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A Model of Ribonuclease Based on Chemical Evidence*

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ABSTRACT: Available chemical and kinetic data have been used to construct a speculative model of bovine pancreatic ribonuclease A. The model is based on several interactions whose existence is suggested by these data.

Protein chemists have been using chemical modification and physicochemical studies to determine internal interactions in proteins in order to obtain information about molecular structure. Bovine pancreatic ribonuclease A has been extensively investigated, and a wealth of experimental results are at hand (*cf.* Scheraga and Rupley, 1962; Hummel and Kalnitsky, 1964). As this information has been accumulated, several attempts have been made to correlate these data in terms of a model, the most recent being that of Saroff (1965). Recently, several new pieces of information about internal interactions in ribonuclease have become available (Cathou and Hammes, 1965; Li *et al.*, 1966; Erman and Hammes, 1966), and it appeared worthwhile to construct a new model on the basis of these and earlier data, including, among others, the covalent structure (Hirs *et al.*, 1960; Potts *et al.*, 1962; Smyth *et al.*, 1963). Although such an attempt is quite speculative, it represents a convenient summary of the known chemistry; further, the ultimate goal of all structural studies is the determination of a three-dimensional model. In addition, such a model may be useful in suggesting other experiments.

In addition to the four disulfide bonds the model is based on the following interactions: (1) Tyr 25-Asp 14, (2) Tyr 92-Asp 38, (3) Tyr 97-Asp 83, (4) Lys 7-Lys 41, and (5) His 12-His 119 (1–3, Li *et al.*, 1966; 4, Marfey *et al.*, 1965; 5, Crestfield *et al.*, 1963). Kinetic data suggest that His 12, His 119, and Asp 121 may be part of the active site (Erman and Hammes, 1966).

With this information, the structure shown in Figures 1 and 2 was constructed from non-space-filling models. The structure was also put together with Pauling–Corey–Koltun models (not shown here) to demonstrate its steric feasibility. No extended α-helical regions appear in the model.

Besides the interactions listed above, the model has most of the polar side-chain groups on the outside and most of the nonpolar side-chain groups on the inside. No attempt has been made to elucidate the details of side-chain interactions, other than those mentioned above. Tyr 73, 76, and 115 are exposed while the other three are buried (Woody *et al.*, 1966). The C terminal interacts with two of the buried tyrosines (92 and 97), in agreement with the fact that only Tyr 25 is buried in pepsin-inactivated ribonuclease (Fujioka and Scheraga, 1965), a derivative which lacks the C-terminal tetrapeptide (Anfinsen, 1956). The N and C terminals are near each other. However, the location of the first six residues in the N terminal is adjustable. In fact, the over-all shape of the molecule can be varied within limits and still preserve these interactions.

FIGURE 1 (opposite): Non-space-filling model of ribonuclease. See Figure 2 for artist's rendition of model from this view, with essential features indicated. The backbone chain was covered with red crepe paper, and the four disulfide bridges with blue paper. A model of cytidine 2′,3′-cyclic phosphate, covered with blue paper, is placed schematically in the groove at the bottom of the model.

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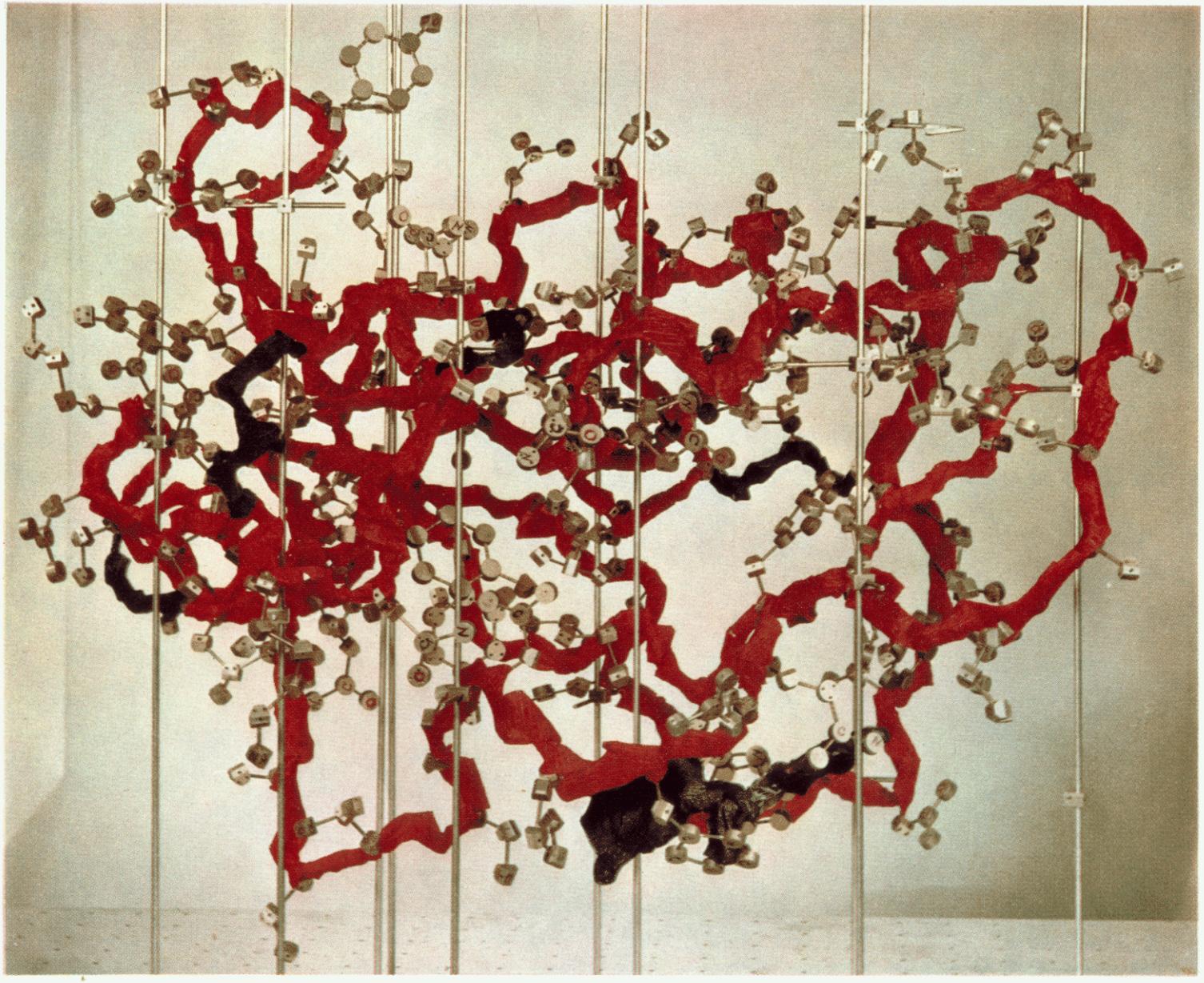

← Top

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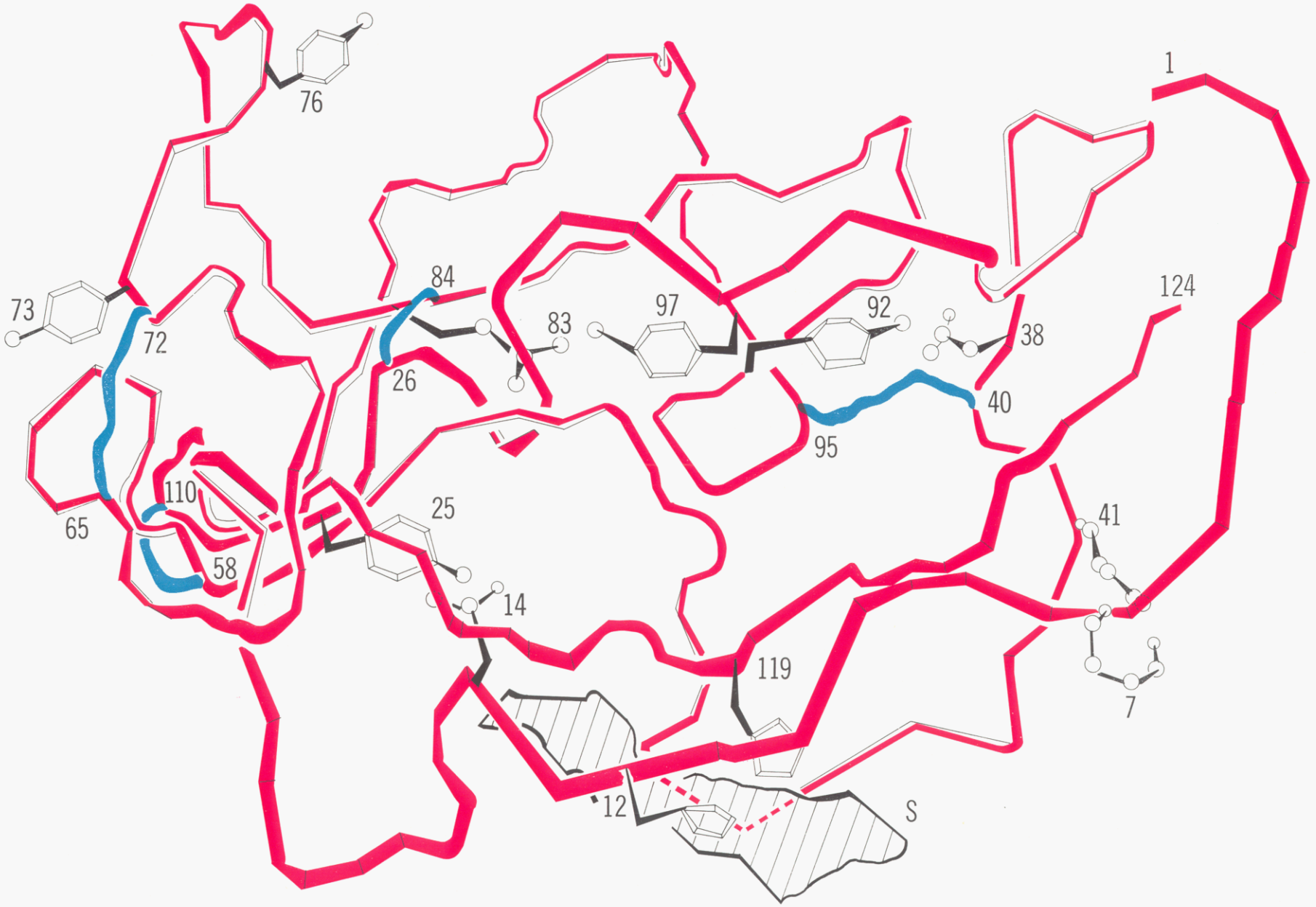
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The molecular dimensions can easily be made consistent with existing crystallographic data (Harker, 1960; Avey *et al.*, 1962). Nevertheless, the positions of the backbone could not be radically altered without disrupting the postulated interactions. However, with such a complex molecule, it is difficult to ascertain whether the proposed structure is unique.

In this model, the active site is in a groove near the bottom of Figures 1 and 2, and models of substrate molecules fit quite comfortably into this groove, although there is considerable latitude in the precise location of the substrate S. A cytidine 2′,3′-cyclic phosphate molecule could simultaneously interact with the side chains of His 12, His 119, Asp 121, and Arg 10. The distance between the two imidazole side chains of His 12-His 119 and the amino groups of Lys 7-Lys 41 is about 10 A. Figure 2 is a schematic drawing of the model which indicates the most important structural features used as a basis for the construction of the model. Tyr 73 and 76 are on the surface of the model, which is in agreement with the fact that they are readily iodinated (Cha and Scheraga, 1963). The schematic drawing of Figure 2 can be conveniently used to follow the course of the backbone chain in the actual model shown in Figure 1.

Chemical and physicochemical studies have provided information about the interactions incorporated in this model. It is only recently, with the availability of X-ray information, that we are in a position to evaluate the validity of conclusions based on chemical and physicochemical methods (Kendrew *et al.*, 1960; Perutz *et al.*, 1960; Blake *et al.*, 1965). Hopefully, it will be possible to make such a comparison in the near future when the X-ray structure of ribonuclease becomes available.

FIGURE 2 (opposite): Schematic drawing (by Karen Vournakis) of the model, from the same view as in Figure 1. The perspective of the drawing distorts some of the relative positions of the model; therefore, the drawing should be used as a guide to follow the chain in the photograph of Figure 1. The numbers refer to amino acid residues, 1 and 124 being the N and C terminals, respectively. The symbol S indicates the location of the substrate.

Addendum

After this paper was submitted, a communication by Hartman and Wold (1966) appeared. Using a bifunctional reagent, these workers found that Lys 31 and Lys 37, and Lys 7 and Lys 37 can each be cross-linked at a distance of $\leqslant$8.6 A. The derivative is enzymically active. These findings are consistent with the proposed model. In Figure 1, Lys 31 is near the top of the model, about one-third of the distance from the right; Lys 37 is near the upper right-hand corner of the model; Lys 7 (near the bottom of the right side of the model) can easily be brought to within 8.6 A of Lys 37 (by moving the N terminal slightly) without disrupting the Lys 7-Lys 41 interaction.

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