STUDIES WITH SOYBEAN PROTEIN AND FIBER FORMATION[1]

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ABSTRACT

Exposure of acid-precipitated soy protein to sodium hydroxide at high pH levels markedly increases its relative viscosity. This effect is accompanied by a rapid shift in the sedimentation constants of the 2, 7, 11, and 15S ultracentrifuge components to essentially 3S. The shape and magnitude of the viscosity curve is dependent upon the concentration of protein and sodium hydroxide as well as time, and is affected by reagents which influence sulfhydryl and disulfide groups. The effects of pH, urea, and mercaptoethanol on the solubility and ultracentrifugal patterns of acid-precipitated protein and the solubility characteristics of precipitated soy protein previously exposed to strong alkali were examined. The relationship of these data to the process by which acid-precipitated soy protein is converted to edible spun fibers is postulated.

The potential world market for high-protein foods has stimulated considerable research in the uses and properties of soybean protein (1,2,3). A facet of this research is the conversion of soy protein into coagulated fibers which can be formulated into textured food products (4,5,6).

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Briefly, the process for producing monofilaments from seed protein is as follows.

Defatted meal is extracted with dilute caustic to solubilize a maximum amount of protein. Since the extract also contains carbohydrates, pigments, and other organic matter, the protein fraction is isolated by isoelectric precipitation at pH 4.5. After washing and dilution, the acid-precipitated protein is redissolved in a strong alkaline solution to yield a viscous preparation. This "dope" solution, as it is commonly called, is then extruded through spinnerettes into an acid-salt coagulation bath, thereby forming the fibers. After being washed, these fibers are ready to be processed into food products.

Investigations of a fundamental nature have been carried out with peanut protein in relation to spinning of textile fibers (7,8,9), but detailed studies with soy protein are rather limited (10,11). In this study, physical and chemical methods were employed to determine the effects of alkali, urea, and mercaptoethanol on soy protein so that the mechanisms involved in fiber formation might be better understood.

Materials and Methods

Soybean protein was extracted from dehulled, hexane-defatted meal containing approximately 50% protein (N × 6.25).

The pH 7.6, $\Gamma/2 = 0.5$ phosphate buffer contained 0.0325*M* dipotassium monohydrogen phosphate, 0.0026*M* monopotassium dihydrogen phosphate, and 0.40*M* sodium chloride.

The soluble fractions, at protein concentrations between 1 and 2%, were examined at room temperature in a Spinco Model E analytical ultracentrifuge. In all cases, a 12-mm. single-sector cell was used at a speed of 59,780 r.p.m. Protein samples were prepared for ultracentrifugal analysis by overnight dialysis at room temperature against the desired buffer.

Viscosities were determined in a nitrogen atmosphere at 28°C. in a Brabender Plasti-Corder having a sigma-bladed measuring head operated at a speed of 34 r.p.m.

Results

1. Changes in Viscosity

The suitability of a dope solution for spinning fibers is determined by its viscosity, which increases rapidly after alkali and protein are mixed. The magnitude and shape of the viscosity curve depend upon a number of factors such as time, protein concentration, alkali concentration, and the presence of additives.

Effect of Protein Concentration. The viscosity of a dope solution containing 13.5% isolated soy protein in 0.95% sodium hydroxide (a pH 12 suspension) increases rather sharply during the first 15 min. of reaction and then levels off (Fig. 1). Comparison of the three curves

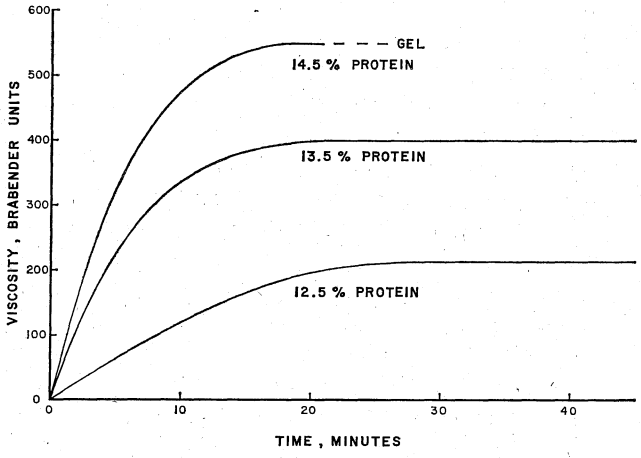

Fig. 1. Effect of time and concentration of soy protein, in 0.95% sodium hydroxide, on viscosity of dope solution.

in Fig. 1 indicates that the concentration of protein determines the magnitude of the curves as well as the time at which the solutions reach maximum viscosity. Protein concentrations of 14.5% or higher result in formation of gels which cannot be spun; concentrations lower than about 12.5% yield solutions having viscosities too low for spinning.

Effect of Sodium Hydroxide Concentration. The concentration of sodium hydroxide during dope formation governs the practical limits within which the protein content can be varied. The viscosity curves in Fig. 2 depict a suitable concentration (0.95% sodium hydroxide) along with higher and lower levels. At 0.81% sodium hydroxide, solutions of low viscosity, which increases slowly with time, are produced. In contrast, a concentration of 1.1% causes rapid attainment of maximum viscosity, which then slowly decreases, presumably because of protein degradation.

Effect of Additives. Factors which influence sulfhydryl and disulfide groups have a marked effect on dope viscosity. For example, mixing the dope solutions in an oxygen atmosphere results in a twofold increase in viscosity compared with mixing in nitrogen. Similarly, low concentrations of bromate and iodate (5 mmoles/800 ml. of solution)

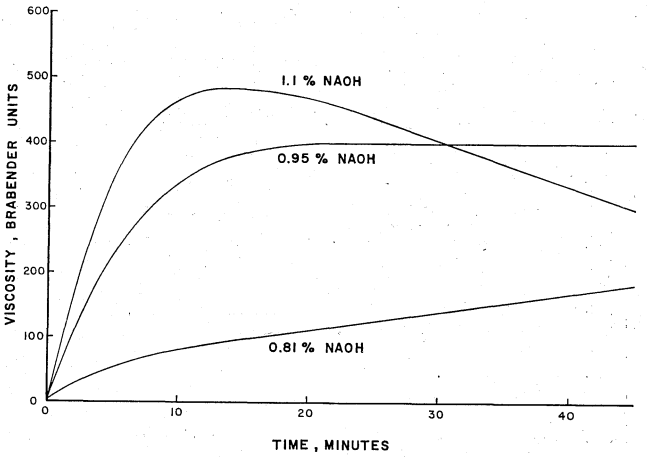

Fig. 2. Effect of time and concentration of sodium hydroxide on viscosity of dope solution. The protein concentration was 13.5% in all three samples.

significantly increase viscosity. Conversely, disulfide bond cleaving agents such as sodium sulfite and thioglycolic acid at very low concentrations reduce maximum viscosity (Fig. 3).

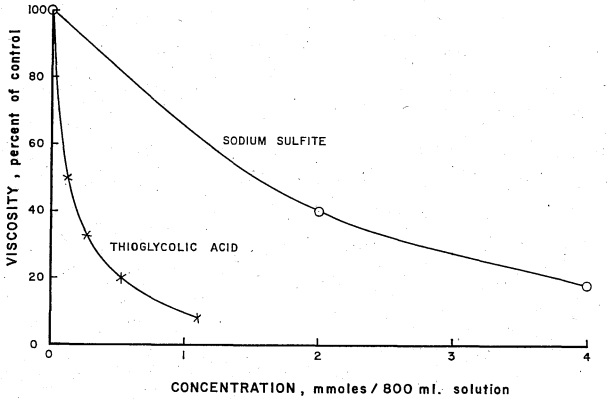

Fig. 3. Effect of sodium sulfite and thioglycolic acid on viscosity of dope solution. The control sample contained 13.5% protein and 0.95% sodium hydroxide, and gave a viscosity of 400 Brabender Units (see Fig. 1).

2. Changes in Sedimentation

Effects of Mercaptoethanol and Urea. The acid-precipitated protein was next examined with respect to its solubility and sedimentation behavior in mercaptoethanol, a disulfide bond cleaving agent, and in urea, a hydrogen bond cleaving agent.

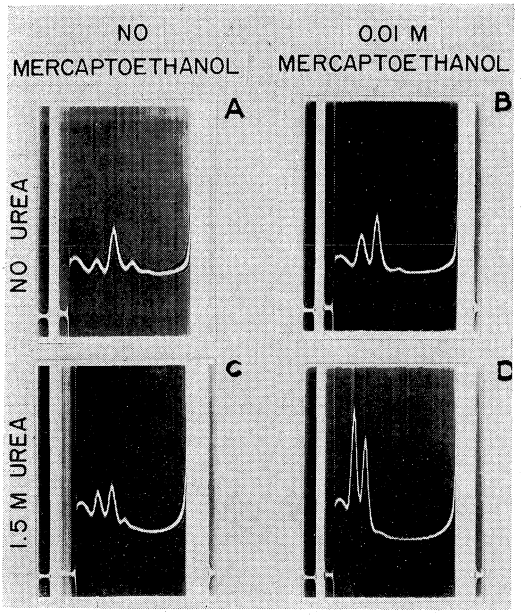

Fig. 4. Effect of mercaptoethanol and urea on ultracentrifugal patterns. The ultracentrifugal analyses were conducted in the following buffer systems: A, phosphate buffer, pH 7.6 $\Gamma/2 = 0.5$; B, phosphate buffer containing 0.01*M* mercaptoethanol; C, phosphate buffer containing 1.5*M* urea; D, phosphate buffer containing 0.01*M* mercaptoethanol and 1.5*M* urea.

Figure 4, A, shows the ultracentrifuge analysis of an acid-precipitated protein sample which had been dialyzed against phosphate buffer (that is, it contained neither mercaptoethanol nor urea). From left to right, the $s_{20,w}$ values of the peaks are 2, 7, 11, and 15S. The addition of 0.01*M* mercaptoethanol to the buffer prior to dialysis had the effect of increasing the relative concentration of the 7S material and, to some extent, the 11S component (Fig. 4, B). This agent also caused an increase in the solubility of this acid-precipitated protein from 68 to 80%. Wolf (12,13) previously noted that the relative distribution of 7 and 11S components from water extracts of soy meal was similarly altered in the presence of mercaptoethanol. The effect was due to dissociation of 7 and 11S polymers which were present in the aqueous extracts.

Dialysis of acid-precipitated protein in 1.5*M* urea caused the change in the protein distribution pattern seen in Fig. 4, C. The observed sedimentation values in this buffer were 2, 6, 9, and 12S. (The 6 and 9S components in the urea analysis correspond to the 7 and 11S components of the upper two diagrams.) Again, the addition

of 0.01*M* mercaptoethanol caused both an increase in solubility (from 73 to 85%) and a change in the relative distribution of the 6 and 9S components (Fig. 4, D). Wolf (12) also noted a partial breakdown of pure 11S to slower-sedimenting material, presumably 7S, upon dialysis in this concentration of urea.

Dialysis of the urea-containing sample (Fig. 4, C) against phosphate buffer containing mercaptoethanol to remove the urea restored the ultracentrifugal pattern to its original distribution. This was true with both 1.5*M* and 3*M* urea.

The effect of exposing soy protein to 6*M* urea is shown in Fig. 5.

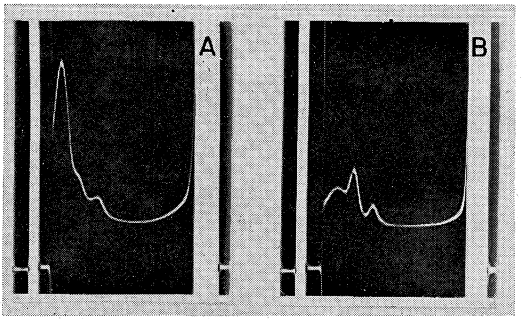

Fig. 5. Effect of 6*M* urea on ultracentrifugal patterns of acid-precipitated protein. A, analysis in phosphate buffer, pH 7.6, $\Gamma/2 = 0.5$, containing 0.01*M* mercaptoethanol and 6*M* urea; B, analysis of sample A in phosphate buffer, pH 7.6, $\Gamma/2 = 0.5$, containing 0.01*M* mercaptoethanol, after removal of urea by dialysis.

Analysis in this buffer (pattern A) reveals a preponderance of material in the 1 to 2S region and a small amount of a 4S component. Removing the urea in this case did not restore the protein pattern to its original distribution: pattern B (Fig. 5) is quite different from that of an untreated sample (e.g., compare with Fig. 4, B); the 15S component is missing, the 11S peak is greatly reduced, and the 7S peak is also somewhat diminished.

Effect of pH. Considerable information has been accumulated regarding the effect of pH on the extractability of soybean protein (14,15). In this case, however, we wished to examine the effect of alkaline treatment on acid-precipitated protein as determined in the analytical ultracentrifuge.

Suspensions of acid-precipitated protein were adjusted to the desired pH with sodium hydroxide, diluted to 4% protein concentration, and stirred for 30 min. at room temperature. Following centrifugation, an aliquot of the supernatant solution was diluted with an equal volume of 1*M* sodium chloride and examined, without prior

dialysis, in the ultracentrifuge. A second aliquot was diluted with an equal volume of phosphate buffer (pH 7.6, $\Gamma/2 = 0.5$) containing 0.01*M* mercaptoethanol, and dialyzed overnight prior to analysis.

Figure 6 illustrates the results of this study. Patterns in the top

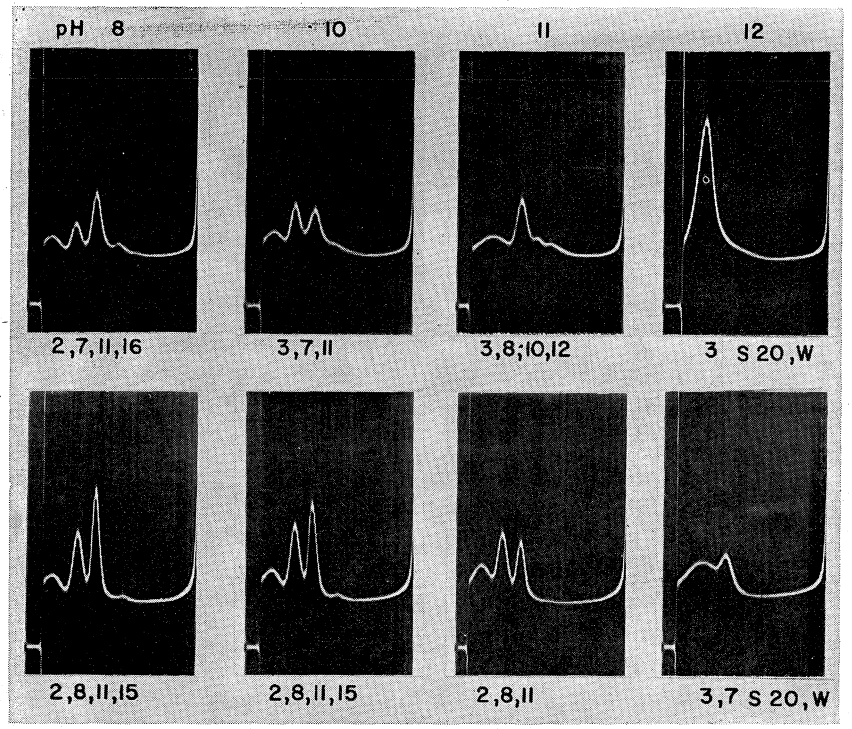

Fig. 6. Effect of pH on ultracentrifugal patterns of hydrochloric acid-precipitated protein. Top row: samples analyzed in 0.5*M* sodium chloride at stated pH. Bottom row: samples analyzed in phosphate buffer, pH 7.6, $\Gamma/2 = 0.5$.

row represent those analyses carried out in 0.5*M* sodium chloride at the stated pH; the bottom row depicts analyses in phosphate buffer.

At pH 8, a distribution pattern containing 2, 7, 11, and 16S components was observed. As the pH of the suspension was increased, the analysis in sodium chloride disclosed a change in distribution to essentially 3S material. The analyses in phosphate buffer, on the other hand, show the major "native" components to be present up to the analysis at pH 11; there is, however, a progressive decrease in the relative amount of the 11S component. Only two components having $s_{20,w}$ values of 3S and 7S were observed in the analysis of the pH 12 suspension.

Effect of Time on Protein Distribution at pH 12. As described in the preceding sections, the viscosity of soy protein increases in strong alkali, and the "native" 2, 7, 11, and 15S ultracentrifugal components are converted to essentially 3S material. To further explore these phenomena, a pH 12 suspension containing 13.5% protein solids in 0.95% sodium hydroxide was prepared (see Fig. 1 for viscosity curve). At intervals of 3, 15, and 30 min., aliquots were diluted with water to approximately 3% protein solids and quickly adjusted to pH 4.5 with 2.5*N* hydrochloric acid to precipitate the protein. This procedure stopped the alkaline reaction at the desired times. The alkali-treated, acid-precipitated samples were then dialyzed against phosphate buffer (pH 7.6, $\Gamma/2 = 0.5$) containing 0.01*M* mercaptoethanol, and analyzed in the ultracentrifuge. Acid-precipitated protein which had not been exposed to alkali was the zero-time sample in this experiment. The sedimentation patterns of these various samples are shown in Fig. 7.

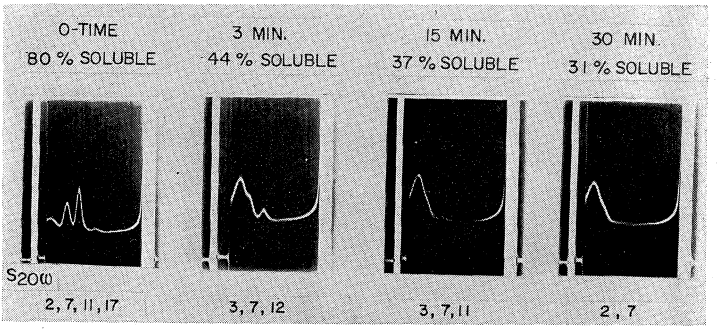

Fig. 7. Effect of "hold" time on ultracentrifugal patterns. The samples were analyzed in phosphate buffer, pH 7.6, $\Gamma/2 = 0.5$, containing 0.01*M* mercaptoethanol.

At zero time, a typical distribution pattern was evident. In this case, the $s_{20,w}$ values were 2, 7, 11, and 17S. Following a 3-min. exposure to alkali, however, the main portion of the 11S peak was converted to 3S material. (At 3 min. the viscosity of the dope solution is still increasing; see Fig. 1.) Also, whereas 80% of the zero-time sample was soluble in phosphate buffer containing mercaptoethanol, only 44% of the 3-min. sample was soluble. The protein samples exposed to alkali for 15 and 30 min. showed a similar decrease in solubility as well as a further shift to the slow-sedimenting material.

Solubility Experiments. The solubility differences noted in urea and mercaptoethanol buffers led to the undertaking of a more detailed study of the solubility of both alkali-treated and nonalkali-treated protein in various phosphate buffers. Preparations of soy protein were

suspended in phosphate buffer alone, or in buffer containing 0.01*M* mercaptoethanol and/or urea. These suspensions were then dialyzed at room temperature against the same solvent system for approximately 24 hr. with occasional stirring, and the amount of protein solubilized was determined. The results are summarized in Table I.

TABLE I
SOLUBILITY OF VARIOUS PREPARATIONS OF SOY PROTEIN

SAMPLE	BUFFER	AMOUNT SOLUBLE
		%
1. Acid-precipitated protein	P.B.[a]	68
	P.B. + m.e.[b]	80
	P.B. + 1.5*M* urea	73
	P.B. + m.e. + 1.5*M* urea	85
2. Acid-precipitated protein; 30 min. at pH 12; reprecipitated	P.B.	16
	P.B. + m.e.	31
	P.B. + 1.5*M* urea	23
	P.B. + m.e. + 1.5*M* urea	70
	P.B. + 6*M* urea	>68
	P.B. + m.e. + 6*M* urea	>75
3. Soybean fiber	P.B.	5
	P.B. + m.e.	30
	P.B. + 1.5*M* urea	30
	P.B. + m.e. + 1.5*M* urea	61
4. Acid-precipitated protein; 30 min. at pH 12; not reprecipitated	P.B.	94

[a] P.B. = phosphate buffer, pH 7.6, $\Gamma/2 = 0.5$.
[b] m.e. = 0.01*M* mercaptoethanol.

The first group, labeled "acid-precipitated protein," refers to protein extracted from meal with dilute caustic and then precipitated with hydrochloric acid at pH 4.5. Phosphate buffer alone (labeled P.B.) solubilized approximately 68% of the preparation. The addition of 0.01*M* mercaptoethanol to the buffer (labeled P.B. + m.e.) increased this value to about 80%. Phosphate buffer containing 1.5*M* urea solubilized 73% of the sample; the combination of urea and mercaptoethanol dissolved 85% of the sample. In the tests in which urea was the solubilizing agent, protein solubilities were determined as follows: the samples were dialyzed in urea buffers for at least 6 hr. at room temperature; after centrifugation to remove insoluble material, the samples were extensively dialyzed against phosphate buffer containing mercaptoethanol to remove all traces of urea, and the amount of protein soluble in this latter buffer was determined.

As mentioned earlier, exposure of acid-precipitated soy protein to pH 12, even for 3 min., considerably reduced its solubility when phos-

phate buffer containing mercaptoethanol was the solvent. Another sample of acid-precipitated protein was exposed to sodium hydroxide at pH 12 for 30 min. The sample was precipitated with hydrochloric acid to arrest the reaction and then dialyzed against a series of phosphate buffers to test for changes in solubility (see Table I, sample 2). Only 16% of this sample was solubilized in phosphate buffer, whereas 68% of sample 1 was soluble. Buffer plus mercaptoethanol solubilized 31% of the protein; 23% was dissolved in buffer plus 1.5*M* urea. In this case, however, the combination of urea and mercaptoethanol dissolved 70% of the sample; that is, the combined action of the disulfide bond cleaving agent and the hydrogen bond breaking agent was needed to cause substantial solubility. More protein could be solubilized by increasing the level of urea to 6 molar. Phosphate buffer plus 6*M* urea dissolved more than 68% of the protein, and the addition of 0.01*M* mercaptoethanol increased the solubility to more than 75%. The latter two values are uncertain, because some material was precipitated upon removal of the urea.

When samples which had been exposed to strong alkali for 30 min. were dialyzed directly in phosphate buffer (precipitation by hydrochloric acid being omitted), greater than 90% solubility was achieved (see sample 4). This result is analogous to that obtained by Wolf (12), who noted that precipitation at cold temperatures resulted in polymerization of soy protein; that is, the protein is more sensitive to changes in solubility or polymerization when it is in the precipitated state.

Fibers prepared from a hydrochloric acid-precipitated extract of soy meal exhibited solubility properties similar to those of the acid-precipitated dope samples; both a hydrogen bond cleaving agent and a disulfide bond reducing agent were required to achieve substantial solubility (see sample 3). In this instance, 61% of the protein was solubilized by the combined agents, *vs.* 30% solubilized by each agent alone.

Discussion

The scheme illustrated in Fig. 8 is suggested to depict the reaction that soybean protein sustains during fiber formation.

The 2, 7, 11, and 15S ultracentrifugal components present in extracts of soy meal are represented by the four figures in the upper left-hand corner of the figure. Exposure of soy protein to strong alkali causes two events.

First, the native globular protein is converted to unfolded polypeptide chains, as shown in the upper right-hand corner of the figure.

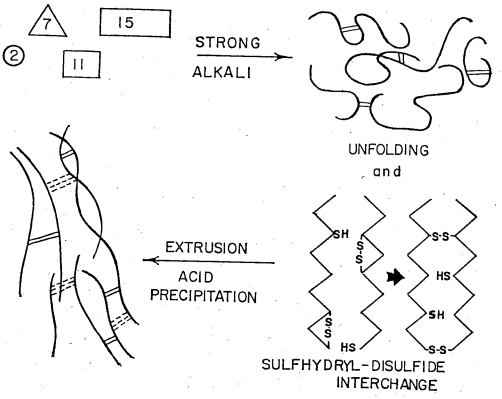

Fig. 8. Schematic representation of fiber formation.

(The double lines in the unfolded structures represent disulfide bonds.) Some dissociation to material of lower molecular weight may also occur at this point. This unfolding and dissociation is evidenced both by the increase in relative viscosity which the protein undergoes when exposed to strong alkali and by the shift in the 2, 7, 11, and 15S ultracentrifuge components to 3 to 5S material.

Second, alkaline conditions also favor sulfhydryl-disulfide interchange reactions as diagrammed in the lower right-hand corner. (Using the procedure of Sokol *et al.* (16), we determined that acid-precipitated soy protein contains approximately 0.6 sulfhydryl equivalents/10^5 g.)

Therefore, prior to and during acidification of the dope samples, either by direct precipitation by hydrochloric acid or else by extrusion through spinnerettes into an acid-salt coagulation bath to make fibers, *new* disulfide bonds can be formed.

Acidification also brings the many polypeptide chains close together, favoring hydrogen and ionic bonding. The extrusion of the dope sample through the spinnerettes would in particular favor this alignment. The spun fibers are depicted in the lower left-hand corner of the figure. They are shown containing both disulfide bonds, represented by the solid lines, and hydrogen or ionic bonds, as pictured by the triple sets of dotted lines.

Once such bonds are formed, phosphate buffer (pH 7.6, $\Gamma/2 = 0.5$) alone is not adequate to solubilize the preparations, although mercaptoethanol is capable of cleaving the newly formed disulfide bonds *if they are accessible.* Table I illustrates, for example, that mercaptoethanol plus buffer solubilized 31% of the acid-precipitated dope

sample, whereas only 16% was soluble in buffer alone. Buffer containing both mercaptoethanol and 1.5*M* urea, however, solubilized 70% of the protein. (This level of urea did not destroy the distribution pattern of the native soy protein.)

On the other hand, although more than 68% of the acid-precipitated dope sample was soluble in 6*M* urea, such a concentration caused irreversible changes in protein structure. This indicates the importance of hydrogen bonds in these samples. Disulfide bonds also play some role in the system, since the addition of mercaptoethanol to the buffer containing 6*M* urea increased protein solubility.

Although much more remains to be learned concerning the basic reactions involved in fiber formation, it is felt that many of the properties which these spun fibers possess may be attributed to the action of hydrogen, ionic, and disulfide linkages.

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