

Gas Phase Stability of Protein-Protein Complexes

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ESI-MS has been used extensively over the last decade to study protein-protein interactions [1]. Serine proteases and their inhibitors provide a valuable model for the study of protein complexes as a great deal is known about solution binding and about X-ray crystal structures of such systems. It is generally accepted that hydrophobic interactions are weakened in the gas phase and that hydrophilic interactions are strengthened. We have recently shown that a series of complexes of chymotrypsin and eglin c mutants containing variation in hydrophobic residues showed no difference in collision induced dissociation curves even though solution binding constants ranged from weak (μM) to strong (pM). Solution binding constants could only be correlated with results in MS, and not MS/MS, mode [2]. Here, we further that study by examining the binding of a select group of these mutants to the serine protease subtilisin Carlsberg. Eglin c has similar inhibition constants for subtilisin Carlsberg and chymotrypsin, but shows vastly different dissociation in the gas phase.

ESI-MS analysis was performed on complexes of eglin c and various mutants with the serine proteases chymotrypsin and subtilisin Carlsberg using both MS only and CID methods. 1:1 complexes were observed in all cases. For the subtilisin-eglin c complexes, an additional 38 Da was observed corresponding to a tightly bound Ca^{2+} that is also observed for subtilisin Carlsberg under non-denaturing conditions. The +11, +12, and +13 charge states were observed for all complexes with the +12 state being most abundant (Figure 1). The +12 charge state was chosen for CID experiments.

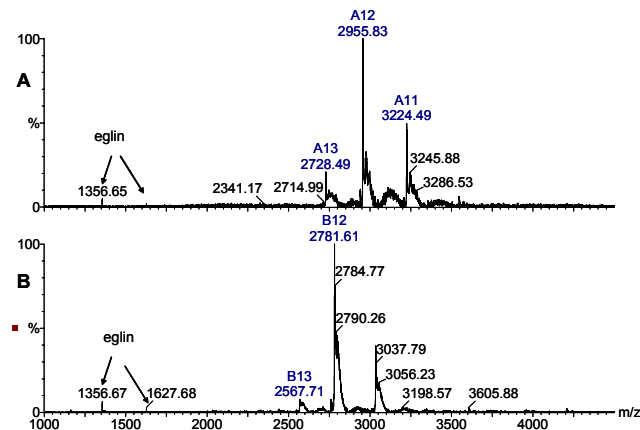


Figure 1. ESI-MS of wild-type eglin c bound to A) subtilisin Carlsberg (solution $K_d = 1.1 \text{ pM}$ [2]) and B) bovine chymotrypsin (solution $K_d = 1.4 \text{ pM}$ [2]). In each case protease and inhibitor were mixed in a 1:1 ratio ($10 \text{ }\mu\text{M}$) each. A small amount of free eglin c is observed possibly due to in source dissociation. Expected masses for each complex are A) 35 459 Da (27288 Da+8133 Da+38 Da) and B) 33 367 Da (25 234 Da + 8133 Da). Observed masses were 35 458 and 33 367 Da respectively.

The gas phase stability of these protease inhibitor interactions was probed by collision-induced dissociation (in the collision cell) of the +12 charge state. Subtilisin and chymotrypsin showed considerably different dissociation profiles with much more collision energy being required to break apart subtilisin-eglin c complexes (Figures 2 to 4). There was no apparent difference in dissociation observed between a single protease and the various mutants supporting our previously observed results [2]. Relative amount of remaining complex plotted as a function of collision energy shows a very sharp decrease in the amount of non-covalent complex for chymotrypsin-eglin with a bimodal distribution for subtilisin-eglin c. While it cannot be concluded that there is only one type of gas phase species present for chymotrypsin-eglin c, it can be argued that there are a minimum of two distinct species for subtilisin-eglin c. At low collision energy, dissociation of subtilisin-eglin c gives rise to the +6/+7 charge states of subtilisin and the +5/+6 charge states of eglin c while at high collision energies, dissociation generates the +9/+10 charge states of subtilisin and the +3 (and a small amount of the +2) species of eglin c (Figure 5). We have additional evidence suggesting that subtilisin binds at the N-terminus of eglin c as well as the accepted binding region.

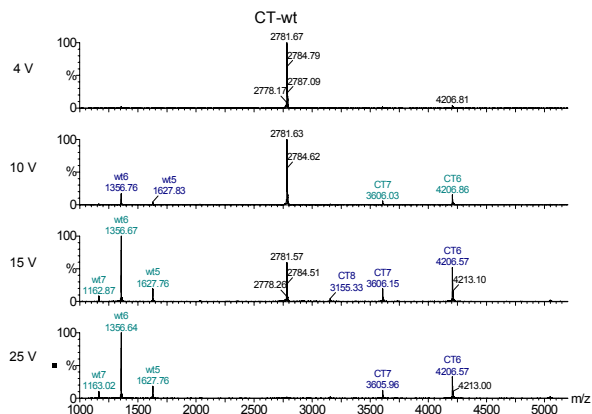


Figure 2. Gas phase dissociation of the chymotrypsin-eglin c complex +12 charge state. Dissociation is almost complete at 25 V.

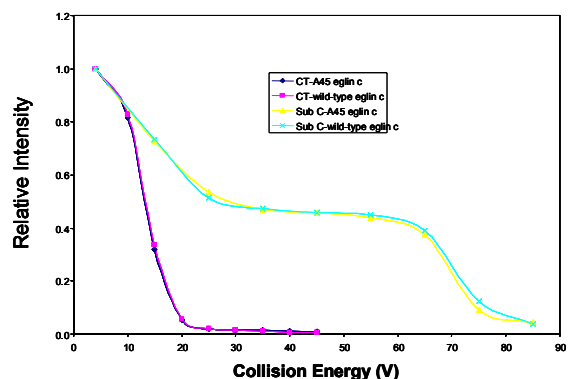


Figure 3. Dissociation of A45-eglin and wild-type eglin from chymotrypsin and subtilisin Carlsberg as a function of collision energy (CE). Chymotrypsin shows a much steeper curve with only 50 % of the complex being present at at CE of 13 V. This value is approximately 25 V for subtilisin-eglin c.

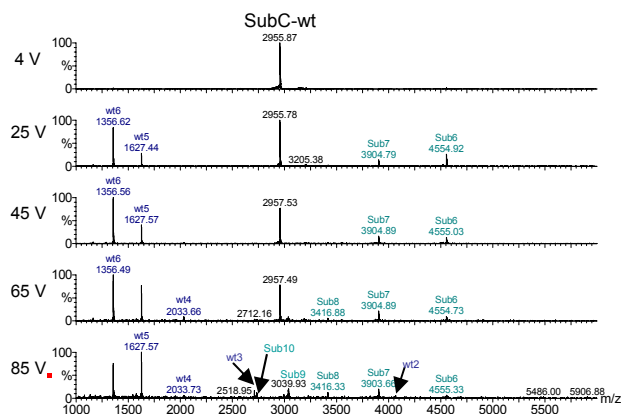


Figure 4. Gas phase dissociation of the subtilisin Carlsberg-eglin c complex +12 charge requires substantially more collision energy for full dissociation.

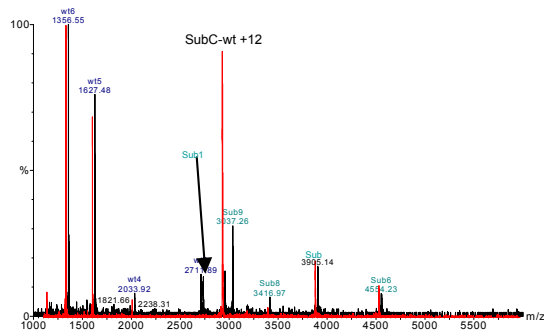


Figure 5. Overlaid spectra showing the difference in species observed for the gas phase dissociation at 55 V (red) and 75 V.

We also examined the possibility that the strength of gas phase binding could correspond to the amount or percentage of hydrophilic interactions at the binding region by calculating the change in solvent accessible surface area between the complex and the free components based on X-ray structure data. Subtilisin-eglin c complexes have less overall surface area interacting than the corresponding chymotrypsin complex, but the absolute and percentage of hydrophilic contributions is greater with subtilisin. At this point, the data set is too small to come to a formal conclusion concerning this property.

The strong solution binding of eglin c to the serine proteases chymotrypsin and subtilisin Carlsberg is reflected in the MS spectra of the complexes. The energy required for dissociation in the collision cell does not correspond to the solution energy of the complexes, but may be related to the nature of the binding region. The dissociation of the subtilisin-eglin c complexes is bimodal suggesting at least two different binding modes in the gas phase. Additional evidence indicates interaction at both the N-terminus and at the accepted binding region.

1. Loo, J. A. (2000) *Int. J. Mass Spectrom.* **200**, 175-186.
2. Doherty-Kirby, A. L. and Lajoie, G. A. (2002) *Proc. of the 50th ASMS Conf. on Mass Spectrometry and Allied Topics, Orlando, Florida.*