C1 Inhibitor Serpin Domain Structure Reveals the Likely Mechanism of Heparin Potentiation and Conformational Disease

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C1 inhibitor (C1-inh), a member of the serpin family, is a major downregulator of inflammatory processes in blood. C1-inh is the only inhibitor known that acts on enzymes involved in early steps of the classical and lectin pathways of complement activation [1]. Genetic deficiency of C1-inh results in hereditary angioedema, a dominantly inheritable, potentially lethal disease [2]. Activities of many serpins are modulated by ligand binding. Antiinflammatory activity of heparin a naturally occurring polyanion is realized through increasing inhibitory activity of C1-inh [1]. There are other examples of serpin-heparin-protease interactions: Heparin enhances the inhibitory effect of the serpin antithrombin by “bridging” it to thrombin [3,4] and by allosteric effects. Polyanion potentiation of protein C inhibitor against protein C by the “co-occupation” mechanism [5] is also based on migration of proteins along the heparin chain.

We expressed the serpin domain of human C1-inh in \textit{Pichia pastoris} and crystallized it. X-ray diffraction data were collected on EMBL beamline X11. (Space group: P6\(_5\), unit cell dimensions: a=98.9 Å, c=94.7 Å resolution limit: 2.35Å.) The structure was solved by molecular replacement using prealigned structures of various serpins as search model. The \(R\)\textsubscript{work} and \(R\)\textsubscript{free} values of the refined model are 0.174 and 0.218, respectively [6].

The structure shows latent conformation. The protein is uncleaved with the reactive center loop (RCL) buried in the central β-sheet of the protein and the cleavage site is stabilized by hydrogen bonds. The C-terminal portion of the RCL unusually forms a 7\(^{th}\) strand of the central β-sheet. The unique conformation of the C-terminal six residues suggests its potential role as a barrier in the active-latent transition (Figure 1).

Based on the structure we explained the effect of two common mutations of C1-inh. The polymorphism V458M affects 30% of people. This mutation has no functional effect, although the conserved hydrophobic central β-sheet is involved. The crystal structure reveals residue 458 is in a large buried cavity, which explains why this steric change does not disrupt internal packing. The A436T mutation affects a residue whose side chain becomes buried upon RCL incorporation. Unlike similar mutants of other serpins, this mutant is not cleavable by proteinases. From the structure, it is evident that the mutant residue packs better in the loop-inserted latent C1-inh than the wild type residue, which explains its observed preference for the more stable latent conformation.

To explore the structural background of heparin potentiation of C1-inh we carried out blind docking calculations with a disaccharide naturally occurring in heparin. The two binding sites found are in a positively charged patch in the proximity of the enzyme-binding region (Figure 1A inset). Earlier mutation experiments [7] and our affinity measurements confirm the heparin binding site. Furthermore the effect of heparin on the C1-inh - protease reaction correlates with the net charge of the binding region: the more positive the enzyme surface is, the more enhanced is the accelerating effect. Based on these results we propose a novel mechanism of the effect of polyanions in serpin action (Figure 1B inset). In this ”sandwich” model the role of the polyanion is charge compensation and no allosteric effect is needed. This model is consistent with the low dependence of the effect on the polyanion chain length.
Figure 1: Structure of C1-inh serpin domain (left). The RCL region is shown in black. A: Result of blind docking. The RCL in the active conformation is shown as black dashed line. B: “Sandwich” mechanism of the effect of heparin on the protease-C1-inh interaction.

These results provide the explanation of heparin’s antiinflammatory activity and may help improving therapeutic C1-inh preparations in treatment of common inflammatory diseases, such as organ transplant rejection and heart attack.

References

[1] I. G. A. Bos, C. E. Hack, and J. P. Abrahams, Immunobiology 205, 518 (2002)
[2] S.A. Cumming, D.J. Halsall, P.W. Ewan and D.A. Lomas, J. Med. Genet. 40, 114 (2003)
[3] W. Li, D.J. Johnson, C.T. Esmon and J.A. Huntington, Nat. Struct. Mol. Biol. 11, 857 (2004)
[4] A. Dementiev, M. Petitou, J.-M. Herbert, and P. G. W. Gettins, Nat. Struct. Mol. Biol. 11, 863 (2004)
[5] J.A. Huntington, M. Kjellberg and J. Stenflo, Structure 11, 205 (2003)
[6] L. Beinrohr, V. Harmat, J. Dobó, Z. Lörincz, P. Gál and P. Závodszky, J. Biol. Chem. 282, 21100 (2007)
[7] I.G.A. Bos, (2003) C1-Inhibitor potentiation by glycosaminoglycans, CIPGegevens, Koninklijke Bibliotheek, Hague, The Netherlands

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