Molecular dynamics and docking simulation of the G216D mutation in the catalytic domain of activated protein C

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Motivation

Activated Protein C (PC) is an anticoagulant trypsin-like serine protease whose deficiency is associated with an increased risk for venous thrombosis. We present here a computationally based analysis of a new variant, G216D, identified in heterozygous patients with normal concentration and reduced activity of the protein. G216 is located on a surface loop in the vicinity of the catalytic S195, H57, D102 triad: it is highly conserved in serine-proteases family and has an important role in determining the specificity of substrate recognition. Ligand recognition in trypsin-like serine proteases is driven by the insertion of a positively charged side-chain into a specificity pocket. The ligand residue establishes electrostatic interactions with an aspartic in position 189 while two highly conserved glycines (216 and 226) shape the binding cavity to accomodate the ligand. Multiple alignment of trypsin-like family members indicated that the loop including G216 is strictly conserved. However it was interesting to observe that the substitution G216D is present in alphatryptase,and not in its beta counterpart. The two tryptases share 90% of identical residues but ashows a reduced activity and a different substrate affinity. X-ray structure comparison shows that the main difference falls within the segment of loop 215-225, involved in substrate binding. In alfa, a kink of the loop, prevents the insertion of the ligand in the binding pocket. Based on the overall structure conservation in trypsin-like family members and the high similarity of binding pockets of PC with beta-tryptase, we have simulated by molecular dynamics the structural effects of G216D substitution in PC. We also tested, by docking approach, the ability of the mutant to correctly bind the substrate. The reduced functionality of PC variant is interpreted in comparison with tryptase model.

Methods

The molecular model of the G216D variant was prepared from the heavy chain of the X-ray PC structure (pdb entry: 1AUT) by residue substitution. Molecular dynamics (MD) simulations were performed for native and variant PC in the canonical ensemble (NPT) using the program CHARMM with the all-atom force field CHARMM22. The proteins were solvated in a tetrahedral box of about 25000 water molecules. The system was relaxed with molecular mechanics and then MD calculations were started. The system was heated to 300°K for 25000 steps, equilibrated for 50000 steps and let to evolve for 6.5 ns with a time-step of 2ft. Docking analyses were carried out on final conformations from MD and on X-ray structures of alpha and beta-tryptase (1LTO and 1A0L respectively) with the corresponding co-crystallized ligands (PPACK and APA). Docking was performed with Autodock 3.05 using the Lamarkian Genetic Algorithm with a grid of 40X40X40 points, a spacing of 0.375Å and centered on the binding site.

Results

The objective of the MD was to explore the effect of mutation on the conformation of functional loop 215-225. Analysis of RMS deviation from the initial structures showed a local conformational change in the mutant associated with the distortion of the loop which is not observed in wt protein. The distorsion is responsible for the displacement of the D216 side chain compared to starting modelled structure and a separation of aspartic C-alpha of ~3Å. This effect is similar to that observed in alpha vs. beta-tryptase where a Ca's separation of ~4Å was found. As for alphatryptase, whose X-ray structure was obtained in absence of ligand (Marquardt Uet al. J.Mol.Biol. 2002, 321:419-502), the altered conformation of the loop 215-225 in PC variant, seems to be incompatible with a correct insertion and presentation of the ligand. To test this hypothesis, we have

simulated by docking approach the binding of the PPACK inhibitor (co-crystallized with PC) to the mutant model. Docking results showed that the ligand is localized at the external surface of the protein probably due to the shape and electrostatic modification of the substrate binding pocket. As a consequence the ligand arginine cannot reach and establish favorable interactions with aspartic 189 localized at the base of the pocket as it seems to be deviated by the negative and the bulky side chain of D 216. We conclude that, in the G216D mutant, ligand interaction, if any, does not produce a correct orientation in the binding pocket and prevent the peptide proteolysis. This observation is in agreement with the reduced functionality observed in heterozygous patients carrying this mutation. It also confirms the key role of the loop 215-225 and highlights the importance of glycine in this specific position for serine-proteases function.

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Supplementary informations

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