Characterization of the HPA-1 polymorphism by MD simulations and FRET measurements

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The human platelet antigen (HPA)-1 is a diallelic alloimmune system carried by the megakaryocyte/platelet-specific integrin $\alpha_{IIb}\beta_3$, which mediates platelet adhesion and aggregation; $\alpha_{IIb}\beta_3$ is essential for hemostasis but can also induce pathological thrombus formation. The HPA1 polymorphism of $\alpha_{IIb}\beta_3$ is characterized by a leucine-to-proline exchange at residue 33 of the mature β_3 subunit. Consequently, the HPA-1 pattern can be expressed as either HPA-1a (Leu33) or its variant isoform HPA-1b (Pro33) [1]. This mutation is clinically relevant, since patients with coronary artery disease, who carry the HPA-1b allele, experience their myocardial infarction 5.2 years earlier than HPA-1a/1a patients [2]. Thus, HPA-1b is a prothrombotic variant of $\alpha_{IIb}\beta_3$, as also shown by increased adhesion, increased thrombus stability, and increased outside-in signaling. However, the underlying mechanism by which the mutation contributes to the prothrombotic properties of the variant integrin has remained elusive so far.

Integrins exist in two main and mutually exclusive conformations: The bent, closed form and the unbent, open structure. Local and global structural rearrangements are required in going from the closed to the open form, thereby leading to integrin activation. In the present study, a combined strategy, integrating large-scale all-atom molecular dynamics (MD) simulations with FRET measurements, was used to characterize the consequences of the Leu33$\rightarrow$Pro33 exchange on the structural dynamics of $\alpha_{IIb}\beta_3$ at an atomic level. For MD simulations, the ectodomains of the two $\alpha_{IIb}\beta_3$ variants in the closed conformation were used as model systems, and simulations of in total 3 µs length were carried out. For the FRET measurements, transfected HEK293 cells stably expressing either the Leu33 or Pro33 isoform of $\alpha_{IIb}\beta_3$ were generated; cyan or yellow fluorescent proteins (CFP; YFP) were cloned to the C-termini of the α_{IIb} or β_3 subunits prior to transfection, respectively. FRET measurements were applied to explore conformational changes in the cytoplasmic tails upon integrin activation.

Comparative analyses of the MD trajectories revealed that Leu33 is involved in stabilizing interactions connecting the PSI domain in the head region and the nearby EGF-I and EGF-II domains in the leg region of the β_3 subunit. The absence of this network of interactions in the Pro33 variant gives rise to an instability that gradually affects the entire structure, thus leading to the system being globally less stable. In agreement to our findings, upon integrin activation, FRET analyses indicated a more extended spatial separation (> 100 Å) of the cytoplasmic tails in Pro33 than in Leu33 cell clones ($p = 0.003$). Taken together, these results provide an explanation how the fine-tuned conformational equilibrium of the integrin can be allosterically influenced by a single-point mutation located more than 90 Å away from any ligand binding sites of the integrin.

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