Structure Preview

The Gentle Grip of a Helping Hand

Author: Andrew R. Pickford

Affiliation: Biophysics Laboratories, Institute of Biomedical and Biomolecular Sciences, University of Portsmouth, PO1 2DY, United Kingdom

E-mail: andy.pickford@port.ac.uk

Telephone: (+44) 2392 842055

Fax: (+44) 2392 842070

Summary: *Using NMR spectroscopy, Zhao and co-workers, in this issue, have modelled the short-lived complex formed between the MT1-MMP hemopexin domain and a synthetic triple-helical collagen mimetic. Their model is consistent with two alternative mechanisms for the breakdown of collagen by the enzyme.*

The matrix metalloproteinases (MMPs) are a family of enzymes involved in proteolysis of extracellular matrix components (Nagase et al., 2006). Membrane-type 1 (MT1-) MMP is one of a subset that are able to cleave interstitial collagens (types I-III), a process termed "collagenolysis" (Fields, 2013). The initial cleavage of the intact collagen triple helix into ¾- and ¼-length fragments is the rate-determining step in its breakdown. Furthermore, collagenolysis of an assembled quarter-staggered fibril (the naturally-favoured state of interstitial collagens) provides the energy for biased diffusion of the enzyme along that fibril surface (Collier et al., 2011). Hence, the precise mechanism of collagenolysis is of considerable biological interest.

In each collagenolytic MMP, the hydrolytic apparatus resides within the catalytic (CAT) domain but, with the exception of MMP-12, efficient collagen cleavage also requires the hemopexin (HPX) domain to act as a "helping hand" (Bode, 1995). In MT1-MMP, this domain enhances collagenolysis 3-4 fold, whilst in most other collagenolytic MMPs it is absolutely required (Nagase et al., 2006). Therefore, elucidating the specific role of the HPX domain is crucial to fully understanding the process.

To date, the only high-resolution investigations of MMP-collagen interactions have been derived from crystallographic and solution NMR analyses of MMP-1 in complex with a triple helical peptide (THP), a collagen-like mimetic, that encompasses the MMP cleavage site (Manka et al., 2012; Bertini et al., 2012). MMP-1 is the best-characterised collagenolytic MMP to date, but is significantly different to the "ectodomain" (the extracellular portion) of MT1-MMP, most notably in possessing a relatively short polypeptide linker between the CAT and HPX domains that keeps them in close proximity when their partially stable "ball-and-socket" interface transiently dislocates (Arnold et al., 2012; Cerofolini et al., 2013). In contrast, the MT1-MMP linker is twice the length of that in MMP-1 and is also O-glycosylated, so is considered to be highly flexible. How then do the

doi:10.1016/j.str.2015.01.006

two domains of MT1-MMP co-operate in bringing about collagen breakdown when the enzyme is so dynamic?

Here, Zhao and co-workers describe the structure elucidation of a short-lived complex formed between the MT1-MMP HPX domain and a homotrimeric THP using solution NMR spectroscopy. The transience of the interaction prevents traditional NMR structure determination based on inter-molecular nuclear Overhauser enhancements (NOEs) which can only be observed when a complex has a relatively long lifetime. Instead, the authors determined the most likely conformation in solution by "soft-docking" a THP homology model onto the previously-solved HPX crystal structure (Tochowicz et al., 2011) using inter-molecular restraints derived predominantly from paramagnetic relaxation enhancements (PREs). PREs are through-space effects, emanating from a paramagnetic centre (in this case, the nitroxide spin-label TOAC), which are detectable even when a complex forms only transiently. By utilising two THP samples with differing TOAC positions, the authors gained sufficient restraints to successfully derive a structure that not only both locates and orients the HPX domain on the THP, but also demonstrates a preference for binding to one of the three THP chains (Figure 1).

To those unfamiliar with the triple helical structure, such a preference for a protein to bind a particular chain in a homotrimer may seem strange. However, the THP has only quasi-three-fold symmetry, since each of its three chains is axially-offset with respect to its neighbours; hence, the "leading", "middle", and "trailing" chain nomenclature. This places equivalent residues in the three chains in subtly different environments despite each chain having essentially the same fold, that of a polyproline II helix. The result is three distinct "faces" of the homotrimeric THP between which binding partners can discriminate.

The collagen binding site on the MT1-MMP HPX domain involves residues conserved amongst collagenolytic MMPs, and is similar to that of MMP-1 in that it involves blades I and II of the four-bladed β-propeller. However, despite MT1-MMP and MMP-1 both cleaving the collagen chains at the same location (the Gly775-Ile776 peptide bond), the position of their HPX domains on the THP differ markedly, with that for MT1-MMP displaced approximately 25 Å N-terminally along the helical axis. Intriguingly, this places the MT1-MMP HPX domain virtually alongside the scissile bond (Figure 1), and thus appears to rule out the possibility that the productive complex between collagen and intact MT1-MMP involves a side-by-side arrangement of the CAT and HPX domains as seen for MMP-1 (Bertini et al., 2012; Manka et al., 2012).

How then does this transient HPX-triple helix interaction lead to collagenolysis? The authors present two valid hypotheses. Firstly, they consider a "sliding model" in which the HPX domain undergoes an axial displacement towards the C-terminus of the triple helix, thus allowing the CAT domain to associate with the triple helix alongside it and so access the scissile bond (i.e. analogous to MMP-1). In this model, the reported transient association would serve to pre-concentrate the MT1-MMP in the vicinity of the cleavage site, and thus accelerate the rate of collagenolysis.

As an alternative, the authors also consider a "clasping model" in which the reported complex does indeed represent the final position of the HPX domain, and thus the CAT domain must wrap around the triple helix in order to access a scissile bond on the opposite side. Such a mechanism would necessitate a highly flexible domain arrangement, a property previously suggested by the nature of the interdomain linker, and evidenced by the presented atomic force

doi:10.1016/j.str.2015.01.006

microscopy data which confirms that there is no stable non-covalent interaction between the two domains. However, whilst this model is attractive for the hydrolysis of an isolated triple helix, it is difficult to imagine it occurring on the surface of an interstitial collagen fibril without localised fraying of triple helices from the surface to allow their unhindered access by the CAT domain of the enzyme.

The impressive study by Zhao and co-workers provides an excellent platform for future research to discriminate between these mechanistic possibilities by examining the collagen-binding activity of the intact MT1-MMP ectodomain, but the enzyme's size, flexibility and post-translational modifications mean that it will remain a particularly challenging target for biophysical analyses.

References

1. Arnold, L.H., Butt, L.E., Prior, S.H., Read, C.M., Fields, G.B., and Pickford, A.R. (2011). J. Biol. Chem. *286*, 45073-45082.

2. Bertini, I., Fragai, M., Luchinat, C., Melikian, M., Toccafondi, M., Lauer, J.L., and Fields, G.B. (2012) J. Am. Chem. Soc. *134*, 2100-2110.

3. Bode, W. (1995) Structure *3*, 527-530.

4. Cerofolini, L., Fields, G.B., Fragai, M., Geraldes, C.F., Luchinat, C., Parigi, G., Ravera, E., Svergun, D.I., and Teixeira, J.M. (2013). J. Biol. Chem. *288*, 30659-30671.

5. Collier (2011) Collier, I.E., Legant, W., Marmer, B., Lubman, O., Saffarian, S., Wakatsuki, T., Elson, E., and Goldberg, G.I. (2011). PLoS ONE *6*, e24029.

6. Fields, G. B. (2013) J. Biol. Chem. *288*, 8785-8793.

7. Manka, S.W., Carafoli, F., Visse, R., Bihan, D., Raynal, N., Farndale, R.W., Murphy, G., Enghild, J.J., Hohenester, E., and Nagase, H. (2012). Structural insights into triple-helical collagen cleavage by matrix metalloproteinase 1. Proc. Natl. Acad. Sci. U. S. A. *109*, 12461-12466.

8. Nagase, H., Visse, R., and Murphy, G. (2006). Cardiovascular Res. *69*, 562-573.

9. Tochowicz, A., Goettig, P., Evans, R., Visse, R., Shitomi, Y., Palmisano, R., Ito, N., Richter, K., Maskos, K., Franke, D., *et al.* (2011). J. Biol. Chem. *286*, 7587-7600.

10. Zhao, Y., Marcink, T.C., Gari, R.R.S., Marsh, B.P., King, G.M., Stawikowska, R., Fields, G.B., and Van Doren, S.R. (2015). Structure (this issue)

doi:10.1016/j.str.2015.01.006

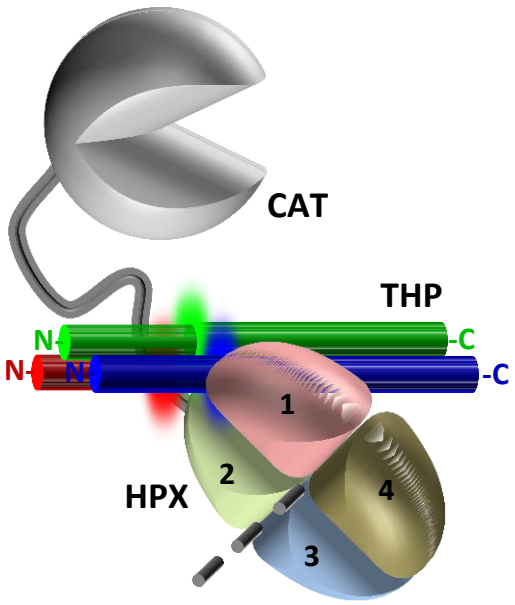

Figure 1. The MT1-MMP HPX domain in a transient complex with a collagen THP. A highly-stylised representation of the complex is shown, with the four blades of the HPX domain β-propeller coloured independently and labelled. In the complex, the β-propeller axis (marked with a dashed line) is at approximately 45° to that of the THP. The CAT domain and interdomain linker (which were absent from the construct used for the NMR studies, but present in the collagenolysis assays) are shown in grey; their orientation and position here are arbitrary. For simplicity, the three THP polypeptides are shown as cylinders (rather than helices) and coloured red, green and blue for the leading, middle and trailing chains, respectively, and the approximate positions of their scissile bonds (Gly775-Ile776) are highlighted with halos.

doi:10.1016/j.str.2015.01.006