Studies on the Mucilage of the Root of "Tororo-aoi" (*Abelmoschus manihot*, MEDIC). II. Effect of Heating on the Colloidal Characteristics of the Mucilage

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In the previous paper[1] it has been shown that the elasticity, spinnability, and filterability of the crude mucilage and the elasticity of the purified mucilage decreased remarkably by heating at 80°C for 1~3 hr. Taking an interest in this characteristic of the mucilage, the present investigation was undertaken in order to observe the effect of heating on the mucilage by electron micrography, sedimentation in an ultracentrifuge, and electrophoresis.

Experimental

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

Crude Mucilage and Purified Mucilage.—These preparations were similar to those described in the previous paper[1].

Electron Microscope.—The sample mucilage was mounted on a specimen holder for electron microscopy with a formvar supporting-fiim and dried. After chromium-shadowing the specimen was observed by an electron microscope of the type Shimadzu SM-T4.

Ultracentrifuge.—Sedimentation diagrams of an approximately 0.4% solution of mucilage in the presence of a 0.7% sodium chloride were taken by an ultracentrifuge of the type Spinco E under the centrifugal force of 259700 ***g*** at speed of 59780 r. p. m..

Electrophoresis.—Electrophoresis patterns of an approximately 0.3% solution of mucilage in a phosphate and sodium chloride buffer of ionic strength 0.02 and 0.18 respectively, were taken by a Tiselius electrophoresis apparatus of the type Hitachi HT-B.

Elasticity and Intrinsic Viscosity.—These determinations were the same as those described in the previous paper[1].

Results and Discussion

Observations from Electron Micrographs.—The microscopic observation of network structure, which appears by the addition of methylene blue solution to the crude mucilage, is reported[2]; but the structure has been presumed[3] not to exist originally in the mucilage

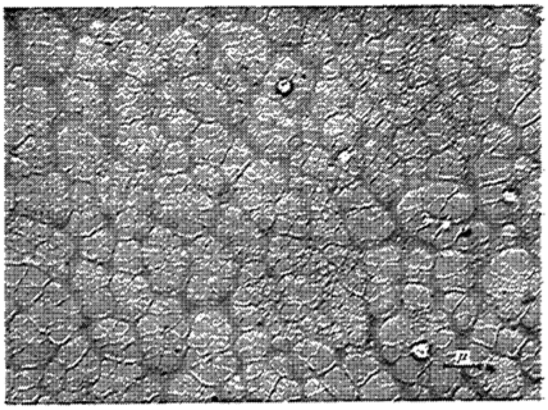

Fig. 1. Electron micrograph of the crude mucilage. (0.2% aqueous solution) (Cr-shadowing)

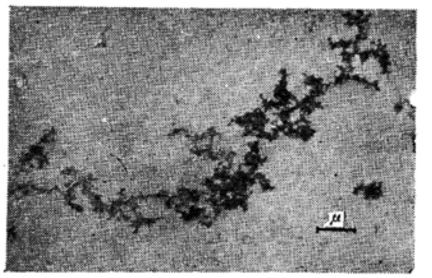

Fig. 2. Electron micrograph of the mucilage heated at 80°C for 3 hr. (0.2% aqueous solution) (Cr-shadowing)

1) S. Inokawa, This Bulletin, 33, 1473 (1960).
2) S. Oguri and K. Tomamezi, *J. Soc. Chem. Indust. Japan*, (*Kogyo Kagaku Zassi*), 46, 146 (1943).
3) S. Oguri, I. Shinohara, and T. Ono, *J. Chem. Soc. Japan, Ind. Chem. Sec.* (*Kogyo Kagaku Zassi*), 58, 227 (1955).

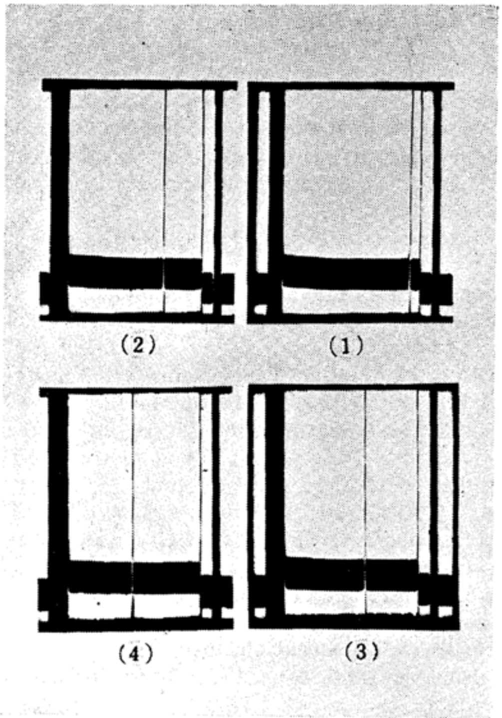

Fig. 3. Sedimentation diagram of the purified fresh mucilage in 0.7% NaCl solution. Speed 59700 r. p. m.; approximate concentration 0.4%; times for each measurement, 16 (1), 90 (2), 112 (3) and 144 (4) min. after reaching full speed.

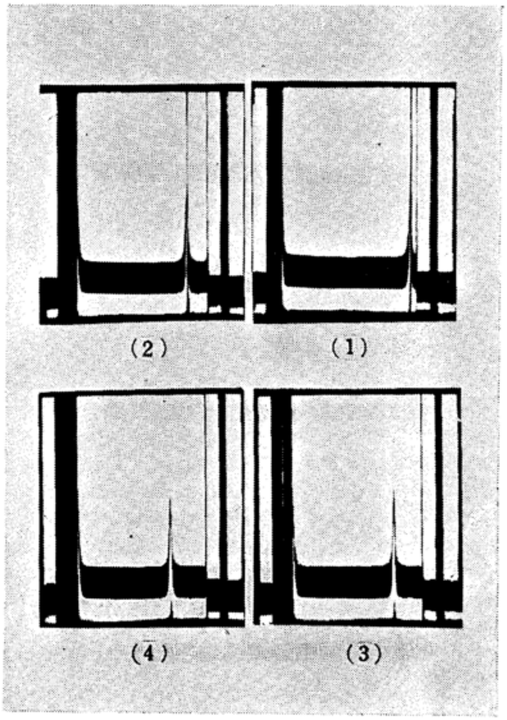

Fig. 4. Sedimentation diagram of the mucilage heated at 80°C for 2 hr. in 0.7% NaCl solution. Speed 59700 r.p.m.; Approximate concentration 0.4%; times for each measurement, 16 (1), 56 (2), 80 (3) and 104 (4) min. after reaching full speed.

but to be formed by an aggregation of composing substances by the addition of the reagent. The present writer has observed that the electron micrograph of the crude mucilage showed a typical network structure as shown in Fig. 1, which by heating at 80°C for 2 hr., was broken to a granular structure as shown in Fig. 2.

Observation from Sedimentation Diagrams in an Ultracentrifuge.—Sedimentation diagrams of the purified fresh mucilage and the mucilage heated at 80°C for 2 hr. are shown in Fig. 3 and in Fig. 4 respectively. The shape of the initial strikingly sharp boundary, as a line, of the fresh mucilage, remained perfectly unchanged through sedimentation. The sedimentation boundary of the heated mucilage was also very sharp, but a little spreading of the boundary occurred with the elapse of time. From Figs. 3 and 4 it may be considered that in the fresh mucilage, a network built by a very strong interaction between the mucin molecular chains moves as a whole under the influence of the centrifugal field, and the considerable weakening of the intermolecular force between chains occurs by heat treatment[4].

Observation from Electrophoresis Patterns.—Electrophoresis patterns of the purified fresh mucilage and the mucilage heated at 80°C for 2 hr. are shown in Fig. 5 and in Fig. 6, respectively. The initial strikingly sharp ascending pattern[5] of the fresh mucilage remained unchanged through electrophoresis similar to the

TABLE I. RELATIONS BETWEEN pH AND MOBILITY AT 0°C IN PHOSPHATE AND NaCl BUFFER OF IONIC STRENGTH 0.02 AND 0.18

	pH	2.1	5.3	6.9	9.6
Mobility	Purified fresh mucilage	1.1×10^{-5}	1.2×10^{-4}	1.3×10^{-4}	2.2×10^{-5}
(cm^2/sec. V.)	Heated mucilage	3.0×10^{-5}	2.6×10^{-4}	2.4×10^{-4}	5.0×10^{-5}

4) In the heated mucilage it was observed that there was something which sedimented rapidly in the initial few minits.

5) The pronounced boundary disturbance in the descending boundary was caused by the adhering of the mucilage on the electrophoresis cell-wall.

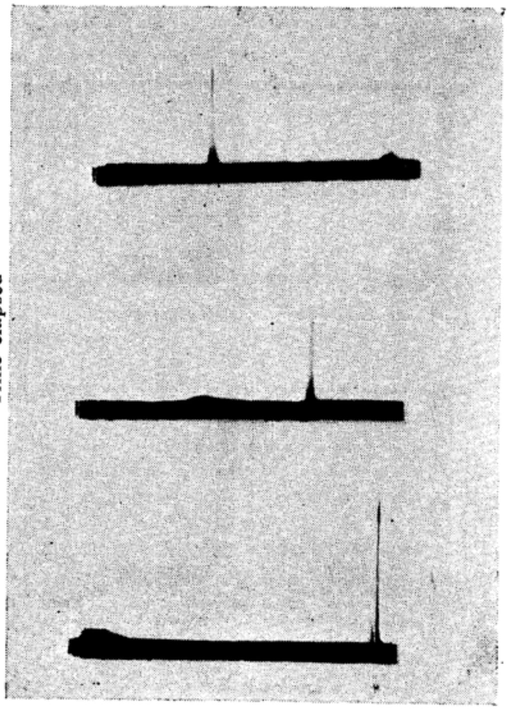

Fig. 5. Electrophoresis pattern of the purified mucilage. Approximate concentration 0.3%; phosphate-NaCl buffer; pH 6.5.

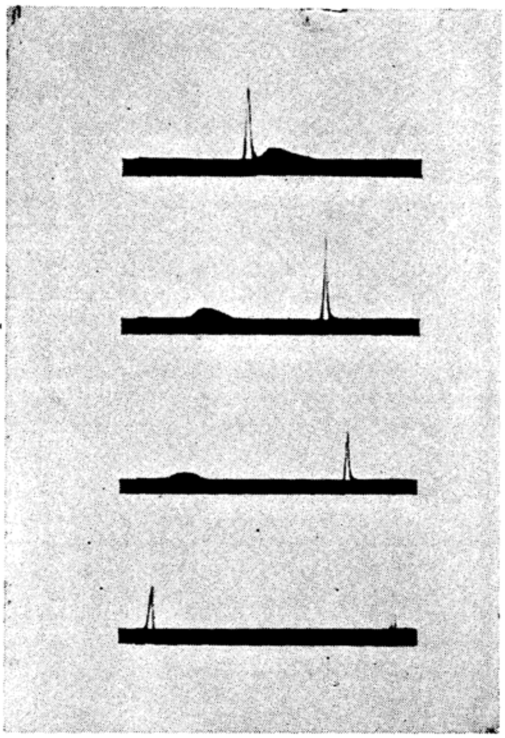

Fig. 6. Electrophoresis pattern of the mucilage heated at 80°C for 2 hr. Approximate concentration 0.3%; phosphate-NaCl buffer; pH 6.5.

centrifugal boundary (Fig. 4). The ascending pattern of the heated mucilage became somewhat broader as time went by. From Figs. 5 and 6 it may also be considered that there exists a very strong interaction between the mucin molecular chains in the fresh mucilage, and that the interaction decreases considerably by heating.

Measurements of the electrophoresis patterns over pH 2.1~9.6 were carried out without observing any change of the shape, and the relations between pH and mobility are tabulated in Table I.

Effect of Heating Temperature on Elasticity and Intrinsic Viscosity of the Mucilage.—Relations between heating temperature and decrease of elasticity and intrinsic viscosity of the purified mucilage were observed at heating time of 30, 60 and 90 min. as shown in Fig. 7. The decrease of the elasticity was gradual up to approximately 50°C and then sharp at the higher temperature, but that of intrinsic viscosity was nearly constant. The results seem to indicate that the decrease of elasticity by heating is not caused simply by degradation of mucin molecules. This is also supported by the previous experimental results[6] that

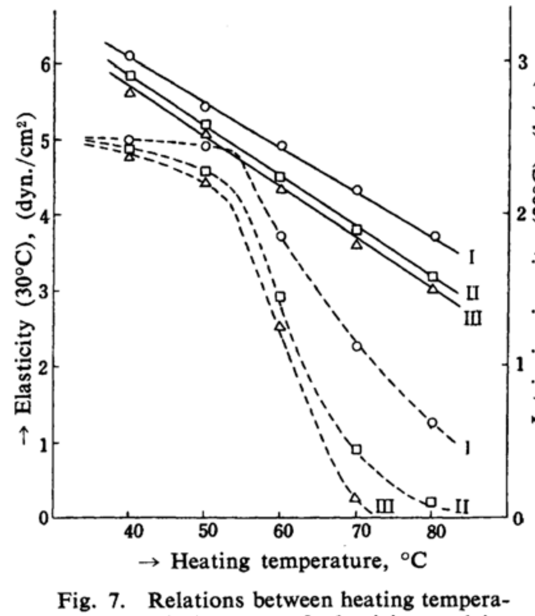

Fig. 7. Relations between heating temperature and decrease of elasticity and intrinsic viscosity.
Mucilage concentration 0.37%
—— Intrinsic viscosity
---- Elasticity
Curve I Heating time 30 min.
Curve II Heating time 60 min.
Curve III Heating time 90 min.

6) S. Inokawa, R. Goto and I. Morimoto, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **81**, 783 (1960).

there was no considerable difference in molecular weight between the fresh and the heated mucilage.

Summary

1) From electron micrographs it was observed that in the crude mucilage there exists a network structure, but its structure is broken by heating.

2) From sedimentation diagrams and electrophoresis patterns it seems reasonable to assume that in the purified fresh mucilage there exist very strong intermolecular forces like those in gel and these forces decrease markedly by heating.

3) It is conceivable that the degradation of the mucin macromolecule does not play an important role in the marked decrease of the elasticity of the purified fresh mucilage by heating.

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 taking the electron micrographs, to Dr. K. Fukai of Osaka University for taking the sedimentation photographs, to Professor S. Tanaka of Kyoto University for his help in taking electrophoresis photographs. It is also a pleasure to thank Koshin Kogyo Co. for their generous supply of the root of "*Tororo-aoi*".

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