SILVER ION INHIBITION OF SERINE PROTEASES:

CRYSTALLOGRAPHIC STUDY OF SILVER-TRYPSIN

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Summary

Silver ion is a potent inhibitor of trypsin and chymotrypsin, with K_I's of 4×10^{-5} M. and 3×10^{-5}, respectively. A crystallographic study shows that the primary silver ion binding site on trypsin is at the active center between the carboxyl group of Asp 102 and the δ-nitrogen of His 57. This result is correlated with the fact that Ag^+ interferes primarily with the acylation rate constant, k_2, and does not significantly affect the binding constant, K_s. The location of this site explains the potent inhibitory effect of silver (I) ions on trypsin activity: The imidazole ring of His 57 is repositioned 1.8 Å further out into the solvent to accommodate the silver ion, preventing its normal interaction with the hydroxyl group of Ser 195. Consequently, His 57 cannot directly assist the proton transfer in the catalyzed reaction.

Since silver ion binds to the catalytic site in this highly specific manner, silver may be used as a specific probe of the active site of serine proteases.

This communication reports the 2.7 Å resolution structure of the isomorphous silver derivative of bovine trypsin inhibited by diisopropyl-fluorophosphate (DIP). Martinek et al.[1,2] found that silver ion is a potent trypsin (or chymotrypsin) inhibitor with a K_I 4×10^{-5} M. They concluded, first, that silver ion prevents the acylation of the enzyme while not appreciably interfering with substrate binding. Second, silver ions compete with protons for the binding site and the silver binding depends on a group with an apparent pK_a of 7.1, which they suggested was the imidazole of His 57. In light of more recent evidence, however, the pK_a reflected in those experiments is more likely that of Asp 102[3,4].

EXPERIMENTAL

To prepare the silver (I) derivative crystals of DIP-trypsin[5] were soaked in solutions containing 0.012 M $AgNO_3$ for periods of four to eight days. Three-dimensional, 2.7 Å data sets for both the native DIP-trypsin

and the silver (I) derivative were collected using a Syntex $P\bar{1}$ automated diffractometer. Data reduction and scaling were accomplished using standard techniques.[5]

RESULTS AND DISCUSSION

From our data and the phases previously determined for DIP-trypsin,[5] a difference Fourier map was obtained. The region of this map in the area of the active site is shown in Fig. 1. The large peak "A" corresponds to the position of the fully occupied, primary silver ion binding site. In addition, there is a smaller region of positive density, "B", above and behind the primary site. This peak results from a movement of the imidazole ring of the catalytic site residue His 57 into the solvent by approximately 1.8 ±(≤ 0.2) Å. A secondary silver ion binding site of 35% refined occupancy was found elsewhere on the surface of the molecule, in the vicinity of His 40.

Fig. 2 shows an ORTEP[6] representation of the catalytic site in the silver (I) trypsin derivative. The silver ion is coordinated in an approximately linear fashion between the lower carboxyl oxygen of Asp 102 and the δ-nitrogen of His 57. Bond distances are: Oδ1 (Asp 102)-Ag^+, 2.3 ±0.2 Å; Ag^+-Nδ (His 57), 2.3 ±0.2 Å. This suggests that the silver ion is in a two coordinate sigma-bonded complex characteristic of silver (I)[7]. This configuration is also structurally very similar to complexes of silver (I) with free amino acids.[8]

The silver DIP-trypsin structure provides a model for the mechanism of the silver (I) inhibition of trypsin. The distance between the ε-nitrogen of His 57 and the position of the γ-oxygen of Ser 195 found previously for benzamidine-trypsin (where the serine oxygen was hydrogen bonded to the εN of His 57) is 4.2 ±0.2 Å in the silver derivative. This long distance, coupled with the unfavorable directionality between these two atoms, prevents proton transfer from the hydroxyl group of Ser 195 to the His 57 imidazole. The effect of Ag^+ on k_2, the acylation rate constant, thus gives an indication of the contribution of His 57 and Asp 102 to enhancement of the catalytic rate of serine proteases.

As well as defining the mechanism of the silver ion inhibition, the silver derivative data have been incorporated into the phase refinement

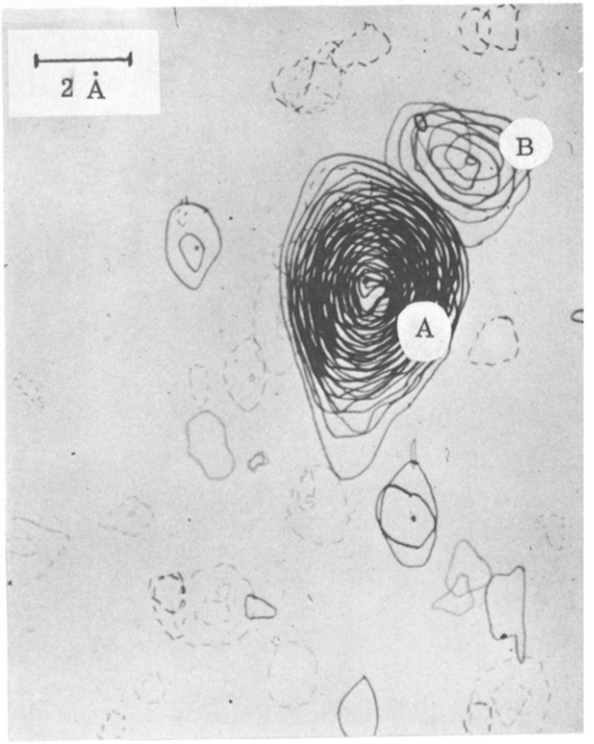

Figure 1. A view parallel to the crystallographic a-axis of the active site region of a difference Fourier map calculated using coefficients $|F_{silver}| - |F_{native}|$. The view shown is a projection through 12 sections, each 1.1 Å apart. Solid contours enclose regions of positive density; the broken lines, regions of negative density. Peak "A" corresponds to the location of the main silver ion binding site. Peak "B" results from the movement of the His 57 imidazole ring. Contours begin at ± 2.5 standard deviations and the contour interval is one standard deviation in the difference electron density.

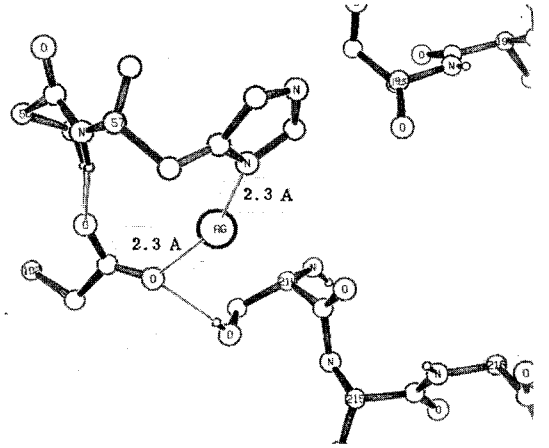

Figure 2. An ORTEP drawing of the catalytic site of silver DIP-trypsin, viewed approximately down the crystallographic b-axis. The DIP group has been omitted for clarity. The ϒ-oxygen of Ser 195 is in the position found for DIP-trypsin, close to that found in tetrahedral intermediates or in acyl enzymes.

of the 2.7 Å structure of DIP-trypsin. The overall mean figure of merit of the 2.7 Å phases increased to 0.83 during this refinement. The details of this refinement will be discussed elsewhere.

One of the key problems in the assignment of microscopic pK_a's to residues at the active center of serine proteases lies in the requirement that one should be examining the "native" enzyme rather than a modified derivative of it. Two familiar problems are thus apparent: 1) presence of a covalent label near the active site perturbs the system under study in an unknown fashion; 2) spectroscopic techniques which study the native enzyme often have problems of assignment to particular residues.

Silver ion can be used to assign peaks to the active center residues by perturbation of the native enzyme spectrum, primarily affecting peaks due to Asp 102 and His 57. We are currently applying this technique to assign peaks associated with carboxylic acid groups in the difference infrared titration spectra of native serine proteases.[9]

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