

The following is an abstract for a talk that was given by Cynthia B. Peterson at the 17th International Congress of the International Society for Fibrinolysis and Proteolysis in Melbourne, Australia on March 22, 2004.

A Model of the Structure of Vitronectin from Small-Angle Scattering and NMR Measurements. Gary S. Lynn[†], Anand Mayasundari^{*}, Nancy Horn^{*}, William Heller[†], Neil Whittimore^{*}, Engin Serpersu^{*} and Cynthia B. Peterson^{*}, [†]Chemical and Analytical Sciences Division, Oak Ridge National Laboratories, and the ^{*}Biochemistry and Cellular and Molecular Biology Department, the University of Tennessee, Knoxville, TN.

Human vitronectin is a 72K circulating protein that regulates fibrinolysis, tissue remodeling and tumor progression. These functions are largely mediated via interactions with the serine protease inhibitor, plasminogen activator inhibitor-1 (PAI-1). We recently predicted a model for three vitronectin domains, and we now report analyses using small-angle scattering and NMR. The N-terminal domain of vitronectin has been identified as a primary binding site for PAI-1. We have collected one- and two-dimensional NMR spectra in both H₂O and D₂O on a 51-residue N-terminal fragment isolated from a CNBr digest. Non-exchangeable proton assignments have been completed and used to identify exchangeable protons from spectra obtained in 90% H₂O/10% D₂O. Secondary structure has been derived from sequential near-neighbor inter-proton NOEs and scalar couplings. Three-bond scalar couplings defining the ϕ -dihedral angle were obtained from 2D DQF-COSY spectra. Integrated peak volumes defining strong, medium, and weak NOEs have defined 372 distance constraints, which have been used along with 22 dihedral restraints in distance geometry, simulated annealing, and refinement calculations. We observe an elongated structure for this domain, with a core stabilized by 3 of its 4 disulfides. Small-angle x-ray scattering (SAXS) measurements on full-length vitronectin were carried out at ORNL and at the National Synchrotron Light Source. Vitronectin samples were monodisperse at concentrations from 1 to 10 mg/mL. Guinier plots of the SAXS data yield straight-line fits corresponding to an R_g of 26.6 ± 0.3 Å. The consensus envelope of vitronectin developed from the SAXS data using GA_STRUCT is ellipsoidal, with evidence for a large and small domain. A model for vitronectin has been calculated using the NMR structure for the N-terminal domain and threaded models for the central and C-terminal domains fit within the three-dimensional consensus envelope.