of 15.18 g (0.15 mol) of triethylamine in 15 mL of methanol. During the addition period which was 10 min, the temperature was kept at 5 °C by means of an ice–salt bath. After being stirred for 1 h without being cooled, the reaction mixture was poured into 500 mL of ether and 400 mL of ice-cold 0.25 N hydrochloric acid. The aqueous layer was extracted with 150 mL of ether. The combined etheral extracts were washed 3 times with 150 mL of cold water, dried over magnesium sulfate, and evaporated at 50 °C/20 torr, giving 36.8 g of crude **3** as a yellow oil which could not be distilled without decomposition. This oil was dissolved in 350 mL of methanol and 2 mL of 48% hydrobromic acid and refluxed for 19 h. The cold reaction mixture was then stirred with 1.5 g of finely powdered anhydrous potassium carbonate for 2 h and afterward freed from the solvent (50 °C/20 torr). The residue was dissolved in methylene chloride and filtered. After evaporation of the solvent, the residue was distilled through a Vigreux column, giving 8.94 g (37.8%) of pure 1-methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane (**4**), bp 69–73 °C/0.2 torr.

The IR, MS, ^{13}C NMR, and ^{1}H NMR (100 MHz) spectra are identical with those of an authentic sample derived from thiamin by UV degradation. In addition, a 360-MHz ^{1}H NMR spectrum was recorded: δ 1.85 (s, 3 H, CH_3), 2.08 (dddd, $J_{C_6H'}$ = 13 Hz, J_{C_7H} = 7 Hz, $J_{C_7H'}$ = 3 Hz, J_{C_6H} = 3 Hz, C_6H), 2.45 (dddd, J_{C_6H} = 13 Hz, J_{C_7H} = 8.5 Hz, $J_{C_7H'}$ = 8.5 Hz, J_{C_6H} = 8 Hz, C_6H'), 3.74 (dd, J_{C_6H} = 3 Hz, $J_{C_6H'}$ = 8 Hz, C_5H), 3.80 (d, $J_{C_3H'}$ = 11 Hz, C_3H), 4.02 (ddd, J_{C_7H} = 8.5 Hz, $J_{C_6H'}$ = 8.5 Hz, J_{C_6H} = 3 Hz, C_7H'); 4.17 (ddd, $J_{C_7H'}$ = 8.5 Hz, $J_{C_6H'}$ = 8.5 Hz, J_{C_6H} = 7 Hz, C_7H), 4.24 (d, J_{C_3H} = 11 Hz, C_3H').

DISCUSSION

The synthetic substance **4** is identical with the material resulting from the UV degradation of thiamin (^{1}H NMR, ^{13}C NMR, IR, MS, packed-column GLC, and capillary GLC). With respect to the ring linkage, two isomers of **4** are possible. The 360-MHz ^{1}H NMR spectrum which is of first order clearly shows the presence of only one isomer.

The second isomer which would be helpful for spectral comparison could not be found. Although all coupling constants can be determined, it is difficult to decide whether the two rings are cis or trans fused because of the fact that the Karplus equation may not be applied to five-membered rings containing two heteroatoms (Wilson and Bazzone, 1974).

Odor Description of 4. Synthetic material: sulfurous, onionlike, fried onionlike, leeklike, meaty, and slightly metallic. Material resulting from thiamin degradation: onionlike, cerallike, leeklike, and meaty.

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LITERATURE CITED

Boehme, H.; Bezzenberger, H.; Clement, M.; Dick, A.; Nürnberg, E.; Schlephack, W. *Justus Liebigs Ann. Chem.* **1959**, *623*, 92.
Dwivedi, B. K.; Arnold, R. G. *J. Agric. Food Chem.* **1973**, *21*, 54.
Mueller et al., Eds. *Methoden Org. Chem. (Houben-Weyl)* **1977**, *7/2c*, 2331.
Seifert, R. M.; Buttery, R. G.; Lundin, R. W.; Haddon, W. F.; Benson, M. *J. Agric. Food Chem.* **1978**, *26*, 1173.
Wilson, G. M., Jr.; Bazzone, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 1465.

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Preferential Retention of Benzo[*a*]pyrene in Tobacco Smoke by β-Lactoglobulin in the Cigarette Filter Structure

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Preferential retention of benzo[*a*]pyrene in tobacco smoke by β-lactoglobulin, added to the cigarette filter structure, was studied. The retention rate of benzo[*a*]pyrene in tobacco smoke by the cigarette filter varied, dependent upon the dose of β-lactoglobulin which was introduced to the filter, while those of smoke condensate and nicotine in tobacco smoke remained at the constant level throughout the doses of β-lactoglobulin in the present study. Twenty milligrams of β-lactoglobulin in a cigarette filter tip was estimated to be capable of reducing the benzo[*a*]pyrene concentration in the dry total particulate matter to a level of half that in intact tobacco smoke.

Cigarette commodities, which were assembled with the filter structure by acetylcellulose fiber tow, have been extensively merchandized. The recent trend for sophistication of the cigarette filter led to an introduction of charcoal granule into the multilayered filter structure successfully. Hygienic criteria for competency of the cigarette filter have been focused on removal of the smoke condensate and nicotine from cigarette smoke.

Wynder and Hoffmann (1964) evaluated a relative importance of tobacco smoke constituents in experimental tobacco carcinogenesis. Benzo[*a*]pyrene (B[*a*]p) and polyaromatic hydrocarbons were proposed to be indicators for tumor initiator while volatile phenol and long-chain fatty acids were to be those for tumor promotors. Recent progress on the studies of experimental tobacco carcinogenesis detected a tobacco-specific carcinogen, *N'*-nitrosonornicotine (U.S. Department of Health, Education

Central Research Laboratory, Morinaga Milk Industry Company Ltd., Meguro-4, Meguro-ku, Tokyo, Japan (J.O. and K.O.), and Central Research Institute, Japan Tobacco and Salt Public Corporation, 6-2, Umegaoka, Midori-ku, Yokohama, Japan (K.M. and K.N.).

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and Welfare, 1975). It is significant, in the present situation, to evaluate cigarette filters from the viewpoint of the retention of the carcinogenic indicators described above.

Ono et al. (1975) observed that β-lactoglobulin (βLG), a major protein species in bovine milk whey, preferentially associated in aqueous solution with B[a]p and 7,12-dimethylbenz[a]anthracene which were the strong carcinogens against experimental animals. However, affinity of this protein to a noncarcinogenic analogous polyaromatic hydrocarbon, chrysene, was limited. This aspect prompted the present authors to examine the application of βLG in the cigarette filter which might effect a retention of B[a]p in tobacco smoke. The present article describes the preferential retention of B[a]p in tobacco smoke with βLG which was incorporated into the cigarette filter structure.

MATERIALS AND METHODS

Preparation of Filter Materials. Commercial filter tips for cigarette, prepared from acetylcellulose fiber of 3.3 and 46 000 filaments per denier and total denier, respectively, were cordially donated by Nihon Filter Co., Ltd., Tokyo, Japan. The length and the circumference of a tip were 17 and 24.7 mm, respectively, and the packing density of acetylcellulose fiber in a tip was 10%. βLG, prepared in accordance with the procedure by Aschaffenburg and Drewry (1957), was dissolved in water to a concentration of 5%. The βLG solution was then dispersed in the filter tips by means of microsyringe in order to place 2 or 4 mg of βLG in a tip by such a manner as to introduce the syringe through the center of the cross section of the tip and to slide the syringe to a direction longitudinal to the tip while the suspension was injected into the tip quantitatively. Tips were then allowed to stand for overnight at 60 °C to remove moisture. The pressure drop was determined against an individual tip, and those tips which revealed the pressure drop of 60 ± 6 mmH_2O were exclusively employed for the experiment. This consideration was to exclude an artifactual retention of the smoke constituents by tips which were mechanically distorted at the moment of introduction of the microsyringe. For determination of the retention of B[a]p by the filter structure with 6 mg of βLG, the triple-layered filters were prepared as described below. Commercial filter tips for cigarettes of 7.5 mm in length constituted two extreme layers, and an intercalary recess of 5 mm was placed with βLG-coated granules which were prepared according to the following procedure. Pulverized acetylcellulose, purchased from Daicel Ltd., Japan, was added with water and acetone, followed by pellet formation by means of a conventional pelleting extruder machine and a flash mill. The preparation was dried at 50 °C, and the granule preparation, falling to the size range of 16–32 mesh, was harvested for the carrier granule of βLG. βLG was separately macerated in water with mortar to a density of 15%. The suspension was mixed with the carrier granule to achieve the test granule which was coated with βLG to 5% of the weight of the carrier granule. The βLG-coated granule was then desiccated at 50 °C, and 120 mg of the granule, which corresponded to 6 mg of βLG, was placed in a recess of the triple-layered filter to achieve a pressure drop of 80 ± 10 mmH_2O. The triple-layered filter of 7.5 and 2.0 mm in the lengths of the extreme layers and the intercalary recess where 20 mg of genuine crystalline βLG was placed, respectively, was subjected to determination of retention of the smoke constituents by 20 mg of βLG. Here the βLG was previously sieved by means of the standard screens of 32 mesh to exclude fine crystals which might cause an unfavorable pressure drop, and the pressure drop of the resulting triple-layered filter was 60 ± 10 mmH_2O. The reason for diversity of the filter structure through the present study was that distortion in the filter structure was observed when they were introduced with the βLG suspension which corresponded to a quantity of βLG exceeding 5 mg and that the introduction of the βLG-coated granule exceeding 120 mg caused an extreme rise in the pressure drop. The filter structures employed were designed to achieve the range of pressure drop in smoking of 60–80 mmH_2O.

Harvesting Smoke Constituents. The filter tips described above or the equivalent plain acetylcellulose filter tips of 17 mm in length were assembled with the cigarette rods of a commercial cigarette commodity, and they were subjected to the machine smoking which simulated human smoking patterns in order to harvest the inclusive particulate matter of the smoke as termed by smoke condensate (SC). Injection of 0.1 mL of water into the filter tips of 17 mm in length, followed by being dried, in accordance with the procedure for introduction of 2 and 4 mg of βLG, was previously confirmed not to significantly affect the retention profile of the smoke constituents. Cigarette samples, which were not assembled with any filter structure, were also subjected to the smoking for calculation of the retention rate. The machine smoking was conducted in a fashion of 2 s of smoking and 58 s of pausing, which resulted in 30 mm of butt length. SC, derived from the smoking of five cigarettes, was harvested on a Cambridge filter disk, CM-113, of 4.4 cm in diameter (Cambridge Filter Corp.). The weight of SC, collected from 60 cigarettes, was determined.

Determination of Dry Total Particulate Matter. SC, collected from 10 cigarettes, was subjected to determination of dry total particulate matter (TPM). Recovery of TPM was determined by subtracting the moisture content of SC, which was assayed by gas chromatographic analysis (Tokura et al., 1968), from the weight of SC.

Determination of Nicotine. Alkaloids in SC, collected from 10 cigarettes, were inclusively assayed by means of steam distillation and optical absorption spectroscopy (Tokura and Furukawa, 1963), and the analytical values were expressed as a recovery of nicotine.

Determination of Benzo[a]pyrene. Quantitative analysis of B[a]p in SC, harvested by the smoking of 50 cigarettes, was achieved in accordance with the procedure of Davis et al. (1966) except that the reagents for fluorometric analysis were purchased from Wako Pure Chemical Ind. Ltd., Japan. Fluorescence emission spectra of an authentic B[a]p and the B[a]p isolate from SC by optical excitation at 376 nm were determined with a Hitachi fluorescence spectrophotometer, 204R. Calculation by the narrow base line on the fluorescence emission spectrum for determination of B[a]p was achieved by the procedure described by Saito et al. (1978). The rate of recovery of authentic perylene, which was added to SC for an internal standard in the procedure for B[a]p isolation, was determined on the fluorescence emission spectrum by optical excitation at 427 nm and it ranged from 60 to 70%. Authentic standards were recrystallized before use. Assays were made in quadruplicate except the assay for 6 mg in the dose of βLG in which the determinations were made in duplicate, and the mean values and the standard deviations were calculated. Values beyond the range, higher or lower than the mean values by 15%, were excluded, and the mean values were recalculated in the quadruplicate determinations.

Calculation. Retentions on the filter and retention rates of TPM, nicotine, and B[a]p in percent were calcu-

Table I. Profile of Retention of Dry Total Particulate Matter on the Filter as a Function of the Dose of β-Lactoglobulin

dose[a] of βLG, mg/tip	pressure drop, mmH$_2$O	recovery, mg	retention on filter, mg	retention rate, %
(−)		33.2 ± 0.2		
0	60	19.1 ± 0.1	14.1	42.5
2		18.9 ± 1.2	14.3	43.0
4		19.0 ± 0.1	14.2	42.8
(−)		31.1		
6	80	15.5	15.6	50.0
(−)		37.8 ± 1.8		
20	60	21.0 ± 1.2	16.8	44.4

[a] The values for the symbol (−) stand for the quantities of smoke condensate recovered by smoking cigarettes which are not assembled with a filter.

Table II. Profile of Retention of Nicotine on the Filter as a Function of the Dose of β-Lactoglobulin

dose[a] of βLG, mg/tip	pressure drop, mmH$_2$O	recovery, mg	retention on filter, mg	retention rate, %
(−)		1.68 ± 0.02		
0	60	1.10 ± 0.02	0.58	34.5
2		1.12 ± 0.08	0.56	34.6
4		1.11 ± 0.08	0.57	34.1
(−)		1.56		
6	80	1.00	0.56	35.9
(−)		1.62 ± 0.08		
20	60	1.07 ± 0.10	0.55	34.0

[a] The values for the symbol (−) stand for the quantities of nicotine recovered by smoking cigarettes which are not assembled with a filter.

Table III. Profile of Retention of Benzo[a]pyrene on the Filter as a Function of the Dose of β-Lactoglobulin

dose[a] of βLG, mg/tip	pressure drop, mmH$_2$O	recovery, ng	retention on filter, ng	retention rate, %
(−)		38.4 ± 3.3		
0	60	25.1 ± 2.8	13.3	34.6
2		21.1 ± 2.8	17.3	45.0
4		18.5 ± 1.3	19.9	51.8
(−)		37.1		
6	80	13.2	23.9	64.4
(−)		36.2 ± 2.4		
20	60	13.0 ± 2.1	23.2	64.1

[a] The values for the symbol (−) stand for the quantities of benzo[a]pyrene recovered by smoking cigarettes which are not assembled with a filter.

Table IV. Concentrations of Benzo[a]pyrene in Dry Total Particulate Matter as a Function of the Dose of β-Lactoglobulin in the Filter

	concn of B[a]p, ppm
no filter	1.16 ± 0.10
dose of βLG, mg/tip	
0	1.31 ± 0.15
2	1.12 ± 0.15
4	0.97 ± 0.07
6	0.85
20	0.62 ± 0.10

lated by an equation of $a - x$ and $(a - x)/a \times 100$, respectively. Here, a stands for the quantity of the smoke constituents which were harvested on the Cambridge filter disks by smoking cigarettes without being assembled with any filter structure while x stands for those quantities of the smoke constituents which were harvested by smoking cigarettes assembled with respective filter varieties.

RESULTS AND DISCUSSION

Recoveries of TPM, nicotine, and B[a]p, which were harvested on the Cambridge filter disks in the machine smoking of one cigarette, were tabulated as a function of the dose of βLG in a filter tip in Tables I, II, and III. Retentions on filter and retention rates of the smoke constituents were also shown in Tables I–III. Concentrations of B[a]p in TPM as a function of the dose of βLG were calculated from the values shown in Tables I and III and were tabulated in Table IV. Standard deviations were presented for values of recoveries and concentrations of the smoke constituents except those values for the case in which 6 mg of βLG was dosed in the filter structure. Concentrations of B[a]p and nicotine in TPM were also illustrated as a function of the dose of βLG (Figure 1). Retention rates of TPM and nicotine remained at comparable levels regardless of the doses of βLG which were

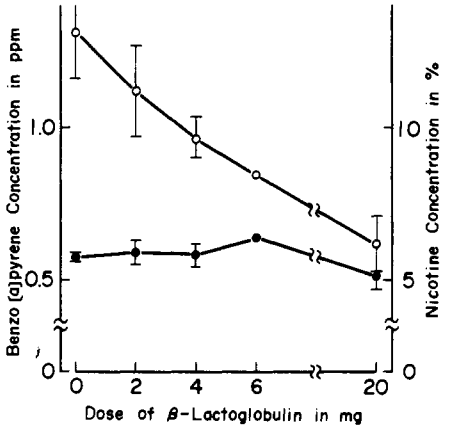

Figure 1. Benzo[a]pyrene (open circles) and nicotine (closed circles) concentrations in dry total particulate matter of tobacco smoke as a function of the dose of β-lactoglobulin in the filter structure.

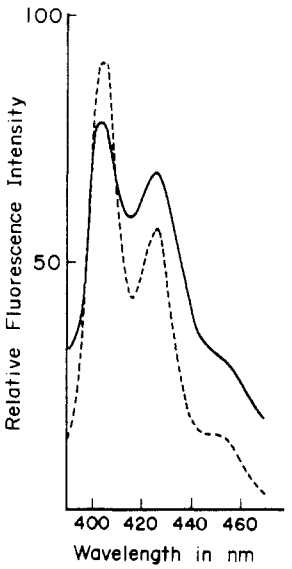

Figure 2. Fluorescence emission spectra of authentic benzo[*a*]pyrene (broken line) and benzo[*a*]pyrene isolate from tobacco smoke (solid line) by optical excitation at 376 nm.

used in the experiment of the present study. On the contrary, the retention rate of B[*a*]p and the concentration of B[*a*]p in TPM significantly varied, dependent upon the dose of βLG as shown in Tables III and IV and Figure 1. The experimental results in Tables I and III and Figure 1 were compatible with a hypothesis of preferential retention of B[*a*]p in tobacco smoke by βLG.

It was noted that the plain acetylcellulose fiber filter by no means affected the preferential retention of B[*a*]p but might have concentrated B[*a*]p in recovered TPM since concentration of B[*a*]p in TPM, harvested by smoking cigarettes without being assembled with any filter structure, was 1.16 ± 0.10 ppm while the concentration was apparently elevated to 1.31 ± 0.15 ppm in the case of smoking those with plain acetylcellulose fiber filters (Table IV).

TPM, which is harvested on the Cambridge filter disk in the machine smoking, is an entity to be inhaled by smoker individuals. Therefore, it is evident that inhalation of B[*a*]p by cigarette smokers is preferentially reduced by applying βLG to the filter structure, while cigarette smokers might inhale TPM in which B[*a*]p is appreciably concentrated when the conventional plain acetylcellulose fiber filter is assembled with the cigarette. The B[*a*]p concentration in TPM in the control test, by smoking cigarettes which were assembled with the plain acetylcellulose fiber filter, was 1.3 ± 0.15 ppm while the concentration was lowered to 0.62 ± 0.10 ppm when cigarettes were assembled with the βLG-processed filter structure of the present study (Table IV). This leads to the conclusion that inhalation of B[*a*]p by cigarette smokers is reduced by ~50% without significant reduction in TPM inhalation when 20 mg of βLG is applied to the cigarette filter structure as compared to the case of omission of βLG.

Typical fluorescence emission spectra of the authentic B[*a*]p solution of 0.05 μg/mL in methanol and B[*a*]p isolate which was obtained from SC of 50 cigarettes and arbitrarily diluted with methanol for quantitative analysis were illustrated in Figure 2. Agreement between the wavelengths at the maximum fluorescence emission intensity in the authentic B[*a*]p and the B[*a*]p isolate was a reflection of the significance in the analytical procedure of the present study, while discrepancy was observed in the relative fluorescence emission intensity of them at the wavelengths of the two peaks in the fluorescence emission spectrum. A similar discrepancy was observed by Shiraishi et al. (1973).

The pressure drop of the filter up to 60–80 mmH_2O was a requisite for achievement of preferential retention of B[*a*]p in the tobacco smoke since the filter structure with lower than 50 mmH_2O in the pressure drop failed to exhibit the preferential retention as described above. The dual function of the moelcular specificity of βLG and the higher pressure drop in the filter structure is, therefore, postulated to be required for preferential retention of B[*a*]p.

Preferential retention of B[*a*]p in tobacco smoke by a filter structure has by no means been documented, and mechanisms for preferential association of B[*a*]p with βLG are still obscure. A pronounced hydrophobicity of βLG, which has been observed extensively (Takenaka et al., 1970; Robillard and Wishnia, 1972; Shangbag and Axelsson, 1975; Axelsson, 1978; Birdi and Steinhardt, 1978; Keshavarz and Nagai, 1979), is seemed to be involved in the mechanisms for association with B[*a*]p. B[*a*]p in tobacco smoke is postulated to be localized at the inner area in the particulate structure of the aerosol of tobacco smoke in which the constituents of the higher to the lower boiling points are oriented in a direction of the inner to the exterior layer. It might be the case that smoke aerosol is adsorbed once by βLG and then desorbed and substituted with the smoke constituents which are subsequently introduced into the filter structure while B[*a*]p might remain exclusively associated with βLG by the hydrophobic affinity.

The present authors also observed a preferential retention of the volatile phenolic compounds in tobacco smoke, which was presumed to be a tumor promotor (Wynder and Hoffmann, 1961), by the filter structure with βLG. These observations are compatible with a hypothesis [proposed by Wynder and Hoffmann (1964)] that the cigarette filter with βLG results in lower risk to the smoker individuals exposed to the carcinogenic indicators.

Commercial application of βLG in cigarette filters is a subject of the present authors' interest. While milk whey is a surplus product which is discharged from the milk processing industry, the isolation process of βLG from milk whey is prohibitively expensive. The dried protein concentrate of milk whey, which contains βLG and other protein species to a concentration of 35%, is currently merchandized at ~6 dollars for 1 kg in the dairy community. Twenty milligrams of βLG is required for reduction of B[*a*]p concentration in TPM, generated by smoking one piece of cigarette, by ~50% according to the experimental results shown in Table III. This quantity of βLG corresponds to 57 mg of the dried protein concentrate of milk whey, and its application to a cigarette filter tip causes elevation of the price of cigarette commodities by ~0.5%. An article on the experimental results with respect to the dried protein concentrate of milk whey will appear elsewhere.

LITERATURE CITED

Aschaffenburg, R.; Drewry, J. *Biochem. J.* **1957**, *65*, 273.
Axelsson, C.-G. *Biochim. Biophys. Acta* **1978**, *533*, 34.
Birdi, K. S.; Steinhardt, J. *Biochim. Biophys. Acta* **1978**, *534*, 219.
Davis, H. J.; Lee, L. A.; Davidson, T. R. *Anal. Chem.* **1966**, *38*, 1752.
Keshavarz, E.; Nagai, S. *Biochim. Biophys. Acta* **1979**, *576*, 269.
Ono, J.; Doi, K.; Nagasawa, T. *Agric. Biol. Chem.* **1975**, *39*, 2149.
Robillard, K. A., Jr.; Wishnia, A. *Biochemistry* **1972**, *11*, 3835.
Saito, Y.; Sekita, H.; Takeda, M.; Uchiyama, M. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 129.
Shangbag, V. P.; Axelsson, C.-G. *Eur. J. Biochem.* **1975**, *60*, 17.

Shiraishi, K.; Shiratori, T.; Takabatake, E. *J. Hyg. Soc. Jpn.* **1973**, *14*, 173.
Takenaka, O.; Nishimura, Y.; Takenaka, A.; Shibata, K. *Biochim. Biophys. Acta* **1970**, *207*, 1.
Tokura, C.; Furukawa, Y. *Kenkyu Hokoku—Nippon Senbai Kosha Chuo Kenkyusho* **1963**, *No. 105*, 291.
Tokura, C.; Sugawara, S.; Tanno, M.; Kosugi, A.; Ike, H.; Kobashi, U.; Kaburaki, Y. *Kenkyu Hokoku—Nippon Senbai Kosha Chuo Kenkyusho* **1968**, *No. 110*, 135.
U.S. Department of Health, Education and Welfare *DHEW Publ. (CDC) (U.S.)* **1975**, *76*.
Wynder, E. L.; Hoffmann, D. *Cancer (Amsterdam)* **1961**, *14*, 1306.
Wynder, E. L.; Hoffmann, D. *Adv. Cancer Res.* **1964**, *8*, 249.

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Soybean Protein Agglomeration: Promotion by Ultrasonic Treatment

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Aggregate formation in soybean proteins after ultrasonic treatment was studied by gel filtration, disc gel electrophoresis, and ultracentrifugation. Ultrasonic-treated soybean proteins contained more aggregates than unsonicated samples. Gel filtration with Sephadex G-200 showed that the aggregates eluted near the void volume, ahead of 7 S and 11 S, and appeared to be stable above a critical protein concentration. Disc gel electrophoresis indicated that a large amount of protein from sonicated samples did not enter the stacking gel. Protein separation in the gel suggested transformational changes of 7 S proteins. Ultracentrifugal analyses also indicated conversion of 7 S into 40–50 S aggregates. Ultrasonic action seemed to promote protein aggregation rather than dissociation in water extracts prepared from defatted soybean flakes.

It has been shown previously that ultrasonic treatment increased soybean protein extractability in water from defatted flakes (Wang, 1975) and from commercial isolates, concentrates, and defatted flakes which were either heated or alcohol-washed before sonication (Wang, 1978). Sonication has been widely used in solubilizing animal and plant tissue components. However, its application to extracting soybean proteins is relatively new.

It is generally surmised that globular proteins in an ultrasonic field may be broken down or transformed into new molecules. Ultrasound induces cavitation which causes proteins to undergo either physical disruption and/or chemical transformations (El'Piner, 1964). The conditions under which proteins are transformed can be controlled in most cases. Changes in protein molecules may thus be studied. A recent example includes studies of the effect of ultrasonic irradiation on several proteins (O'Shea and Bradbury, 1973). Mayoglobin, apomyoglobin, and proteins from wool were sonicated and the modified proteins studied by gel permeation chromatography. Only myoglobin was aggregated, while apomyoglobin split into half-molecules and others showed little or no changes after sonication. It appears that the breakdown is a result of a physical rather than chemical process. The chemical process that is associated with cavitation may produce secondary effects such as formation of bilayers, micelles, and aggregates. Aggregation was also observed under various conditions in ultrasonic-treated samples including serum albumin (Searcy, 1966), apolipoprotein in a mixture of trioleins and lecithin (Forte et al., 1974), blood platelets (Miller et al., 1979), and goat immunoglobulin (Huang and Kennel, 1979).

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Reports on ultrasonic-treated soybean proteins are scanty. Thus, we have extended our earlier studies of ultrasonic effects on soybean proteins (Wang, 1975, 1978).

This paper reports a phenomenon of ultrasonic promoted agglomeration of soybean proteins as demonstrated by results obtained from gel filtration, disc gel electrophoresis, and ultracentrifugational analyses.

MATERIALS AND METHODS

Materials. Kanrich variety soybeans purchased locally were cracked, dehulled, flaked, and defatted. The defatted flakes contained 46.4% protein (dry basis) and had a nitrogen solubility index of 91 (Smith et al., 1966). The flakes were refrigerated until used. Fine-grade Sephadex G-200 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Acrylamide for electrophoresis was purchased from Eastman Kodak Co., Rochester, NY. A commercial preparation of soybean trypsin inhibitor (TI) for identification purposes in gel filtration was bought from Sigma Chemical Co., St. Louis, MO.

Protein Extractions. Protein extraction in a 1:10 ratio of flakes to water was used as described previously (Wang, 1975). Sonication was carried out for 8 min with a sonifier (Heat Systems-Ultrasonics Inc., Model J32A, Plainview, NY). The samples (10 g of meal; 100 mL of H_2O) in a 250-mL beaker were chilled in ice during sonication. After sonication, the mixture was centrifuged at 10000*g* for 15 min, and the supernatant was poured through a thin layer of glass wool to obtain a clear filtrate. The proteins were lyophilized and stored in a refrigerator for further use. Protein fractions (7 S, 11 S, and whey proteins) were prepared according to Thanh and Shibasaki (1976), dialyzed, lyophilized, and stored at 4 °C for future use in gel filtration and disc gel electrophoresis analysis.

Gel Filtration of Proteins. Lyophilized protein samples (125 mg or less) were dissolved in 5 mL of sodium phosphate buffer (pH 7.6; $\mu = 0.1$) and applied to the top