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JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
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Macromolecular Crowding and Protein Stability

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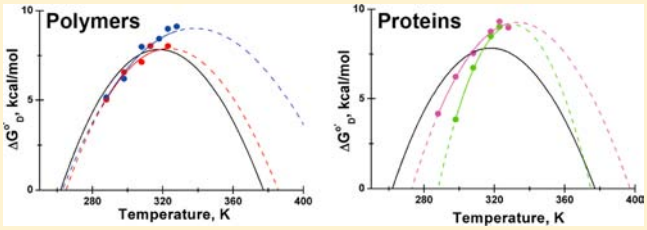
Supporting Information

ABSTRACT: An understanding of cellular chemistry requires knowledge of how crowded environments affect proteins. The influence of crowding on protein stability arises from two phenomena, hard-core repulsions and soft (i.e., chemical) interactions. Most efforts to understand crowding effects on protein stability, however, focus on hard-core repulsions, which are inherently entropic and stabilizing. We assessed these phenomena by measuring the temperature dependence of NMR-detected amide proton exchange and used these data to extract the entropic and enthalpic contributions of crowding to the stability of ubiquitin. Contrary to expectations, the contribution of chemical interactions is large and in many cases dominates the contribution from hardcore repulsions. Our results show that both chemical interactions and hard-core repulsions must be considered when assessing the effects of crowding and help explain previous observations about protein stability and dynamics in cells.



INTRODUCTION

The cellular interior is an exceptionally complex environment where macromolecules can reach concentrations of 300 g/L and occupy 30% of the cellular volume.[1] This crowded environment is vastly different from the dilute, idealized conditions usually used in most biophysical studies. The consequences of this macromolecular crowding[2] arise from two phenomena: hard-core repulsions and nonspecific chemical interactions.[3] First, we describe the equilibrium thermodynamics of globular protein stability and then discuss the resulting thermodynamic parameters in terms of crowding.

The stability of globular proteins can be defined as the standard-state free energy change, $\Delta G^{\circ}_{D}{}'$, of the reaction:[4,5]

$$N \rightleftarrows D \tag{1}$$

where N is the biologically active native state and D is the denatured state. $\Delta G^{\circ}_{D}{}'$ can be dissected into its enthalpic, $\Delta H^{\circ}_{D}{}'$, and entropic, $\Delta S^{\circ}_{D}{}'$, components:

$$\Delta G^{o'}_{D,T} = \Delta H^{o'}_{D,T} - T\Delta S^{o'}_{D,T} \tag{2}$$

where T represents the absolute temperature. For globular proteins, $\Delta H^{\circ}_{D,T}{}'$ and $\Delta S^{\circ}_{D,T}{}'$ are temperature dependent:[6]

$$\Delta H^{o'}_{D,T} = \Delta H^{o'}_{D,T_{ref}} + \Delta C_p(T - T_{ref}) \tag{3}$$

$$\Delta S^{o'}_{D,T} = \Delta S^{o'}_{D,T_{ref}} + \Delta C_p \ln\left(\frac{T}{T_{ref}}\right) \tag{4}$$

where T_{ref} is a reference temperature and ΔC_p is the heat capacity change upon denaturation. Substituting eqs 3 and 4 into eq 2 gives

$$\Delta G^{o'}_{D,T} = \Delta H^{o'}_{D,T_{ref}} - T\Delta S^{o'}_{D,T_{ref}} + \Delta C_p\left[(T - T_{ref}) - T\ln\left(\frac{T}{T_{ref}}\right)\right] \tag{5}$$

$\Delta G^{\circ}_{D}{}'$ is zero at T_g, where the concentrations of N and D are equal. Inspection of eq 2 shows that at T_g, $\Delta S^{\circ}_{D,Tg}{}'$ equals $\Delta H^{\circ}_{D,Tg}{}'/T_g$, such that eq 5 can be simplified to

$$\Delta G^{o'}_{D,T} = \Delta H^{o'}_{D,T_g}\left(1 - \frac{T}{T_g}\right) + \Delta C_p\left[(T - T_g) - T\ln\left(\frac{T}{T_g}\right)\right] \tag{6}$$

Figure 1 shows a plot of $\Delta G^{\circ}_{D}{}'$ versus T for the small globular protein, ubiquitin. The curvature arises because ΔC_p is nonzero, leading to a temperature of maximum stability. The downward curvature leads to two values for T_g. The higher one is more commonly assessed because the lower T_g is usually below the freezing point of the sample. Few studies have examined the enthalpic and entropic components of crowding, yet, as discussed next, such data are required to understand crowding effects.

The effects of crowding arise from two phenomena, hard-core repulsions and soft (i.e., chemical) interactions. The hard-core repulsions arise because the crowders decrease the space available to the protein being studied. Application of Le Chatelier's principle leads to the conclusion that repulsions favor N because this form is more compact than D. The hard-core repulsive effect is entirely entropic because it involves only the arrangement of molecules, not their interaction. The original formulation of macromolecular crowding theory[7] and most work in the area has stressed only hard-core repulsions.

Received: June 1, 2012
Published: September 6, 2012

   dx.doi.org/10.1021/ja305300m | J. Am. Chem. Soc. 2012, 134, 16614−16618

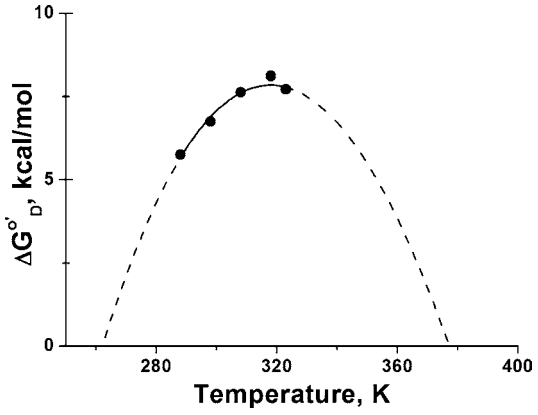


Figure 1. Stability of ubiquitin as a function of temperature. The curve is a fit of the data to eq 6. The solid curve indicates the range measurable by NMR-detected amide proton exchange. Experiments were performed in 50 mM sodium acetate, pH 5.4.

Soft, or chemical, interactions can be attractive or repulsive. Repulsive chemical interactions are stabilizing because they reinforce the hard-core repulsion. Attractive interactions are destabilizing for the same reason urea is destabilizing; favorable but nonspecific interactions with the protein backbone favor exposure of more surface, which leads to unfolding. Attractive interactions are known to have an enthalpic component.[8]

Little is known about the relative importance of hard-core repulsions and chemical interactions because there are few studies about how crowding affects $\Delta H^{o}_{D}{}'$ and $\Delta S^{o}_{D}{}'$. Methods that use heat or destabilizing cosolutes to assess the effect of protein crowders on stability are problematic. Thermal denaturation at high protein concentrations is usually accompanied by irreversible aggregation, obviating the use of simple equilibrium thermodynamic models. Even when aggregation is avoided, heat and destabilizing cosolutes complicate data interpretation because, unless the proteins have very different stabilities,[9] both the test protein and crowder denature. An advantage of NMR-detected amide proton exchange is that stability can be measured at room temperature in the presence of any non ^{15}N-containing cosolute.[10] The advantage arises because nearly all the test protein remains in the native state.[11]

We chose ubiquitin (pI 6.4) as the test protein because it folds in a two-state manner,[12] and its unusually high temperature of maximum stability allowed estimation of ΔC_p. For synthetic crowders, we chose the uncharged polymers polyvinylpyrrolidone (PVP, 40 kDa) and Ficoll (70 kDa). We also used two globular proteins as crowding agents, bovine serum albumin (BSA, 67 kDa, pI 4.7) and lysozyme (15 kDa, pI 11.0).

EXPERIMENTAL SECTION

Protein Expression and Purification. The pET-46 plasmid (Novagen) containing the gene for histidine-tagged ubiquitin[13] was transformed into BL-21 (DE3-Gold) competent *Escherichia coli* cells (Stratagene). The transformants were spread onto Luria broth agar plates containing 0.1 g/L ampicillin. Liquid Luria−Bertani (LB) media (100 mL containing 1 g bacto-tryptone, 0.5 g bacto-yeast extract, and 1 g NaCl in H_2O) containing 0.1 g/L ampicillin was inoculated with a single colony of ubiquitin-expressing *E. coli* cells and incubated overnight at 310 K with shaking at 250 rpm. The next morning, this preculture was pelleted (Sorvall RC-3B, H6000A rotor, 1600 *g*). One L of ^{15}N enriched M9 media (6 g Na_2HPO_4, 2 g glucose, 3 g KH_2PO_4, 0.5 g NaCl, 1 g $^{15}NH_4Cl$, 2 mM $MgSO_4$,10 μM $CaCl_2$) containing thiamine HCl (1 mg/L) and ampicillin (0.1 g/L) was used to resuspend the cell pellet. This culture was incubated at 310 K with shaking until its optical density at 600 nm reached 0.8. Induction was then initiated by adding isopropyl β-D-1-thiogalactopyranoside to a final concentration of 1 μM. Induction was allowed to proceed for 4 h, whereupon the culture was centrifuged at 1600 *g*, and the pellet frozen.

The pellet was resuspended in 20 mL of buffer (50 mM Na_2HPO_4, 500 mM NaCl, 30 mM imidazole, pH 7.6). Cells were lysed by sonic dismembration for 10 min (Fisher Scientific, Sonic Dismembrator Model 500, 14% amplitude, 2 s pulse, 3 s rest). The lysate was centrifuged at 14 000 *g* for 30 min, and the supernatant retained. Streptomycin sulfate (0.2 g) was added with stirring on ice for 30 min, followed by centrifugation at 14 000 *g* for 30 min. The supernatant was forced through a sterilized 0.22 μm filter. The ubiquitin was purified by Ni^{2+}-affinity chromatography on an AKTA FPLC (GE Healthcare). The column was washed with 60 mL of low-imidazole buffer (50 mM Na_2HPO_4, 500 mM NaCl, 30 mM imidazole, pH 7.6), and then eluted with 80 mL of high imidazole buffer (50 mM Na_2HPO_4, 500 mM NaCl, 500 mM imidazole, pH 7.6). The pure fractions (as assessed by SDS-PAGE) were pooled, dialyzed against H_2O, and subjected to size exclusion chromatography (Superdex 200 10/300) using water as eluent. The protein was then lyophilized (Labconco).

NMR. Amide proton exchange experiments were performed as described by Miklos et al.[10] on a 600 MHz Varian Inova spectrometer equipped with a standard triple-resonance HCN probe and three axis gradients. The ^{1}H dimension was acquired with a sweep width of 12 000 Hz and comprised 1024 complex points. The ^{15}N dimension was acquired with a sweep width of 2500 Hz and comprised 64 complex increments. Each experiment required two samples, an optimization and an exchange sample. Optimization samples of 1 mM ubiquitin in 50 mM sodium phosphate, pH 5.4, with 15% D_2O were used for shim adjustment and pulse width calibration. pH values were obtained from direct meter readings, uncorrected for the isotope effect.[14] Exchange samples contained 1 mM ubiquitin and 50 mM sodium acetate buffer, pH 5.4, and were made with 99.9% D_2O. Denaturation of BSA is not a problem because its T_g (≈338 K)[15] is well above the highest temperature used (323 K).

Ficoll, BSA, and lysozyme were exchanged in D_2O prior to use (PVP has no exchangeable protons). One gram of each was suspended in 10 mL of D_2O. Exchange for 36 h at 310 K was followed by lyophilization overnight. The dried samples were again suspended in 10 mL of D_2O, and the process repeated.

Per exchange sample, 20−24 consecutive HSQC spectra[16,17] were acquired. Processing was performed with NMRPipe.[18] Assignments have been described.[19] Crosspeak volumes were plotted against time and fit to exponential decays by using NMRViewJ[20] to yield values of k_{obs}, the rate of exchange for a particular residue.

Amide ^{1}H Exchange and Protein Stability. Exchange occurs via the scheme shown in eq 7:[11,21]

$$\mathrm{cl}-{}^{1}\mathrm{H} \underset{k_{cl}}{\overset{k_{op}}{\rightleftarrows}} \mathrm{op}-{}^{1}\mathrm{H} \xrightarrow{k_{int}} \mathrm{op}-{}^{2}\mathrm{H} \underset{k_{op}}{\overset{k_{cl}}{\rightleftarrows}} \mathrm{cl}-{}^{2}\mathrm{H} \tag{7}$$

where cl−^{1}H is the amide proton in N, which opens and closes with rate constants k_{op} and k_{cl} and op−^{1}H is the open, exchange competent state. For ubiquitin at pH values <8.5, k_{cl} is much larger than k_{int},[22,23] such that the free energy required to expose an amide proton ($\Delta G^{o}_{op}{}'$) can be determined using the equation

$$\Delta G^{o'}_{op} = -RT \ln K_{op} = -RT \ln \frac{k_{op}}{k_{cl}} = -RT \ln \frac{k_{obs}}{k_{int}} \tag{8}$$

where R is the gas constant, k_{obs} is the observed rate constant of exchange, and k_{int} is the rate constant for an amide proton in a peptide lacking stable structure. Values of k_{int} were calculated using the program SPHERE (www.fccc.edu/research/labs/roder/sphere/).[24,25] The crowders do not change k_{int}.[10,26]

Each $\Delta G^{o}_{D}{}'$ value in Figures 1−3 is the mean $\Delta G^{o}_{op}{}'$ for the 10 residues that undergo exchange only upon global unfolding.[22,23,27] The standard deviation of this mean varies between 0.1 and 0.8 kcal/mol with an average of ∼0.3 kcal/mol (Supporting Information). This value matches the reproducibility of $\Delta G^{o}_{op}{}'$, which is defined as the standard deviation of the mean from three trials of a single condition.[10,26]

dx.doi.org/10.1021/ja305300m | J. Am. Chem. Soc. 2012, 134, 16614−16618

Least-squares fits to eq 6 incorporating Monte Carlo error analysis using a measured uncertainty of 0.3 kcal/mol were performed with Mathematica 8 (Wolfram Research). For each condition, 200 trials were used, but the parameters and their uncertainties remain constant beyond ~20 trials. The fitted parameters along with their uncertainties are shown in Tables 1−3.

Table 1. Thermodynamic Parameters at the low T_g

cosolute[a]	T_g,K	$\Delta H_g^{o\prime}$, kcal/mol	ΔC_p, kcal/mol·K
dilute	262 ± 3	−76 ± 7	1.5 ± 0.3
PVP	265 ± 4	−74 ± 11	1.4 ± 0.4
Ficoll	263 ± 3	−66 ± 7	1.0 ± 0.2
BSA	288 ± 1	−127 ± 9	3.1 ± 0.5
lysozyme	273 ± 2	−87 ± 8	1.6 ± 0.3

[a]Cosolute concentrations are 100 g/L.

RESULTS

Amide ^{1}H exchange data were analyzed to yield the free energy required to expose an amide ^{1}H to solvent, $\Delta G_{op}^{o\prime}$.[11] Our focus is on the global stability, $\Delta G_D^{o\prime}$, which we define as the average of $\Delta G_{op}^{o\prime}$ values from the 10 residues that are exposed only on global unfolding. These residues were identified previously by combining data from stopped flow and NMR experiments.[22,23,27] Experiments were performed at pH 5.4 in 50 mM sodium acetate buffer. The crowding agents did not change the positions of the ubiquitin crosspeaks, indicating that stable ubiquitin−crowder complexes do not form. Each solution condition was assessed at up to 6 temperatures from 288 to 323 K. The exchange rates are too fast to measure at higher temperatures and too slow to measure at lower temperatures. The standard deviation of the mean for $\Delta G_D^{o\prime}$ is ±0.3 kcal/mol as determined from replication of experiments.[26,28] The data sets are given in the Supporting Information. The maximum crowder concentration was limited to 100 g/L because higher concentrations of protein crowders broaden the spectra or decrease the stability so much as to prevent quantification of rates.[29]

The temperature dependence of the stability (Figures 1−3) was used to quantify the enthalpic and entropic components. The fitted parameters from the least-squares fitting to eq 6 with Monte Carlo error analysis are shown in Table 1. The value of ΔC_p, 1.5 ± 0.3 kcal/mol·K in dilute solution, agrees with the value from calorimetry (1.4 kcal/mol·K).[12,30] The value of high T_g in dilute solution, 378 K (Figure 1), is close to that from calorimetry (373 K).[31,32] The deviation is probably due to the difference in conditions: pH 7.0 vs 5.4, our use of D_2O (which increases T_g)[33] and our use of a his-tagged protein. The parameters for ubiquitin in 100 g/L solutions of 40 kDa PVP, 70 kDa Ficoll, BSA, and lysozyme (Table 1) were then used to generate the smooth curves in Figure 2 and 3. Lastly, we assessed $\Delta G_D^{o\prime}$, $\Delta H_D^{o\prime}$, and $-T\Delta S_D^{o\prime}$ under crowded conditions at the low and high T_g of ubiquitin in the absence of crowders (262 and 378 K, respectively) using least-squares fitting to eq 6 with Monte Carlo error analysis (Tables 2 and 3).

DISCUSSION

Uncovering the origin of macromolecular crowding effects requires analysis of the temperature dependence so that $\Delta S_D^{o\prime}$ and $\Delta H_D^{o\prime}$ can be assessed. Most studies focused on only the high-temperature limb of the stability curve.[34−36] Such a narrow focus prevented the determination of ΔC_p, and hence, the enthalpy and entropy changes in dilute and crowded solutions could not be compared at a common temperature. Ubiquitin's high temperature of maximum stability allowed us to observe the curvature in stability versus temperature plots (Figures 1−3) and to estimate ΔC_p.

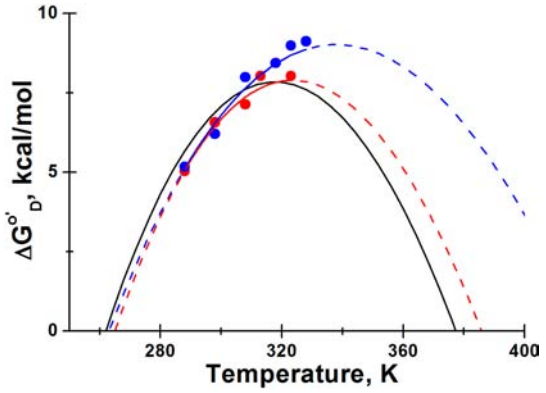


Figure 2. Thermal stability curves of ubiquitin in solutions (50 mM sodium acetate, pH 5.4) containing 100 g/L PVP (red) and 100 g/L Ficoll (blue). The black curve shows the stability of ubiquitin in dilute solution (from Figure 1).

Table 2. Thermodynamic Parameters at 262 K, where $\Delta G^{o\prime}$ = 0 in Dilute Solution[a]

cosolute	$\Delta G_{262K}^{o\prime}$	$\Delta H_{262K}^{o\prime}$	$-T\Delta S_{262K}^{o\prime}$
dilute	0 ± 1	−80 ± 10	80 ± 10
PVP	−1 ± 1	−80 ± 20	78 ± 20
Ficoll	−0.4 ± 0.8	−70 ± 10	70 ± 10
BSA	−15 ± 4	−210 ± 20	190 ± 20
lysozyme	−4 ± 1	−106 ± 10	100 ± 10

[a]Thermodynamic parameters are in kcal/mol, and cosolute concentrations are 100 g/L.

As explained in the Introduction, the simplest interpretation of theory predicts that crowding will stabilize proteins because hard-core repulsions favor the native state. The crowding induced increase in $\Delta G_D^{o\prime}$ arises because of the decrease in $\Delta S_D^{o\prime}$; $\Delta H_D^{o\prime}$ is expected to remain constant. Inspection of Figures 2 and 3 and Tables 2 and 3 shows that the real situation is more complex.

Contrary to expectations, the effects of crowding on stability at the low T_g (Table 2) are either insignificant (PVP and Ficoll)

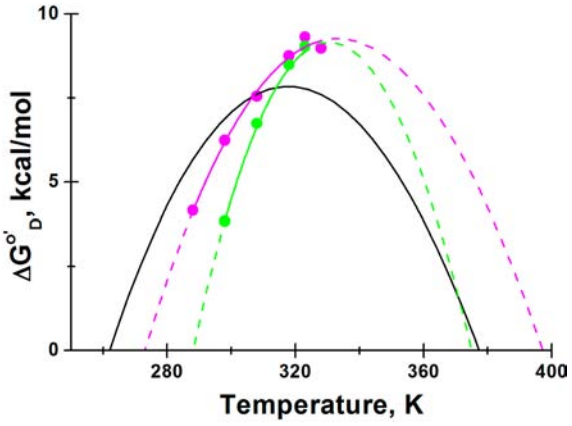


Figure 3. Thermal stability curves of ubiquitin in solutions (50 mM sodium acetate, pH 5.4) containing 100 g/L BSA (green) and 100 g/L lysozyme (magenta). The black curve shows the stability of ubiquitin in dilute solution (from Figure 1).

dx.doi.org/10.1021/ja305300m | J. Am. Chem. Soc. 2012, 134, 16614−16618

or deleterious (BSA and lysozyme), exactly the opposite of what is expected if hard-core repulsions were the most important effect. The destabilization arises from a decrease in $\Delta H^{o}_{D}{}'$ compared to dilute solution, again counter to expectations. Turning to the high T_g (Table 3), only Ficoll stabilizes ubiquitin

Table 3. Thermodynamic Parameters, at 378 K, where $\Delta G^{o}{}' = 0$ in Dilute Solution[a]

cosolute	$\Delta G^{o}_{378K}{}'$	$\Delta H^{o}_{378K}{}'$	$-T\Delta S^{o}_{378K}{}'$
dilute	0 ± 3	100 ± 20	−100 ± 30
PVP[b]	3 ± 3	80 ± 30	−80 ± 30
Ficoll[b]	8 ± 2	50 ± 20	−40 ± 20
BSA	0 ± 4	150 ± 40	−150 ± 40
lysozyme	4 ± 2	90 ± 20	−80 ± 20

[a]Thermodynamic parameters are in kcal/mol, and cosolute concentrations were 100 g/L. [b]Using a sugar-based polymer, Christiansen et al.[36] showed that smaller polymers are more stabilizing than larger ones. Miklos et al.[26] showed the same trend using PVP. Analysis of our data indicates that 70 kDa Ficoll is more stabilizing than 40 KDa PVP, which seems contradictory. However, Christiansen et al.[36] used sugar-based polymers and Miklos et al. used PVPs. We used one sugar-based polymer (Ficoll) and one nonsugar-based polymer (PVP). The chemical difference between Ficoll and PVP precludes detailed interpretation in terms of size.

compared to buffer alone. As predicted by theory, the stabilization arises from a decrease in $\Delta S^{o}_{D}{}'$, but counter to expectations, the entropic stabilization is almost completely offset by a decrease in $\Delta H^{o}_{D}{}'$.

The observation of strong enthalpic contributions of both signs leads to our major conclusion: Chemical interactions play a key role in crowding. Note that 100 g/L solutions of BSA or lysozyme do not affect the backbone chemical shifts of ubiquitin, indicating that the crowders do not form stable complexes with ubiquitin. The lack of chemical shift changes, however, does not rule out the presence of weak attractive interactions because their fleeting nature would result in fast exchange, which would maintain the chemical shifts.

Focusing on the protein crowders, neither BSA nor lysozyme significantly affects stability at the high T_g. The larger ΔC_p in BSA may reflect stronger interactions of this crowder with the denatured state, increasing the surface of ubiquitin exposed on unfolding.[37] The lack of stabilization is consistent with work on chymotrypsin inhibitor 2[28] and studies of protein stability in cells.[38−41] It is important to bear in mind that the hard-core repulsions are still present; all the crowders used here occupy 7−8% of the solution volume.[26,42,43]

The present results from assessing $\Delta S^{o}_{D}{}'$ and $\Delta H^{o}_{D}{}'$ at two common temperatures provide strong experimental evidence supporting our suggestion from isothermal studies[28] that attractive interactions can cancel the stabilizing effect of hard-core repulsions. Other observations reinforce this idea. Simulations aimed at explaining in-cell stability data[38,39] indicate an important role for attractive interactions.[3b] NMR relaxation studies[29,44] and molecular dynamics simulations[45] show that chymotrypsin inhibitor 2 interacts with BSA and lysozyme. Sedimentation equilibrium and stability studies of cytochrome c in highly concentrated sugar solutions (the monomer of Ficoll)[46,47] and NMR studies of this protein in polyethylene glycol[46] also indicate the presence of attractive interactions.[47,48] The fact that high concentrations of these solutes affect ubiquitin, chymotrypsin inhibitor 2, and cytochrome c in the same way suggests that chemical interactions are generally important. In summary, stabilizing hard-core repulsions can be completely offset by chemical interactions between the crowders and the protein being studied.

These chemical effects probably arise from a variety of sources, including charge−charge interactions, hydrogen bonding, and the hydrophobic effect. Both lysozyme (pI 11.0) and ubiquitin (pI 6.4) are polycations under our conditions (pH 5.4), which means the molecules repel each other. This repulsion should reinforce the stabilizing effect of hard-core repulsions. On the other hand, BSA (pI 4.7) and ubiquitin (pI 6.4) have complementary charges. NMR relaxation studies of interactions between the electrostatically similar protein chymotrypsin inhibitor 2 (pI 6.5) and the BSA indicate that they attract one another.[44] $\Delta H^{o}_{378K}{}'$ is larger for BSA than it is for lysozyme, consistent with the idea that complementary charge−charge interactions between the BSA and ubiquitin must be broken for ubiquitin to unfold. Additional indirect support for the charge−charge interactions comes from the observation that destabilization of chymotrypsin inhibitor 2 by BSA can be alleviated by increasing the salt concentration.[28] Evidence for the existence of crowder-test protein hydrogen bonding comes from the cytochrome c studies discussed in the previous paragraph.[47,48] It is also likely that favorable hydrophobic interactions play a key role, as has been suggested from in-cell NMR data on ubiquitin[40] and chemical cross-linking studies.[49]

For more than 30 years, macromolecular crowding effects were considered mostly entropic. We have shown that both hard-core repulsions and weak chemical interactions must be considered to understand the effects of macromolecular crowding. The final outcome depends on the winner of a closely matched battle between hard-core repulsions and nonspecific chemical interactions. This conclusion is quite general because it applies to synthetic polymers, proteins, and membrane proteins[50] as crowding agents and has important implications for understanding cellular chemistry.

Protein stability in cells can be tuned by surrounding proteins.[40] Our discovery of a key role for enthalpic effects points to the importance of chemical interactions and helps explain several confusing observations, including why most globular proteins cannot be observed by $^{15}N-^{1}H$ in-cell NMR and why the crowded intracellular environment can fail to stabilize or even destabilize proteins.[13,38−40,51] Quoting Spitzer and Poolman,[52] "the cytoplasm is a highly anisotropic and structured environment, in which many proteins carry out their functions as multimeric complexes at specific subcellular locations". Given the competition between hard-core repulsions and nonspecific chemical interactions, altering the intracellular environment at certain "addresses" could be used to regulate key protein functions, such as transcription, translation, replication, and segregation.[53−56]

■ ASSOCIATED CONTENT

Supporting Information

Tables of $\Delta G^{o}_{op}{}'$ values for globally exchanging ubiquitin residues in dilute solutions, PVP, Ficoll, lysozyme, and BSA can be found. 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.

■ ACKNOWLEDGMENTS

We thank Elizabeth Pielak, Matthew Redinbo, Terrence Oas, and William Monteith for helpful discussions and Marc ter Horst for spectrometer maintenance. This research was supported by the National Science Foundation grant MCB-1051819.

REFERENCES

(1) Zimmerman, S. B.; Trach, S. O. *J. Mol. Biol.* **1991**, *222*, 599−620.
(2) Minton, A. P.; Wilf, J. *Biochemistry* **1981**, *20*, 4821−4826.
(3) (a) Elcock, A. H. *Curr. Opin. Struct. Biol.* **2010**, *20*, 196−206. (b) McGuffee, S. R.; Elcock, A. H. *PLoS Comput. Biol.* **2010**, *6*, e1000694.
(4) Hermans, J.; Scheraga, H. A. *J. Am. Chem. Soc.* **1961**, *83*, 3283−3292.
(5) Becktel, W. J.; Schellman, J. A. *Biopolymers* **1987**, *26*, 1859−1877.
(6) Privalov, P. L.; Khechinashvili, N. N. *J. Mol. Biol.* **1974**, *86*, 665−684.
(7) Minton, A. P. *Biopolymers* **1981**, *20*, 2093−2120.
(8) Makhatadze, G. I.; Privalov, P. L. *J. Mol. Biol.* **1992**, *226*, 491−505.
(9) Denos, S.; Dhar, A.; Gruebele, M. *Faraday Discuss.* **2012**, DOI: 10.1039/C2FD20009K.
(10) Miklos, A. C.; Li, C.; Pielak, G. J. *Methods Enzymol.* **2009**, *466*, 1−18.
(11) Englander, S. W.; Kallenbach, N. R. *Q. Rev. Biophys.* **1983**, *16*, 521−655.
(12) Wintrode, P. L.; Makhatadze, G. I.; Privalov, P. L. *Proteins: Struct., Funct., Genet.* **1994**, *18*, 246−253.
(13) Barnes, C. O.; Monteith, W. B.; Pielak, G. J. *ChemBioChem* **2011**, *12*, 390−391.
(14) Schowen, K. B.; Schowen, R. L. *Methods Enzymol.* **1982**, *87*, 551−606.
(15) Michnik, A.; Michalik, K.; Drzazga, Z. *J. Therm. Anal. Calorim.* **2005**, *80*, 399−406.
(16) Kay, L. E.; Keifer, P.; Saarinen, T. *J. Am. Chem. Soc.* **1992**, *114*, 10663−10665.
(17) Bodenhausen, G.; Ruben, D. J. *Chem. Phys. Lett.* **1980**, *69*, 185−189.
(18) Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A. *J. Biomol. NMR* **1995**, *6*, 277−293.
(19) Schneider, D. M.; Dellwo, M. J.; Wand, A. J. *Biochemistry* **1992**, *31*, 3645−3652.
(20) Johnson, B. A.; Blevins, R. A. *J. Biomol. NMR* **1994**, *4*, 603−614.
(21) Berger, A.; Linderstrom-Lang, K. *Arch. Biochem. Biophys.* **1957**, *69*, 106−118.
(22) Benitez-Cardoza, C. G.; Stott, K.; Hirshberg, M.; Went, H. M.; Woolfson, D. N.; Jackson, S. E. *Biochemistry* **2004**, *43*, 5195−5203.
(23) Sivaraman, T.; Arrington, C. B.; Robertson, A. D. *Nat. Struct. Biol.* **2001**, *8*, 331−333.
(24) Bai, Y.; Milne, J. S.; Mayne, L.; Englander, S. W. *Proteins: Struct., Funct., Genet.* **1993**, *17*, 75−86.
(25) Connelly, G. P.; Bai, Y.; Jeng, M. F.; Englander, S. W. *Proteins: Struct., Funct., Genet.* **1993**, *17*, 87−92.
(26) Miklos, A. C.; Li, C.; Sharaf, N. G.; Pielak, G. J. *Biochemistry* **2010**, *49*, 6984−6991.
(27) Gladwin, S. T.; Evans, P. A. *Folding Des.* **1996**, *1*, 407−417.
(28) Miklos, A. C.; Sarkar, M.; Wang, Y.; Pielak, G. J. *J. Am. Chem. Soc.* **2011**, *133*, 7116−7120.
(29) Wang, Y.; Li, C.; Pielak, G. J. *J. Am. Chem. Soc.* **2010**, *132*, 9392−9397.
(30) Ibarra-Molero, B.; Loladze, V. V.; Makhatadze, G. I.; Sanchez-Ruiz, J. M. *Biochemistry* **1999**, *38*, 8138−8149.
(31) Makhatadze, G. I.; Lopez, M. M.; Richardson, J. M., III; Thomas, S. T. *Protein Sci.* **1998**, *7*, 689−697.
(32) Grasso, D. M.; Sciacca, M. F. M.; Milardi, D.; Pappalardo, M.; La Rosa, C. *J. Therm. Anal. Calorim.* **2006**, *86*, 311−314.
(33) Makhatadze, G. I.; Clore, G. M.; Gronenborn, A. M. *Nat. Struct. Biol.* **1995**, *2*, 852−855.
(34) Zhou, H. X.; Rivas, G.; Minton, A. P. *Annu. Rev. Biophys.* **2008**, *37*, 375−397.
(35) Waegele, M. M.; Gai, F. *J. Chem. Phys.* **2011**, *134*, 095104.
(36) Christiansen, A.; Wang, Q.; Samiotakis, A.; Cheung, M. S.; Wittung-Stafshede, P. *Biochemistry* **2010**, *49*, 6519−6530.
(37) Myers, J. K.; Pace, C. N.; Scholtz, J. M. *Protein Sci.* **1995**, *4*, 2138−2148.
(38) Ghaemmaghami, S.; Oas, T. G. *Nat. Struct. Mol. Biol.* **2001**, *8*, 879−882.
(39) Ignatova, Z.; Gierasch, L. M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 523−528.
(40) Inomata, K.; Ohno, A.; Tochio, H.; Isogai, S.; Tenno, T.; Nakase, I.; Takeuchi, T.; Futaki, S.; Ito, Y.; Hiroaki, H.; Shirakawa, M. *Nature* **2009**, *458*, 106−109.
(41) Schlesinger, A. P.; Wang, Y.; Tadeo, X.; Millet, O.; Pielak, G. J. *J. Am. Chem. Soc.* **2011**, *133*, 8082−8085.
(42) Lavrenko, P. N.; Mikriukova, O. I.; Okatova, O. V. *Anal. Biochem.* **1987**, *166*, 287−297.
(43) Squire, P. G.; Himmel, M. E. *Arch. Biochem. Biophys.* **1979**, *196*, 165−177.
(44) Li, C.; Pielak, G. J. *J. Am. Chem. Soc.* **2009**, *131*, 1368−1369.
(45) Feig, M.; Sugita, Y. *J. Phys. Chem. B* **2012**, *116*, 599−605.
(46) Crowley, P. B.; Brett, K.; Muldoon, J. *ChemBioChem* **2008**, *9*, 685−688.
(47) Weatherly, G. T.; Pielak, G. J. *Protein Sci.* **2001**, *10*, 12−16.
(48) Saunders, A. J.; Davis-Searles, P. R.; Allen, D. L.; Pielak, G. J.; Erie, D. A. *Biopolymers* **2000**, *53*, 293−307.
(49) Kim, Y. H.; Stites, W. E. *Biochemistry* **2008**, *47*, 8804−8814.
(50) Hong, H.; Bowie, J. U. *J. Am. Chem. Soc.* **2011**, *133*, 11389−11398.
(51) Crowley, P. B.; Chow, E.; Papkovskaia, T. *ChemBioChem* **2011**, *12*, 1043−1048.
(52) Spitzer, J. J.; Poolman, B. *Trends Biochem. Sci.* **2005**, *30*, 536−541.
(53) Gralla, J. D. *Mol. Microbiol.* **2005**, *55*, 973−977.
(54) Toro, E.; Shapiro, L. *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, a000349.
(55) Spitzer, J. *Microbiol. Mol. Biol. Rev.* **2011**, *75*, 491−506.
(56) Shapiro, L.; Losick, R. *Science* **1997**, *276*, 712−728.

NOTE ADDED IN PROOF

A recent paper showed that the synthetic crowders polyethylene glycol and dextrans also have enthalpic effects on protein stability. Sukenik, S.; Sapir, L.; Gilman-Politi, R.; Harries, D. *Faraday Discuss.* **2012**, DOI: 10.1039/C2FD20101A, in press.