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Elucidation of the molecular interaction mechanism between PAI-1 and a PAI-1 inhibiting antibody fragment Fab-55F4C12

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Introduction: Plasminogen activator inhibitor-1 (PAI-1) is a member of the serine protease inhibitor (serpin) superfamily and is the principal inhibitor of the plasminogen activators tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) *in vivo*. In healthy individuals, PAI-1 is found at low levels in the plasma, but is elevated significantly in a number of diseases, including atherosclerosis, deep vein thrombosis, and non-insulin dependent diabetes mellitus.
Objective: Elucidation of the molecular interaction mechanism between PAI-1 and a PAI-1 inhibiting antibody fragment Fab-55F4C12.
Methods: Fab-55F4C12 was generated by papain digestion of MA-55F4C12, followed by protein A and gelfiltration purification. The purified Fab-55F4C12 was concentrated to OD = 10. Different crystallisation screens were tried, and initial crystal clusters were obtained in condition 19 of Structure Screen 1 of Molecular Dimensions. Small needles (0.2 x 0.1 x 0.1 mm) were obtained after intensive optimisation of the crystallisation condition. The crystal structure of Fab-55F4C12 was determined by X-ray crystallography at cryogenic temperature. The data set was collected at DESY (Hamburg, Germany) to a resolution of 2.7 Å. Data processing was done using MOSFLM and SCALA. The space group was assigned to be $P2_12_12$ with unit-cell parameters a = 52.04 Å, b = 98.66 Å, c = 191.68 Å, with two molecules in the asymmetric unit. The data set is 99.72 % complete. Initial phases were obtained with molecular replacement. The structure was refined using Coot and Refmac5. Crystallisation of the Fab-55F4C12 / PAI-1 complex has been unsuccessful so far. Therefore, the complex is being modelled through docking of the crystal structures of the two subunits of the complex using the rigid-body docking programs DOT and ZDOC. Potential interactions between the two subunits will be deduced from the models and compared with epitope information gathered from mutagenesis studies.
Conclusions: Characterization of the complex of Fab-55F4C12 with PAI-1 may provide valuable information on the molecular interactions between the Fab-fragment and PAI-1, leading to a better understanding of the mechanism of inhibition. The elucidation of the binding site of inhibitory monoclonal antibodies may contribute to the rational design of PAI-1 modulating therapeutics.

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Associations between Human Paraoxonase and Human Phosphate Binding Protein

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While the involvement of the calcium-dependent, HDL-associated paraoxonase 1 (PON1), in atherosclerosis prevention is well established [1], its physiological function remains unknown. PON1 is known for its ability to hydrolyze paraoxon. Current research is focused on its capacity to inactivate various organophosphorous compounds, including nerve gases and pesticides. In preparing PON1 for functional studies, we have interestingly isolated another 38 kDa protein which co-purifies with it at the same time. Thus, we have successfully solved the structure of this protein, which is a human plasma solute-binding protein (SBP) that binds phosphate by X-rays diffraction. Its structure is similar to the prokaryotic phosphate solute-binding proteins (SBPs) associated with ATP binding cassette transmembrane transporters, though phosphate-SBPs have never been characterized or predicted from nucleic acid databases in eukaryotes. Human Phosphate Binding Protein (HPBP) crystallizes as a homo-dimer that we assume to be physiologically relevant because its interface, which is mostly hydrophobic, has an area of about 790 $Å^2$, a typical value for transient protein complexes. This area is postulated to interact with the hydrophobic HDL-associated HPON1. The conformational changes induced by the composition of the crystallization liquor could have favoured the dissociation of the complex and privileged crystallization of HPBP alone. Indeed, it has been shown that, upon substrate binding, SBPs undergo a conformational change concerning the two domains that are connected by a flexible hinge (Felder et al., 1999). This movement, described as a ″Venus fly trap motion ″ was also predicted for HPBP using the program ElNemo [2]. Recent studies obtained by gel filtration chromatography indicate that oligomeric composition of HPON1, HPBP and HPON1/HPBP complex(es) are dependent on the calcium, phosphate and detergent concentrations. Here we report the study of this association by gel filtration chromatography approach. The knowledge of these oligomeric reorganizations of the HPON1/HPBP complex is essential to establish the potential physiological role of HPBP, potentially involved in atherosclerosis prevention, by preventing growth of calcium phosphate crystals.

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