Studies on the Mucilage of the Root of "Tororo-aoi" (Abelmoschus manihot, MEDIC). III. On the Mechanism of the Characteristic Colloidal Nature of the Mucilage

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(Received April 28, 1960)

In the previous paper[1] it has been shown that in the freshly purified mucilage there exists a network structure built by a very strong interaction between the mucin molecular chains, and that an irreversible marked loss of the interaction occurs by heat treatment although the change in average molecular weight measured by osmotic pressure is small[2]. The present investigation was undertaken in order to explain the mechanism of the network structure in the fresh mucilage and its breaking by heat treatment. Details are shown as follows.

Experimental

1) Material.—Roots of "*Tororo-aoi*" were stored in 0.5% formalin.

2) Crude Mucilage, Purified Mucin, and Purified Mucilage.—These preparations were similar to those described in the previous paper[3].

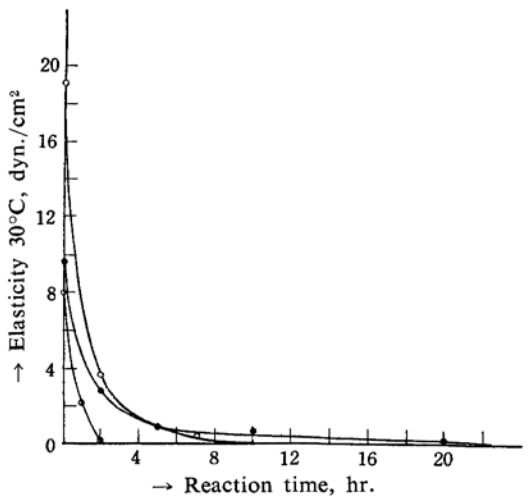

Fig. 1. Effect of addition of H_2O_2, H_2SO_4, or H_2O_2–H_2SO_4 on the elasticity of the purified mucilage.
Concentration of mucilage: 0.53%
○, H_2O_2 (0.12%)
●, H_2SO_4 (9.8%)
◑, H_2O_2–H_2SO_4 (0.06% : 9.8%)

3) Elasticity and Intrinsic Viscosity.—These determinations were the same as those described in the previous paper[3].

4) Action of Hydrogen Peroxide on the Mucilage.—The elasticity changes (at 15°C) in the purified mucilage in the presence of hydrogen peroxide, sulfuric acid, or the mixture of both, are shown in Fig. 1.

Acetone was added into the mucilage to precipitate the mucin, just when the elasticity could hardly be observed in the above experiments. The mucin thus obtained was washed with acetone, dried up in vacuo and redissolved in water. In the experiment using sulfuric acid, the redissolved mucilage was neutralized with a dilute sodium hydroxide solution. Table I gives the results of measurement of intrinsic viscosity of the redissolved mucilage.

TABLE I. INTRINSIC VISCOSITY OF THE PURIFIED MUCILAGE JUST WHEN THE ELASTICITY COULD HARDLY BE OBSERVED BY ADDITION OF HYDROGEN PEROXIDE, SULFURIC ACID, OR BOTH

Treatment by	Intrinsic viscosity l./g.				Mean value l./g.
H_2O_2	1.7	1.2	1.8	1.6	1.6
H_2SO_4	0.47	0.55	0.58	0.50	0.53
H_2O_2–H_2SO_4	1.8	1.7	1.4	1.7	1.7
Heating at 80°C for 2 hr.	1.5	1.6	1.4	1.4	1.5

The intrinsic viscosity of the mucilage treated with hydrogen peroxide or hydrogen peroxide-sulfuric acid is about the same as that of the heated mucilage. However, that of the mucilage treated with sulfuric acid is 0.52, and is considerably smaller than the above-mentioned values.

5) Effect of Oxygen in the Air upon the Mucilage and the Mucin.—a) The purified mucins, one in nitrogen atmosphere and the other in the air, were

TABLE II. EFFECT OF OXYGEN UPON THE INTRINSIC VISCOSITY LOSS OF THE PURIFIED MUCIN BY HEAT TREATMENT

Heated in	Intrinsic viscosity, l./g.	
	Before heating	After heating at 80°C for 120 min.
N_2 atmosphere	2.86	2.77
Air	2.86	1.83

1) S. Inokawa, This Bulletin, 33, 1476 (1960).
2) S. Inokawa, R. Goto and I. Morimoto, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagagu Zasshi*), 81, 783 (1960).
3) S. Inokawa, This Bulletin, 33, 1472 (1960).

TABLE III. EFFECT OF OXYGEN UPON THE INTRINSIC VISCOSITY LOSS OF THE PURIFIED MUCILAGE BY HEAT TREATMENT

Condition	Method	Intrinsic viscosity, l./g.		
		Before heating	After heating 80°C, 90 min.	After heating 80°C, 150 min.
Oxygen-free mucilage	High vacuum	2.64	2.23	2.10
	N_2-blowing	2.55	2.20	2.08
Air-containing mucilage		2.80	1.47	1.38

heated at 80°C for 120 min. by the high vacuum method described in the previous paper[4]. Both the heated mucins were dissolved in water and the viscosity was measured. As shown in Table II the intrinsic viscosity of the mucin heated in nitrogen atmosphere does not decrease practically. b) The purified oxygen-free mucilage prepared by the high vacuum method[4] was heated at 80°C and the intrinsic viscosity was measured. As shown in Table III the intrinsic viscosity loss of the purified oxygen-free mucilarge by heating is much smaller than that of the usual air-containing mucilage.

For the purpose of obtaining oxygen-free mucilage more simply, oxygen-free nitrogen[5] was blown for 10 hr. into the purified mucilage in the glass tube shown in Fig. 2, and then the glass tube was sealed off first at A and next at B. The viscosity loss of the obtained oxygen-free mucilage by heating was similar to that of oxygen-free mucilage prepared by the high vacuum method as shown in Table III. Then, the effect of oxygen upon the elasticity loss of the purified mucilage by heating was examined

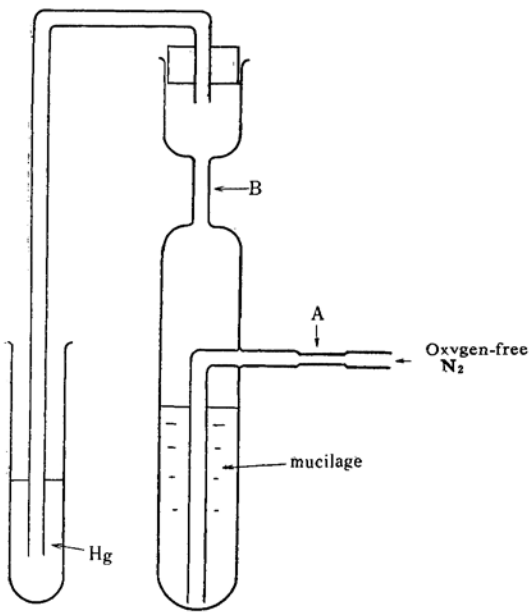

Fig. 2. Apparatus for the blowing method.

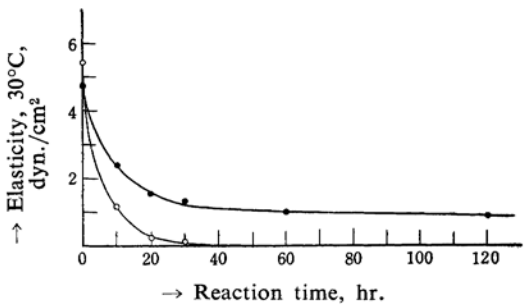

Fig. 3. Effect of oxygen on the elasticity fall of the purified mucilage by heating.
Concentration of mucilage: 0.31%.
○, air-containing mucilage
●, oxygen-free mucilage

by this nitrogen-blowing method. The elasticity loss of nitrogen-blowing mucilage by heating as shown in Fig. 3 is much smaller than that of the air-containing mucilage.

6) Muddy Matter Produced by Heating.—It has been reported[1], from the observation of sedimentation diagrams of the heated purified mucilage in an ultracentrifuge that there was something which sedimented rapidly in the initial few minutes. A precipitate[6] (ca. 1.1 wt. % of mucin), which was dispersed easily by irradiation of ultrasonic wave, was obtained from the purified mucilage heated at 95°C for 16 hr. The dispersed precipitate was mounted on a specimen holder for electron microscopy with a formvar supporting-film and dried.

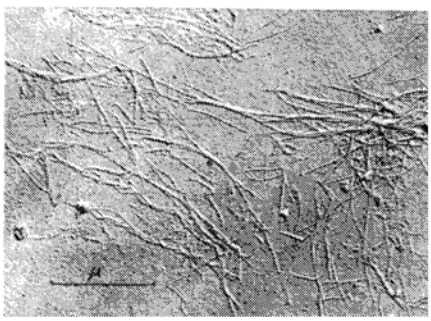

Fig. 4. Electron micrograph of the precipitate produced by heating the purified mucilage.
(dispersed by ultrasonic wave)
(Cr-shadowing)

4) S. Inokawa, R. Goto and E. Fujimoto, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **81**, 678 (1960).

5) By blowing of carbon dioxide instead of nitrogen, similar results were obtained.

6) This precipitate did not show reactions of starch.

After chromium-shadowing the specimen was observed by an electron microscope of the type Akashi TRS-50 B, showing a fine fiber-like picture as shown in Fig. 4. The nitrogen content of the precipitate was 1.9%. After hydrolysis of the precipitate in 2 N hydrochloric acid at 100°C for 22 hr. in a sealed tube, the hydrolyzed solution was diluted with water, filtered, passed through a cation-exchange resin Amberlite IR-112, evaporated, and dried, in vacuo. From the residue only glucose was detected by paper chromatography using the solvent system: ethyl acetate: pyridine: water (12 : 5 : 4).

7) Effect of Potassium Thiocyanate, Sodium Lauryl Sulfate, Guanidine Hydrochloride, and Dichloro acetic Acid on the Elasticity of the Purified Mucilage.—Fig. 5 shows the elasticity curves plotted against concentrations of four sorts of reagents. It is observed that the elasticity is practically uninfluenced by the addition of these reagents[7].

8) Periodate Oxidation of the Mucilage.—The mucilage (0.448 g.) was dissolved in water (in 500 ml. bottle), and sodium periodate solution (0.2 M, 10 ml.) was added, followed by water to the requisite volume. Formic acid was yielded, and uptake of periodate of the solution followed. The results are tabulated in Table IV. It is clear that 1 M of formic acid was produced by consuming 2 M of periodate.

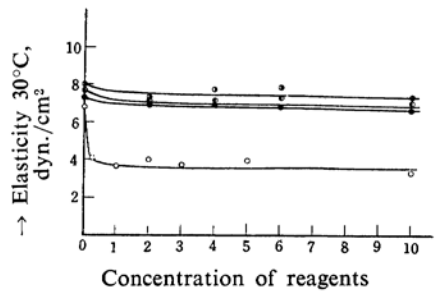

Fig. 5. Effect of addition of potassium thiocyanate sodium lauryl sulfate, guanidine hydrochloride, and dichloroacetic acid on the elasticity of the purified mucilage.
Concentration of mucilage: 0.36%.
●, potassium thiocyanate
◐, sodium lauryl sulfate
◑, guanidine hydrochloride
○, dichloroacetic acid

TABLE IV. PERIODATE OXIDATION OF THE MUCILAGE AT 15°C[8]

Time, hr.	Formic acid produced mol./G mol.[9]	Consumed IO_4^- mol./G mol.
0	0	0
10	0.72	1.21
24	1.09	1.64
52	1.19	2.02
75	1.23	2.07
125	1.24	2.14

Discussion

It is said that the mucin of "*Tororo-aoi*" is a polysaccharide consisting of rhamnose and galacturonic acid linked in straight chains[10] and the molecular weight is very high, that is, the value is 150000~200000[2]. The relative amounts of the components vary, depending on the source. However, it is shown from the results of periodate-oxidation (experiment 8) that the mucin molecule has branched chains. Thus, it seems to be plausible that the mucin molecule is a long chain polysaccharide with many short branched chains. Also, it has been shown that in the fresh purified mucilage there exists a network structure like a gel[1]. Considering that the elasticity is little changed by the electrolysis[3] or the addition of sodium chloride[3], the network structure may not be built by intermolecular ionic bonds of the mucin. Moreover, the elasticity is little influenced by the addition of potassium thiocyanate, sodium lauryl sulfate, guanidine hydrochloride, and dichloroacetic acid (experiment 7). Therefore the network structure may not be built by hydrogen bonds between mucin molecules. Also, the agitation of mucilage causes an irreversible diminution of the elasticity[3]. From the consideration of these experimental results, it seems to be most reasonable to suppose that the network structure is built by interlocking or entanglement of mucin molecules.

By heating, the elasticity of the purified mucilage diminishes and at the same time muddy matters consisting of fibrous glucosans and proteins were produced (experiment 6) but the molecular weight change is slight[2]. These experimental results mentioned above would lead to the conclusion that the mucin molecules are entangled between short branched chains of the mucin molecules, fibrous glucosans and proteins. On heating, the entanglement is broken by oxidation (experiment 5).

Summary

1) The colloidal characteristics of the mucilage from "*Tororo-aoi*" such as the elasticity, spinnability, and so on are caused by the network built by interlocking between short branched chains of the mucin molecules, fibrous glucosans and proteins.

7) It seems to be reasonable that a little loss in elasticity by the addition of dichloroacetic acid was caused by a change of pH in the mucilage because the elasticity loss was identical with the case in which acetic acid or oxalic acid was added.

8) P. F. Fleury and J. Lange, *J. pharm. Chim.*, [8], **17**, 107, 196 (1933).

9) The value of G mol. was assumed to be 161, which is the mean value of molecular weight of rhamnose and galacturonic acid.

10) S. Machida and N. Uchino, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **74**, 183 (1953).

2) The remarkable change of the colloidal nature by heating is caused by breaking down the interlocking by oxidation.

Finally the writer wishes to express his hearty thanks to Professor R. Goto, Kyoto University, for his invaluable advice and encouragement throughout this work. He is also indebted to Professor E. Suito and Dr. K. Takiyama of Kyoto University for their help in the preparation of the electron micrographs, and further to Mr. A. Sera, Mr. A Takatsuki, Mr. I. Morimoto and Mr. E. Fujimoto for their kind co-operation in this research. It is a pleasure to thank Koshin Kogyo Co. for the generous supply of roots of "*Tororo-aoi*".

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