Direct Observations of Shifts in the β-Sheet Register of a Protein-Peptide Complex Using Explicit Solvent Simulations

Maria T. Panteva,[△] Reza Salari,[△] Monica Bhattacharjee, and Lillian T. Chong*
Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania

ABSTRACT Using explicit solvent molecular dynamics simulations, we were able to obtain direct observations of shifts in the hydrogen-bonding register of an intermolecular β-sheet protein-peptide complex. The β-sheet is formed between the FHA domain of cancer marker protein Ki67 (Ki67FHA) and a peptide fragment of the hNIFK signaling protein. Potential encounter complexes of the Ki67FHA receptor and hNIFK peptide are misregistered states of the β-sheet. Rearrangements of one of these misregistered states to the native state were captured in three independent simulations. All three rearrangements occurred by a common mechanism: an aromatic residue of the peptide (F263) anchors into a transient hydrophobic pocket of the receptor to facilitate the formation of native hydrogen bonds. To our knowledge, these simulations provide the first atomically detailed visualizations of a mechanism by which nature might correct for errors in the alignment of intermolecular β-sheets.

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[△]Maria T. Panteva and Reza Salari contributed equally to this work.

*Correspondence: ltchong@pitt.edu

Intermolecular β-sheets are found in many protein-protein complexes (1). The formation of the native bound structure is likely to involve encounter complexes (2) with nonnative hydrogen-bonding registers, and these misregistered states may then either dissociate or rearrange to the correct register. Intersheet rearrangements within aggregates of amyloid peptides were previously detected by isotope-edited IR spectroscopy (3). In several studies, misregistered states were observed in atomistic molecular dynamics (MD) simulations of peptides in either implicit (4,5) or explicit solvent (6); however, their rearrangements to the native states were not resolved in these studies. To date, investigators have only been able to capture rearrangements using implicit solvent simulations artificially accelerated by low solvent viscosity (7), explicit solvent simulations employing high temperature with replica exchange (8), and Monte Carlo simulations considering only torsional degrees of freedom (9,10).

In this work, we obtained direct observations of shifts in the register of a β-sheet using all-atom, explicit solvent MD simulations at room temperature. The β-sheet involves the formation of four backbone hydrogen bonds between the forkhead-associated domain of the cancer marker Ki67 (Ki67FHA) and a peptide fragment (residues 260–266) of the hNIFK signaling protein (11). This is an ideal model system for simulating rearrangements of β-sheets not only because of its small size (106 residues; Fig. S1 of the Supporting Material) but also because of its weak binding affinity ($K_D = 42 \pm 5$ mM) (11), which may facilitate the rearrangements. We explored the rearrangements of two potential encounter complexes to the native state. These complexes involve either a "+2" or "−2" register shift in the β-sheet, in which the peptide is displaced by two residues in the direction of the N- and C-termini of the Ki67FHA receptor, respectively (Fig. 1 and Table S1).

In a previous study, Pande et al. (12) showed that it is possible to simulate protein folding kinetics using a large ensemble of trajectories that are much shorter than the folding time; therefore, we wondered whether this approach might also be useful for capturing rearrangements of the misregistered states. To address this issue, we first performed a large ensemble (>300) of short (20–40 ns), explicit solvent simulations starting from each of the misregistered states (+2 and −2 states) using the Folding@Home distributed computing network. We then extended the simulations that resulted in partial rearrangements (<2.5 Å C^{α} root mean square deviation (RMSD) of the β-sheet from the native state or at least one native hydrogen bond) to a much longer timescale (300 ns) using the TACC Ranger supercomputer. Because of the high computational cost (~10 ns/day using 32 cores on Ranger), we did not extend the remaining simulations (for simulation details, see the Supporting Material).

A small number of the short Folding@Home simulations (11 starting from +2 state and 10 starting from the −2 state) resulted in partial rearrangements. Only four out of the hundreds of short simulations performed resulted in dissociation of the peptide, suggesting that the +2 and −2 states are at least metastable.

Of the 11 300-ns simulations that started from the +2 state, three resulted in complete rearrangements to the native state

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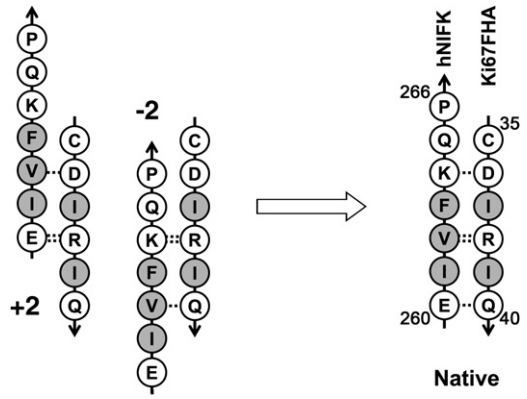

FIGURE 1 Rearrangements of misregistered states (*left*) to the native state of the β-sheet between the Ki67FHA receptor and hNIFK peptide were explored by simulations. Only two-residue shifts (+2 and −2) were considered, because they maintain alignment of hydrophobic residues (*gray*) between the strands.

(Fig. 2 *A*). The C^{α} RMSDs of the intermolecular β-sheet from the native state were within 1 standard deviation of the average value during a 300-ns simulation starting from the native state (1.5 ± 0.3 Å). Of the remaining eight simulations, seven remained bound in a nonnative state and one resulted in unbinding of the peptide. None of the 10 300-ns simulations starting from the −2 state rearranged. In one of these simulations, the peptide partially dissociated, and in the other nine, receptor residue F20 sterically blocked translation of the peptide toward the native register (Movie S1).

All three rearrangements from the +2 state share a common mechanism (Movie S2, Movie S3, and Movie S4). We illustrate this mechanism for the most rapid rearrangement (*green* in Fig. 2 *A*) by monitoring the χ_1 angle of receptor residue F20, percent burials of F20 and peptide residue F263, and number of native hydrogen bonds in the β-sheet (Fig. 2 *B*; plots for the other two rearrangements and the native complex are provided in Fig. S2). As shown by the snapshots in Fig. 2 *C*, rearrangement begins with partial dissociation of the peptide at 23.4 ns. At 42.5 ns, F20 swings out into solution (χ_1 angle of −160° to 60° in the *upper panel* of Fig. 2 *B*), exposing a hydrophobic pocket (pocket 1) in the receptor. The subsequent anchoring of peptide residue F263 into this transient pocket (*middle panel* of Fig. 2 *B*) seems to facilitate the formation of two more native hydrogen bonds at 56.1 ns (*lower panel* of Fig. 2 *B*). The hydrogen bonds form sequentially starting from the N- to C-terminal ends of the peptide until all four have formed at 72.8 ns. Because the mechanism in Fig. 2 *C* involves partial dissociation of the peptide, it is distinct from the reptation-like mechanism (13) previously observed in implicit solvent simulations of amyloid peptides (7,9,10). To our knowledge, our simulations provide the first direct views of β-sheet rearrangements that involve anchoring into a transient pocket. Of interest, the use of the targeted MD approach (14) to accelerate rearrangements from the +2 state did not result in the same mechanism (Fig. S3).

To investigate the importance of pocket 1 in the rearrangements, we mutated F20 to an alanine, thereby leaving

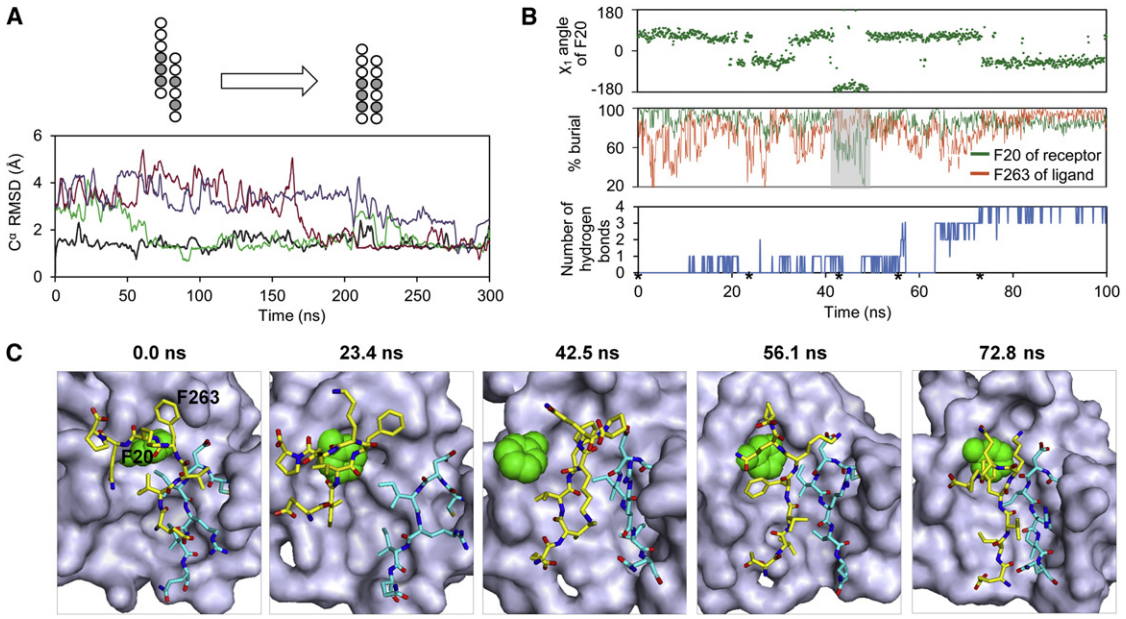

FIGURE 2 Mechanism of rearrangement from the +2 state to the native state. (*A*) Plot of the C^{α} RMSD of the β-sheet from the native state versus time for simulations starting from the +2 (*green*, *red*, and *purple*) and native (*gray*) states. For each simulation, the Folding@Home network was used for the first 20–40 ns, and the simulation was then extended to 300 ns on the Ranger supercomputer. (*B*) Plots of the χ_1 angle of receptor residue F20, % burials of F20 and peptide residue F263, and number of native hydrogen bonds in the β-sheet versus time for the most rapid rearrangement. As highlighted by the gray box in the % burial plot, F263 anchors into a transient hydrophobic pocket of the receptor. (*C*) Snapshots at times indicated by asterisks in *B*, tracking the positions of the F20 side chain (*green*), peptide (*yellow*), and receptor β-strand (*cyan*); the rest of the receptor is represented by its molecular surface (*gray*).

the pocket open. Out of 10 100-ns simulations starting from the mutant +2 state, one rearranged to the native state (Fig. S4 *A* and Movie S5). In this rearrangement, however, F263 does not anchor into pocket 1; instead, it uses another pocket (pocket 2) that is blocked when F20 swings out to expose pocket 1 (Fig. S4, *B* and *C*). Pocket 2 is also used as an anchor point by F263 during the slowest rearrangement of the wild-type +2 state before F263 settles into pocket 1 (Fig S2 *B* and Movie S4). From simulations of the unbound receptor, it appears that the F20 gating of both pockets 1 and 2 is intrinsic to the receptor (Fig. S2 *D*).

In closing, we have reported the first (to our knowledge) direct observations of shifts in the β-sheet register of a protein-peptide complex using explicit solvent MD simulations. In particular, rearrangements of the +2 misregistered state to the correct register of the Ki67FHA-hNIFK peptide complex were captured in three independent simulations. All three rearrangements share a common mechanism: the anchoring of peptide residue F263 into a transient pocket of the receptor facilitates the "crawling" of the peptide along the receptor surface to the native alignment. These rearrangements suggest that MD simulations can correct for errors in the register of nonlocal β-sheets. This may be useful in the area of structure prediction, where the prediction of β-sheet registers remains a challenge (15).

Given that protein binding interfaces are much richer in aromatic residues than the average protein surface (16), the anchoring of aromatic residues into transient pockets may be a general mechanism of "dynamic" induced-fit binding. This general mechanism may be relevant for various proteins in which alternate registers play important roles in their biological functions (17–21). Our results also demonstrate that MD simulations can identify transient pockets that could potentially be used to develop new classes of pharmaceuticals, particularly for targets that appear "undruggable" (22). In this work, we simulated only three β-sheet rearrangements; however, the generation of a large ensemble of these kinds of simulations will become more practical as computational power improves.

SUPPORTING MATERIAL

Methods, references, four figures, one table, and five movies are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(11)00383-3.

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