J|A|C|S COMMUNICATIONS

Published on Web 01/27/2009

Intrinsically Disordered p53 Extreme C-Terminus Binds to S100B($\beta\beta$) through "Fly-Casting"

Jianhan Chen*

Department of Biochemistry, Kansas State University, Manhattan, Kansas 66506

Received December 6, 2008; E-mail: jianhanc@ksu.edu

Intrinsically disordered proteins (IDPs) are functional proteins that can exist as dynamical ensembles of disordered conformations under physiological conditions.[1] Frequently involved in crucial areas such as regulation and cellular signaling, many IDPs can undergo disorder−order transitions upon target recognition. The disordered nature of IDPs is believed to offer many unique advantages including the ability to allow high specificity coupled with low affinity and structural plasticity for binding multiple targets. The disordered ensembles are not necessarily random. Instead, residual structures often persist and are believed to have important implications in function.[2] Nonetheless, their specific roles in the coupled binding and folding process during IDP function are not well understood. It has been proposed that preformed structural elements that resemble the folded conformations in complexes might serve as initial contact points and facilitate the folding of flexible regions on the substate surface.[2] However, recent NMR and coarse-grained molecular simulation studies of the recognition of the KIX domain of coactivator CBP by the disordered KID domain of transcription factor CREB suggested that binding occurred through formation of nonspecific encounter complexes and increasing the amount of structure in the unbound states would actually reduce the binding rate.[4] Such a "fly-casting"-like process facilitates binding through a larger capture radius.[3] These two contrasting scenarios are analogous to the conformational selection or induced-fit (or folding) mechanisms commonly invoked in understanding protein−ligand interactions. In either case, the amount of residual structures in the unbound state modulates the binding affinity through the entropic cost of concomitant folding.

The extreme C-terminus of the tumor suppressor p53 (residues 367−392) is one of the few examples that have been experimentally shown to be capable of adopting multiple folded conformations upon binding to different targets, including the α-helix, β-strand, and two distinct loops.[5] Atomistic simulations indicate that the free peptide appears to sample each of these folded conformations with small but significant probabilities (see Supporting Information (SI), Figures S1 and S2). Such a pre-existence of folded-like conformational substates is often viewed as evidence of conformational selection, and one might thus be tempted to postulate that this is how the p53 extreme C-terminus recognizes its targets. Here we investigate this hypothesis by calculating the multidimensional potentials of mean force (PMFs) of coupled folding and binding of the p53 extreme C-terminus to protein S100B($\beta\beta$) using physics-based atomistic simulations. Examination of the free energy surfaces along appropriate folding and binding reaction coordinates should provide a rigorous thermodynamic clarification of the underlying recognition mechanism (e.g., see Figure S3).

Upon binding to Ca^{2+}-loaded dimeric S100B($\beta\beta$), p53 extreme C-terminus folds into a short helix that spans residues 376−387 (see Figure 1a).[6] Given the fast time scales of typical helix-coil transitions, we calculated the PMFs using a combination of umbrella sampling, with harmonic restraints applied along the distance between centers of mass (CMs) of two proteins (the binding reaction coordinate), and replica exchange molecular dynamics (REX-MD) simulations, for sampling the (helix) folding/unfolding transitions near a given CM separation distance. Several simplifications were made to further reduce the computational cost. (1) Solvent effects were described using a consistent generalized-Born implicit solvent protein force field, which was previously optimized to balance solvation and intramolecular interactions and had been shown to be capable of accurately describing peptide conformational equilibria under stabilizing and destabilizing conditions.[7] (2) A minimal construct was built by only including protein segments that are directly involved in the p53/S100B($\beta\beta$) interactions (detailed in SI). Shown in Figure 1b, the construct includes 63 residues with 1077 atoms, a reduction of ∼50% compared to a system with full-length proteins. (3) The p53 peptide was constrained to move along a straight line defined by the initial CM positions in the PDB structure. At the end, a total of 18 umbrella sampling windows were equidistantly placed from 11 to 28 Å CM separations with a restraint force constant of 5.0 kcal/mol/Å^2. At each window, a 45 ns restrained REX-MD simulation was carried out with 16 replicas spanning 270−500 K using the MMTSB Toolset[8] and CHARMM.[9] PMFs were calculated using the weighted histogram analysis method (WHAM).[10] Note that the current protocol relies on REX-MD for sufficient sampling of p53 peptide conformation equilibria, which appears to be adequate along all the folding reaction coordinates examined here (see Figure S5).

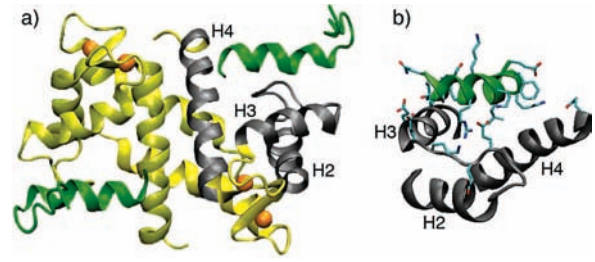

Figure 1. Cartoon representations of (a) p53 extreme C-terminus (in green) in complex with Ca^{2+}-loaded S100B($\beta\beta$) dimer (in yellow) (PDB ID: 1dt7). Calcium ions are shown as orange spheres, and the segments of the 100B($\beta\beta$) monomer included in the simulations are colored in gray. (b) The minimal molecular construct for the p53/S100B($\beta\beta$) interactions, with the side chains of p53 and charged residues of S100B($\beta\beta$) at the interface shown in stick mode.

The 2D free energy surfaces of p53/S100B($\beta\beta$) interaction computed from the last 20 ns of the restrained REX-MD simulations are shown in Figure 2a and 2b. The binding reaction coordinate is defined as the CM separation distance, and the helix-coil conformation equilibria of the p53 extreme C-terminus are described by the number of helical residues and end-to-end distance. Cross sections of the 2D PMF of Figure 2a at several important CM separations are shown in Figure S5-c. The free energy surfaces clearly show that unbound p53 peptide at large CM separations samples a wide

10.1021/ja809547p CCC: $40.75

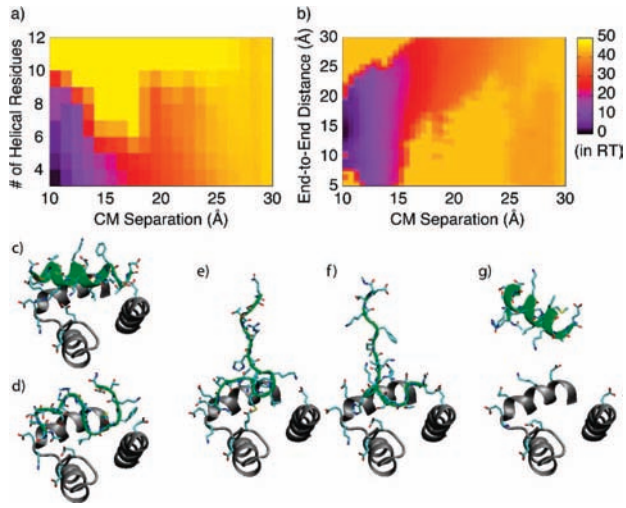

Figure 2. 2D PMFs of coupled folding and binding of p53 extreme C-terminus to S100B($\beta\beta$). The helical residues were identified by (i,i+4) backbone hydrogen bonding patterns. By definition, a helical segment consists of at least four residues. Snapshots with no helical residue ($N_{\text{helix}} = 0$) were assigned with $N_{\text{helix}} = 3$ for continuity in the WHAM analysis. End-to-end distance was defined as the distance between C_α atoms of residues 375 and 388. Representative structures are shown for important regions: (c) a bound complex with N_{helix}=8 (a local minimum identified in the cross section of the 2D surface at 11 Å CM separation; see Figure S5-c); (d) a bound complex with an unfolded p53 peptide; (e−f) nonspecific complexes with interactions through N- or C-terminus of p53 peptide at ∼17 Å CM separations; (g) a folded conformation at ∼28 Å CM separation ($N_{\text{helix}} = 11$).

range of conformational states including those similar to the folded structures observed in the p53/S100B($\beta\beta$) complex (e.g., see Figure 2g). Upon binding to S100B($\beta\beta$), the accessible conformational space is greatly reduced, leading to a much narrower end-to-end distance distribution observed in Figure 2b at small CM separations. The global minimum of the 2D PMF shown in Figure 2a corresponds to a bound state with largely unfolded p53 peptide (e.g., see Figure 2d), and the bound state with folded p53 peptide (e.g., see Figure 2c) is only a local minimum that is ∼4 RT higher in free energy (also see Figure S5-c). The inability of the current force field to predict the correct global free energy minimum might be attributed to two main factors, a systematic basis in the current protein force fields toward overstabilizing protein−protein interactions[11] and limitations in current surface-area based treatment of nonpolar solvation in implicit solvent models.[12] The same factors are likely responsible for the apparent overestimation of the binding affinity in the current calculation (experimental K_D ∼23 μM or ΔG_D ∼ −11 RT[13]). Despite these pitfalls due to force field limitations, the calculated 2D free energy surfaces clearly point to a necessity of the p53 extreme C-terminus unfolding at intermediate CM separation distances (∼17 Å). In contrast to the initial conformational selection hypothesis based on simulations of the free peptide, the p53/ S100B($\beta\beta$) recognition actually appears to occur through a "fly-casting"-like process similar to that of the KID/KIX interaction.[4] The p53 peptide is quite extended (large end-to-end distance) with minimal structures (low helical content) in the nonspecific complexes and can interact with S100B($\beta\beta$) through either the N- or C-terminus (e.g., see Figure 2e and 2f). Closer inspections show that the hydrophobic pocket of S100B($\beta\beta$) at the interface is surrounded by charged residues including six glutamic acids and two lysines, which are complementary to the predominantly positive-charged p53 peptide with three lysines, one arginine, and one glutamic acid. Salt-bridge interactions between them not only provide stabilization effects but also likely facilitate the formation of the nonspecific complexes en route to the folded specific complex through electrostatic steering. The long-range electrostatic forces are likely driving the peptide unfolding. Such a concerted involvement of electrostatic steering and fly casting effects could maximize the efficiency of binding.[14] We note that these kinetic aspects would require further validation such as through transition state analysis.[4]

In summary, the multidimensional free energy surfaces of coupled binding and folding reveal that the p53 extreme C-terminus recognizes protein S100B($\beta\beta$) through a "fly-casting"-like process, even though the free peptide appears to sample conformations that resemble various folded structures observed when in complex with different targets. Such a pre-existence of folded-like conformational states is thus not sufficient evidence of conformational selection. Accordingly, the primary role of the residual structures in unbound p53 extreme C-terminus appears to be to modulate the binding affinity, while the intrinsic flexibility is critical for the binding rate. The current work also demonstrates that, despite various existing limitations, physics-based atomistic simulations, coupled with advanced sampling, can be useful for structural and functional characterizations of IDPs.

Acknowledgment. We thank Drs. Keith Dunker and Debabani Ganguly for helpful discussions. This work is contribution 09-164-J from the Kansas Agricultural Experiment Station.

Supporting Information Available: Additional details regarding simulation of free p53, minimal molecular construct, and convergence are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Dyson, H. J.; Wright, P. E. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 197–208. (b) Dunker, A. K.; Brown, C. J.; Lawson, J. D.; Iakoucheva, L. M.; Obradovic, Z. *Biochemistry* **2002**, *41*, 6573–6582. (c) Tompa, P. *Trends Biochem. Sci.* **2002**, *27*, 527–533.
(2) (a) Receveur-Brechot, V.; Bourhis, J. M.; Uversky, V. N.; Canard, B.; Longhi, S. *Proteins* **2006**, *62*, 24–45. (b) Fuxreiter, M.; Simon, I.; Friedrich, P.; Tompa, P. *J. Mol. Biol.* **2004**, *338*, 1015–1026.
(3) Shoemaker, B. A.; Portman, J. J.; Wolynes, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 8868–8873.
(4) (a) Sugase, K.; Dyson, H. J.; Wright, P. E. *Nature* **2007**, *447*, 1021–U11. (b) Turjanski, A. G.; Gutkind, J. S.; Best, R. B.; Hummer, G. *PLoS Comput. Biol.* **2008**, *4*, e1000060.
(5) Oldfield, C. J.; Meng, J.; Yang, J. Y.; Yang, M. Q.; Uversky, V. N.; Dunker, A. K. *BMC Genomics* **2008**, *9 Suppl 1*, S1.
(6) Rustandi, R. R.; Baldisseri, D. M.; Weber, D. J. *Nat. Struct. Biol.* **2000**, *7*, 570–574.
(7) (a) Chen, J. H.; Im, W. P.; Brooks, C. L. *J. Am. Chem. Soc.* **2006**, *128*, 3728–3736. (b) Chen, J. H.; Brooks, C. L.; Khandogin, J. *Curr. Opin. Struct. Biol.* **2008**, *18*, 140–148.
(8) Feig, M.; Karanicolas, J.; Brooks, C. L. *J. Mol. Graphics Modell.* **2004**, *22*, 377–395.
(9) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187–217.
(10) Kumar, S.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A.; Rosenberg, J. M. *J. Comput. Chem.* **1992**, *13*, 1011–1021.
(11) Kang, M.; Smith, P. E. *J. Comput. Chem.* **2006**, *27*, 1477–85.
(12) Chen, J. H.; Brooks, C. L. *Phys. Chem. Chem. Phys.* **2008**, *10*, 471–481.
(13) Wilder, P. T.; Lin, J.; Bair, C. L.; Charpentier, T. H.; Yang, D.; Liriano, M.; Varney, K. M.; Lee, A.; Oppenheim, A. B.; Adhya, S.; Carrier, F.; Weber, D. J. *Biochim. Biophys. Acta* **2006**, *1763*, 1284–1297.
(14) Levy, Y.; Onuchic, J. N.; Wolynes, P. G. *J. Am. Chem. Soc.* **2007**, *129*, 738–739.

JA809547P