Reply to the "Comment on 'Urea-Mediated Protein Denaturation: A Consensus View'"

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · FEBRUARY 2011

Impact Factor: 3.3 · DOI: 10.1021/jp106305w · Source: PubMed

2 AUTHORS:

Atanu Das
University of Texas at Austin
13 PUBLICATIONS **140** CITATIONS
SEE PROFILE

Chaitali Mukhopadhyay
University of Calcutta
81 PUBLICATIONS **955** CITATIONS
SEE PROFILE

THE JOURNAL OF PHYSICAL CHEMISTRY B

COMMENT

pubs.acs.org/JPCB

Reply to the "Comment on 'Urea-Mediated Protein Denaturation: A Consensus View'"

Atanu Das† and Chaitali Mukhopadhyay*

Department of Chemistry, University of Calcutta, 92, APC Road, Kolkata 700 009, India

The conclusion that has been drawn in the previous paper[1] regarding the importance of the electrostatics interaction of urea has been drawn on the basis of the distribution of interaction energies as well as on the hydrogen bonding interactions between the protein backbone and urea and the radial distribution functions of urea atoms around the charged side chain of protein at initial and final stages of the simulation. We have observed nearly identical situations for three different proteins namely ubiquitin, G311, and GB1. While the distribution energies differed from that reported by Berne's group,[2] the observations regarding the hydrogen bonds and RDF's matched fairly well.

As has been very rightly pointed out by Berne's group in their Comment, the main difference lies in the values of bulk-water energies. While different cutoffs, simulation temperature, and strategies can account for 2−3 kcal/mol, we think the main difference arises from the difference in the definition of the interacting systems. While Bernes' group had calculated the "potential energy" of a particular set of species [FSS-U, FSS-W, bulk-W, and bulk-U], we have calculated the "interaction energy" of a particular species with the "rest of the system". In the presence of protein for individual snapshots from the trajectories we had

(i) defined the FSS-U, FSS-W, bulk-W, and bulk-U subsets
(ii) for each individual subset, computed the "INTERACTION energy" of the subset with the "REST of the system followed by averaging over per molecule of urea/water, as we had clearly mentioned in the Methods of our original paper.[1]
(iii) used the average INT-energies to generate the distributions.

Please note that in the INTE command of CHARMM the interaction energy is calculated between the sets and not INTRA-SET. For example, interaction energy between a particular water in FSS will be calculated with atoms of protein, urea [both in FSS and bulk], water in bulk [which comes within the applied cutoff] but not with another water in FSS. So the distributions [both FSS and BULK] do not include the self-interaction energies averaged as per molecules of water or urea. In other words, the distributions that have been given in Figure 3 of ref 1 implicitly assume a common E_o for urea/water in both bulk and FSS. Hence our distributions will emphasize more on the differences in the environment.

The main difference lies in the Figure 3 of our main paper[1] as we were using a different definition of interaction energy from that of Hua et al. We have done an estimate of the self-interaction energies for water in FSS and bulk [only for ubiquitin and also for a single trajectory]. For FSS and bulk water the self-electrostatic contributions are −15.62 and −16.28 kcal/mol, respectively, while for van der Waals the contributions are 0.02 and 0.16 kcal/mol, respectively. Hence including those will shift our original distributions by nearly equal amounts, leaving previously drawn conclusions unchanged. Addition of these values to our previous distributions [−1.5, −0.51, and −1.25, −0.57 kcal/mol for electrostatic and van der Waals interaction of FSS and bulk water, respectively with "rest of the system"] leads to the total electrostatic interaction energies as −17.12 and −17.53 kcal/mol for FSS and bulk water, which are very similar to those reported by Berne's group.[2] The calculated average values of respective potential energies are given in Table 1. We agree that the contribution of electrostatics toward the stabilization of a single urea molecule from bulk to FSS is now decreased heavily (from −12.09 to −3.03 kcal/mol) compared to that of VDW (−2.0 to −2.36 kcal/mol); however, the contribution of electrostatic stabilization energy −3.03 kcal/mol is higher than the corresponding VDW contribution of −2.36 kcal/mol. As a result, our conclusion of ref 1 remains unaltered. Figure 1 here can replace Figure 3 of the original paper.

Table 1. Interaction Energies in kcal/mol

	FSS-U	bulk-U	FSS-W	bulk-W
elec	−21.64	−18.61	−17.12	−17.53
VDW	−9.77	−7.41	−0.49	−0.41

Another source of difference could be the protein-conformational state sampling. While our trajectories are shorter [∼35 ns each and truncated after the protein lost most of the secondary structures], the protein conformations that have been sampled include native, near-native intermediates, intermediates, and the denatured forms. In the Figure 1C of ref 2 it is shown that while the native protein has a radius of gyration around 15 Å, in the presence of urea [in a 1000 ns simulation] the most probable distribution of the radius of gyration of the protein lies in the range 29−33 Å. This will not only control the number of water/urea molecules in the FSS/bulk but also control the amount of exposed hydrophobic residues. The fact that this will indeed affect the overall conclusion has recently been shown by Garcia's lab.[3] They found that "the denaturation is driven by favorable direct interaction of urea with the protein through both electrostatic and van der Waals forces and quantified their contributions. Though the magnitude of direct electrostatic interaction of urea is larger than van der Waals, the difference between unfolded and folded ensembles is dominated by the van der Waals interaction." Hence the distribution of the interaction energy will also be biased by the amount of folded/unfolded states sampled. We believe that our shorter trajectories are capturing the early kinetic stages of the denaturing process where protein is more globular and has predominantly polar/charged groups exposed, while

Received: July 8, 2010
Revised: December 17, 2010
Published: January 19, 2011

dx.doi.org/10.1021/jp106305w | J. Phys. Chem. B 2011, 115, 1327–1328

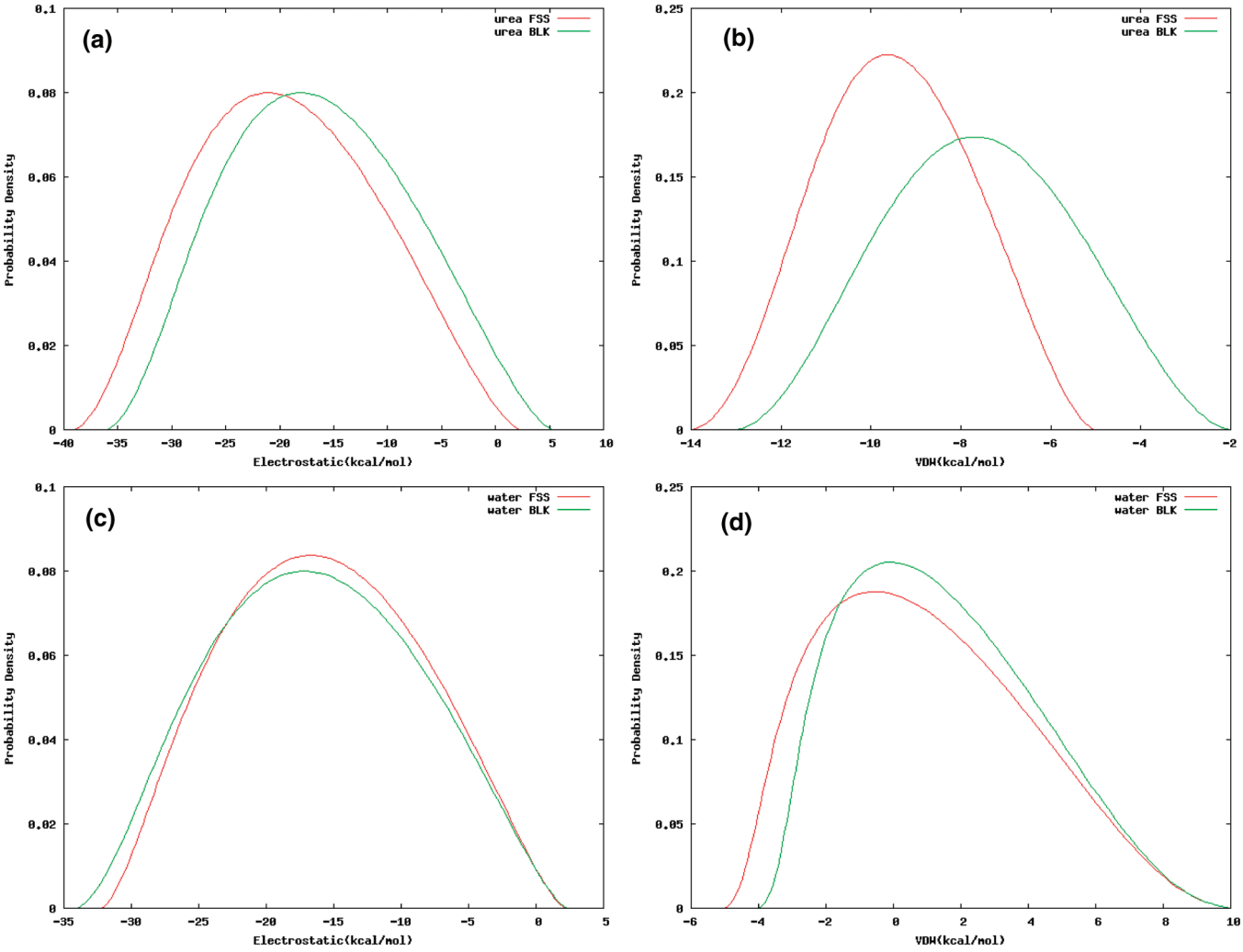

Figure 1. Interaction energy distribution. The probability distribution function of VDW and electrostatic interaction energy of urea and water in the first solvation shell of protein and in the bulk region with the rest of system. The first solvation shell (FSS) is defined as within 5.0 Å of protein, and the bulk region is defined as not within 6.0 Å of protein (ubiquitin): (a) urea-electrostatic; (b) urea-VDW; (c) water-electrostatic; (d) water-VDW.

longer trajectories [1000 ns] will involve the majority of the unfolded states with the nonpolar groups exposed. Then the majority of the time the interaction between protein and urea will be dominated by urea-nonpolar atoms rather than urea-polar atoms, and this will affect the distribution profile and [as suggested by Canchi et al.[3]] might shift the balance toward the van der Waals interaction. Thus, we suggest that at early stages of the unfolding where urea is interacting mostly with the exposed polar groups of the protein [which our shorter trajectories capture] which is reflected from the backbone-urea hydrogen bond formation time series, radial distribution function around polar/charged side chains within first 10 ns.[1,2] However a well-equilibrated unfolded state [characterization of that was beyond the scope of our shorter trajectories] will be stabilized by dispersion interaction as suggested by Hua et al.,[2] where the trajectory length is 1000 ns.

AUTHOR INFORMATION

Corresponding Author

*E-mail: chaitalicu@yahoo.com, cmchem@caluniv.ac.in.

Present Addresses

[†]Department of Physics and Astronomy, University of British Columbia, Hennings Building #404, 6224 Agricultural Road, Vancouver, B.C. V6T1Z1, Canada.

REFERENCES

(1) Das, A.; Mukhopadhyay, C. *J. Phys. Chem. B* **2009**, *113*, 12816–12824.

(2) Hua, L.; Zhou, R.; Thirumalai, D.; Berne, B. J. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 16928–16933.

(3) Canchi, D. R.; Paschek, D.; Garcia, A. E. *J. Am. Chem. Soc.* **2010**, *132*, 2338–2344.