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The Implication of the Sequence of Disulfides Breaking in the Reductive Unfolding Pathways of Ribonuclease

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Time-resolved disulfides breaking of bovine pancreatic ribonuclease A (protein was from Dr. Scheraga group at Cornell) were observed by Fitscale, a novel data analysis method through a conventional single wavelength data collection. The study of the sequence of the disulfide bonds breaking from the native state and its mutants reveals that the disulfide bonds breaking due to x-ray irradiation is related to the disulfide bond unfolding pathways. The order of the disulfides breaking in native RNase A is consistent with the reductive unfolding pattern that the (40-95) and (65-72) disulfide bonds takes parallel reduction resulting from these two disulfide bonds have very similar accessibility to the solvent and more accessibility than the other two (58-110) and (26-84) disulfide bonds. By in silico mutation of the residue tyrosine (Y) 92 to glycine (G) and leucine (L), which tyrosine 92 is considered as a burial of the (40-95) disulfide bond to the solvent, the observation of the change of the sequence of the disulfide bonds breaking indicates the (40-95) disulfide is affected by the intramolecular interactions which demonstrated by its reduction unfolding pathway.