

Preliminary Crystallographic Data for Cross-linked (Lysine⁷-Lysine⁴¹)-Ribonuclease A

A cross-linked derivative of ribonuclease A, *N^ε,N^{ε'}*-(2,4-dinitrophenylene-1,5)-(lysine⁷-lysine⁴¹)-RNase A, has been crystallized by dialysis against 30% (v/v) ethanol/water mixtures buffered at high pH. Single crystals belong to the orthorhombic space group *P*2₁2₁2₁, *a* = 37.2 Å, *b* = 41.2 Å, *c* = 41.2 Å, with one molecule in the crystallographic asymmetric unit.

Chemically modified proteins have served as the basis for many biochemical studies. In the interpretation of the experimental results, it is generally assumed that the native protein conformation is essentially preserved and may be altered only in the local region of the modification. In a few cases, the crystallographic structures of the modified proteins have been studied by X-ray crystallography (Allewell *et al.*, 1973; Beddell *et al.*, 1975).

Recently, the thermodynamic properties of a chemically cross-linked derivative of ribonuclease A have been reported (Lin *et al.*, 1984). Reaction of 1,5-difluoro-2,4-dinitrobenzene with bovine pancreatic ribonuclease A resulted in the introduction of a cross-link between lysine residues 7 and 41. The molecule retained the immunochemical reactivity and circular dichroic spectra of the native protein. However, refolding experiments showed that the cross-linked derivative exhibited a reversible thermal transition at a temperature 25 deg.C higher than that of native ribonuclease A at pH 2.0. The observed increase in thermal stability has been attributed to a loss in entropy of the protein in its unfolded state. Inherent in this interpretation is the assumption that the native conformation is unaltered by the cross-linking reagent (Johnson *et al.*, 1978; Lin *et al.*, 1984). In order to test this hypothesis, we have initiated structural studies of the cross-linked form of ribonuclease A.

Cross-linked ribonuclease A was prepared as described (Lin *et al.*, 1984) and lyophilized from 0.1 M-acetic acid. Lyophilized protein was dissolved in doubly distilled water to a protein concentration of 20 mg/ml. Crystals were grown in 20 μl cells by dialysis against 30% (v/v) ethanol/buffer mixtures at 7°C. Both 50 mM-Tris·HCl (pH 7.5) and 50 mM-imidazole (pH 8.0) were suitable buffers for crystallization. The protein crystallized in aggregates of rounded "petals" that usually gave disordered diffraction patterns. However, single petals occasionally grew large enough to allow single crystal X-ray diffraction studies.

Crystals belong to the orthorhombic space group *P*2₁2₁2₁ with *a* = 37.2 Å, *b* = 41.2 Å, *c* = 75.6 Å. X-ray precession photographs show diffraction intensities to at least 2.0 Å resolution. The volume per

unit mass, *V_m*, is 2.1 Å³/dalton, which is in the range observed for most protein crystals (Matthews, 1968). Assuming a protein volume similar to that of ribonuclease S (Richards, 1974), a single molecule occupies 53.6% of the crystallographic asymmetric unit. Crystals are quite stable at room temperature in the X-ray beam, and an initial three-dimensional set of diffraction intensities to 2.1 Å resolution has been collected. Attempts are currently underway to solve the structure by molecular replacement methods (Rossmann, 1972), using the refined structure of ribonuclease A as a starting model (Borkakoti *et al.*, 1982; Wlodawer *et al.*, 1982).

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