High-resolution crystal structures of recombinant wild type and selenomethione labeled bovine trypsin (S195A) mutant reveals no electron density for three surface loops that includes the C191 - C220 disulfide.

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Crystals of bovine trypsin (UniProt ID P00760) is used by SER-CAT as a quality control standard for determining beamline optimization and performance. However, a selenomethionine labeled standard for MAD/SAD energy optimization and other studies was also needed. To provide this MAD/SAD standard a S195A mutant of bovine trypsin with selenomethione labeling was expressed, purified and crystalized. Crystals of the recombinant protein had a similar habit and diffraction quality compared to native crystals. An 18 sec data set to 1.5 Å resolution was collected on beamline 22ID at the selenium absorption edge using a Rayonix MX300HS 10 Hz CCD detector. The data set (99.1% complete) consisting of 180 one-degree images each exposed for 0.1 seconds was collected. Following SER-CAT protocols the data were auto processed using cmdKylin. Phases were then generated using phenix.autosol and phenix.autobuild placed 190 of the 223 amino acids giving R and Rfree values of 0.2122 and 0.2332, respectively. Refinement (3 rounds) of the auobuilt model was carried out using phenix.refine and converged to give R and Rfree values of 0.1994 and 0.2187, respectively with good stereochemistry. However, inspection of the refined model (COOT) showed that the electron density of three surface loops (totaling 27 residues) was missing with the loss of the C191 - C220 disulfide. The presentation will provide details of the production of selenomethione labeled protein, its crystallization, data collection and structure solution. It will also explore possible causes for the missing loop density. Work supported in part by funds from the SER-CAT Member Institutions, the University of Georgia Foundation, and the National Institutes of Health (S10 RR25528 & 1S10 RR028976).