Abstract

Thrombin contains electropositive patches at opposite poles of the molecule which represent potential exosites for the binding of macromolecular ligands. The function of anion-binding exosite I, the fibrin(ogen) recognition site, has been well described. Anion-binding exosite II, located near the carboxyl terminus of the molecule, has been proposed to bind heparin on the basis of chemical modification studies. To define the functional heparin-binding site on thrombin, purified recombinant alpha-thrombins were prepared with glutamic acid substitution for selected basic amino acid residues in exosite II or exosite I. Heparin affinity was assessed by NaCl gradient elution from heparin-agarose, and second-order rate constants for inhibition by antithrombin III were determined in the absence and presence of heparin. Affinity for heparin-agarose was reduced markedly by selected mutations in exosite II (R89E, R245E, K248E, and K252E, numbered from the amino terminus of the B chain) but not by other mutations in exosite II (K174E, K247E) or by mutations in exosite I (R68E, K154E). All recombinant thrombins had similar rate constants for inhibition by antithrombin III without heparin. However, affinity for heparin-agarose correlated directly with the rate of inhibition by antithrombin III with heparin. These results demonstrate that selected mutations in anion-binding exosite II define a functional heparin-binding site and support the template mechanism of heparin action.

PMCID: PMC44027 **Free PMC Article**