456e Atomic Molecular Dynamical Modeling of a Large Protein Complex: Stf-Fviia

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In this work we report the results of atomistic molecular dynamics (MD) simulations performed over 22 ns for a large protein complex, soluble Tissue Factor- Factor VIIa (sTF-FVIIa), in the presence of explicit solvent. We elected to use as study case the sTF-FVIIa complex, a key initiating system in thrombosis and hemostasis. The simulations contained more than 134,000 atoms total (624 residues + TIP3P water molecules) with long-range interactions computed by Particle Mesh Ewald summations. Additionally a relatively small integration time step (1 fs) was used in the most exacting simulations. We analyze the resulting conformational fluctuations, comparing against the X-ray crystallographic structure of the complex and unbound structures (PDB entries: 1DAN, 1BOY). We performed systematic MD simulations of this complex, using the AMBER suite, for over 22 ns each at different conditions. We studied the following: a) the importance of sampling space by parallel computing through ‘duplicate' simulations started from a common initial state to explore statistical convergence; b) the influence of the length of the solvation shell (or box size); c) the influence of the integration step (1, 2 and 5 fs); and d) the conformational fluctuations of the unbound state of FVIIa and TF. An analysis of the results show that at least 10 ns ‘production runs' with relatively small integration steps (1fs) are required to obtain sound biochemical information of the dynamics for this system. Specifically, we have used MD simulations to predict the solution structure of a complete refined solution equilibrated model for TF(Ser1-Met218)-FVIIa(Ala1-Pro406) based on the crystal structure (PDB entry: 1DAN). We observed significant Gla-EGF1 and EGF1-EGF2 inter-domain motion of FVIIa in the present simulation, where the Gla-EGF1 has the major contribution to the overall motion. Also, we observe minimal inter-domain movement associated with the EGF2 and SP domains. The EGF2 domain maintains structural integrity that is likely required for substrate binding. The predicted solution structure of the SP domain is compared with the available X-ray crystal structures determined at different conditions. No significant restructuring for this domain is observed. The methodology employed in this work suggests what is required to have realistic predictions for dynamical solution conditions of protein complexes. The equilibrated solution structure developed here of the key complex that initiates coagulation should provide insight into the details of how tissue factor enhances the action of FVIIa and could provide crucial impact for drug design.