Understanding the Molecular Basis for Differential Binding of Integrins to Collagen and Gelatin

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ABSTRACT Integrin-mediated cell adhesion plays a central role in cell migration and signaling. Overexpression of integrins is also associated with cancer invasion and metastasis. Although a number of problems in integrin-matrix interactions have been studied in detail, the molecular specificity, which increases integrin adhesion to native collagen but results in poor integrin-gelatin interaction, is not understood. In this report, we study the role of individual amino acids in integrin-collagen and integrin-gelatin interactions using long-term (>100 ns) molecular simulations. The results, which are force-field independent, show that denatured collagen induces helical conformations in integrin amino acids and significantly reduces the poly-proline II content, which stabilizes the integrin-collagen interactions. Our simulations provide a possible explanation of the molecular specificity in integrin binding and suggest new targets for regulating integrin-mediated invasion and metastasis.

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Integrin-matrix interactions are critical for cell adhesion, migration, signaling, and survival (1,2). Recent studies have linked overexpression of integrins with the progression of a number of fatal diseases, including pancreatic cancer (3). Although the role of integrins in migration, adhesion, and signaling has been studied through a number of experimental approaches, only very few computational studies have addressed the molecular basis of these complex interactions. Although integrin-fibronectin complexes have been studied using steered molecular dynamics (4–6), no study, to the best of our knowledge, has addressed the dynamic and conformational properties of integrin-collagen complexes. Such a study of molecular interactions between integrin and collagen will provide much needed information on the role of individual amino acids in complex formation and the effects of various mutations in stabilizing or destabilizing the integrin-ECM complex. Using long time atomistic simulations, we address the molecular basis of specificity in integrin-collagen interactions.

It has been shown that a number of different cell lines show strong adhesion and migration on native collagen surfaces but are unable to attach on denatured collagen. (gelatin) (7–10). So far, no quantitative hypothesis has been presented for this behavior. A detailed molecular level understanding of integrin-collagen and integrin-gelatin interactions will therefore serve two key purposes: 1), to quantitatively answer the question of molecular specificity in collagen-integrin and collagen-gelatin interactions; and 2), to suggest possible mutations that may help in decreasing integrin-collagen adhesion and thus provide targets for invasion and metastasis control in numerous cancer treatments.

In this study, we present long-time Langevin dynamics (LD) simulations (>100 ns) of I domain of $\alpha 2\beta 1$ complexed with a triple helical collagen peptide containing a critical GFOGER motif (Protein Data Bank: 1DZI) (11). Simulations were performed with the LD algorithm using the solvent-accessible surface area solvation potential described elsewhere (12). The LD simulations with solvent-accessible surface area potential have shown very good agreements with explicit solvent simulations as well as experimental studies (12–14). To ensure that our results were not affected by the structural and energetic biases in force fields (14,5), simulations were performed with two distinctly different force fields, namely the all-atom OPLS 2001 force field (16) and the Garcia/Sonbonmatsu modified Amber 94 force field (17). For each force field, each simulation was carried out for 3–10 ns at 298 K, after an initial equilibration of at least 1 ns and was repeated at least 10 times with different initial conditions. The different initial conditions were generated by starting the simulation with the structures that had been equilibrated for different lengths of time (between 1 and 2ns).To study the interactions of integrins with denatured collagen, the collagen triple-helix peptide was mechanically unfolded using a force of 500 pN until a completely stretched structure was obtained.

To capture some of the aspects of the unfolded ensemble, the unfolded collagen chain was equilibrated for different lengths of time (between 1 and 2ns), and 10 distinct starting unfolded structures were generated for each force field. This unstructured collagen (gelatin) was then placed in place of the native collagen peptide in the original Protein Data Bank structure. The integrin-gelatin complex was then equilibrated for another 1–2 ns, and based upon the length of equilibration, different starting structures were obtained. Once again,

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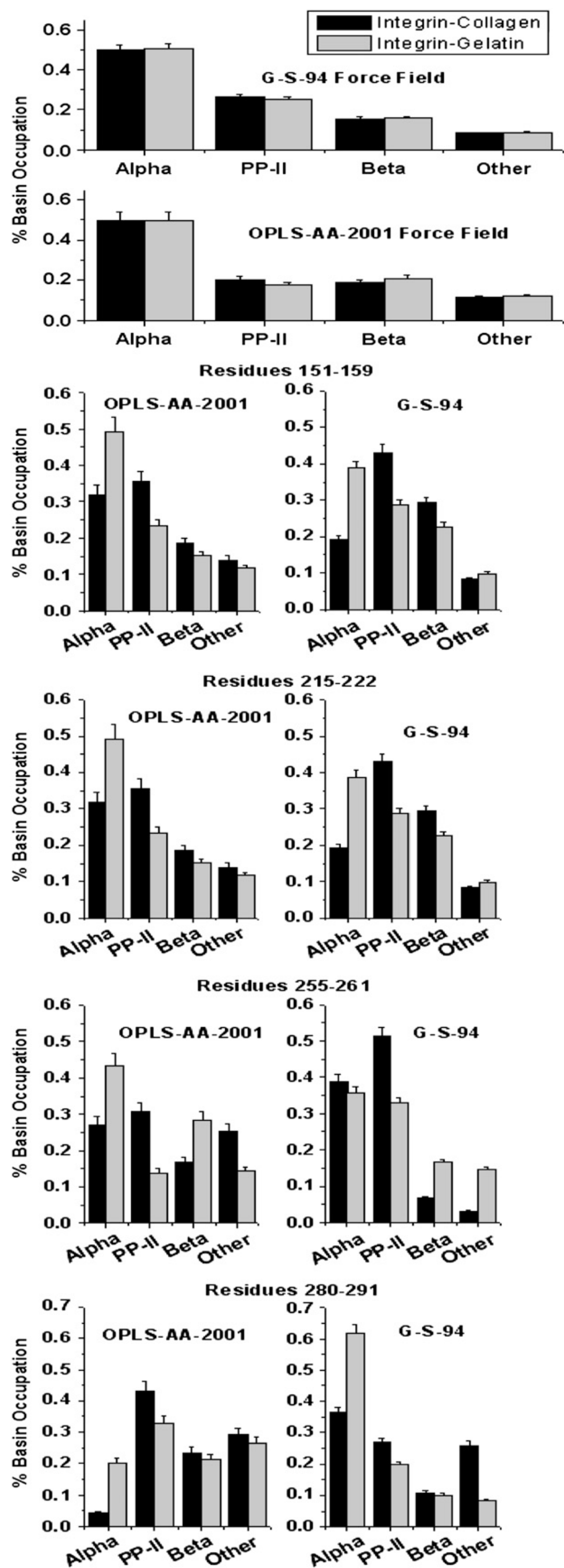

FIGURE 1 Percent occupation of individual amino acids in a given basin. (*A*) The percent occurrence of all integrin amino acids in both force fields. Both force fields show that upon unfolding of collagen, the global conformational preferences remain the same. However in all regions of the integrin molecule close to the ligand (i.e., amino acids 151–159, 215–222, 255–261, and 280-291) the α-conformations increase whereas PP-II conformations decrease. No particular trend is observed in β-propensities.

at least 10 different simulations were performed for both force fields and length of the simulation time varied between 3 and 10 ns.

To study the molecular specificity of collagen and gelatin interactions with $\alpha 2\beta 1$ integrin, the conformational preferences of all amino acids in the integrin molecule were recorded every 10 ps. We then focused on conformations of all amino acids in the integrin molecule that were <10 Å away from the collagen triple helix (Supplemental Fig. 1). Four such regions of amino acids were identified in the integrin molecule, namely amino acids 151–159, 215–222, 255–261, and 280–29. These amino acids represented regions of integrin most likely to interact with the collagen triple-helix peptide. Although the hydrogren-bond operating distance is much smaller, we wanted to include all possible amino acids that had the slightest possibility of a direct interaction with the collagen peptide. Based on the ϕ/ψ-angle, the conformations of each amino acid at each interval were classified as α, β, PP-II, or other by a previously published method (14).

Detailed analysis of conformational preferences of integrin amino acids in the vicinity of collagen triple helix suggested that 1), overall conformational preference of the integrin molecule remains the same regardless of the presence of collagen or gelatin in the integrin vicinity (Fig. 1 and supplemental figure); 2), deconstructing the conformational preferences shows that the PP-II conformations decreased significantly in all four regions of amino acids close to collagen upon unfolding (Fig. 1 and supplemental figure); and 3), on the other hand, regardless of the force field, there was a significant increase in α-helical conformations of amino acids in the vicinity of the ligand upon unfolding of collagen. We also note that α-helical preference either increased significantly (in 80% of the amino acids in the collagen/gelatin vicinity) or remained the same (within the margin of error), but never decreased in the regions close to the ligand. On the other hand, the PP-II conformation always decreased upon folding. The β-conformations showed no particular trend. These results are independent of force fields. Further tests with shorter simulations employing the CHARMM 27 force field also showed similar trends (data not shown).

Using long time simulations, our force-field independent results demonstrate two key aspects of integrin-ligand interactions that were previously unknown. First of all, we show that unfolded collagen induces α-helical conformations in the residues in its vicinity while decreasing the PP-II conformations.

As a high percentage of PP-II structure has been shown in a number of experimental and theoretical studies focusing on unfolded peptides (18,19), our results suggest that a proline rich collagen triple helix decreases the PP-II structure in its binding partner upon unfolding while increasing the helical content; however, the overall structural preferences remain conserved (Fig. 1). Thus a high PP-II content is desirable for increased binding.

These results suggest that although the global propensity of integrin structural preferences is intact, local changes lead to decrease in binding. We conclude that for integrin collagen interactions, formation of PP-II structure in integrin significantly increases the stable interactions and the loss of PP-II structure is associated with poor binding. The observation also suggests a possible mutation strategy for decreased integrin adhesion desired in many cancer therapies. Decreasing the PP-II propensity by single or multiple mutations may lead to a strong decrease in integrin-mediated cell adhesion in cancers where increased invasion and migration rates are observed due to integrin overexpression.

SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at http://www.biophysj.org.

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