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Title Role of Hydrophobic Amino Acids in Heparin Binding and Conformational Activation of Antithrombin III
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Abstract To, date most work on heparin binding and activation of Antithrombin III (ATIII) has focussed on basic ATIII residues and anionic groups on heparin. Salt dependence studies of heparin binding to ATIII indicate that ionic and non-ionic interactions contribute ~40% and ~60% of the binding free energy, respectively. Thus, a predominant nonionic component of the binding interaction remains to be identified and investigated. F121 and F122 were selected for investigation based on their proximity to positively charged residues involved in pentasaccharide binding and their conservation in ATIIIs from different vertebrate species, but not in different branches of the serpin family. We made Phe 122 Leu (F122L) and Phe 121 Ala (F121A) substitutions on a beta-ATIII background (N135A) and characterized variant inhibition, heparin binding and activation properties. Dissociation equilibrium constant (Kd) studies showed ~2000- and ~13-fold decreased affinities for full-length heparin (HAH) binding to F122L and F121A respectively. Ionic strength dependence studies revealed that, the decrease in affinities of pentasaccharide and HAH for F122L were exclusively due to the loss of non-ionic interactions, with no loss in number of ionic interactions. F121 and F122 together contribute 43% of the total binding free energy and 77% of the energy of non-ionic binding interactions. Rapid kinetics analysis of heparin binding showed that F122 is critical for promoting a normal rate of conformational change and stabilizing the high affinity, activated binary complex. Structural analysis suggests that F122 and F121 contributions to binding energy are mediated indirectly, through contacts between their phenyl rings and the nonpolar stems of positively charged heparin binding residues. Kinetic and structural considerations suggest that, although hydrophobic residues F122 and F121 make minimal contact with the pentasaccharide, they play a critical role in heparin binding and activation of ATIII by coordinating the P-helix-mediated conformational change and organizing a network of ionic and nonionic interactions between positively charged heparin binding site residues and the cofactor. Hydrophobic residues towards C-terminal end of D-helix may similarly be imporant.