The mutation Ser2117Leu, in the second hydrophobic spike of the factor V C2 domain, increases phospholipid affinity and specific activity

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Factor VIII (fVIII) binds to phospholipid (PL) membranes, to von Willebrand factor (vWf) and to factor IXa via motifs localized to the C2 domain. We have shown that PL binding and vWf binding are mediated by two pairs of hydrophobic residues, each displayed at the tips of beta-hairpin turns. The homologous hydrophobic residues in the C2 domain of factor V also contribute to PL binding. We hypothesized that these hydrophobic residues of the fVIII C2 domain make specific contacts with PL that may explain the different PL binding properties of the two proteins. To test this hypothesis, we have prepared fVIII/fV hybrid mutants in which either amino acid(s) of the fVIII C2 domain were changed to the homologous residues of fV (Mutants #1-M/F 2199/2200 W/W, #2-L/L 2251/2252 L/S, and #3-M/F/L 2199/2200/2252 W/W/S) or the complementary fV/FVIII hybrid mutations in which amino acids of the fV C2 domain were changed to the homologous residues of fVIII (Mutants #4 W/W 2063/2064 M/F, #5 L/S 2116/2117 L/L, and #6 W/W/S 2063/2064/2117 M/F/L). Mutants were expressed in COS-1 cells and purified by immunoaffinity chromatography and/or FPLC. The fVIII/fV hybrid mutants #1-3 had specific activities that equalled or exceeded wild type fVIII in both 1-stage and 2-stage commercial aPTT assays that contain a large excess of PL. In a PL-limiting Xase assay (sonicated vesicles of PS : PE : PC 4 : 20 : 76, 0.15 µM PL), the mutants had 80-95% reduction in specific activity. Phospholipid titration indicated that the phospholipid affinities were >fivefold higher, >fivefold lower, and unchanged for mutants #1-3. The apparent affinity for factor IXa was decreased for all mutants. The specific activity of the fV/FVIII hybrids exceeded those of wild type factor V in a prothrombin time assay with factor V deficient plasma. PL affinites are 9.8 and 21-fold higher than wild type factor V for mutants #5 and #6 which contain the S[rarr]L change in the second hydrophobic spike. Together, these results indicate that Leu vs. Ser in the second hydrophobic spike (fVIII-2252, fV-2117) enhances PL affinity at least ninefold for both factor VIII and factor V. While the Trp-Trp pair constituting the 1st hydrophobic spike appears to confer a somewhat higher PL affinity but reduced factor IXa affinity than the native Met-Phe pair of factor VIII. These results

suggest that the specific amino acids of the hydrophobic spikes contribute to the particular membrane binding properties of factor VIII and factor V.

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