars). That is, we effectively remove the influence of different free energy surfaces with different force fields by always considering the same set of peptide configurations. By running sufficiently long constant pressure simulations with each of these configurations, restrained to their initial position, we can accurately determine the average system volume, as a function of the number of helical residues. The results of these simulations for the Amber ff03* and ff03w force fields are summarized in Fig. 5. We find that this simple analysis captures the reaction

FIG. 4. Dependence of Connolly volume on helicity. The Connolly volume averaged over configurations with the same number of helical residues is shown for (a) Amber ff03* and (b) Amber ff03w.

volume effects inferred from the thermodynamic fits. Namely, at 1 bar pressure, the reaction volume for helix formation is positive with ff03* and negative with ff03w (Figs. 5(a) and 5(c)), while at 4 bars pressure the change of volume upon helix formation becomes negative even for TIP3P (Figs. 5(b) and 5(d)). The large scatter in the individual system volumes (black data points) indicates that other factors besides helicity are important for determining system volume. Nonetheless, when the volume of a given configuration with ff03w is subtracted from that with ff03*, these effects are largely eliminated, leaving helicity as the main determinant of the difference between the two water models (Figures 5(e) and 5(f)). This result highlights the importance of solvation in determining the difference in apparent volume between the helical and less helical states.

Although Amber ff03* with the TIP3P water model may fail to describe the correct qualitative pressure-dependence of helix stability, it should be noted that the differences in reaction volume under consideration are extremely tiny: a change of volume $\Delta V = 1 \text{ cm}^3 \text{ mol}^{-1}$ per residue, or $1.66 \times 10^{-3} \text{ nm}^3$ per molecule per residue can be related to a change of apparent helix radius ΔR using a helix pitch of $\Delta L \sim 0.15 \text{ nm}$ and helix radius $R \sim 0.45 \text{ nm}$ via $\Delta R \approx \Delta V / 2\pi R \Delta L$, yielding a change of apparent radius $\Delta R \approx 0.0039 \text{ nm}$. While this is a simplified model calculation, it serves to illustrate the subtlety of the effect that must be captured.

FIG. 5. System volumes determined for a common set of peptide configurations. (a) Amber ff03*, 1 bar; (b) Amber ff03*, 4 kbar; (c) Amber ff03w, 1 bar; (d) Amber ff03w, 4 kbar; (e) and (f) difference between volumes using Amber ff03w and Amber ff03* at 1 bar and 4 kbar, respectively. Black data points are the average volumes determined for individual configurations (20 per number of helical residues) and red symbols are average system volumes for all configurations with the same number of helical residues.

IV. CONCLUSION

The dependence of globular protein stability on pressure has been found to be mainly determined by cavities in the folded structure, masking other possible effects of interest. In the case of helix formation, in fact the opposite trend is found to that for protein folding, namely pressure stabilization of helices. We have shown here that simulations with an accurate water model, TIP4P/2005, are capable of capturing the pressure dependence of helix formation. Further, in agreement with earlier work, we find that using the TIP3P water model leads to pressure-induced destabilization of helices, a qualitatively incorrect result. We further show here that this difference is not merely due to the different peptide conformations sampled with that water model. Instead there is a critical role for solvent structure in determining the reaction volume changes. Taken together, our results emphasize the importance of using an accurate water model for capturing the folding of peptides and proteins under different thermodynamic conditions.

The experimental results obtained for helix formation have proved to be a stringent test for simulation models. In future, it would be very interesting to compare simulation results with experimental data for other model peptides such as β -hairpins, should such data become available. Experimental kinetics results for the pressure-dependence of helix¹⁵

and protein folding^{34,35} kinetics are also a rich source of information for future detailed comparison with molecular simulation.

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