Denaturation of Soybean Proteins by Organic Solvents[1]

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ABSTRACT

A systematic study was made of the denaturing ability of about 30 kinds of organic solvents toward soybean proteins. In general, the denaturing ability of organic solvents depended on their hydrophobicities and their degree of dilution by water. Highly hydrophobic solvents possessed little denaturing power toward proteins, even at high temperature. The denaturing power of solvents increased with addition of water, whereas that of water also increased with addition of solvents. Consequently, the water-solvent mixture had high denaturing abilities that could not be attained by the individual components alone. Lower alcohols were much stronger denaturants than other solvents examined. The denaturing ability of alcohols at low concentration increased with the hydrophobicities of alcohols; the reverse was found at high concentrations. The denaturation mechanisms of soybean proteins with organic solvents and with water are discussed from the standpoint of the three-dimensional structure of soybean protein molecules.

Several workers have investigated the denaturation of soybean proteins with organic solvents (1-6). The purpose of the present studies was: (a) to choose oil-extracting solvents that would minimize protein insolubilization; (b) to obtain useful data for choosing conditions that would preclude protein insolubilization during alcohol extraction.

However, enhancing soybean protein denaturation is also important for some aspects of soybean utilization, because denaturation markedly increases susceptibility to proteinase attack (7-10). Traditionally, denaturation by heat and moisture has been used for this purpose, but denaturation by organic solvents should also be of interest because the soybean protein molecules contain some hydrophobic regions not expected to be altered by water (9,10).

In the present study, the denaturing action of various organic solvents on soybean proteins was investigated systematically, and the conditions under which soybean proteins are completely denatured were determined with the solvents having strong denaturing power, that is, the lower alcohols. This work is discussed herein, together with the mechanisms of the denaturation with organic solvents, water, and the solvent-water mixtures from the standpoint of the three-dimensional structure of soybean protein molecules.

MATERIALS AND METHODS

Defatted Soybean Flour

Whole soybeans were dried, dehulled, and ground to 30-mesh size. The resultant flours were air-dried after removal of the oil with n-hexane extraction at 35°C. Total nitrogen was 8.506%.

Denaturation of Soybean Proteins with Solvents

The flour sample corresponding to 85 mg. nitrogen was put into a test tube with 5 ml. of a solvent, except as noted later. The tube was sealed and treated at the desired temperature and time in a constant-temperature bath. The sample was then

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cooled rapidly to about 20°C. and unsealed; the solvent was removed by aeration at room temperature.

Measurement of Native Protein in Treated Samples

The native proteins in the treated samples were measured quantitatively by the method described previously (8).

RESULTS

Denaturation of Defatted Soybean Flour Proteins with Various Organic Solvents

The denaturing abilities of various solvents toward soybean proteins are shown in Tables I, II, III, and IV.

The highly hydrophobic solvents, immiscible with water, possess very slight abilities to denature soybean proteins, even at high temperatures (Table I). On the contrary, in the solvents which have fairly high hydrophobicity but are able to dissolve a little water, the denaturing ability of these solvents is increased markedly by the addition of water, though they hardly possess this ability without addition of water (Table II). On the other hand, the denaturing abilities of water are strikingly increased in the presence of small amounts of these solvents (Table III). With hydrophobic solvents miscible with water in any ratio, there were characteristic ratios of solvent to water, at which maximum denaturation occurred (Table IV). A comparison of the denaturing ability in the maximum denaturing concentration of each solvent indicates that lower alcohols were stronger denaturing agents than acetone or dioxane.

TABLE I. DENATURATION (60 MIN.) OF DEFATTED SOYBEAN FLOUR PROTEINS WITH WATER-IMMISCIBLE ORGANIC SOLVENTS

Solvents	Absorbance(s)[a] at three denaturation temperatures 60°C.	80°C.	100°C.
Benzine	0.275	0.250	0.242
Benzene	0.275	0.241	0.231
n-Hexane	0.270	0.266	0.233
Toluol	0.273	0.260	0.241
Carbon tetrachloride	0.275	0.245	0.271
Isopropyl ether	0.250	0.250	.....
Chloroform	0.252	0.228	.....
Butyl acetate	0.266	0.226	.....
Amyl acetate	0.283	0.270	0.241
Ethyl propionate	0.283	0.263	0.250
Trichloroethylene	0.283	0.242	0.219
Water	0.250	0.202	0.008

[a] Native protein contents shown by absorbance(s) in the soybean flour proteins after the treatments.

It is obvious from these data that water-containing organic solvents have a high denaturing power which cannot be attained by the individual components alone. The denaturing abilities of the series of the lower alcohols having different hydrophobicities were investigated at various concentrations expressed as mol.% (Figs. 1 and 2). According to these figures, there existed striking dependence between the molecular structures of lower alcohols and their denaturing abilities. In the region below the maximum denaturing concentration characteristic of each alcohol, the

TABLE II. DENATURATION (60 MIN.) OF DEFATTED SOYBEAN FLOUR PROTEINS WITH PARTIALLY WATER-MISCIBLE ORGANIC SOLVENTS

Solvent	Concentration *vol.%*	Absorbance(s)[a] at three denaturation temperatures 60°C.	80°C.	100°C.
Methyl ethyl ketone	100	0.304	0.268	0.263
	water-sat'd	0.254	0.211	0.177
Methyl formate	100	0.236	.....	.....
	water-sat'd	0.072	.....	.....
Ethyl formate	100	0.230	0.184	.....
	water-sat'd	0.115	0.096	.....
Propyl formate	100	0.256	0.215	0.089
	water-sat'd	0.226	0.174	0.089
Methyl acetate	100	0.302	0.255	.....
	water-sat'd	0.255	0.226	.....
Ethyl acetate	100	0.290	0.290	0.252
	water-sat'd	0.283	0.270	0.226
n-Butanol	100	0.266	0.255	0.188
	90	0.255	0.210	0.184
	water-sat'd	0.201	0.176	0.080
sec-Butanol	100	0.266	0.263	0.225
	80	0.214	0.152	0.094
	water-sat'd	0.166	0.094	0.006
Isobutanol	100	0.280	0.273	0.210
	90	0.240	0.194	0.176
Water		0.250	0.202	0.008

[a]Native protein contents shown by absorbance(s) in soybean flour proteins after treatments.

TABLE III. EFFECT OF PARTIALLY WATER-MISCIBLE ORGANIC SOLVENTS ON WATER-DENATURATION (60 MIN.) OF DEFATTED SOYBEAN FLOUR PROTEINS

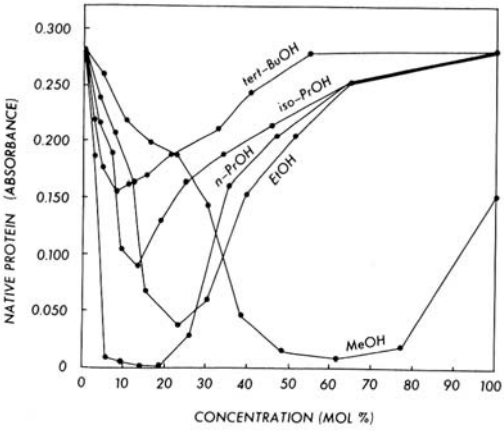
Solvents	Amounts Added *vol.%*	Absorbance(s)[a] at two denaturation temperatures 50°C.	70°C.
Water	.....	0.289	0.219
Methyl ethyl ketone	5	0.230	0.205
	10	0.214	0.138
	20	0.175	0
Methyl formate	5	0.280	0.218
	10	0.254	0.046
	20	0.105	0
Ethyl formate	5	0.250	0.214
Propyl formate	sat'd	0.262	0.238
Methyl acetate	4	0.254	0.232
	10	0.222	0.214
	20	0.214	0
Ethyl acetate	5	0.238	0.250
n-Butanol	4	0.208	0.163
sec-Butanol	5	0.250	0.190
	10	0.212	0.078
Isobutanol	5	0.208	0.205

[a]Native protein contents shown by absorbance(s) in the soybean flour proteins after treatments.

TABLE IV. DENATURATION (60 MIN. AT 50°C.) OF DEFATTED SOYBEAN FLOUR PROTEINS WITH WATER-MISCIBLE ORGANIC SOLVENTS[a]

Concentration vol.%	Absorbance[a]						
	Methanol	Ethanol	n-Propanol	Isopropanol	tert-Butanol	Acetone	Dioxane
0	0.283	0.283	0.283	0.283	0.283	0.283	0.283
10	0.257	0.237	0.188	0.212	0.215	0.231	0.216
20	0.215	0.208	0.007	0.188	0.175	0.204	0.197
30	0.196	0.162	0.004	0.103	0.155	0.192	0.189
40	0.187	0.066	0.002	0.091	0.162	0.187	0.186
50	0.142	0.035	0.001	0.130	0.170	0.186	0.182
60	0.045	0.059	0.027	0.163	0.187	0.192	0.192
70	0.013	0.152	0.160	0.190	0.211	0.201	0.192
80	0.007	0.205	0.205	0.215	0.242	0.226	0.234
90	0.018	0.265	0.266	0.267	0.282	0.272	0.270
100	0.152	0.283	0.284	0.284	0.285	0.284	0.285

[a]Native protein contents shown by absorbance(s) in the soybean flour proteins after the treatments.



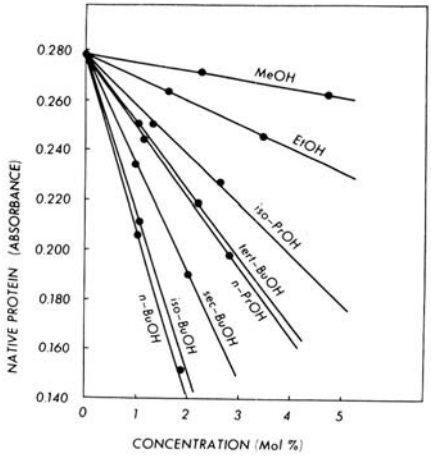


Fig. 1. Denaturation of defatted soybean flour proteins with lower alcohols for 60 min. at 50°C.

Fig. 2. Effect of addition of lower alcohols to water on denaturation of defatted soybean flour proteins by water for 60 min. at 50°C.

denaturing effects increased with increase of the hydrocarbon contents, length of the straight chains, and distance of branching position from the alpha-carbon atom, indicating that the denaturing abilities of alcohols coincided completely with the order of their hydrophobicities. In the region over the maximum denaturing concentration, on the other hand, the order of alcohols was quite the reverse, except for n-propanol. These phenomena will be discussed later from the standpoint of the three-dimensional structure of soybean protein molecules.

Next, the effects of time, temperature, and solvent concentration on the alcoholic denaturation of defatted soybean flour proteins were investigated in detail (Figs. 3, 4, and 5). The velocity of denaturation by aqueous alcohol is very rapid, and the denaturation is completed within several minutes (Fig. 3). Figure 4 shows

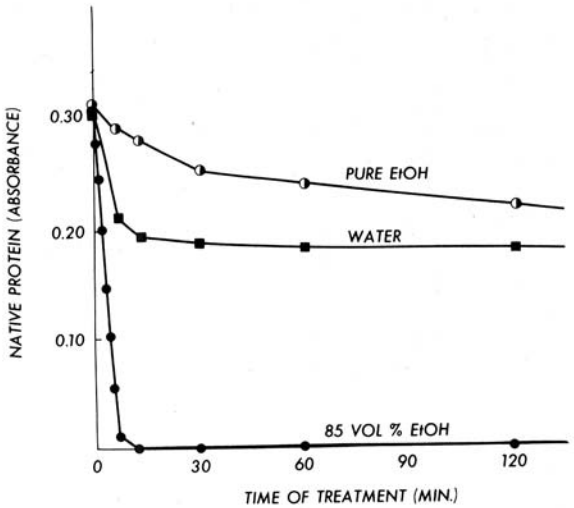


Fig. 3. Effect of denaturation time on denaturation of defatted soybean flour proteins by water and ethanol at 80°C.

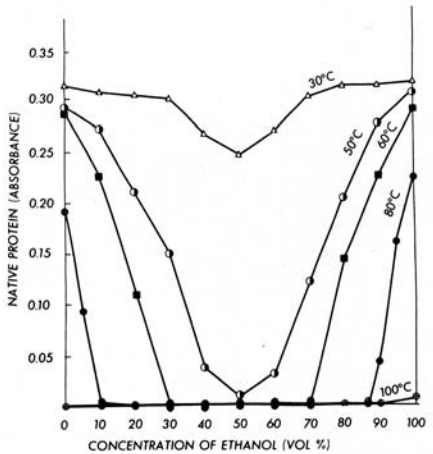


Fig. 4. Effect of temperature and concentration on denaturation of defatted soybean flour proteins with ethanol for 60 min.

the effect of the temperature and concentration of ethanol on denaturation. When the concentrations of alcohols in the zero native protein intercepts from Fig. 4 are plotted for each temperature, the curve designated as EtOH in Fig. 5 is obtained. For the defatted soybean flour treated under the conditions indicated on the left side of this curve, various amounts of native proteins are present, increasing with the distance from the curve. In the region of the right sides of this curve, however, no native proteins remain. Similar curves for methanol and isopropanol are also shown as the curves designated as MeOH and iso-PrOH, respectively, in Fig. 5. The concentration of methanol, ethanol, and isopropanol at which the native proteins

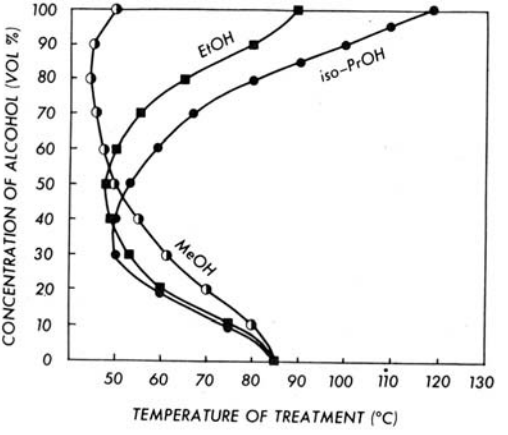


Fig. 5. Denaturing curves of the defatted soybean flour proteins with methanol, ethanol, and isopropanol for 60 min. Explanation in text.

disappear completely in the lowest temperature are 70 to 90, 45 to 55, and 30 to 40 vol.%, respectively, and the lowest temperature for disappearance of native proteins at those alcohol concentrations becomes lower in the order mentioned, indicating that the denaturing power in the maximum denaturing concentration of each alcohol becomes stronger in that order. This is also shown in Table IV.

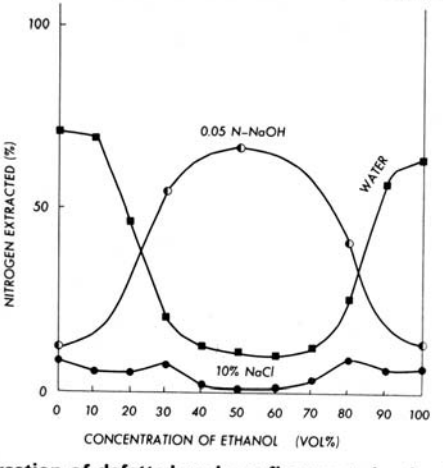


Fig. 6. Successive extraction of defatted soybean flour proteins denatured with ethanol for 60 min. at 50°C. by water, salt, and alkali solutions.

Figure 6 shows that alcohol-denatured soybean proteins are insoluble in water or salt solution but are soluble in dilute alkaline solution.

DISCUSSION

The author reported previously (7-11) that: (a) the major soybean protein molecules are compactly folded, including the interior hydrophobic region; (b) the major structures of polypeptide chains are beta- and disordered forms, though a small amount of alpha-helix is present; and (c) the chains have little susceptibility

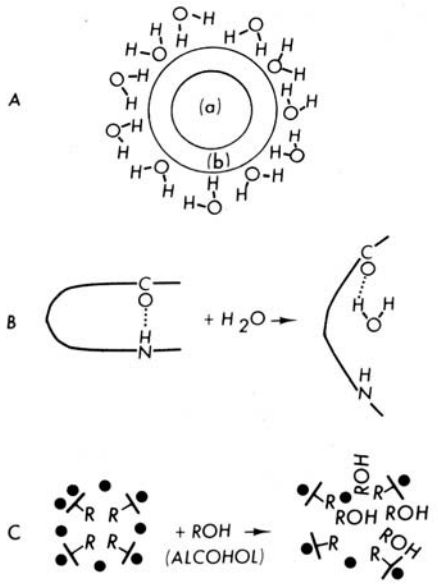


Fig. 7. Schematic representation of the mechanism of protein denaturation with water and alcohols. Top (A), one globular protein molecule surrounded by water (HOH) in which a hydrophobic region (a) and a hydrophilic region (b) are present. B and C, disruption of hydrogen bond by water and of the hydrophobic bond by alcohol. Thick lines in B and C, polypeptide backbone of proteins; ● in C, one molecule of water; -R in C, amino acid side chains.

to proteinases before disruption of the internal structure. Direct information about the spatial position of the amino acid side-chains has not been obtained so far, except for tryptophan and tyrosine, on soybean proteins (9,10). Recently, however, the spatial positions of almost all the amino acid side-chains in the molecules have been determined by means of X-ray analysis for several globular proteins, such as mioglobin (12), hemoglobin (13), and lysozyme (14) molecules. According to these results, most of the hydrophobic side-chain residues were located toward the center of the molecule, forming hydrophobic regions, and the hydrophilic ones were located on the surface of the molecule. On the basis of these results and my results reported previously about soybean protein molecules (9-11), it is most reasonable to consider the soybean protein molecule as an oil drop covered with a shell of hydrophilic chain groups, as shown in Fig. 7. If such a three-dimensional structure of soybean protein molecules is assumed, the various phenomena observed here can be satisfactorily explained as described below.

The lack of denaturing ability by water-immiscible hydrophobic solvents toward soybean proteins, even in high temperature (Tables I and II), can be explained by the idea that the solvents are prevented from entering into the hydrophobic region by the surrounding hydrophilic shell and the outside hydration layer, which cannot be disrupted by hydrophobic solvent alone. On the other hand, water can destroy these hydrophilic shells easily, owing to its strong hydrogen bond-forming ability. The hydrophobic regions of the molecules, however, cannot be disrupted directly by water, because hydrophobic bonds are strengthened in water environments,

though the indirect rupture of the hydrophobic region might occur, accompanying the unfolding of polypeptide chains caused by disruption of the hydrophilic shell. Thus, the hydrophilic shell will be the principal region altered as a result of denaturation with water alone. On the other hand, disruption of the hydrophobic region will be easily brought about by the addition, to water, of water-soluble solvents which possess both hydrophobic and hydrophilic radicals. The hydrophobic portions of the added solvents can penetrate into the hydrophobic region after disruption of the surrounding hydrophilic shell (Fig. 7). In this case, the denaturing abilities of water should increase with the amounts of added solvents, and, further, with the same concentrations of added solvents their effect should increase in the order of hydrophobicities of the added solvents. However, adding too much of these solvents to water will weaken the action of water to breaking the hydrophilic shell of the protein molecules. Thus, the data observed in Tables I, II, III, and IV and Figs. 1 and 2 could be explained quite satisfactorily on the basis of this idea.

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