"The importance of exosite interactions for the cleavage efficiency of thrombin – Analysis of the three cleavage sites in coagulation factor VIII"

Gurdeep Chahal

Thrombin is a critical enzyme in the blood coagulation cascade. This serine protease is known to cleave a number of physiological substrates including fibrinogen, coagulation factor V, factor VIII and several protease activated receptors. In addition to the residues within its active site, the specificity of thrombin for different substrates is determined by the two electropositive exosites I and II. Inactive factor VIII is a heterodimeric protein comprised of a heavy chain (A1-a1-A2-a2-B domains) and a light chain (a3-A3-C1-C2 domains), which is activated through limited proteolysis by thrombin at P1 residues; Arg_{372} (a1-a2 domain junction), Arg_{740} (a2-B domain junction), and Arg_{1689} (a3-A3 junction). There is a cluster of negatively charged amino acids localized N-terminal to these three cleavage sites, which could potentially form electrostatic complementarity with the electropositive exosites in thrombin for efficient cleavage at the individual proteolytic sites.

I have studied the role of such exosite interactions for the cleavage efficiency of thrombin for factor VIII. I compared the efficiency in cleavage by thrombin of just the minimal cleavage site P4-P4' for each of the three cleavage sites to the thrombin consensus cleavage sequence (P4-P4′=LTPRGVRL) which was identified, previously, in our lab. The P4-P4′sequence for R372 cleavage site showed barely any cleavage in comparison to the consensus site, which explains its low cleavage rate. After adding the N terminal region rich in negative charges, the activity of the Arg_{372} cleavage site increased by approximately 30 fold and is now only 3-4 fold less efficient than the consensus site. The importance of these negative charges for this interaction can be analyzed by the fact that when half the negative charges from this region were replaced with neutrally charged amino acids, the activity dropped by 12-15 fold. In contrast, the minimal P4-P4' region of the Arg_{740} cleavage site, which conforms relatively closely to the consensus site, is cleaved nearly at the same rate as the thrombin consensus sequence. The addition of the negatively charged region to the P4-P4' minimal region for Arg_{740} site increased the activity by approximately 6-7 fold. This site was then 2-3 fold more efficient than the consensus site. By replacing more than half of these negatively charged residues with the uncharged residues, the activity dropped by 3-4 times and this site were now almost as efficient as the minimal P4-P4' site. The result from the R1689 site, which also closely conforms to the consensus site, was almost identical to the R740 site.

These results indicate that these negatively charged exosite interacting regions primarily affect the cleavage rate of poor cleavage sites like R372 and only minimally affect sites that conform closer to the consensus site. So, the cleavage at this site could likely constitute a good model for studying exosite interactions for thrombin.

Degree project in biology, Master of Science (2 years), 2012
Examensarbete i biologi 45 hp till masterexamen, 2012
Institutionen för biologisk grundutbildning och Institutionen för Cell- och Molekylärbiologi, Uppsala universitet
Handledare: Professor Lars Hellman