Low Resolution Structure Determination Shows Procollagen C-Proteinase Enhancer to be an Elongated Multi-Domain Glycoprotein

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Procollagen C-proteinases (PCPs) control a variety of morphogenetic events, such as dorso-ventral patterning and extracellular matrix assembly, during development and tissue repair [1]. Procollagen C-Proteinase Enhancer (PCPE) is an extracellular matrix glycoprotein which can enhance the activities of PCPs, on procollagen substrates, by up to 20-fold [2]. Like PCPs, PCPE is a modular CUB domain containing protein, though it lacks intrinsic enzymatic activity. In addition to its acidic N-terminal CUB domains 1 and 2, PCPE has a basic C-terminal NTR (netrin-like) domain, homologous to the active region of TIMPs (Tissue Inhibitors of MetalloProteinases). PCP enhancing activity is a property of the CUB domain region of PCPE. The aim of this study was to determine the overall shape of PCPE in solution.

Active recombinant human PCPE was expressed in 293-EBNA cells and purified as described for the baculovirus expressed protein [3]. When analysed by analytical ultracentrifugation, PCPE sedimented essentially as a single population with sedimentation ($s_{20,w} = 3.17 \times 10^{-13}$ s$^{-1}$) and translational diffusion ($D_{20,w} = 6.5 \times 10^{-7}$ cm$^2$s$^{-1}$) coefficients consistent with the calculated molecular mass of 49 kDa for monomeric PCPE. In addition, the diffusion coefficient was consistent with a prolate ellipsoid (cigar shape) of axial ratio (length:breadth) 5:1. When observed directly by transmission electron microscopy after rotary shadowing, PCPE was frequently rod-like in appearance, with approximate dimensions 200 x 80 Å, consisting of two closely adjacent lobes.

Analysis by small angle X-ray scattering (SAXS) gave further insights into the three-dimensional structure of PCPE. For this, X-ray scattering from solutions of PCPE at concentrations of 3 to 30 mg/ml was measured, as a function of scattering angle, on the X33 camera at EMBL on storage ring DORIS III at DESY. Extrapolation to zero angle confirmed that the protein was monomeric. Direct transformation of the data to yield the pair distribution function $p(r)$ showed the maximum dimension of PCPE to be 150 Å, about 3x greater than that calculated for a sphere of the same molecular mass, and consistent with both the analytical ultracentrifugation and electron microscopy data.

More detailed information about the shape of PCPE was obtained by ab initio model fitting to the SAXS data using the programmes DALAI GA [4], DAMMIN [5]and GASBOR [6]. All three programmes gave similar results. The average of ten best fit structures obtained using DAMMIN is shown (in grey) in Fig. 1. PCPE is clearly highly elongated with a long arm and a short arm connected by a kink. Also shown superimposed on the structure are three-dimensional atomic
models for each domain in PCPE (CUB1, CUB2, NTR) calculated using GENO-3D [7] based on known 3D structures for CUB domain proteins (spermadhesins) and TIMP-2 (for the NTR domain). Using these models, together with structures for connecting and terminal sequences generated by CREDO [8], satisfactory agreement between observed and calculated SAXS data was obtained.

**Figure 1.** Low resolution structure of PCPE based on model fitting to the SAXS data. The average of ten best fit models is shown in light grey, as 3 orthogonal views. Superimposed on the structure (darker grey) are 3D models of the CUB1, CUB2 and NTR domains based on known 3D structures of homologous proteins.

The low resolution structure of PCPE gives insights into the mechanism by which this protein enhances the activity of PCPs. We have recently obtained evidence that PCPE binds not only to the junction region of the procollagen C-propeptide trimer but also to the collagenous region of the procollagen molecule [2]. This might lead to a conformational change in the substrate that facilitates cleavage of procollagens by PCPs. Comparison of the PCPE structure with that recently obtained for the C-propeptide trimer [9] shows that the length of the PCPE molecule is consistent with multiple binding.

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**References**


