TABLE IV

EQUILIBRIUM CONSTANTS OF METATHESIS

Temp., °C.	Equil. const. $\times 10^4$	Concn. chlorfluor at equil., mole %
25	1.69	0.65
50	4.05	1.0
75	7.14	1.3
100	11.4	1.7
125	16.4	2.1

Thus, it is shown that not only is the attainment of equilibrium in reaction 6 slow, but metathesis at equilibrium amounts only to about 0.7 mole % at 25°.

Esterification of Methylphosphonic Halides.—The relative esterification rates of methylphosphonic halides in 2-propanol solution, obtained by heat evolution measurements, are in the order: chlorfluor ≫ dichlor > equimolar dichlor–difluor mixture ≫ isopropyl methylphosphonic chloride > difluor. The esterification of difluor is catalyzed by acids, whereas that of isopropyl methylphosphonic chloride is not. When a mole of 2-propanol was added to a mixture containing a mole each of dichlor, difluor and chlorfluor, a mole of Sarin was produced and the original quantities (within 5%) of dichlor and difluor were recovered by distillation. This indicates the rapidity of chlorfluor reaction relative to the other dihalides and also that neither HF, HCl nor 2-propanol catalyzes the disproportionation of chlorfluor.

In considering the mechanism of esterification of an equimolar mixture (dichlor–difluor), it can be seen that the small equilibrium amounts of chlorfluor that might be present would react first. The only remaining question concerning the participation of chlorfluor in the mechanism is whether attainment of equilibrium in reaction 6 is sufficiently rapid to replenish the supply of chlorfluor reacting.

The esterification of the dichlor–difluor mixture is essentially complete within 30 minutes at 25°. The metathesis of this mixture to chlorfluor would have to be at least as rapid for the alcohol to react to any significant degree with chlorfluor. Because of the small equilibrium constant of metathesis ($K = 1.7 \times 10^{-4}$), the rate could not be measured conveniently. Since the reverse reaction, disproportionation of chlorfluor, should theoretically go almost to completion and is 6000-fold more rapid at equivalent concentrations, it can be followed. The slow change reported by Hoffmann at 60° and the slight changes indicated in the freezing point and infrared spectrum of chlorfluor during 34 months of storage at room temperature attest to the relative unimportance of chlorfluor participation in the mechanism studied.

The relative rate data obtained tend to substantially confirm the Sarin formation mechanism postulated by Perry.

Grateful acknowledgment is given to Mr. J. B. Bouck of these Laboratories for making available to us for discussion the results of the infrared measurements prior to his more detailed publication.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY, ITHACA, NEW YORK]

Structural Studies of Ribonuclease. III. A Model for the Secondary and Tertiary Structure[1,2]

BY HAROLD A. SCHERAGA

RECEIVED SEPTEMBER 8, 1959

A three-dimensional model of ribonuclease has been constructed on the basis of available data on the primary, secondary and tertiary structure. The model will be useful for the design of future experiments to establish the complete structure of this protein.

In the previous papers of this series[3,4] information was obtained about the internal hydrogen bonding in ribonuclease. With the recent availability of the complete amino acid sequence of oxidized ribonuclease[5] and the location of the disulfide bridges in the native molecule,[6] it is now possible to construct a model of ribonuclease which will be consistent with the available data on the primary, secondary and tertiary structure. It must be emphasized at the outset that the model to be described here may not necessarily be a unique one. However, it is believed to be consistent with the known structural information about this protein and, further, it provides a basis for future experiments to prove the presence or absence of interactions between specific side-chain groups. Presumably the complete three-dimensional structure of ribonuclease will be established when a sufficient number of such interactions is located.

Model Building Units.—A description of the Pauling–Corey model building units, and the molecular dimensions on which they are based, has already been presented by Lindley and Rollett in their paper on the structure of insulin.[7] I am indebted to Dr. R. B. Corey for sending me blue-

(1) This investigation was supported by research grant E-1473 from the National Institute of Allergy and Infectious Diseases, of the National Institutes of Health, U. S. Public Health Service, and by research grant G-6461 from the National Science Foundation.

(2) Presented before the Division of Biological Chemistry at the 136th meeting of the American Chemical Society, Atlantic City, New Jersey, September, 1959.

(3) C. Y. Cha and H. A. Scheraga, THIS JOURNAL, **82**, 54 (1960).

(4) C. L. Schildkraut and H. A. Scheraga, *ibid.*, **82**, 58 (1960).

(5) C. H. W. Hirs, S. Moore and W. H. Stein, *J. Biol. Chem.*, **235**, 633 (1960). I am indebted to Dr. Hirs for sending me a copy of the sequence prior to publication.

(6) D. H. Spackman, W. H. Stein and S. Moore, *ibid.*, **235**, 648 (1960).

(7) H. Lindley and J. S. Rollett, *Biochim. et Biophys. Acta*, **18**, 183 (1955).

prints for the construction (by E. F. Cramer) of the aluminum, non-spacefilling type model units on a scale of 1″ = 1Å. In the designation of amino acid residues, the three letter abbreviation system of Brand and Edsall will be used in the text, even though it originally may have been intended for use only in amino acid sequences.

Covalent Structure.—Native ribonuclease consists of a single polypeptide chain of 124 amino acid residues with lysine and valine as the N-terminal and C-terminal groups,[5,8] respectively. There are 8 half-cystine residues at positions 26, 40, 58, 65, 72, 84, 96 and 110,[5] considering the N-terminal lysine as residue No. 1. These 8 half-cystines may be designated I through VIII and are linked by disulfide bridges as follows[6]: I–VI, II–VII, III–VIII, IV–V. The complete amino acid sequence determined by Hirs, Moore and Stein is shown in Fig. 1.

The Model.—The backbone chain was folded so as to take account of the available chemical information which is described in the next section. The right-handed α-helix (with L-amino acids) was used as much as possible, consistent with the data on deuterium–hydrogen exchange.[4] The first obvious structural feature is that the peptide chain between Cys 65 and Cys 72 cannot be in an α-helical form if the S–S bridge IV–V is to be made. Similar considerations required the rupture of potential peptide hydrogen bonds to form the remaining S–S bridges. The four proline residues also contributed to breaks in the helix. The complete molecule is postulated to consist of six helical portions H_1–H_6 and six non-helical regions B_1–B_6. Photographs of the model are shown in Figs. 2 and 3 and a schematic arrangement of the chains in Figs. 4 and 5. The extent of helical folding of the backbone chain is indicated in Table I. As can be seen in the end-view of Figs. 3 and 5, most of the molecule consists of a parallel array of chains except for the N-terminal helical portion H_1 which lies perpendicular to the rest of the molecule.

Chemical Evidence.—First, and most important of all, the model is based on the amino acid sequence of Hirs, Moore and Stein[5] and the location of the disulfide bridges by Spackman, Stein and Moore.[6]

The helical portion H_1 was located as shown in order to bring the N-terminal and C-terminal portions of the molecule close together. The justification is that both ends seem to be required for enzymatic activity, the Asp 121 residue from the work of Anfinsen,[9] the His 119 residue from the work of several investigators[10] and the 20 residue N-terminus from the work of Richards and Vithayathil.[11] In this compact form the molecule, even though not perfectly ellipsoidal, has an axial ratio of approximately 1.5 to 2.5, which would correspond to a value of 2.13×10^6 for the hydrodynamic parameter β.[12] This is in good agreement with the value of 2.09×10^6 found by Harrington and Schellman.[13]

Molecular dimensions of $18 \times 30 \times 48$ Å. have been deduced from X-ray studies of wet ribonuclease crystals.[14] The model has dimensions of about $19 \times 30 \times 43$ Å. From an analysis of Patterson projection maps, Carlisle and Scouloudi concluded that the "molecule may contain 5 parallel 'crystallographic' polypeptide chains arranged in hexagonal close-packing. . . . These 5 chains need not be incompatible with a lesser number of chemical chains of unequal length." In a subsequent paper Carlisle, Scouloudi and Spier[15] proposed 6 rather than 5 'crystallographic' chains but preserved the general feature of a predominantly parallel array of chains. On the other hand, Magdoff, Crick and Luzzati[16] concluded that a structure with all rods parallel is unlikely. In view of the unsettled state of the X-ray situation, we can draw no conclusions as to the compatibility of the model with the X-ray diffraction pattern.

Since there are 124 residues in the molecule, a maximum of 120 (taking break B_7 of Table I into account) backbone hydrogen bonds are possible if the whole molecule could be put in the form of an α-helix. From the data of Table I (breaks B_1–B_6), 30 of these hydrogen bonds are not formed, leaving 90 CO–NH hydrogen bonds in the six helical portions of the backbone. Since we may expect some looseness at the ends of each helical portion,[17] amounting to about 4 hydrogen bonds per helix, we may subtract 24, leaving about 66 fairly strong backbone hydrogen bonds. In deuterium–hydrogen exchange experiments the hydrogens in these bonds would be expected to exchange slowly.[18] Experimentally,[4] 70 hydrogens[19] are found to exchange slowly or not at all at 0°, in good agreement with the model.[21] The exchange studies[4] permitted a breakdown of these 70 hydrogens into three sets of 25, 25 and 20, respectively, according to their rate of exchange. While one can try to distinguish the hydrogens of the model according to their expected rates of exchange, it is premature to make a definite identification of the three sets of hydrogens. Further work is in progress along these lines.

(8) C. B. Anfinsen, R. R. Redfield, W. L. Choate, J. Page and W. R. Carroll, *J. Biol. Chem.*, **207**, 201 (1954).

(9) C. B. Anfinsen, *ibid.*, **221**, 405 (1956).

(10) (a) H. G. Gundlach, W. H. Stein and S. Moore, *ibid.*, **234**, 1754 (1959); (b) W. D. Stein and E. A. Barnard, "Abstracts, IVth International Congress of Biochemistry, Vienna," Pergamon Press. Ltd., London, 1958, p. 21; (c) E. A. Barnard and W. D. Stein, *Biochem. J.*, **71**, 19P (1959).

(11) F. M. Richards, *Fed. Proc.*, **17**, 296 (1958); F. M. Richards and P. J. Vithayathil, *J. Biol. Chem.*, **234**, 1459 (1959).

(12) H. A. Scheraga and L. Mandelkern, THIS JOURNAL, **75**, 179 (1953).

(13) W. F. Harrington and J. A. Schellman, *Compt. rend. trav. Lab., Carlsberg, Ser. Chim.*, **30**, 21 (1956).

(14) C. H. Carlisle and H. Scouloudi, *Proc. Roy. Soc. (London)*, **A207**, 496 (1951).

(15) C. H. Carlisle, H. Scouloudi and M. Spier, *ibid.*, **B141**, 85 (1953).

(16) B. S. Magdoff, F. H. C. Crick and V. Luzzati, *Acta Cryst.*, **9**, 156 (1956).

(17) J. A. Schellman, *Compt. rend. trav. Lab. Carlsberg, Ser. Chim.*, **29**, 230 (1955).

(18) K. Linderstrøm-Lang, "Symposium on Peptide Chemistry," Chemical Society, London, Special Publication **No. 2**, 1 (1955).

(19) See footnote 3 of ref. 20 for further discussion of the total number of slowly exchangeable hydrogens.

(20) H. A. Scheraga, C. Y. Cha, J. Hermans, Jr., and C. L. Schildkraut, "*Amino Acids, Proteins, and Cancer Biochemistry*," Academic Press, Inc., New York, N. Y., p. 31 (1960).

(21) The per cent. helical content, deduced from deuterium-hydrogen exchange experiments, is higher than that suggested by Yang and Doty[22] from optical rotatory dispersion measurements. We have preferred to rely on the exchange data because of difficulties involved in determining "per cent. helical content" from optical rotation data.

(22) J. T. Yang and P. Doty, THIS JOURNAL, **79**, 761 (1957).

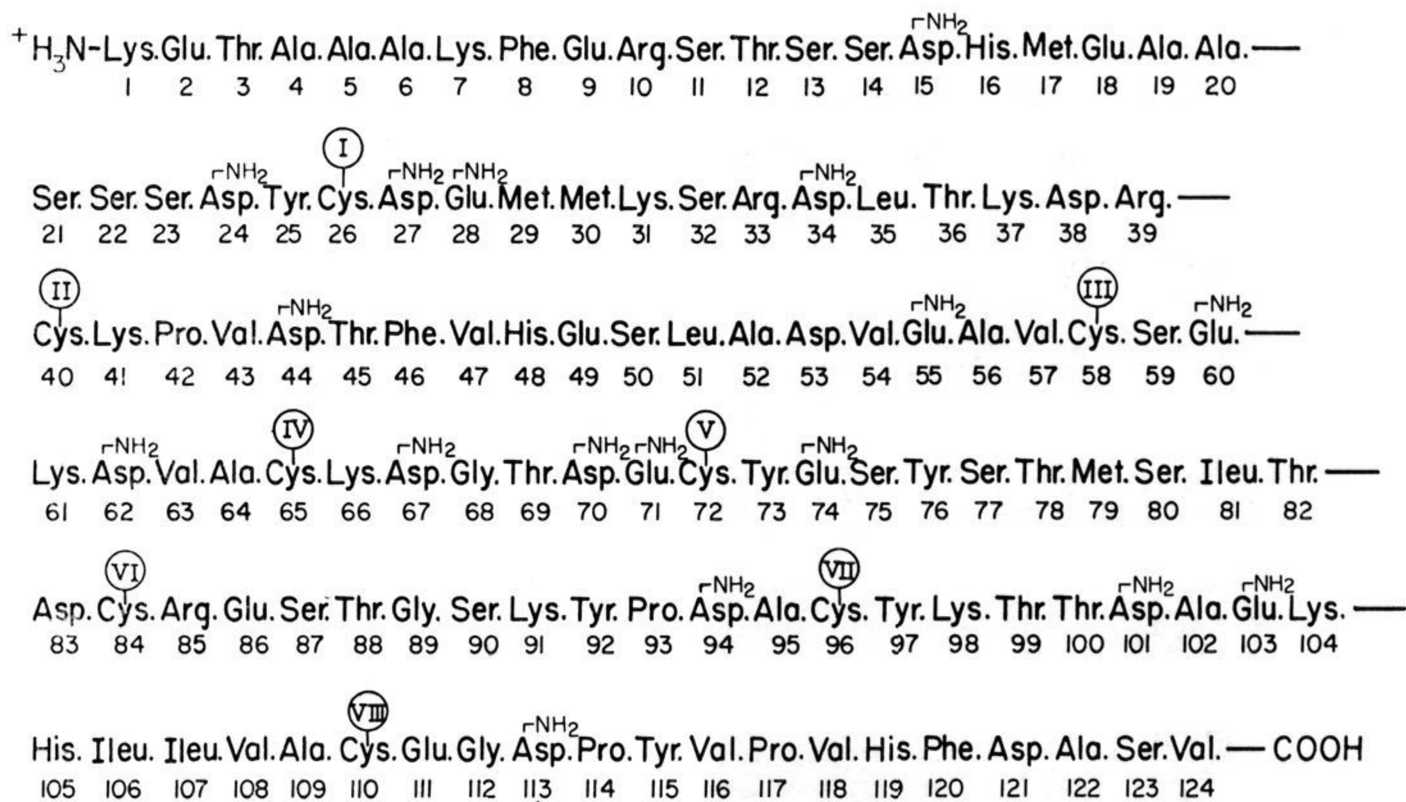

Fig. 1.—Amino acid sequence of oxidized bovine ribonuclease, determined by Hirs, Moore and Stein.

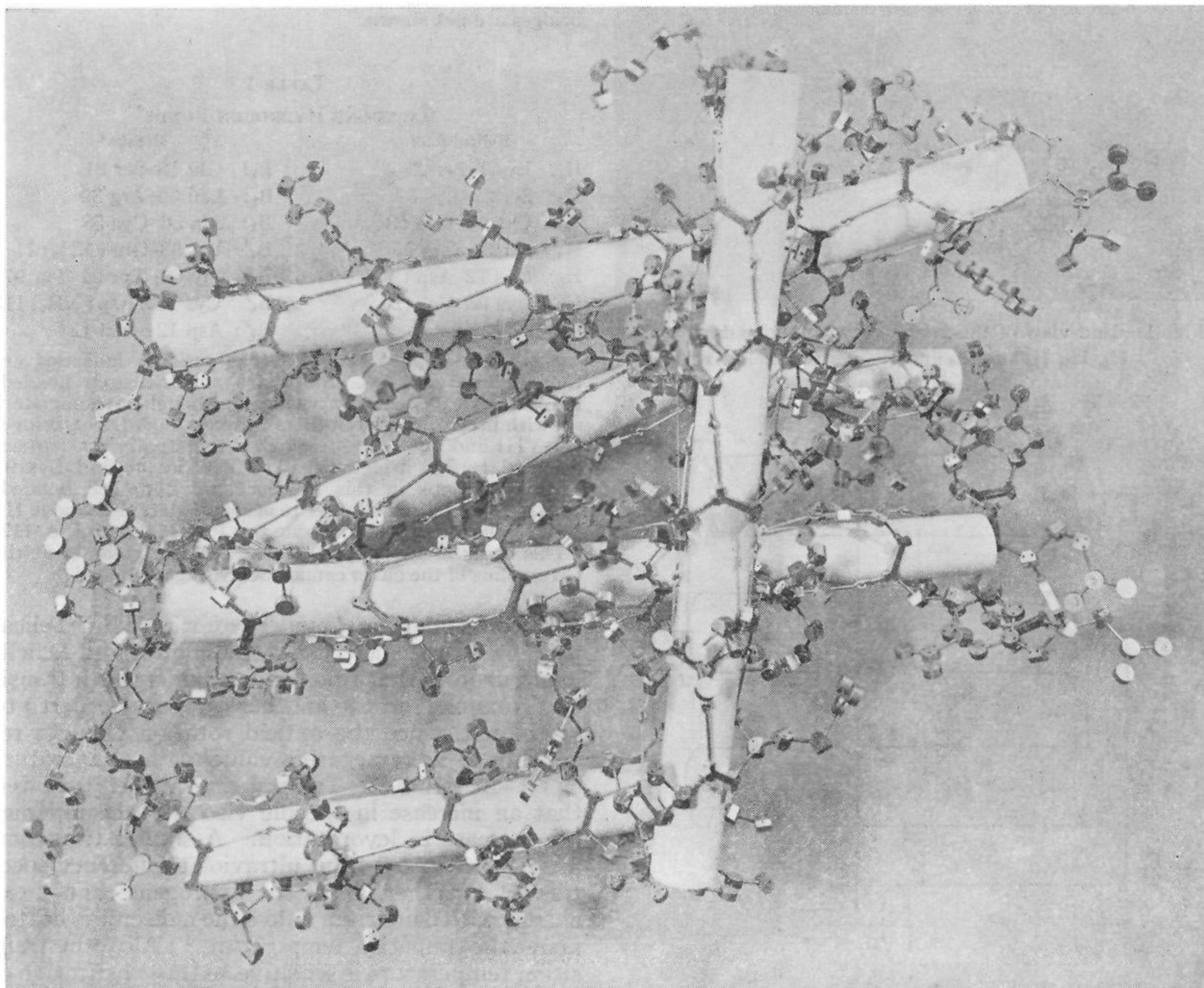

Fig. 2.—Model of ribonuclease showing parallel array of all helical portions except H_1 which is perpendicular to the other chains.

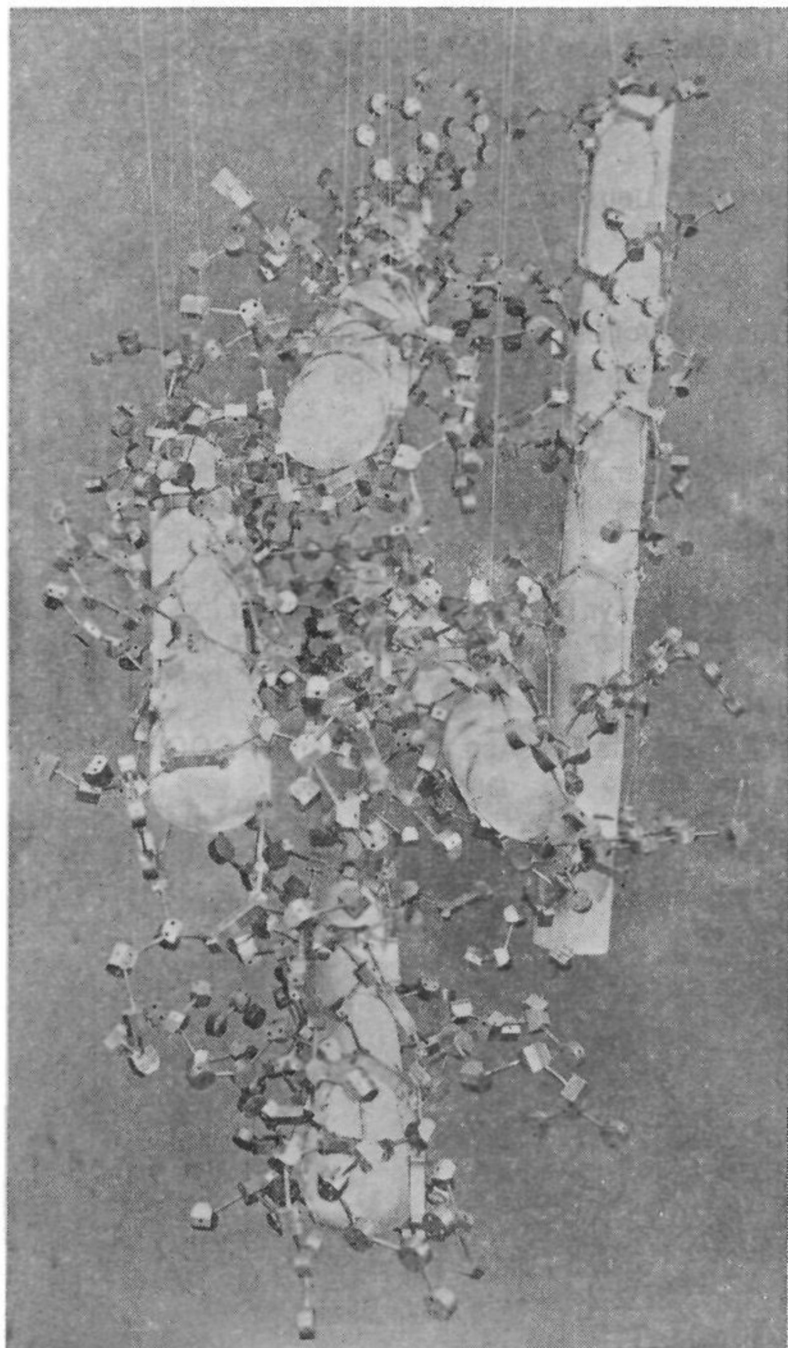

Fig. 3.—End-view of the model showing the ends of helices H_2, H_3, H_5 and H_6 and the vertical helix H_1.

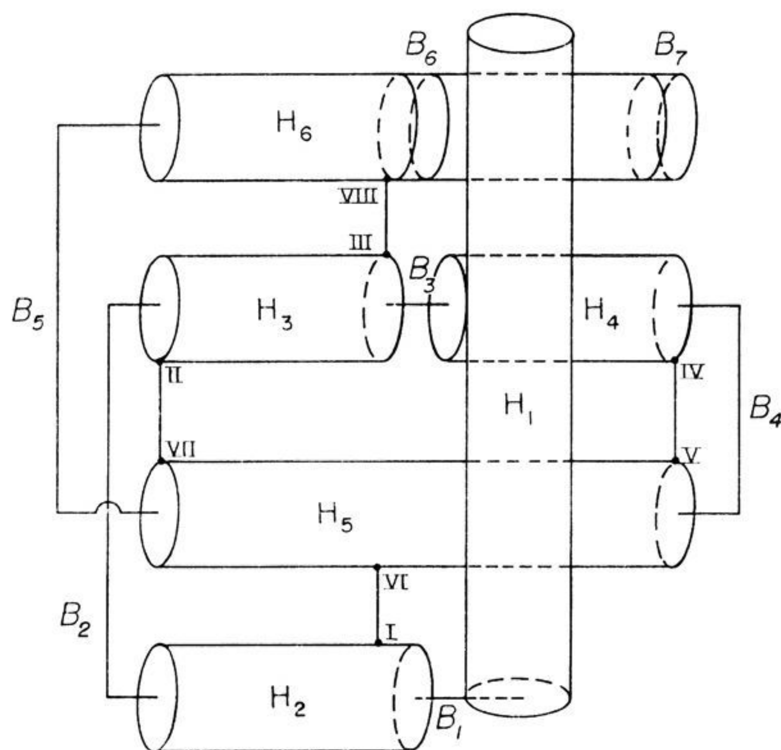

Fig. 4.—A schematic representation of the model, corresponding to the photograph of Fig. 2. The positions of the disulfide bridges are also shown.

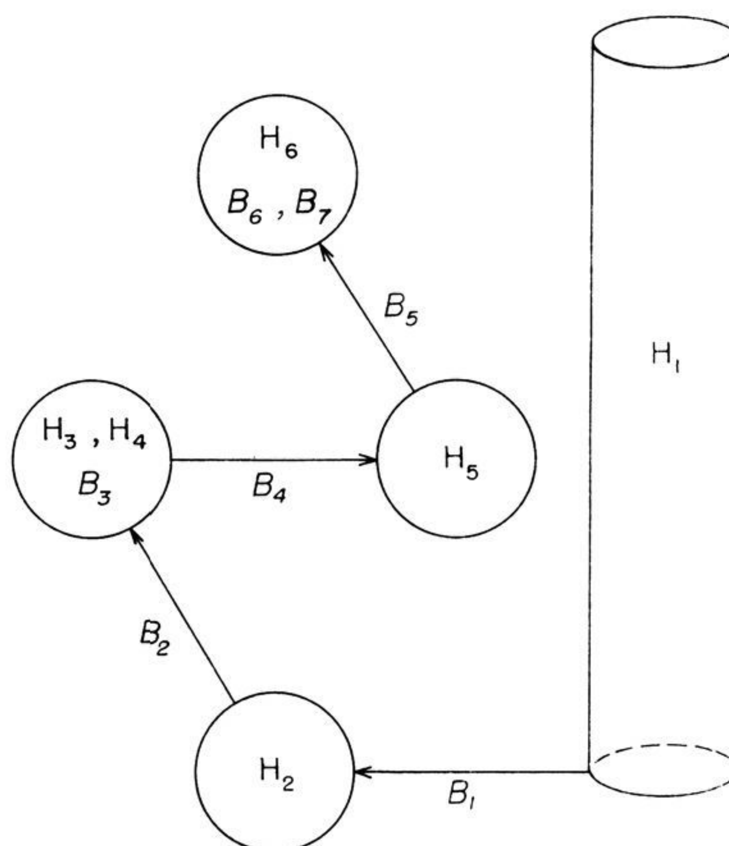

Fig. 5.—A schematic representation of the model, corresponding to the photograph of Fig. 3. The disulfide bridges are not shown.

TABLE I

BACKBONE HYDROGEN BONDS[a]

Helical folding		Breaks[b]	
H_1:	Lys 1–Ser 21	B_1:	Glu 18–Ser 21
H_2:	Ser 22–Lys 37	B_2:	Leu 35–Arg 39
H_3[c]:	Cys 40–Cys 58	B_3:	Ala 56–Cys 58
H_4[c]:	Ser 59–Cys 65	B_4[d]:	Val 63–Glu (NH_2) 71
H_5:	Cys 72–Asp (NH_2) 94	B_5[e]	Gly 89, Tyr 92–Tyr 97
H_6:	Lys 98–Val 124	B_6[f]	Cys 110, Asp (NH_2) 113
		B_7[g]:	Asp 121–Val 124

[a] See also Figs. 4 and 5. [b] The residues indicated are those whose carbonyl groups are not hydrogen bonded. [c] H_3 and H_4 are essentially co-linear, although not completely so, with break B_3 between them to accommodate S–S bridges III–VIII and IV–V. [d] Break B_4 is required in order to accommodate S–S bridge IV–V. [e] The Ser 90 and Lys 91 carbonyl groups in this sequence are hydrogen bonded. Break B_5 is required in order to accommodate S–S bridge II–VII. [f] These breaks are due to Pro 114 and Pro 117. [g] This break arises simply because the four residues at the C-terminus of the chain cannot be hydrogen bonded.

There is additional evidence for including helical portions in the molecule. Harrington and Schellman[13] showed that ribonuclease undergoes a transition from one form to another (probably ordered to disordered) since the optical rotation changes reversibly to more negative values at a temperature of 60° at *p*H 6.5. Tanford and Weber[23] showed that an increase in specific viscosity accompanies the increase in levorotation. A similar transition has been deduced from ultraviolet difference spectra.[24,25] Further, all of the hydrogens cannot exchange with deuterium unless the molecule is heated above the transition temperature.[4] Also, the transition temperature is sensitive to the substitution of

(23) C. Tanford and R. E. Weber, private communication.

(24) H. A. Scheraga, *Biochim. et Biophys. Acta*, **23**, 196 (1957).

(25) J. Hermans, Jr., and H. A. Scheraga, to be published.

deuterium for hydrogen,[26] as was found previously for the helix–random coil transition in poly-γ-benzyl-L-glutamate.[27] The disruption of the helix by urea[28] or guanidine hydrochloride[3] removes the abnormality of the ionization of the tyrosyl and carboxyl groups. If the disulfide bridges are broken (*e.g.*, by oxidation), the helices lose a considerable amount of their free energy of stabilization and the resulting molecule converts to a randomly-coiled form.[13] In confirmation of this, Hvidt found that all of the hydrogens of oxidized ribonuclease exchanged instantaneously with deuterium.[29] The conclusion seems inescapable that a portion of the molecule is in a shielded, organized structure which is postulated here to be in the form of α-helices.

The remaining evidence to be considered concerns the tertiary structure. According to Richards[11] the 20-residue N-terminus is associated to the core even when the Ala 20–Ser 21 peptide bond is split. While no quantitative data are available for the association constant, the binding has been found to be very strong. In the model we have postulated that the N-terminus is in the form of an α-helix (H_1) and that the side-chains perform the dual role of stabilizing this short helix and binding it to the core. Given the freedom of rotation provided by break B_1, it is possible to achieve a significant amount of interaction (both hydrogen and hydrophobic bonding) between H_1 and the rest of the molecule. It must be emphasized that these interactions are *postulated;* it remains for future experiments to establish whether or not these interactions are present. The side-chain bonds are listed in Table II. We may estimate[30,31] that each bond contributes about 0.5 to 1 kcal. to the free energy, or a total of 5 to 10 kcal. per molecule. Most of these bonds involve non-ionizable groups so that this free energy of stabilization would be available over most of the pH range, *unless other parts of the molecule changed configuration with pH.*

Three of the six tyrosyl groups show abnormal ionization behavior in the native molecule[32,33] but titrate normally in urea[28] and guanidine hydrochloride.[3] Presumably these three tyrosyl groups are involved in strong interactions which are disrupted when the molecule is denatured. It has been suggested,[13,24,34] on the basis of ultraviolet difference spectra, that these abnormal groups are involved in tyrosyl-carboxylate ion hydrogen bonds. In the model, tyrosyl residue 25 seems relatively free. A variety of hydrogen bonds (involving the remaining 5 tyrosyl groups) are possible, some of them involving carboxylate ions. Examples of possible bonds are Tyr 73–Glu (NH_2) 71 or 74,[35] Tyr 76–Asp 121, Tyr 92–Arg 33, Tyr 97–Lys 98 (the coöperative interaction involving Tyr 97, Lys 98 and Asp (NH_2) 101 is also possible), Tyr 97–Asp (NH_2) 94, and Tyr 115–Glu 2. The Tyr 76–Asp 121 bond could account for the ultraviolet difference spectrum of pepsin-treated ribonuclease.[34,36,37] It should be noted that this assignment of the hydrogen bonded tyrosyl groups is highly tentative since slight shifts in the positions of the backbone chains can implicate other sets of hydrogen bonded tyrosyls. For this reason it is difficult to say which three are the abnormal ones in the titration curve.

TABLE II

POSSIBLE INTERACTIONS BETWEEN SIDE-CHAINS OF H_1 AND THE REST OF THE MOLECULE

Hydrogen bonds	Hydrophobic bonds
Glu 2–Tyr 115	Ala 6–Val 116
Lys 7–Ser 11[a,b]	Met 17–Ileu 81
Glu 9–Asp (NH_2) 113	
Arg 10–Ser 75	
Ser 13–Thr 82	
Ser 14–Thr 78	
His 16–Arg 85[b]	
Glu 18–Glu (NH_2) 74	

[a] This bond is within H_1 and would contribute to the stabilization of the helix. [b] According to F. M. Richards and P. J. Vithayathil, "Abstract of 136th American Chemical Society Meeting," Atlantic City, N. J. Sept., 1959, p. 23-C, the Lys 7 and His 16 residues seem to be required for activity. The involvement of these groups in hydrogen bonds (in the model) may help maintain the helical folding of H_1.

The amount of exposure to the solvent varies among the four disulfide bridges. Bonds I–VI and III–VIII are relatively protected whereas bonds IV–V and II–VII are quite exposed. Anfinsen[38] has indicated that 2 of the 4 disulfide bridges are more susceptible to reductive cleavage. However, it is difficult to predict which two could be sacrificed and still preserve the enzymatic activity as is observed.[38,39] For example, only a slight enlargement of break B_6 would expose bond III–VIII; also rotations of some of the side chain groups near bond IV–V could protect this bond.

Despite the presence of susceptible bonds the native molecule is attacked only slowly by proteolytic enzymes.[40] This behavior may be accounted for by the fact that practically all of the bonds of *oxidized* ribonuclease attacked by trypsin, chymotrypsin and pepsin[5] are in helical portions of the model of the native molecule and thus are stabilized against enzymatic attack.[17,41,42] However, 3 such

(35) In this interaction the hydrogen of the phenolic hydroxyl group of Tyr 73 can be bound simultaneously to both oxygens of the carboxyl groups of Glu (NH_2) 71 and 74. Such a coöperative bond would raise the pK of the tyrosyl groups considerably.[30] If Glu (NH_2) 74 is involved in this interaction, then it could not be bonded simultaneously to Glu 18 as postulated in Table II.

(42) The C-terminus is represented as a helix at neutral pH. However, at the pH and temperature at which pepsin attacks ribonuclease,[9] the latter is partially denatured.[25] Presumably, under the conditions of pepsin digestion, the C-terminus of ribonuclease is unfolded.

(26) J. Hermans, Jr., and H. A. Scheraga, *Biochim. et Biophys. Acta*, **36**, 534 (1959).
(27) M. Calvin, J. Hermans, Jr., and H. A. Scheraga, THIS JOURNAL, **81**, 5048 (1959).
(28) O. O. Blumenfeld and M. Levy, *Arch. Biochem. Biophys.*, **76**, 97 (1958).
(29) A. Hvidt, *Biochim. et Biophys. Acta*, **18**, 306 (1955).
(30) M. Laskowski, Jr., and H. A. Scheraga, THIS JOURNAL, **76**, 6305 (1954).
(31) H. A. Scheraga, *Ann. Rev. Physical Chem.*, **10**, 191 (1959).
(32) D. Shugar, *Biochem. J.*, **52**, 142 (1952).
(33) C. Tanford, J. D. Hauenstein and D. G. Rands, THIS JOURNAL, **77**, 6409 (1955).
(34) M. Sela and C. B. Anfinsen, *Biochim. et Biophys. Acta*, **24**, 229 (1957).
(36) M. Sela, C. B. Anfinsen and W. F. Harrington, *Biochim. et Biophys. Acta.*, **26**, 502 (1957).
(37) C. C. Bigelow and M. Ottesen, *ibid.*, **32**, 574 (1959).
(38) C. B. Anfinsen, "Symposium on Protein Structure," Ed. by A. Neuberger, John Wiley and Sons, Inc., New York, N. Y., 1958, p. 223.
(39) M. Sela, F. H. White and C. B. Anfinsen, *Science*, **125**, 691 (1957).
(40) C. H. W. Hirs, *J. Biol. Chem.*, **219**, 611 (1956).
(41) M. Laskowski, Jr., and H. A. Scheraga, THIS JOURNAL, **78**, 5793 (1956).

bonds are in non-helical portions and their apparently increased strength may be due to stabilization from side-chain hydrogen bonds[41] or other interactions, *viz.*, the Arg 39–Cys 40 bond by the proximity of positive charges on Lys 37 and Arg 39, the Lys 66–Asp (NH_2) 67 bond by a Lys 66–Asp (NH_2) 67 hydrogen bond, and the Tyr 97–Lys 98 bond by the coöperative hydrogen-bonding interaction involving Tyr 97, Asp (NH_2) 101, and Lys 98. Those peptide bonds near the ends of the helical portions probably are not stabilized as much as those in interior portions of the helices. Presumably, the breakdown of the secondary and tertiary structure (*e.g.*, in oxidized ribonuclease) enhances the susceptibility to enzymatic attack.[41]

A variety of other observations on the effect of various chemical agents on the configuration of ribonuclease has been reported.[38,43] These observations seem to be compatible with the proposed model. Also, numerous interactions, not listed here in detail, exist between the side-chain groups in the model.

There are several ways to envision the association of the enzyme and substrate; for example, the substrate could be attached parallel to H_1 and lying across H_2, H_5 and H_6. If serine and histidine are involved in the "active center," several such pairs are close together, *viz.*, His 16–Ser 13, His 105–Ser 90, His 105–Ser 87 and His 119–Ser 123. However, whereas His 119 may be involved,[10] Ser 123 cannot be since this group can be removed by carboxypeptidase without loss of activity.[9] Obviously further experiments are required to locate the "active center" in the molecule.

In conclusion a model has been constructed which seems to be in accord with the available chemical evidence. Its importance lies in the fact that it provides a basis to plan experiments for the investigation of interactions between side-chain groups, along lines indicated elsewhere.[31] It may also be of help in Fourier analyses of X-ray data on ribonuclease crystals.

Acknowledgment.—I am indebted to Mrs. Joanne Widom for assembling the model.

(43) K. U. Linderstrøm-Lang and J. A. Schellman, "The Enzymes," Ed. P. D. Boyer, H. Lardy and K. Myrbäck, Vol. I, Academic Press, Inc., New York, N. Y., 1959, p. 443.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF SOUTHERN CALIFORNIA, LOS ANGELES 7, CALIFORNIA]

The Effect of Water upon the Rate of Heat Denaturation of Egg Albumin[1]

BY ROBERT L. ALTMAN[2,3] AND SIDNEY W. BENSON

RECEIVED DECEMBER 21, 1959

The rate of heat denaturation of solid egg albumin has been found to be extremely sensitive to the water content of the protein. This sensitivity makes it extremely difficult to obtain precise rate data in the system. This is further complicated by the small amounts of hysteresis observed in the sorption process and the slow approach to sorption equilibrium. Despite these difficulties it is possible to interpret the data in terms of a 12th order dependence ($\pm$ 2) on sorbed H_2O and an apparent activation energy of 75 $\pm$ 10 kcal. These values cannot be interpreted in terms of a simple breaking of hydrogen bonds leading to an unfolding of the peptide chain, nor can they be interpreted in terms of a homopolar fission of a covalent bond. They do indicate that some high energy process involving compensating solvation energy is involved in the denaturation process.

Introduction

Early studies of the heat denaturation of solid egg albumin showed that the denaturation process was greatly accelerated by the presence of water vapor.[4,5] Barker[6] reinvestigated this process in a rather crude manner, measuring the rate of heat denaturation of solid egg albumin as a function of the relative humidity of water. He found the heat denaturation rate exponentially related to the concentration of water vapor. His method, however, did not admit of very precise control or knowledge of the amount of water sorbed by the protein nor did they give any detailed information on the order of the reaction with respect to protein. To further elucidate both of these points the present study was undertaken.

The experimental approach followed in this research was to expose initially dry egg albumin to water vapor at a specific constant relative humidity (P/P_0) and temperature and determine the extent of insolubilization with time as a function of water vapor pressure and temperature. Initial work on determining the effect of relative humidity upon the denaturation rate was undertaken by using salt hydrates as the method of maintaining constant humidity.[7] Vapor pressure tables of those salts useful in the temperature range 80 to 100° appear in the International Critical Tables[8] and the Landolt-Börnstein Tabellen.[9]

From this work using hydrates or saturated solutions of NaCl, NaI, KBr and KI, it was soon found that the rate of denaturation was very sensitive to changing vapor pressure. But while internal re-

(1) This work has been supported by a grant (G-3541) from the U. S. Public Health Service of the National Institutes of Health.

(2) The material in this paper has been included in a dissertation submitted by R. L. Altman to the Graduate School of the University of Southern California in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(3) University of California, Berkeley, California.

(4) S. Lewith, *Arch. Exp. Pathol. Pharmakol.*, **26**, 341 (1890).

(5) H. Chick and C. J. Martin, *J. Physiol.*, **40**, 404 (1910); **43**, 1 (1911); **45**, 61, 261 (1912).

(6) H. A. Barker, *J. Gen. Physiol.*, **40**, 404 (1933).

(7) Barker, ref. 6.

(8) "International Critical Tables," Vol. I, McGraw-Hill Book Co., New York, N. Y., 1926, p. 67.

(9) Landolt-Bornstein, "Physikalisch-Chemische Tabellen," Vol. I, 5th Auflage, Julius Springer, Berlin, 1923, pp. 1288, 1907.