CONFORMATIONAL ANALYSIS OF SERINE PROTEASE INHIBITORS AND ITS APPLICATIONS FOR DRUG DESIGN

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The uncontrolled activity of serine proteases has been implicated in a wide range of pathological conditions (Travis and Salvesen, 1983; Weiss, 1989), and so the ability to design specific inhibitors has considerable therapeutic importance. Most natural inhibitors are relatively large proteins and are unsuitable for use as pharmaceuticals. There is therefore a need for novel peptide inhibitors which are small and stable enough to be formulated more readily as drugs.

The design of such inhibitors is best approached by a consideration of the structure and mechanism of the natural compounds. As a first step towards this goal we have extended and generalised the analyses carried out by previous workers (cf Greenblatt et al 1989), comparing the crystal structures of the natural inhibitors in their free and complexed states. The purpose of this study has been to examine the feasibility of basing the design of a novel inhibitor on the conserved features of the natural ones.

Using data obtained from the literature (Greenblatt et al 1989) and the Brookhaven Protein Data Bank (Bernstein et al 1977) we have compared the main chain torsion angles (ϕ, ψ) of the interacting residues of seven different inhibitors in their free and/or complexed states. The results are summarised in the Ramachandran plots shown in Figs. 1 and 2.

FIGURE 1

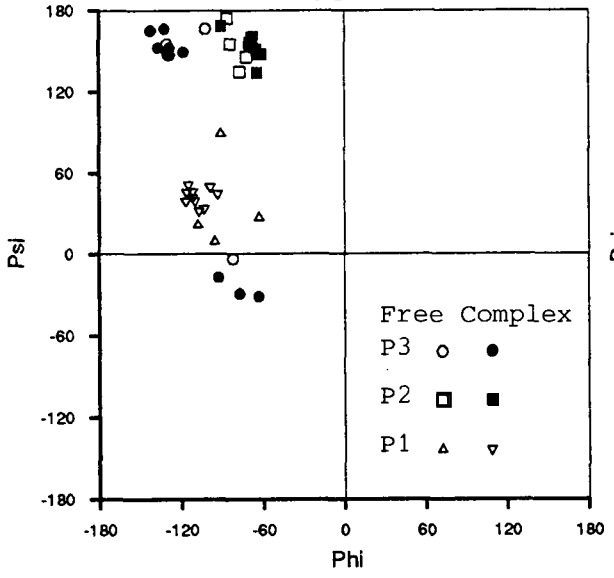
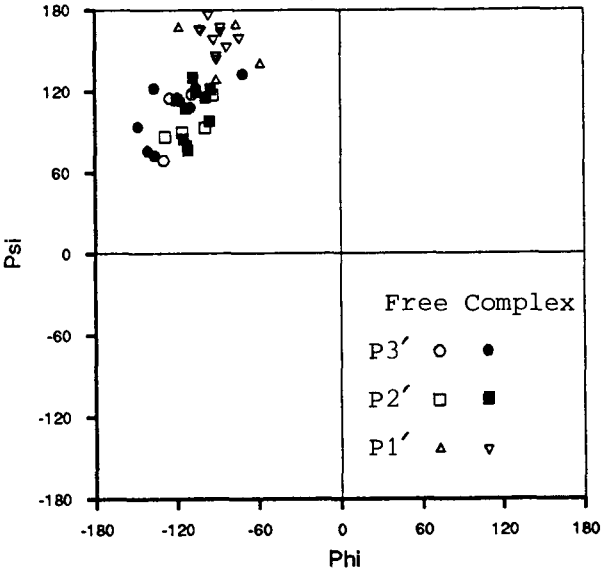


FIGURE 2



ϕ, ψ angles for inhibitor residues, P1→P3 (Fig 1) and P1′ →P3′ (Fig 2). Data is shown for free and 3 complexed forms of bovine pancreatic trypsin inhibitor, free and 2 complexed forms of ovomucoid, free and 1 complexed form of barley seed chymotrypsin inhibitor, free streptomyes subtilisin inhibitor, 1 complexed form of porcine pancreatic secretory inhibitor, 2 complexed forms of eglin-C and a complexed form of Chymotrypsin inhibitor-1 from Russet Burbank potato tubers.

The results show that there is little change in the main chain conformation of the interacting residues upon complexation. This implies a 'lock and key' rather than an induced fit mechanism, and indicates that the main chain conformation in this region determines the inhibitory activity, whereas the specificity is modulated by the nature and conformation of the interacting amino acid side-chains. These observations combined with the fact that the inhibitors from one source can inhibit enzymes from another suggest that the proteases and their partner inhibitors have developed through parallel convergent evolution.

It can be concluded that serine protease inhibitors must have a particular main chain conformation which is fairly rigid and that any novel inhibitor to be designed must meet this basic specification. Since the inhibitor specificity will depend upon the nature of the interacting side-chains, a study of the conformations of these is currently in progress. On the basis of these studies we propose to design novel peptide inhibitors of human neutrophil elastase, for the treatment of pulmonary emphysema.

Travis, J., Salvesen, G.S., (1983) Ann. Rev. Biochem. 52: 655-709
Weiss, S.J., (1989) New England J. Med. 320: 365-376
Bernstein, F.C. et al (1977) J. Mol. Biol. 112: 535-542
Greenblatt, H.M. et al (1989) J. Mol. Biol, 205: 201-228