Physicochemical Aspects of Texturization: Fiber Formation from Globular Proteins

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ABSTRACT

The modification of proteins to produce fibrous or "fiber-like" textured products is a subject of unique importance and interest. Unfortunately, very little is known about the physicochemical properties of texturized "fibers," the mechanisms that produce them, or the forces that hold the protein molecules in a given structure. This paper deals with the following topics: (a) methods for characterizing the physicochemical properties of textured protein products; (b) the structures of naturally occurring fibrous proteins and synthetic polypeptides as possible models for texturized "fibers"; (c) the current understanding of the mechanisms and the forces required to form and stabilize fibrous protein structures; and (d) a brief and generalized survey of the presently available "fiber" forming processes.

INTRODUCTION

Proteins are unsurpassed in their textural versatility and structural variety. Nature has adapted proteins to meet its many different structures in skin, as hard and rigid structures in bones and nails, as textile fibers in silk and wool, as gels in gelatin, as meat, and as cheese. By appropriate manipulations, man has been able to adapt proteins, designed by nature for one specific function, to provide other functions. Soy protein, in particular, has this versatility. This native globular protein has been made by appropriate processing treatments to function in gels, emulsions, pastes, plastic coatings, and fibers.

An important and interesting aspect of protein texturization processes in the modification of globular proteins to produce fibrous or "fiber-like" products. Studies of the mechanisms involved in this process will greatly increase our understanding of the forces that operate to produce globular and fibrous proteins and will improve the technology needed for adapting the readily available globular proteins, as soy, to systems that require fibrous textures such as meats.

TERMS AND CONCEPTS

Protein Structure

Proteins are organized into three-dimensional structures on four structural levels. Proteins are formed from their constituent amino acids through the formation of peptide bonds. The peptide bonds form a backbone joining the amino acid residue in a long chain. The first level or organization, the *primary structure,* is the sequential arrangement of these residues in the chain. Thus, the amino acid composition, the number and sequence of residues in the chain, and the molecular weight of the chain are properties of the primary structure.

Structures that are formed and stabilized through hydrogen bonding represent the second level of organization, the *secondary structures.* Because of the appreciable double bond character in the peptide bond, rotation about the peptide bond is restricted. If there are no hydrogen bonds, disulfide bonds, and other interactions among the side groups, free rotation about all the other single bonds of the chain will be allowed. A protein chain in such a state is called a *random coil.* Formation of hydrogen bonds restrict this rotational freedom. Structures that result from optimum degree of hydrogen bonding are the *α-helix* (Fig. 1) and the *β-pleated sheet* (Fig. 2). In the single stranded α-helix, the hydrogen bonds are within a given chain; whereas in the β-pleated sheet, the hydrogen bonds may be interchain (between adjacent chains) or intrachain (between parts of a folded chain). The chains can be aligned either parallel or antiparallel to each other.

The three-dimensional arrangement of these secondary structures within a given chain corresponds to the third level of organization, *the tertiary structure.* Most proteins have either spherical or rodlike shapes for their tertiary structure. Proteins having spherical or nearly spherical shape are called *globular* (Fig. 3); whereas rodlike single chain structures normally form fibrous structures. A globular structure may contain areas of random coil, α-helix, and β-pleated sheets. For example, the globular 11S soy protein contains ca. 60% random coil, 5% α-helix, and 35% β-sheet (1,2).

The fourth level of organization, the *quarternary structure,* is the three-dimensional arrangement of single chains (called subunits) into the final structure of the multichain unit (Fig. 3). Again, the two major shapes of the multichain units are globular and rodlike. Rodlike fibrous structures are formed by the orderly packing of individual rods. An example is a proposed structure of collagen (Fig. 4) showing the regular packing of triple chain rods to form a microfibril.

Crystalline and Amorphous Solids

Thus far, we have considered the organization of protein structures on a molecular level. On a macroscopic level, these molecular structures are packed into crystalline or amorphous solids. Crystalline solids have regular repeating arrangement of the protein molecular units, whereas the protein molecular units in amorphous solids have a predominately random arrangement.

Fiber

The term fiber has many connotations. In general, it denotes any filament whose length is much greater than its diameter. Figure 5 illustrates some types of molecular arrangements that have been proposed for drawn filaments. In filaments a and b, there is random arrangement of the molecules, which results in amorphous filaments. In filaments c and d, there is ordered molecular arrangement, which results in crystalline fibers. In filament e, there is a mixture of crystalline and amorphous areas. In order to distinguish between crystalline and amorphous filament, we will use the term *fiber to designate only those filaments that have an appreciable degree of crystallinity along their filament axis.*

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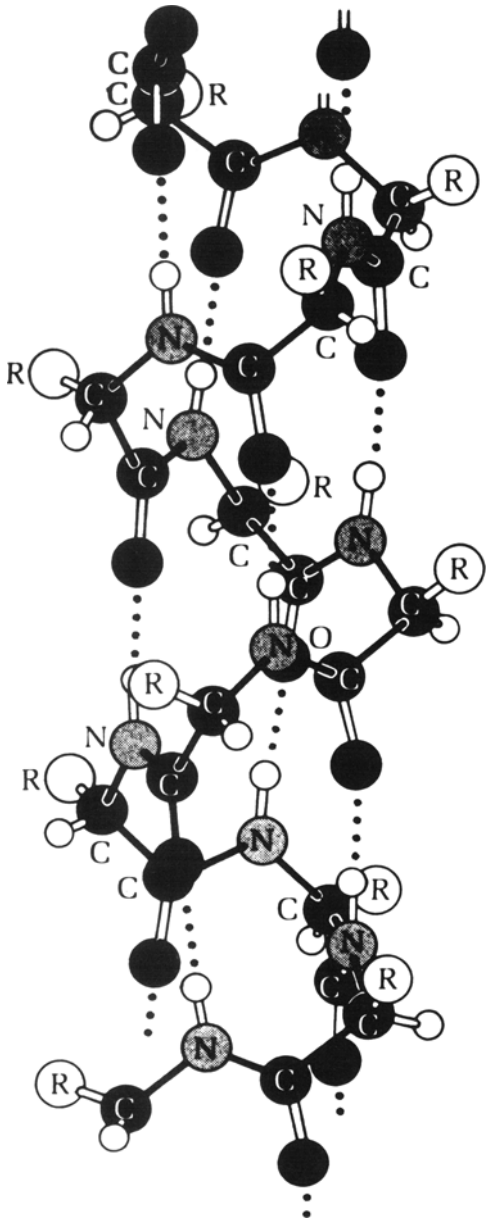

FIG. 1. Ball and stick model of a right-handed α-helix.

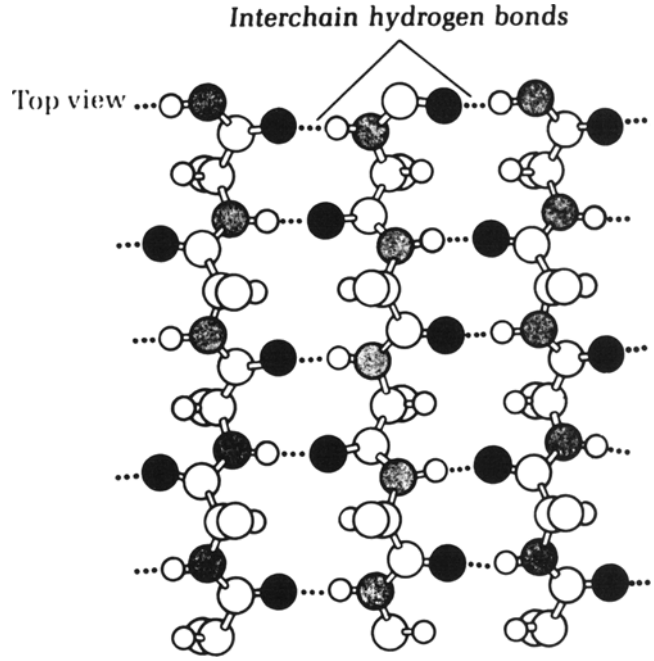

FIG. 2. Ball and stick model of an anti-parallel β-pleated sheet.

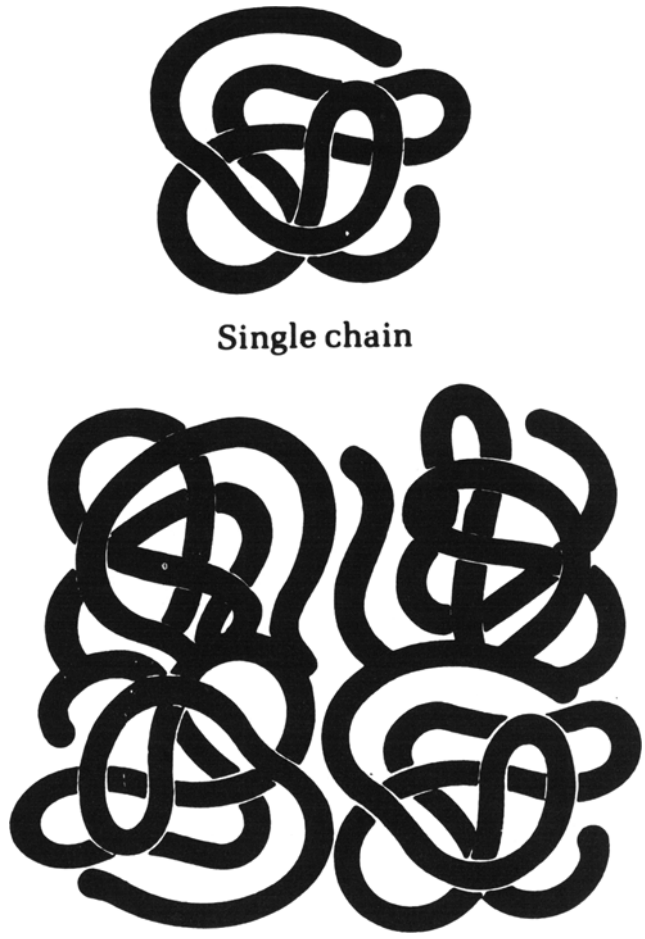

FIG. 3. Models illustrating globular tertiary and quarternary structures.

CHARACTERIZATION OF FIBROUS PROTEINS

There are many physicochemical and functional methods available for characterizing fibrous proteins. The methods include X-ray, electron, and neutraon diffraction; infrared, visible, and untraviolet spectroscopy; light scattering; birefringence; visible and electron microscopy; nuclear magnetic and electron spin resonance; and thermal, electrical, and mechanical analyses. A discussion of many of these techniques can be found in Hearle and Greer (4). We will discuss the application to fiber characterization of four especially useful methods: X-ray, electron microscopy, infrared spectrscopy, and mechanical analysis.

X-ray Diffraction (4,5-7)

The technique of X-ray diffraction is widely used for structural determination. It has been successfully applied to single crystals, crystalline powders, oriented films, and fibers. For single crystals, X-ray diffraction studies can reveal the dimensions of the unit cell, the molecular arrangement within the unit cell, and the atomic arrangement within the molecule. Such detailed studies are difficult. To date, only several dozen biologically important molecules have been fully analyzed.

The X-ray patterns of single crystals are composed of geometric arrays of many spots. Unlike single crystals, fibers exhibit crystalline order along the unique crystal axis but may lack regular order in the directions perpendicular

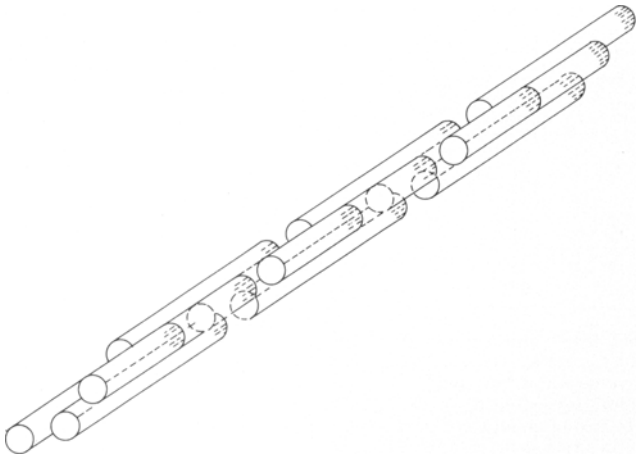

FIG. 4. Proposed model of collagen according to Veis et al. (3).

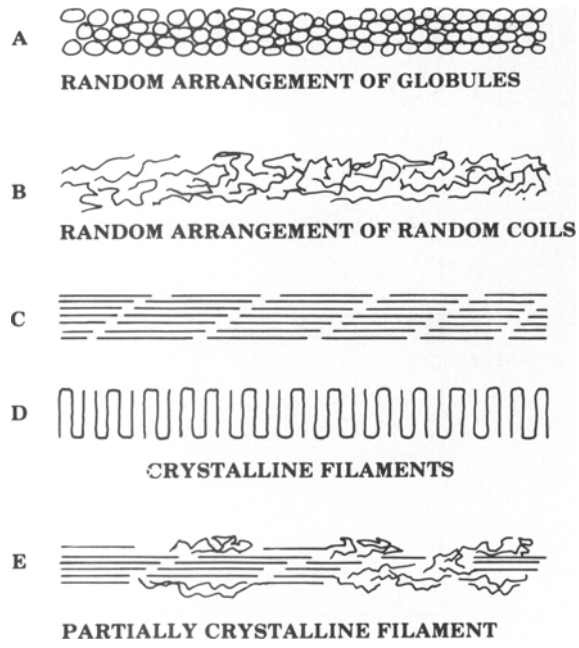

FIG. 5. Proposed molecular arrangements for drawn filaments.

to that axis. Thus, the X-ray pattern of fibers resembles the pattern of single crystals that are rotated about the unique crystal axis. The pattern will be composed of short arcs and diffused spots. Amorphous solids have no repeating order. Their X-ray patterns are composed of continuous rings like those of crystalline powders.

X-ray patterns can reveal the type of fiber structure and the degree of crystallinity. As an illustration of this technique, the X-ray patterns of Fraser et al. (5) for α,β, and amorphous wool keratin are given in Figures 6a, b and c, respectively. Characteristic of the X-ray patterns of α-helical fibers (Fig. 6a) are the symmetrical arcs at 5.15A° and the diffused spots at 9.8A°. In the characteristic β-pleated sheet pattern (Fig. 6b), the spots are shifted to 9.7A° and a pair of additional spots appear at 2.7A°. In the amorphous pattern, (Fig. 6c) only rings at 9.5A° and 4.5A° appear. By comparing the X-ray patterns of the man-made fibers with these typical patterns, the unknown structures of manmade fibers can be determined. Furthermore, by estimating the intensity ratio between the rings of the amorphous pattern and the spots and arcs of the crystlline α or β patterns, the degree of crystallinity can be estimated. In application to man-made soy filaments, this method would be useful in determining the degree of crystallinity, if any, that is

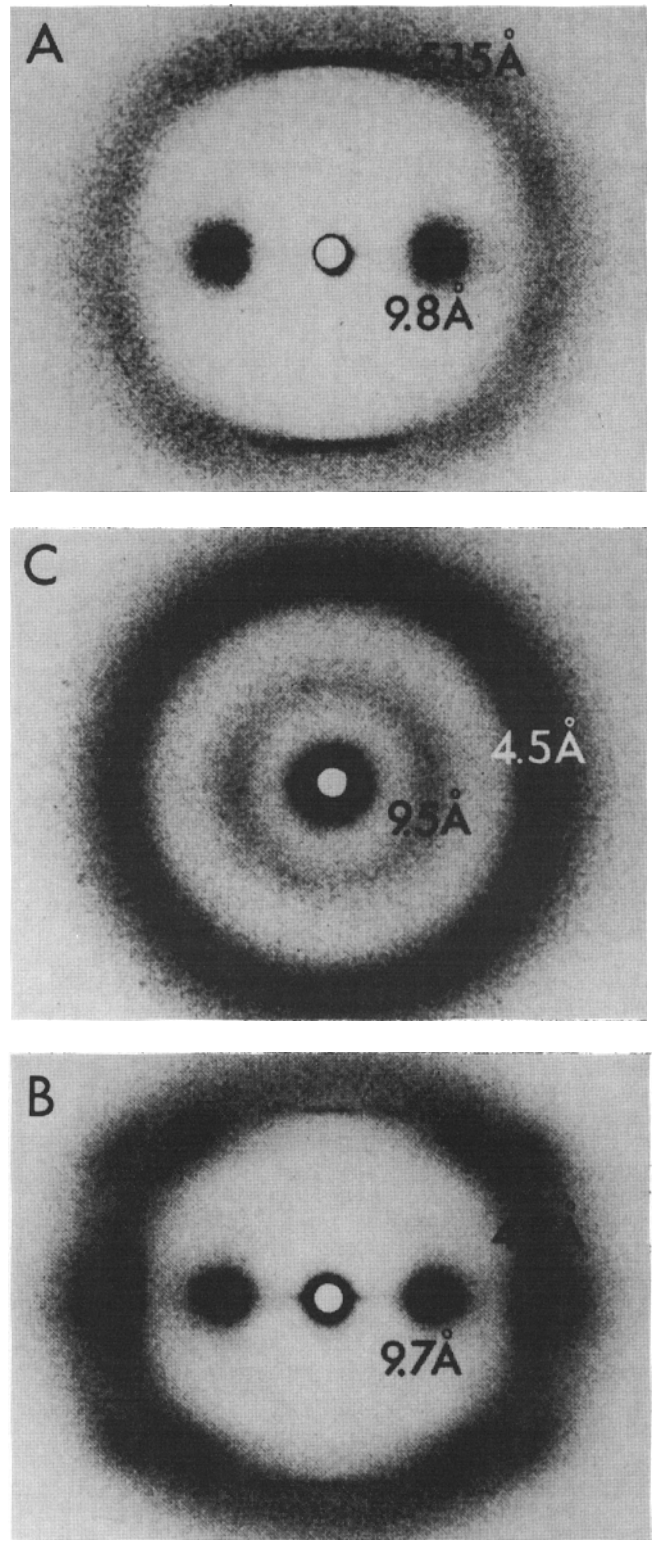

FIG. 6. X-ray patterns for wool keratin according to Fraser et al. (5). (a) α-Keratin. (b) β-Keratin. (c) Amorphous keratin.

produced by the filament formation process. In the case of keratins, this X-ray technique has been used to monitor the $\alpha \rightarrow \beta$ transition that occurs when α-keratin is stretched (6).

Electron Microscopy (4,7,8)

Electron microscopy is a widely used technique that greatly extends the resolution of the optical microscopy. Commercial scanning electron microscopes can achieve a resolution of 150A°. Thus, this technique is useful for looking at the gross structure of large fibers and fiber

TABLE I

Amide I Frequencies[a]

Conformation	Designation	Theoretical frequency	Krimm assignment	Silk (Bombyx Mori)
Random coil	υ_a	υ_0	1658	1660(m)
α-Helix	$\upsilon_{\|\|}$ (0)	$\upsilon_0+D_1+D_3$	1650	1650(w)
	$\upsilon_\perp$ ($2\pi/\eta$)	$\upsilon_0-0.17D_1$ $+0.50D_3$	1646	
Anti-parallel	$\upsilon_{\|\|}$ (0,π)	$\upsilon_0+D_1-D_1'$	1685	1695(m)
β-Sheet	$\upsilon_\perp$ (π,0)	$\upsilon_0-D_1+D_1'$	1632	1634(s)
	$\upsilon_\perp$ (π,π)	$\upsilon_0-D_1-D_1'$	1668	

[a]The notation of Miyazawa and Blout (10) is used. The || and ⊥ designation indicate the absorption of radiation polarized parallel and perpendicular to the fiber axis, respectively. The D_1 D_3, and D_1' symbols are interaction coefficients whose magnetitude depends upon the degree of coupling between the designated interacting vibrating units. The frequency assignments are in cm^{-1}. The s, m, w symbols indicate strong, medium, and weak intensities, respectively.

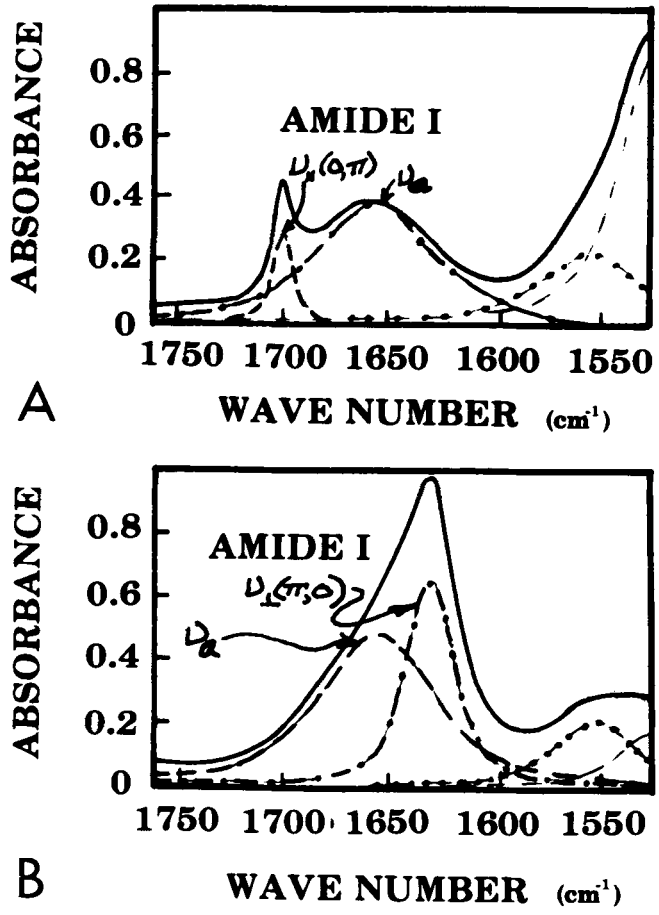

FIG. 7. Parallel (7a) and perpendicular (7b) amide I bands of silk fibroin according to Suzuki (11).

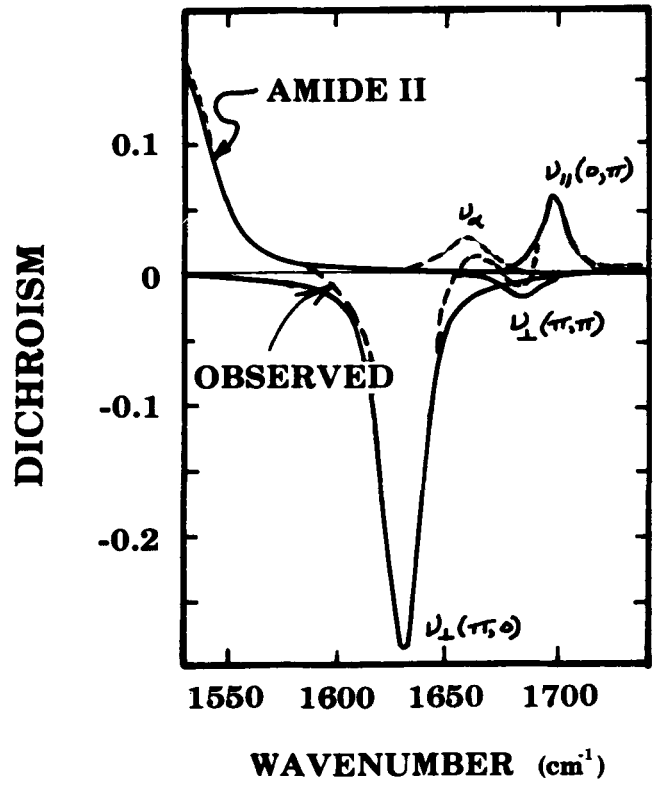

FIG. 8. Dichroism spectrum of porcupine quill (β-keratin) according to Fraser and Suzuki (15).

bundles. The resolution is, however, too poor to pick out individual microfibrils. The advantage of this technique is the ease and versatility of application.

The transmission electron microscope can achieve a resolution of 20A°, which is enough to pick out individual microfibrils. The resultion increases in the order: optical microscopy < scanning electron microscopy < transmission electron microscopy < X-ray diffraction. Thus, these methods are complementary allowing us to view structures at many different orders of magnification.

Infrared (IR) (9-16)

Infrared spectroscopy (IR) can be used to detect the presence of secondary structures. For fibers, the information available from IR confirms and extends the information obtainable from X-ray diffraction. The presence of α-helix or β-sheet structure will split and shift the unperturbed random coil absorption bands. The predicted shifts, the frequency assignments of Krimm (9), and the actual bands found for the amide I vibration of silk fibroin (Bombyx Mori) are tabulated in Table I.

For fibers having a mixture of crystalline and amorphous areas, quantitative analysis of the degree of crystallinity can be achieved by deconvolution of the composite spectra into the component bands. An example of such deconvolution (11) on the parallel and perpendicular amide I bands of silk fibroin is given in Figures 7a and 7b, respectively. Even though silk fibroin has a high content of crystalline β structure, the contribution of the amorphous areas, ν_a, is clearly visible.

Figure 8 is a deconvoluted dichroism spectra of porcupine quill β-keratin. Dichroism spectra measure the difference in absorption ($\nu_{||}$-$\nu_\perp$) of light polarized parallel and perpendicular to the fiber axis. Because the amorphous areas absorb parallel and perpendicular light equally, they

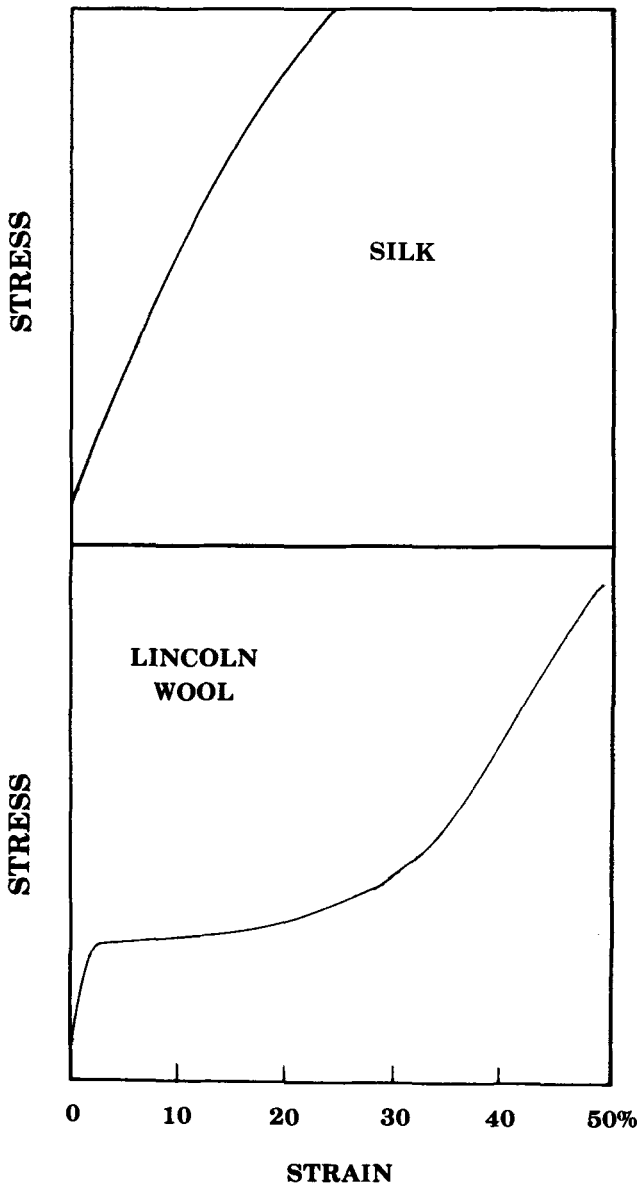

FIG. 9. Comparison of the stress-strain behavior of silk with that of wool (20).

do not contribute to the dichroism. In the absence of the amorphous band, the very small contribution (ν_α) of the α-helical regions can be seen.

Mechanical Analysis (3,17-20)

Types of mechanical measurements that can be made to characterize fibers include stress needed to break the fiber (tensile strength); stress as a function of elongation, temperature, or water activity; and time-dependent response of stress to strain. Besides yielding important functional information, these mechanical measurements in conjunction with model fiber studies and appropriate correlations can yield information about the basic physicochemical structure of the fiber. For example, the difference in the stress-strain behavior of silk and wool in Figure 9 is directly attributable to the differences in structure of the two fibers. Because of its lower crystallinity and higher degree of cross-linking, wool can be much more extended before breaking than can silk. The plateau in the wool curve is attributed to the $\alpha \rightarrow \beta$ transition that occurs in wool but not in silk.

Figure 10 is an example of a thermoelastic study. A regenerated feather keratin is heated in water at constant elongation. The stress is monitored as a function of temperature. As the temperature is increased a sharp cooperative transition from the original crystalline state to a rubber-like state is observed. As the fiber is cooled, the stress does not revert to the original line because the rubber-like state reverts very slowly back to the crystalline state.

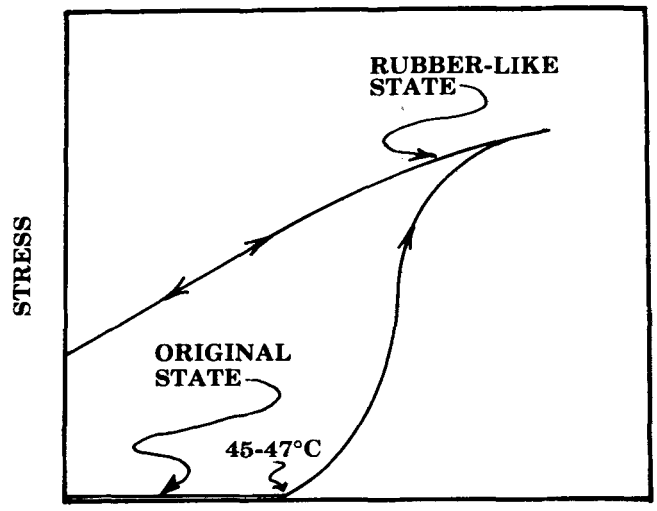

FIG. 10. Thermoelastic behavior of regenerated feather keratin heated in H_2O at pH 5 according to Lundgren (21).

NATURALLY OCCURRING PROTEIN FIBERS

There are large varieties of naturally occurring fibrous proteins exhibiting widely different structural and functional properties. To illustrate this variety and complexity, features of silk and wool, two well-characterized fibrous proteins, will be summarized.

Wool (α-keratins)

Wool is a naturally occurring protein fiber in the keratin family. Keratins are complex sulfur-containing proteins found in the outer protective tissues of vertebrates. Wool is classified as an α-keratin according to its X-ray diffraction pattern. It is composed of α-helical microfibrils embedded in an amorphous matrix.

The crystalline microfibrils, composed of low sulfur proteins, comprise ca. 50% of the total wool protein. The basic unit is a double stranded α-helix. These units join to form microfibrails of 70Å in diameter. Structural analyses data suggest that the microfibril is composed of two central strands surrounded by nine more strands on the outside. These microfibril units are embedded in a high sulfur matrix. End groups from the microfibrils protrude into the matrix and become cross-linked to the matrix through disulfide bonds during the hardening process.

The matrix is an amorphous body of high cystine proteins, which are extensively cross-linked through disulfide bond formation. This cross-linking within the matrix and between the matrix and the microfibrils gives stability and wet strength to the wool fiber, and the unique mixture of crystalline microfibrillar and amorphorous regions gives unique stretching properties to wool (17,18).

Silk (Fibroin)

Silk is composed mainly of a fibrous component called fibroin. Fibroin proteins contain ca. 86% glycine, alanine, and serine residues which have small side chains that pack tightly into crystal lattices. Thus, silk fibroin is highly crystalline. Most of the other bulky residues are outside of the crystalline areas. Silk has ca. 0.2% half cystine. Thus, it has a negligible degree of disulfide cross-linking.

TABLE II

Comparison of Silk and Wool Proteins

Property	Silk	Wool
Percent of amino acids with small side groups	high	low
Half cystine content	0.2%	high
Crystallinity	high	low
Cross-linking	low	high
Solubility (aqueous)	insoluble	insoluble
Elongation at break	low	high
Stabilized by	crystal packing	disulfide cross-links

TABLE III

Comparison of Some Properties of Proteins Derived from Their Amino Acid Compositions

Protein	Molecular weight x 10^{-3}	Hϕ Kcal./ residue	p	Average charge	% Alanine glycine serine	% Half cystine
Silk[a]	100?	480	0.45	0.033	85.9	0.2
Soy Isolate[b]	22[c]	870	1.62	0.37	17.0	0.8
Wool (low sulfur)[d]	50	940	1.24	0.38	21.6	6.1
Lysozyme[a]	15	970	1.18	0.14	26.4	6.2
α_s-casein[a]	24	1200	1.27	0.24	14.5	0.2
β-Lactoglobulin[a]	17	1250	1.09	0.41	14.8	3.1

[a]Bigelow (28).

[b]Amino acid analysis of RAckis et al. (32) was used.

[c]Also higher molecular weight subunits.

[d]Amino acid analysis of Thompson and O'Donnell (33) was used.

Silk fibroin occurs predominantely in the form of β-pleated sheets. Strands are intermolecularly hydrogen bonded to each other to produce the β-sheets. The silk fibrils are produced by nature under conditions that resemble those of an extrusion process. Studies (24) on hydrocarbon polymers as polyethylene and isotactic polystyrene have shown that proper high shear and setting conditions can induce crystallization of the molten polymers. Silk may be an example of such stress-induced crystallization occurring in nature.

The stability of silk, its great tensile strength, and its inertness to water are the result of its high crystallinity. The crystal packing is so stable and extensive that it requires much energy to disrupt it.

Comparison of Wool with Silk

A selected set of properties for wool and silk are compared in Table II to contrast these two fibers. Because of the differences in their primary structure, they have widely different secondary, tertiary, and quarternary structures. And as a result, their functional properties are widely different. These two proteins illustrate the two types of forces that can stabilize a given fibrous structure. In wool, disulfide cross-linking gives wool strength; whereas in silk, crystal packing is the basis for stability. These factors alone or in combination might be used to form soy protein fibers.

FACTORS FAVORING FIBER FORMATION

From our knowledge of naturally occurring fibrous and globular proteins, we can deduce the factors that favor fiber formation and contrast them with those that favor globular structures.

Characteristics Favorable to Fiber Formation

Large molecular weight is a requirement for fiber formation. Fibers from polyamide polymers do not achieve appreciable tensile strength until the chain lengths exceed 60 residues (21). For proteins, this represents a minimum chain molecular weight of 7000 daltons. The tensile strength of the fibers increases with increasing subunit chain length until a limiting plateau is reached at ca. 200 residues. (Subunit chain molecular weight $\sim$22,000 daltons.) Longer subunit chain length does not increase the tensile strength of the resulting fiber. Much longer subunit chains are detrimental to fiber formation (22). While large molecular weight is a requirement for fiber formation, it does not necessarily favor fiber formation. There are well-known proteins, such as soy proteins, containing subunits of 22,000 daltons or larger with native globular quaternary structures (23).

The extended nature of fibers required extended molecular building blocks. These can be single or multiple stranded α-helical strands or extended random coils. Examples of single stranded helices are synthetic polypeptides as poly-L-glutamic acid (7). Wool is an example of a double stranded helix, and collagen is an example of a triple helix. Fully extended random coils can be packed into extended β-sheets as in silk fibroin.

Crystalline molecular packing requires small amino acid side groups that will fit into lattice sites. Large bulky groups will be unable to fit into the sites and will prevent crystal formation (22). It is not necessary that entire chains be composed of small side group residues. It is sufficient to have regions of the chains to be composed primarily of small side groups. Under proper conditions, these segments will crystallize and those segments containing predominantly bulky groups will remain amorphous. Thus, proteins with appreciable amounts of bulky groups may still have an appreciable amount of crystalline areas if such a favorable arrangement of residues exists. On the other hand, random coils that have their sequences regularly interrupted by bulk side groups will not be expected to crystallize. Random coils as isotactic polystyrene with regularly spaced bulky groups that can fit into an ordered lattice are exceptions to this rule.

Fiber formation involves the aligning of α-helices or extended random coils side by side and holding them there to form a crystalline filament. Regularly spaced cohesive forces are needed to hold the aligned chains in place (22). If there are sufficient chain segments not held together by

attractive forces or experiencing repulsive forces, rearrangement out of this aligned configuration to a more favorable nonaligned structure will occur. These cohesive forces include the electrostatic attraction of unlike charges, hydrogen bonding, apolar (hydrophobic) interactions, and cross-linking through disulfide or other groups.

Cross-linking between chains serves to stabilize the aligned structures. Proper degree of interchain cross-linking will make the chains easier to align; and once aligned, the cross-links act as cohesion sites holding the chains together and enhancing crystallization. Intermolecular cross-linking is extensively used in the shear-induced crystallization of hydrocarbon polymers (24). Intrachain or intramolecular cross-links will hold the chain or molecule in a folded fashion making it impossible to be fully extended. Thus, they tend to stabilize folded globular structures.

In an aqueous environment, polar or hydrophilic groups are stabilized by interacting with water; whereas nonpolar or hydrophobic groups are more stable away from water. The hydrophobic bond is the term used by Kauzmann (25) to describe the gain in stability or the lowering of free energy on the transfer of nonpolar residues from an aqueous environment to the nonpolar interior of the molecule. It is widely recognized that hydrophobic bonding makes a major contribution to the stability of globular proteins (25-27).

Estimation of Hydrophobicity

There are a number of methods for estimating the hydrophobicity of a protein (28) and using it to predict its structure. Here, we summarize two of the methods.

Fisher (29) assumed that all the hydrophobic groups of native proteins are buried in the interior and all hydrophilic groups are on the surface. He assumed that the proteins are tightly packed and arbitrarily assigned Arg, Asp, Glu, His, Lys, Ser, Thr, Tyr to be hydrophilic. All other residues are considered to be hydrophobic. Using the above definitions and the specific volumes for each residue, he calculated a parameter p, defined as the ratio of the total volume of the hydrophilic residues to the total volume of the hydrophobic residues.

$$p \equiv (\text{Vol. hydrophilic Res.}) / (\text{Vol. hydrophobic Res.})$$

Given a molecule of fixed total volume, the parameter p determines the shape that the molecule must assume in order to have all its hydrophilic groups on the surface and all its hydrophobic groups buried. Large p's will require extended shapes (rods, cylinders, etc.) that have high surface area to interior volume ratios. Small p's will require spherical shapes that minimize the surface area to interior volume ratio. For extremely small p's such that even spherical shapes are not sufficient to bury all the hydrophobic residues, aggregation will occur to increase to number of buried groups.

Tanford (31) established a numerical hydrophobicity scale by measuring the free energy gained by transferring amino acid side groups from water to ethanol. Ethanol was taken to be representative of the hydrophobic protein interior. Bigelow (28) used Tanford's values to calculate the average hydrophobicity ($H\phi$) for a number of proteins. Defined this way, this average hydrophobicity equals the stabilizing energy per residue resulting from hydrophobic bonding within the protein molecule. High average hydrophobicity favors a globular (spherical) conformation, and low average hydrophobicity favors the extended shapes.

Comparison of Properties Derived from Amino Acid Composition

In Table III, soy protein and casein are compared with some naturally occurring fibrous and globular proteins with regard to the properties discussed above. All the values listed are derived from known amino acid compositions. The average charge was calculated by assuming Arg, Asp, Glu, His, Lys to be charged at pH 7. Of the listed proteins, silk and wool are fibrous; α-casein is a random coil; and all the others are globular in their native states. On the basis of these data, silk is quite different from all the other listed proteins. Except for the cystine content, soy proteins are not too different from wool. On this basis, it might be expected that wool-like fibers but not silk-like fibers can be produced from soy proteins. It might be necessary to introduce some additional cross-links to compensate for the low cystine content of soy protein. By similar comparisons, α_s-casein and β-lactoglobulin seem to be too hydrophobic and to contain too few small residues (Ala, Gly, and Ser) to form wool-like fibers. It must be remembered that the above analyses are based upon amino acid composition. Effects due to amino acid sequence may be much more important. For example, the average hydrophobicity of α-casein is much larger than that of soy proteins. Based on the average hydrophobicity only, the Fisher and Bigelo models would predict α_s-casein to have a higher tendency to assume a globular conformation that would soy proteins. Yet, the native structure of α_s-casein is an extended random coil; and the native structures of soy proteins are globular.

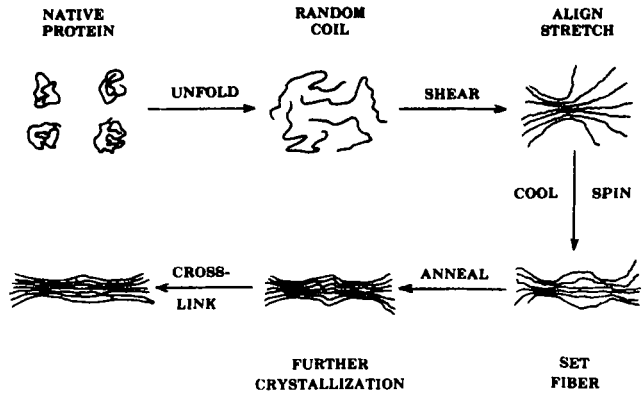

FIG. 11. Schematic representation of the postulated fiber forming process.

TABLE IV

Synthetic and Reconstituted Protein Fibers

Globular proteins	Nonglobular proteins
Cottonseed proteins	Casein
Egg white albumin	Collagens
Hemoglobin	Fibrinogen
Insulin	Fish proteins
β-Lactoglobulin	Keratins
Ovalbumin	Muscle proteins
Peanut proteins	Zein
Soy proteins	

MECHANISM OF FIBER FORMATION FROM GLOBULAR PROTEINS

Figure 11 is a schematic representation of the process of transforming globular proteins into fibers. The major steps of the process have been postulated for some time (21,22). The overall mechanism can be separated into five major steps. One, the protein must be dissolved and denatured to provide a shearable solution of random coils. During this step, intramolecular disulfide cross-links that can prevent complete denaturation to random coils must be reduced. Two, the random coils are sheared to stretch and align them. Three, once the stretched random coils are aligned, they must be fixed before they relax back to a random distribution. Four, after setting, the fiber is annealed by

heating it under stretch to just below its fusion temperature to cause the crystalline areas to grow. A phase transition from a rubber-like state to a crystalline state is sometimes observed during this process. Finally, in the fifth step, the fiber can be cured by cross-linking agents. This curing step is often desirable and necessary in order to harden the fiber and to make it insoluble in water.

FIBER FORMING PROCESSES

Many varieties of processes and equipment have been devised which achieve in varying degrees the desired results of the above five stages of fiber formation. Following is a brief survey of some of these processes and equipment.

Although nonaqueous solvents have been used, the predominent solvent systems used for proteins are aqueous. For soy and other vegetable proteins, high concentration slurries of neutral to alkaline pH are normally used (34). Agents used to increase solubility are often added. Denaturation of the proteins to random coils can be accomplished by heating, by raising the pH, by the action of detergents, and by using urea, guanidine hydrocholoride or certain amides. Any of these methods can be used alone or in combination. Disulfide bond reducing agents are also normally included.

Heating is often done under extrusion conditions. The degree and intensity of heating vary with the protein system used, the type of equipment, and the manufacturer (34). When alkali is used as a denaturing agent, the viscosity and pumpability of the dope solution are important considerations (35). Highly concentrated slurries often gel at high pHs. Urea, guanidine hydrochloride, and SDS are effective unfolding agents; but these agents must be removed during or after the setting stage (36).

Shear has traditionally been achieved by extrusion through dies or spinning through small orifices or capillaries (34). However, the shear forces developed by the conventional food protein extruders may not be sufficient to produce the necessary stretching and aligning of the protein chains to provide optimum degree of crystallinity in the fibers. Recent advances in the field of stress-induced crystallization of hydrocarbon polymers have produced a variety of equipment that can produce much higher and more effective shearing forces.

Setting conditions are determined primarily by the protein and solvent systems used. Fibers can be regenerated from keratin and gelatin solution by cooling. For heated soy and other vegetable protein slurries, rapid cooling with the simultaneous evaporation of water is sufficient to set the filament. Alkaline dope solutions are spun into acid baths or baths containing Ca^{++} or Mg^{++} to coagulate the protein. Some processes add gelling agents to the dope slurry in order to facilitate the setting of the silament. These agents include alginates, gums, and polyacrylic acid (34).

In the normal soy or vegetable protein filament spinning process, the filaments coming from the spinning bath are stretched by a "take-up" wheel moving faster than the extrusion rate. In this way, the filaments are stretched while they are coagulating. The applied stress increases the alignment of the protein chains and retards relaxation of the aligned chains back to a random distribution. After setting, further annealing can also increase the crystallinity of the fiber.

There are many cross-linking agents that can be used to harden the stretched fiber. Available cysteine can be readily converted to disulfide cross-links through oxidation. Formaldehyde and glutaraldehyde are frequently used cross-linking agents. (See reference 19 for a brief discussion.) Some novel up-to-date cross-linking techniques were presented in a recent symposium (37). Hydrocolloids and food binders have also been used.

SYNTHETIC PROTEIN FIBERS

Table IV is a partial list of the proteins that have been made into filaments. Reconstituted keratins and collagens are fibers in that they have an appreciable degree of crystallinity. Insulin and egg white albumin filaments also have appreciable amounts of crystalline structure. These fibers possess a β-pleated sheet structure. Stretched egg white fiber has tensile strength approaching that of wool. The evidence on soy protein filaments is unclear. Scanning electron microscopy show no signs of fibrous structure. Mechanical properties as weak tensile strength indicate amorphous gel structure. There is, however, X-ray evidence of β-pleated sheet structure in stretched films (38).

REFERENCES

1. Koshiyama, I., J. Sci. Food Agric. 23:853 (1972).
2. Koshiyama, I., and D. Fukushima, Cereal Chem. 50:114 (1973).
3. Veis, A., R.S. Bhatnager, C.A. Shuttleworth, and S. Mussell, Biochem. Biophys. Acta 200:97 (1970).
4. Hearle, J.W.S., and R. Greer, Text. Prog. 2:1 (1970).
5. Fraser, R.D.B., T.P. Mac Rae, and G.E. Rogers, "Keratins: Their Composition and Biosynthesis," Thomas, Springfield, IL, 1972.
6. Bendit, E.G., Text. Res. J. 30:547 (1960).
7. Fraser, R.D.B., and T.P. Mac Rae, "Conformation in Fibrous Proteins," Academic Press, New York 1973.
8. Slayter, E.M., in "Physical Principles and Techniques of Protein Chemistry," part A, Edited by S.J. Leach, Academic Press, New York, 1970, Ch. 1.
9. Krimm, S., J. Mol. Biol. 4:528 (1962).
10. Miyazawa, T., and E.R. Blout, J. Am. Chem. Soc. 83:712 (1961).
11. Suzuki, E., Spectrochim. Acta 23A:2303 (1967).
12. Baddiel, C.B., J. Mol. Biol. 38:181 (1968).
13. Bendit,E.G., Biopolymers 4:539 (1966).
14. Timasheff, S.N., H. Susi, and L. Stevens, J. Biol. Chem. 23:5467 (1967).
15. Fraser, R.D.B., and E. Suzuki, Spectrochim. Acta 26A:423 (1970).
16. Bradbury, J.H., in "Physical Principles and Techniques of Protein Chemistry, art B, Edited by S.J. Leach, Academic Press, New York, 1970. Ch. 11.
17. Chapman, B.M., J. Text. Inst. 60:181 (1969).
18. Chapman, B.M., Text. Res. J. 39:1102 (1969).
19. Singleton, R.W., in "Man-Made Fibers," Vol. 3, Edited by H.F. Mark, S.M. Atlas, and E. Cernia, Interscience, New York, 1968.
20. Brown, A.E., and J. Menkart, in "Ultrastructure of Protein Fibers," Edited by R. Borasky, Academic Press, New York, 1970, pp. 9, 15.
21. Lundgren, H.P., Adv. Protein Chem. 305 (1949).
22. Huang, F.F., and C. Rha, Polym. Eng. Sci. 14:81 (1974).
23. Kitamura, K., T. Takagi, and K. Shibasaki, Agric. Biol. Chem. 40:1837 (1976).
24. Peterlin, A., Polym. Prepr. 16:315 (1975).
25. Kauzmann, W., Adv. Protein Chem. 1 (1959).
26. Chothia, C. Nature 248:338 (1974).
27. Ryan, D.S., 1st Chem. Congress of the N. Amer. Continent, Mexico City, 1975, Agrochemistry paper 39.
28. Bigelow, C.C., J. Theor. Biol. 16:187 (1967).
29. Fisher, H.F., Biochemistry 51:1285 (1964).
30. Tanford, C., J. Am. Chem. Soc. 84:4240 (1962).
31. Nozaki, Y., and C. Tanford, J. Biol. Chem. 7:2211 (1971).
32. Rackis, J.J., R.L. Anderson, H.A. Sasame, A.K. Smith, and C.H. Van Etten, J. Agric. Food Chem. 9:409 (1961).
33. Thompson, E.O.P., and I.J. O'Donnell, Aust. J. Biol. Sci. 18:1207 (1965).
34. Hanson, L.P., "Vegetable Protein Processing," Noyes Data Corp., Park Ridge, NJ, 1974.
35. Circle, S.J., E.W. Meyer, and R.W. Whitney, Cereal Chem. 41:157 (1964).
36. Palmer, K.J., and J.A. Galvin, J. Am. Chem. Soc. 65:2187 (1943).
37. Friedman, M., organizer, "Symposium on Nutritional and Biochemical Consequences of Crosslinking," 172nd ACS Natl. Meeting, San Francisco, CA, 1976. See papers 45-48, 56, 69, 75, 99-102, 104.
38. Senti, F.R., C.R. Eddy, and G.C. Nutting, J. Am. Chem. Soc., 65:2473 (1974).

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