A comparison of staphostatin B with standard mechanism serine protease inhibitors

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Staphostatins are the endogenous, highly specific inhibitors of staphopains, the major secreted cysteine proteases from *Staphylococcus aureus* [1, 2]. We have previously crystallized staphostatin B alone and in complex with an inactive variant of staphopain B that had an alanine residue instead of the active site cysteine residue. This work has shown that staphostatins A and B are active site directed inhibitors that span the active sites of their target proteases in the same orientation as substrates [3].

Together with biochemical work, the crystallographic results suggested a number of similarities between staphostatins and standard mechanism serine protease inhibitors: (i) The inhibitors are tight-binding, (ii) they interact with their target enzymes in a manner broadly similar to substrates, (iii) their binding loops are exposed and somewhat flexible in the free inhibitor structures (possibly with the exception of BPTI), (iv) the binding loops rearrange and assume a "canonical" conformation in the presence of the target enzymes, (v) the inhibitors appear largely resistant to cleavage, and (iv) peptides that mimic only the binding loop have much lower affinity than complete inhibitor molecules and behave as substrates, not as inhibitors [3, 4].

There are also obvious differences between staphostatins and standard mechanism inhibitors: (i) canonical mechanism inhibitors target serine proteases, staphostatins target staphopains, i.e. cysteine peptidases of the papain clan. (ii) We are not aware of any standard mechanism inhibitors that would have a fold similar to the lipocalin-like fold of staphostatins. (iii) Cleaved standard mechanism inhibitors retain their structures and can be religated by their target proteases [5]. Cleaved staphostatins precipitate [6], religation has not been observed to the best of our knowledge. (iv) In contrast to canonical mechanism serine protease inhibitors, staphostatins are highly sensitive to mutations in their binding loops [3]. (v) Detection of an acyl-enzyme intermediate has been reported for the complex of at least one standard mechanism inhibitor in complex with its target protease [4], although it remains to be proven that the acyl-enzyme was formed prior to the denaturation step that was required for analysis. No staphopain B-staphostatin B acyl-enzyme intermediate has been detected so far [3].

The aim of this study was to extend the comparison to include detailed geometric features in the vicinity of the active site and close to the potentially scissile amide bond of the inhibitor, that could not previously be studied because of the mutation in the active site cysteine residue. As staphopain B with intact active site is toxic to *E. coli*, we coexpressed the enzyme with staphostatin B. A tight, inactive staphopain B-staphostatin B complex was formed in vivo, and turned out to be very stable in all protein purification steps. The crystal form that was grown with the alanine mutant of protease could not be reproduced with the wild-type enzyme, but related crystallisation conditions allowed to grow staphopain B-staphostatin B crystals that belonged to space group P2(1), contained two complexes in the asymmetric unit, and diffracted to 1.9 Å on BW6, DESY. Native data were collected on this beamline, and the structure was solved by molecular replacement, using the previously solved complex of staphostatin B with an inactive staphopain B mutant (PDB-accession code 1PXV), as the search model.

The crystal structure shows continuous electron density for the binding loop of staphostatin B, and no density connecting the active site cysteine sulfur atom to the scissile peptide bond. Apart from this similarity, there are important differences: In complexes of serine proteases with their standard mechanism inhibitors, (i) the carbonyl oxygen atom of the reactive amide bond points towards the oxyanion hole of protease (ii) there is a short contact between the side chain oxygen

atom of the active site serine residue and the carbonyl carbon atom of the reactive amide bond of the inhibitor, (iii) the O(gamma)…C=O (where O(gamma) is the side chain oxygen atom of the active site serine residue, and C and O are the carbonyl carbon and oxygen atoms of the reactive amide bond) is around 90°, close to the Bürgi-Dunitz angle [4].

In contrast, in the staphopain B-staphostatin B structure, (i) the oxygen atom of the reactive amide bond points away from Gln237Nε and Cys243N, that according to most authors form the oxyanion hole (ii) the distance between the side chain sulfur atom of the active site cysteine residue and the carbonyl carbon atom of the reactive amide bond is longer than the sum of the van der Waals radii, (iii) the O(gamma)…C=O (where O(gamma) is the side chain oxygen atom of the active site serine residue, and C and O are the carbonyl carbon and oxygen atoms of the reactive amide bond is around 170°, and thus very different from the Bürgi-Dunitz angle.

At present, it is not clear how similar the staphopain B-staphostatin B complex is to the complex of a papain-type peptidase with a substrate. The arrangement in our crystal structure is strikingly similar to the "ion-molecule" complex that Kollman and coworkers have proposed for the Michaelis complex of a papain-type peptidase with a substrate [7], but it is significantly different from the conventionally assumed Michaelis complex.

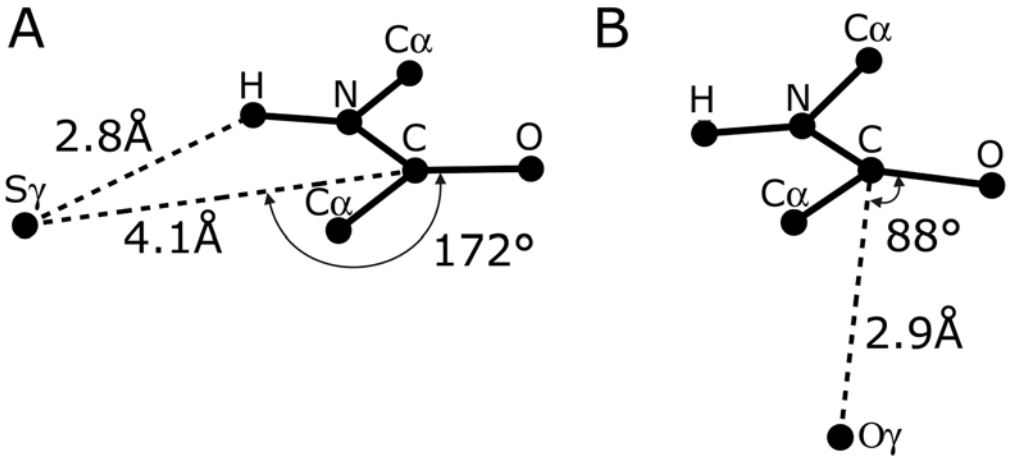

Figure 1: Arrangement of the active site nucleophile and the reactive amide bond in (A) the staphopain B-staphostatin B complex and (B) the complex of a serine protease with a standard mechanism inhibitor.

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