Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Conformations of Biopolymers

C. A. J. Hoeve[a]
[a] Department of Chemistry, Texas A&M University, College Station, Texas

To cite this Article Hoeve, C. A. J.(1981) 'Conformations of Biopolymers', Journal of Macromolecular Science, Part A, 15: 6, 1075 — 1087
To link to this Article: DOI: 10.1080/00222338108066454
URL: http://dx.doi.org/10.1080/00222338108066454

J. MACROMOL. SCI.–CHEM., A15(6), pp. 1075-1087 (1981)

Conformations of Biopolymers

C. A. J. HOEVE

Department of Chemistry
Texas A&M University
College Station, Texas 77843

ABSTRACT

Discussion of the history of biopolymers is focused on proteins and polypeptides. Rubber elasticity is discussed from the early days onward, when the first and second laws of thermodynamics were established. Insight in the elasticity of elastin, an amorphous protein occurring in ligaments and arteries, is followed against this background. Denatured proteins also fit in this category. At present, the random-coil state that underlies the elasticity is rather well understood as a result of the new methods of analyzing the dimensions in terms of the conformations of the residues. Subsequently, the discovery of the α-helix, as well as that of the other helical structures of DNA and collagen, is described. The conversion to random coils is followed with emphasis on our insight into the cooperative nature of the transition. Finally, the least understood globular proteins are considered. Major progress was made with the successful analysis of x-ray patterns. The native state is characterized by closely packed residues in complicated, but unique, patterns. The conversion to random coils (denaturation) is sharp, not unlike a phase transition. Although the native state is rather closely packed, some mobility still exists. The implication of this mobility for enzymatic action is hinted at.

INTRODUCTION

Reviewing the history of biopolymers is an arduous task at best in view of the vast expansion that has occurred in this century. Necessarily, anyone bold enough to undertake this task is open to criticism of being biased in his choice of subjects. I shall not pretend to be an exception.

Before the twentieth century, work on biopolymers was carried out under the collective name of colloidal chemistry, since the very existence of macromolecules was not established. In these times hair, collagen, ligaments, wood, as well as milk and blood proteins were visualized as physical associations. We shall here be concerned with the conformations of biopolymers as chemical entities and we shall thus not describe this early work. An exception is made, however, for the early thermodynamic developments of rubber elasticity. As we shall see, the concept of random coils, and thereby practically the entire field of polymers, can be directly traced to these studies. It is perhaps less known that, although mainly gases were taken as subjects for investigating the first and second law of thermodynamics, rubber also deserves its place in history.

Of course, conformations of biopolymers is so huge a field that limitations must be imposed. In view of the central role that proteins have played, I shall focus attention on proteins and polypeptides. In order not to lose sight of the meandering path of the emergence of our present knowledge, we have to abandon all attempts at a comprehensive coverage. Instead, only the milestones along the road toward our understanding will be highlighted.

RUBBERLIKE ELASTICITY AND RANDOM COILS

Long before the existence of polymer molecules was realized, thermodynamic measurements were performed on natural rubber that came from India. It is quite impressive to read the first accounts of rubber elasticity, as early as 1805, by John Gough [1]. He described, surprisingly accurately although qualitatively, the essential experimental results of rubber elasticity as follows. His first experiment: "Hold one end of the slip [of rubber] . . . between the thumb and forefinger of each hand; bring the middle of the piece into slight contact with the lips; . . . extend the slip suddenly; and you will immediately perceive a sensation of warmth in that part of the mouth which touches it. . . . For this resin evidently grows warmer the further it is extended; and the edges of the lips possess a high degree of sensibility, which enables them to discover these changes with greater facility than other parts of the body. The increase in temperature, which is perceived upon extending a piece of Caoutchouc, may be destroyed in an instant, by permitting the slip to contract again; which it will do quickly by virtue of its own spring, as oft as

the stretching force ceases to act as soon as it has been fully exerted." His second experiment: "If one end of a slip of Caoutchouc be fastened to a rod of metal or wood, and a weight be fixed at the other extremity . . . ; the thong will be found to become shorter with heat and longer with cold." What is truly surprising is that these crucial observations were made before the first and second law of thermodynamics had been formulated. The work of Carnot was published in 1824 [2]. In this ingeneous work with the objective to estimate the efficiency of engines, he realized that two reservoirs of different temperatures were required. He emphasized that a quantity of heat had to be withdrawn from the higher temperature and be given off at the lower temperature. Moreover, he reasoned that the maximum efficiency does not depend on the working substance, but only on the two temperatures. Despite the clear logic, his ideas went virtually unnoticed until the principles of the first and second law were perceived by Joule and Kelvin in the 1850s. It was only natural that these investigators, particularly Joule, were eager to investigate these principles. Besides gases, natural rubber was also made subject of their investigations. Lord Kelvin [3] derived the following general equation (in present notation) for the elasticity of any solid

$$C_p(\partial T/\partial f)_{adiab} = -T(\partial L/\partial T)_f \qquad (1)$$

This equation relates the adiabatic temperature change accompanying the application of a force to the length-temperature coefficient at constant force. Joule [4] tested this relationship by measuring separately the left- and right-hand sides of a slight variant of Eq. (1) and showed an excellent (for those days) agreement. We must realize that these experiments were conducted to test a prediction of what is now known as the second law and not to investigate the material properties of rubber. Equation (1) is a general thermodynamic equation. The negative sign of $(\partial L/\partial T)_f$ for rubber found by Joule, and earlier by Gough, was unique, however, since it was opposite to the generally observed positive thermal expansion coefficient for other materials. It became known as the Gough-Joule effect.

Also Joule could not appreciate the molecular interpretation of his finding, since in those days the existence of polymer molecules was unknown. It is remarkable, however, that he was aware of the close analogy between the heat effects accompanying adiabatic compression of gas and adiabatic extension of rubber. He already hinted [4] that in both cases these effects were due to the motion of the constituent particles. Of course, Joule could not grasp what these particles were. The prevailing idea was that rubber, wood, and fibrous proteins, such as tendon and ligaments, were physical, instead of chemical, associations between relatively small molecules. These associations were called colloids in general, and when known to be crystalline, micelles [5]. Only in the twentieth century did the concept of polymer molecules emerge.

Staudinger [6] finally proved convincingly that long molecules do exist by drawing attention to the high viscosity of their solutions. He proposed that the intrinsic viscosity is proportional to the degree of polymerization. Furthermore, he associated insolubility of some polymer samples with network structures of these long molecules. He did, however, not yet appreciate the flexibility of the chains, but considered them to be long, stiff rods. For the first time Meyer et al. [7] stated clearly that, although carbon bond lengths and bond angles are fixed, rotations around C–C single bonds are possible and that this rotation is sufficient to convey a high mobility to the bonds, not unlike that of molecules in a gas. Furthermore, they made the interesting observation that the elasticity of ligaments (from the neckbands of a cow) was more reversible than that of rubber, since in rubber the sulfur cross-links were sliding. Although qualitative, the concept of mobility must have had a great impact. From then on interest in polymer elasticity was renewed in a burst of activity and quantitative treatments developed rapidly.

Guth and Mark [8] and, simultaneously and independently, Kuhn [9] treated the flexibility of a chain by considering each chain link to be randomly oriented in space, independent of its neighbors. Each link represented a number of consecutive C–C bonds in a polymer molecule. In this manner the links could be considered to be independently oriented, whereas the bonds could not. Through this device, called a statistical element, the chain end-to-end distribution was proved to be Gaussian. It followed that the mean-square end-to-end distance was proportional to the number of statistical elements. These concepts were extremely powerful. At once, in complete generality for all polymers, it was established that the chains were not static but should be considered as dynamic structures with ever-changing shapes. Any measurement reflected an average over a distribution of shapes. Moreover, these dimensions could be estimated. Since rotation around single bonds was considered to be free, that is without any change in energy, deformation of chains by a force was likewise considered to be without any change in energy and thus a purely entropic effect. It was thus of paramount importance to measure these entropy effects on stretching rubber. How could this be done?

Wiegand and Snyder [10] developed a Carnot engine that converted heat into work with the working substance not a gas but a rubber band. As Carnot had already envisioned, such an engine should work with any working substance. Indeed, the former authors built an engine that worked. More importantly, however, they developed a most useful equation to measure the energy and entropy contributions to the retractive force. They wrote

$$f = (\partial F/\partial L)_{P,T} = (\partial H/\partial L)_{P,T} - T(\partial S/\partial L)_{P,T} \qquad (2)$$

where F is the Gibbs free energy. From the first and second law of thermodynamics they showed that

$$(\partial S/\partial L)_{P,T} = -\partial(f/\partial T)_{L,p} \quad (3)$$

combining Eqs. (2) and (3), they found

$$(\partial H/\partial L)_{P,T} = f - T(\partial f/\partial T)_{P,L} \quad (4)$$

Thus, if the force and its temperature dependence at fixed length were measured, the force could be decomposed in its energy and entropy components. These experiments were carried out by Meyer and Ferri [11]. They found that the enthalpy change was indeed negligible, confirming the earlier ideas that the retractive force is an entropy effect. Encouraged by this success, the same authors [12] performed similar experiments with ligaments (ox ligamentum nuchae), mainly consisting of the protein elastin. Indeed, from 42 to 62°C they obtained the same result, $(\partial H/\partial L)_{P,T} = 0$. At lower temperatures, however, they found $(\partial H/\partial L)_{P,T} < 0$. This meant that the enthalpy decreased with length, an effect opposite to that for a steel spring. Fortunately, however, a readily available analog was natural rubber, which displayed the same effect at high elongations. In that case crystallization was known to occur. It was thus quite natural that these authors proposed partial crystallization in elastin when stretched at temperatures lower than 40°C. This concept was reinforced by Wöhlisch and co-workers [13] who carried out similar measurements. They went even so far as to calculate values for the heat of crystallization of elastin.

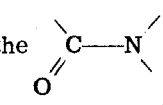
Much later [14-19] it was shown that elastin is indeed rubberlike, but that no crystallization occurs on stretching. The source of the earlier misinterpretation was the failure to distinguish between $(\partial U/\partial L)_{V,T}$ and $(\partial H/\partial L)_{P,T}$. The former coefficient should be used as the basis for determining the energy change in chains. The latter coefficient, experimentally obtained, also includes the effects of volume expansion on stretching the sample. Elastin was stretched while immersed in water. It was shown that the water uptake on stretching is responsible for the negative sign of $(\partial H/\partial L)_{P,T}$. When proper corrections are made for this water uptake, no appreciable energy changes occur during stretching.

Recent considerations [20-22] have shown that description in terms of the statistical element is not satisfactory. The rotations around single bonds are known to be restricted and to involve energy differences. Sophisticated methods have been developed [22], avoiding altogether the statistical elements, by starting with the conformations and their energy differences. If differences in energy exist, stretching the chain is accompanied not only be entropy changes but by energy changes as well. These energy changes can be measured by using Eq. (2) and by applying the proper corrections to obtain $(\partial U/\partial L)_{V,T}$. In turn, this coefficient can be related to $d \ln \overline{r_0^2}/dT$, the temperature

coefficient of the unperturbed mean-square end-to-end distance of the free polymer chains. Besides this coefficient, the value of r_0^2 is obtainable from light-scattering and viscosity measurements in dilute solution. These measurements, combined with calculations of the most stable conformations, have yielded copious results and insights in the conformations of a wide range of polymers [22]. For polypeptides the conformations have been shown to be relatively simple in that the energy of each peptide residue can be described as a function of only two rotational angles ϕ and ψ around C^α–N and C^α–C bonds, respectively. For a number of homopolymeric polypeptides the value for the characteristic ratio, $r_0^2/n\ell^2$, is in the range of 9.0 ± 0.5 [23]. In this expression n is the number of peptide units and the distance, ℓ, between two consecutive C^α atoms equals 3.80 Å. A range of denatured globular proteins, in which S–S links are broken by reduction, also occurs in random coil form. Despite the large variety of proteins, the value of the characteristic ratio was found to be remarkably constant, equal to 4.15 [24], in good agreement with calculated values [25]. Besides elastin, however, few native proteins occur as random coils; most fibrous proteins are crystalline and will be discussed in the next section.

FIBROUS PROTEINS

Foremost in the x-ray analysis of fibrous proteins was the work of Astbury [26]. He showed that silk molecules crystallized in practically extended zigzag chains called the β-form. Most other fibrous proteins, such as keratin, myosin, and collagen, crystallized in folded chains called α-forms. Some of these, notably keratin, could be converted into the β-form by stretching. The folds that he proposed for the α-forms were later shown to be incorrect. In Astbury's defense it must be remarked, however, that the quality of the patterns leaves much to be desired. The diffraction spots are few in number and considerably smeared. It was thus impossible to derive much information from these diagrams alone, without other data.

It was not until the work of Pauling and co-workers [27, 28] appeared in 1951 that great progress was made. By systematically studying a variety of amino acids and their analogs, they determined bond angles and bond lengths of the peptide group. A most important finding was that the  group was planar. With these data, combined with the requirement of a maximum number of H-bonds, they established that only a few preferred conformations should occur. One of these is the β-form in which the polypeptide chains are slightly folded into pleated sheats. In this form hydrogen bonds are formed

between the C=O group of one chain and N–H group of the neighboring chain. Hence hydrogen bonding is intermolecular. The most important structure proposed by Pauling et al. was not planar, as was invariably proposed before, but helical; it was the now celebrated α-helix. This is a structure with a translation along the helical axis of 1.5 Å per peptide unit. One complete rotation around the helical axis is performed after 3.6 units. This proposal violated a previously deemed rigid rule, that the number of units per repeat had to be a small integer. The concepts that helices were possible and that this rule could be violated opened up an entirely new method [29] of analyzing x-ray diagrams. It was realized that these helices derive their stability primarily from intramolecular H-bonds and only in a minor way from intermolecular forces. Consequently, these helical molecules can be dissolved without shape changes.

In 1953 Watson and Crick [30] proposed the double-stranded helix for DNA. In this structure bases of one strand form hydrogen bonds with those of the other. The bases are complementary in that adenine of one strand binds exclusively to thymine of the other strand; another complementary pair is that of guanine and cytosine. In this manner, in their entirety, the strands in DNA molecules are complementary. It was immediately recognized that this was the key to understanding duplication in genes. Vivid accounts of the history of this important discovery, that was to revolutionize biology, can be found in a monograph by Watson [31]. Discussion of this important work falls outside the scope of this review. In the context of conformational stability, it should be remarked that, even more than the α-helix, the double-stranded DNA structure is stabilized by intramolecular interactions.

Collagen is another fibrous protein that was analyzed by x-ray methods. Preliminary studies of Ramachandran and Kartha [32], much refined by Rich and Crick [33], showed that collagen consists of triple-stranded helices. These three strands are mutually stabilized by hydrogen bonds between them. All these helices, whether single or multiple stranded, were found to be stable in solution. The question arose: Can these rodlike structures be converted into the random coil state so characteristic for synthetic polymers?

HELIX COIL TRANSITIONS

Doty and co-workers [34-36] reported that poly-γ-benzyl-ℓ-glutamate was rodlike in most organic solvents, but that it was a random coil in dichloroacetic acid. With its analog, polyglutamic acid, Doty and Yang [35] showed that a sharp, reversible, helix-coil transition occurred with changes in pH, solvent composition, or temperature. As they realized, the sharpness of the transition indicates a cooperative effect.

It was not immediately recognized that in quite a different field of science, ferromagnetism, the theory for this cooperative effect

had already been developed by Ising [37]. He treated the case of a one-dimensional row of spins that could be up or down. Furthermore, the energy of two neighboring spins was assumed to be dependent on whether they were parallel or antiparallel. It is true that this was an oversimplified model for describing the three-dimensional case of ferromagnetism, but this one-dimensional problem could be solved whereas the three-dimensional problem could not. With the discovery of the helix-coil transition, essentially one-dimensional transitions became available experimentally.

Almost simultaneously, largely unaware of Ising's work, a number of investigators [38-44] treated the helix-coil transition. The simplest theory, able to explain the essential features, is that of Zimm and Bragg [38]. They showed that, to a good approximation, the problem could be treated in terms of parameters s and 1 for residues in the hydrogen-bonded and free form, respectively. Furthermore, they expressed the cooperativity parameter σ as a measure for the difficulty to change a nonhydrogen bonded sequence of residues (random coil sequence) into a hydrogen-bonded sequence (helical sequence). If $\sigma = 0$, the entire chain is either completely helical or completely random coil; the transition is then "all or none." No mixture of sequences is possible in the same molecule and cooperativity is a maximum. If, on the other hand, $\sigma = 1$, the probability for a residue to be hydrogen-bonded is independent of the state of its neighbors. In this case no cooperativity exists. Similar treatments can be given for the helix-coil transitions for DNA and collagen. In these cases the "coil state" represents two and three random coils, respectively.

GLOBULAR PROTEINS

Unlike randomly coiled and rodlike protein molecules, globular proteins do not have synthetic analogs. In view of the support derivable from simple structures, this is a serious disadvantage. It was early recognized that globular proteins are much more compact than random coils, since their solutions are much less viscous. Einstein [45] derived that for spheres the relative viscosity, $(\eta - \eta_0)/\eta_0$, is dependent only on the volume fraction of the spheres and not on their size. In this equation η and η_0 are the viscosity of the solution and the solvent, respectively. This result means that the intrinsic viscosity for compact spherical molecules is independent of molecular weight. Since many globular proteins followed this rule, the concepts of compactness and nearly spherical shape became quickly established. Nevertheless, larger intrinsic viscosity values were found and it was only natural that these deviations were attributed to nonspherical shapes. The simplest nonspherical shapes were ellipsoids, and thus theoretical work was carried out on the effect of the axial ratio of ellipsoids on various frictional properties [46]. As was

pointed out by Scheraga [47], however, no guarantee exists that the shape is indeed ellipsoidal. As a matter of fact, later x-ray work showed this assumption to be rather poor in general.

In view of the relatively large number of nonpolar residues usually present in globular proteins, Kauzmann [48] proposed that, to a large extent, the shape of the globule is determined by the tendency of these residues to associate and thereby to reduce their unfavorable (high free energy) interaction with water. These interactions are called hydrophobic interactions. As Kauzmann pointed out, these interactions are similar to those underlying the insolubility of hydrocarbons in water; they are unusual in that the enthalpy of dissolution in water is negative. The lowering in entropy is the reason for the low solubility. These losses in both enthalpy and entropy are consequences of the increased order induced in the structure of water in contact with the hydrocarbon compared to that of bulk water. Frank and Evans [49] coined the name "icelike" for these structures, although it is now recognized that they are much more mobile than ice. It followed from these ideas that the protein molecules would tend to form rather compact spheres with exclusion of water. Of course, x-ray analysis allows, in principle, a more precise determination of these shapes. Unfortunately, the analysis of the x-ray diagrams proved to be a gigantic, nearly impossible, task.

Already in the 1930s Bernal and Crowfoot [50] obtained the first x-ray diagram of pepsin. The sharpness and wealth of spots indicated that, despite their large size, all molecules were folded quite similarly. In the words of Perutz [51]: "It caused a sensation at a time when proteins were still widely regarded as 'colloids' of indefinite structure." It took, however, several decennia before a complete analysis was accomplished.

The first globular enzyme structure determined from x-ray patterns was myoglobin by Kendrew et al. [52]. It consists of a chain of 153 residues with a molecular weight of 18,000. In order to determine the phases of the x-ray reflections, isomorphous substitutions were carried out with heavy atoms, such as mercury compounds, according to methods developed by Perutz. In total, five different substituted analogs were analyzed. The 9600 reflections had to be measured, not only for myoglobin itself, but also for all of its analogs. Hundreds of hours of computing time (and many more man-hours) were required before the structure was resolved. The result showed that 65 to 72% of the residues formed runs of α-helices interrupted by sharp bends. Perutz et al. [53] showed that hemoglobin consists of four subunits of the myoglobin type. Later, Perutz [54] investigated the changes occurring during oxygenation and showed that small but definite changes occur in the myoglobin conformations around the heme group, which is in the active area of the enzyme. He furthermore emphasized that an enzyme is not a static, but a dynamic system.

With similar methods, x-ray diagrams of many other globular proteins have been analyzed. Few obvious similarities exist between

their structures. Several common elements are the following. The α-helix content is generally on the order of 20%, lower than in myoglobin. Almost invariably, the nonpolar residues are on the inside and the polar ones on the outside, just as Kauzmann [48] had predicted. An interesting analysis along these lines was given by Fisher [55]. He divided all residues into two groups: polar and nonpolar. Assuming that all residues are of equal volume and that all proteins are spherical, he calculated the ratio of polar to nonpolar residues as function of molecular weight for more than 30 proteins. The experimentally observed ratio, based on his subdivision in two groups, and the calculated ratio were generally in excellent agreement. If the experimental ratio was too large, corresponding to a larger number of polar residues, deviations from spherical shapes were observed. As expected, the deviations are particularly large for the fibrous proteins fibrinogin and myosin. On the other hand, for small values of the experimental ratio (low contents of polar residues), a strong tendency existed toward aggregation. By association of the nonpolar regions the unfavorable contact with water is reduced, and thus better agreement between the theoretical and experimental ratio was then obtained. Although these considerations are, of course, only semiquantitative, they nevertheless serve to emphasize the principle that hydrophobic residues are located on the inside of the globular proteins. Thus, unlike random coils, where the conformation of each residue is independent of that of others, globular proteins acquire their stability through interactions between many residues. It must further be recognized that, although few repeating patterns are detectable in the path followed by the tortuous chain, the sequence of rotational angles ϕ and ψ are unique for each globular protein. Otherwise, rich x-ray diagrams with sharp spots could not have been obtained. Just like the helix-coil transition, much has been learnt from the transition of globular proteins to random coils. In this case the transition is usually called denaturation.

CONFORMATIONAL CHANGES

Under a variety of conditions, such as changes in pH or temperature, the globular protein can be denatured. Amazingly, sometimes this denaturation can be made to reverse itself. Apparently, at least to a large extent, after the chain has been converted into a random coil, the chain can be folded in the same unique conformation that it had when in the native globular form. A beautiful and representative example is the denaturation of chymotrypsinogen by raising the temperature [56]. The extinction coefficient at 2950 Å served to monitor the transition. The transition region extends over only 10°. The sharpness was reminiscent of the helix-coil transitions observed before. Flory [57] emphasized, however, that this apparent similarity is treacherous. Unlike a helix, which is essentially a one-dimensional structure, a globular protein is a three-dimensional

structure. In the transition zone of the helix-coil transition, helical and random coil sequences occur in the same molecule since $\sigma > 0$. Any intermediates in denaturation of globular proteins are expected to be of extremely high free energy, since these intermediates would not be stabilized by the unique manner of packing representative of the native state and, on the other hand, they would not benefit from the large entropy enjoyed by random coils. Experiments [58] have largely confirmed that intermediates are undetectable. The uniqueness of the native conformation should not be interpreted as a complete immobilization, however.

Linderstrøm-Lang and co-workers [59] found that, although slower than in the denatured state, the peptide H-atoms throughout the entire native protein molecule can be exchanged by D-atoms. Thus the interior of the globule cannot be, at the same time, closely packed and immobile. Furthermore, Perutz's method of isomorphous substitution with heavy atoms is based on at least some mobility without denaturation. In some cases the heavy substituents penetrate deeply into the globule. Indeed, in biochemistry the ideas of Koshland [60] have gained wide acceptance. Based on reaction rate studies, he proposed that enzymatic action entails conformational changes in the enzyme which are induced by the substrate. Recent refined x-ray work of Sternberg et al. [61], designed to study internal displacements in lysozyme molecules in the crystalline state, suggest that internal motions occur. In general, the displacements increase with the distance from the center of the globule. It will be a great challenge to explain the rather subtle conformational changes occurring in this and other globular proteins and the manner in which these changes influence enzymatic activity. Although, as history teaches us, predictions are fallible, it seems fairly safe to predict rich harvests from future research efforts in this field.

ACKNOWLEDGMENTS

Support by the National Science Foundation grant number PCM78-08580 and by grant N00014-79-C-0267 of the Office of Naval Research is gratefully acknowledged.

REFERENCES

[1] J. Gough, Proc. Lit. Philos. Soc., Manchester, 2nd ser., 1, 288 (1805).
[2] S. Carnot, Réflexions sur la puissance motrice du feu, 1824 Facsimile Reprint, Dawsons of Pall Mall, London, 1966.
[3] Lord Kelvin, Q. J. Math., 1, 57 (1857).
[4] J. P. Joule, Trans. R. Soc. (London), A149, 91 (1859).
[5] C. von Nägeli, Theorie der Gärung, Munich, 1879, p. 121.
[6] H. Staudinger, Z. Angew. Chem., 42, 67 (1929).

[7] K. H. Meyer, G. von Susich, and E. Valkó, Kolloid-Z., 59, 208 (1932).
[8] E. Guth and H. Mark, Monatsh. Chem., 65, 93 (1934).
[9] W. Kuhn, Kolloid-Z., 68, 2 (1934).
[10] W. B. Wiegand and J. W. Snyder, Trans. Inst. Rubber Ind., 10(3), 234 (1934).
[11] K. H. Meyer and C. Ferri, Helv. Chim. Acta, 18, 570 (1935).
[12] K. H. Meyer and C. Ferri, Pflüger's Arch., 238, 78 (1936).
[13] E. Wöhlisch, E. Weitnauer, W. Grüning, and R. Rohrbach, Kolloid-Z., 104, 14 (1943).
[14] C. A. J. Hoeve and P. J. Flory, J. Am. Chem. Soc., 80, 6523 (1958).
[15] F. Mistrali, D. Volpin, G. B. Garibaldo, and A. Ciferri, J. Phys. Chem., 75, 142 (1971).
[16] C. A. J. Hoeve and P. J. Flory, Biopolymers, 13, 677 (1974).
[17] W. Grut and N. G. McCrum, Nature, 251, 165 (1974).
[18] K. L. Dorrington, W. Grut, and N. G. McCrum, Ibid., 255, 476 (1975).
[19] K. L. Dorrington and N. G. McCrum, Biopolymers, 16, 1201 (1977).
[20] M. V. Volkenstein and O. B. Ptitsyn, Zh. Tekh. Fiz., 25, 662 (1955).
[21] P. J. Flory, C. A. J. Hoeve, and A. Ciferri, J. Polym. Sci., 34, 337 (1959).
[22] P. J. Flory, Statistical Mechanics of Chain Molecules, Wiley-Interscience, New York, 1969.
[23] D. A. Brant and P. J. Flory, J. Am. Chem. Soc., 87, 2788 (1965).
[24] S. Lapanje and C. Tanford, Ibid., 89, 5030 (1967).
[25] W. G. Miller and C. V. Goebel, Biochemistry, 7, 3925 (1968).
[26] W. T. Astbury, Ann. Rev. Biochem., 8, 113 (1939).
[27] L. Pauling, R. B. Corey, and H. R. Branson, Proc. Natl. Acad. Sci., U.S., 37, 205 (1951).
[28] L. Pauling and R. B. Corey, Ibid., 37, 241, 729 (1951).
[30] J. D. Watson and F. H. C. Crick, Nature, 171, 737, 964 (1953).
[31] J. D. Watson, The Double Helix, Atheneum, New York, 1968.
[32] G. N. Ramachandran and G. Kartha, Nature, 174, 269 (1954).
[33] A. Rich and F. H. C. Crick, J. Mol. Biol., 3, 483 (1961).
[34] P. Doty, A. M. Holtzer, and J. H. Bradbury, J. Am. Chem. Soc., 76, 4493 (1954).
[35] P. Doty and J. T. Yang, Ibid., 78, 498 (1956).
[36] P. Doty, A. Wada, J. T. Yang, and E. R. Blout, J. Polym. Sci., 23, 851 (1957).
[37] E. Ising, Z. Phys., 31, 253 (1925).
[38] B. H. Zimm and J. K. Bragg, J. Chem. Phys., 28, 1246 (1958).
[39] J. H. Gibbs and E. A. DiMarzio, Ibid., 28, 1247 (1958).
[40] S. A. Rice, A. Wada, and E. Geiduscheck, Discuss. Faraday Soc., 25, 130 (1958).
[41] J. H. Gibbs and E. A. DiMarzio, J. Chem. Phys., 30, 271 (1959).
[42] B. H. Zimm and J. K. Bragg, Ibid., 31, 526 (1959).

[43] T. L. Hill, Ibid., 30, 383 (1959).
[44] L. Peller, J. Phys. Chem., 63, 1194 (1959).
[45] A. Einstein, Ann. Phys.(4), 19, 289 (1906); 34, 591 (1911).
[46] L. Perrin, J. Phys. Radium (7), 5, 497 (1934).
[47] H. A. Scheraga, Protein Structure, Academic, New York, 1961, Chap. 1.
[48] W. Kauzmann, Adv. Protein Chem., 14, 1 (1959).
[49] H. S. Frank and M. W. Evans, J. Chem. Phys., 13, 507 (1945).
[50] J. D. Bernal and D. Crowfoot, Nature, 133, 794 (1934).
[51] M. F. Perutz, Sci. Am., 211(5), 64 (1964).
[52] J. C. Kendrew, R. E. Dickerson, B. E. Standberg, R. G. Hart, D. R. Davies, D. C. Phillips, and V. C. Shore, Nature, 185, 422 (1960).
[53] M. F. Perutz, M. G. Rossmann, A. F. Cullis, H. Muirhead, G. Will, and A. C. T. North, Ibid., 185, 416 (1960).
[54] M. F. Perutz, Ibid., 228, 726 (1970).
[55] H. F. Fisher, Proc. Natl. Acad. Sci., U.S., 51, 1285 (1964).
[56] J. F. Brandts, J. Am. Chem. Soc., 86, 4291 (1964); 87, 2759 (1965).
[57] Ref. 22, Chap. 7.
[58] J. F. Brandts, in Structure and Stability of Biological Macromolecules (S. N. Timasheff and G. D. Fasman, eds.), Dekker, New York, 1969, Chap. 3.
[59] A. Hvidt and K. Linderstrøm-Lang, Biochim. Biophys. Acta, 14, 574 (1954); 16, 168 (1955); 18, 308 (1955).
[60] D. Koshland, Proc. Natl. Acad. Sci., U.S., 44, 98 (1958).
[61] M. J. E. Sternberg, D. E. P. Grace, and D. C. Phillips, J. Mol. Biol., 130, 231 (1979).