THE MECHANISM OF THE SLIME FORMATION ON SUGARED
KAMABOKO. III. IDENTIFICATION OF DEXTRAN BY
MEASURING THE VELOCITY CONSTANT OF ACID
HYDROLYSIS AND BY OXIDATION WITH SODIUM
META-PERIODATE FOR 'NETO' COMPOUND

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In the preceding papers,^{1,2)} a problem of surface spoilage of Kamaboko producing slime or "Neto" was described as to the mother substance and causative bacteria being considered to promote this particular spoilage, in which the writers assumed that the slime or "Neto" would be dextran, a kind of glucose polymer characterized with its 1,6 glucosidic linkage. The facts that brought this conclusion could be as follows; the "Neto" is unexceptionally formed on Kamaboko containing cane sugar as a seasoning stuff, which is never produced on the food added by glucose, fructose, xylose and arabinose instead of sucrose. In addition, neither mixture of glucose and fructose nor the phosphorylated form of these sugars do fulfil requirement of the problem organism for dextran production, even both hexoses ought to be considered as satisfactory energy source. On the other hand, with progress of formation of "Neto" on sugared Kamaboko, sucrose in the Kamaboko is consumed rapidly accompanied with the increase of reducing sugar as degradation product of sucrose by bacteria. From these experimental results, the writers presumed that a polymerative decomposition of sucrose would take place in sugared Kamaboko and this slimy, transparent "Neto" should be a certain carbohydrate polymer like dextran. Besides this, a strain of *Leuconostoc*, a popular dextran forming bacteria has been isolated from spoiled sugared Kamaboko. This "Neto" material, purified by a repeated precipitation with methanol for three times, was as well hydrolized with dextranase prepared from *Penicillium funiculosum*, as done for clinical pure dextran. The end products from the "Neto" decomposed by the dextranase, were identified as isomaltose and isomaltotriose by paper chromatography.

In order to add further proof for our conclusion, in this report, another identification test specific for dextran by using sodium-metaperiodate and measuring the velocity constant of hydrolysis for a standard clinical dextran will be described.

In 1952, KOBAYASHI³⁾ has reported that the rate of hydrolysis of polysacchararides like starch and dextran with 10 N H₂SO₄ under various temperatures, in which he mentioned the velocity constant of hydrolysis calculated on the basis of unimolecular reaction, was about 10 times at 40°C and about 6 times at 60°C larger for amylose,

which involves 1, 4 glucosidic linkage, than dextran. And, in 1953, KOBAYASHI⁴⁾ also reported that the ratio of amount between 1,4 and 1,6 glucosidic linkage among different kinds of dextran, by means of measuring the amount of formic acid derived from dextran due to oxidation with sodium meta-periodate which distinguished the 1,6 linkage from the 1,4 linkage.

Meanwhile, McCLESKEY et al⁵⁾ (1947) showed that most commonly encountered species which forms dextran from sucrose was *Leuconostoc mesenteroides* including over a hundred strains, and he grouped these organisms into four types; A, B, D and F chiefly by their colony characteristics. In 1956, JEANES⁶⁾ examined the ratio of 1,6 and 1,4 linkage in samples of dextran obtained from the culture of these four types of *Leuconostoc mesenteroides* and disclosed there should be a wide variation in the number of α -1,6 glucosidic linkage.

The idea in these findings mentioned above was adopted as a means to scrutinize the nature of 'Neto' produced by Kamaboko spoilage.

Materials and Methods

'Neto' sample

'Neto' sample used was prepared in the same method described in the previous reports.

Amylose and Amylopectin

Amylose and amylopectin used in this experiment were supplied by the courtesy of Dr. J. NIKUNI, Prof. of Osaka University. These staffs were said to be prepared from potato and sweet potato.

Determination of reducing sugar obtained by acid hydrolysis of 'Neto' sample.

Acid hydrolysis of both 'Neto' and amylose was carried out at the temperature of $60 \pm 0.1^\circ\text{C}$. kept in a water bath. After 'Neto' sample and sulphuric acid were heated to this temperature separately, both materials were mixed in an Erlenmeyer flask of 100 ml. with glass plug. The reaction was proceeded in a condition of 0.2 per cent of 'Neto' sample with 10 N sulphuric acid. At the interval of every 60 minutes, the mixture subjected to hydrolysis was pipetted from the flask and placed in a beaker and then neutralized with 10 per cent sodium hydroxide solution, and reducing sugar thus remained in the sample was determined by HANES method and figured out in terms of glucose.

In another experiment, total amount of reducing sugar in both 'Neto' and amylose was determined after complete hydrolysis with 1 N H_2SO_4 solution for the account of calculating the rate of hydrolysis. In the case of 'Neto', the hydrolysis was undertaken by heating in a oil bath kept between 120 and 130°C for 8 hrs. and for amylose, in a boiling water bath for 2 hrs. After both 'Neto' and amylose was hydrolized completely, the hydrolyzate was neutralized with sodium hydroxide solution and also

reducing sugar derived in such a way was determined by HANES method.

Calculation of the velocity constant of hydrolysis of 'Neto'

The value of velocity constant of acid hydrolysis of 'Neto' and amylose was computed by the following formula,

$$k = \frac{1}{t} \log_{10} \frac{c}{c-x}$$

where c is the amount of total reducing sugar in 'Neto' or amylose, x for the amount of reducing sugar released from polymers at a certain time during hydrolysis under the given condition, t for the time in minutes.

Determination of formic acid produced by oxidation of 'Neto' with sodium meta-periodate

Formic acid produced due to periodate oxidation of the polysaccharide was determined by the procedure described by SMITH et al.⁷⁾ 'Neto', amylose, amylopectin and clinical pure dextran are dissolved in 2 per cent solution of sodium chloride separately and each solution was cooled to 5–6°C by standing in a refrigerator. To this solution, sodium meta-periodate was added in an amount of four times more than the amount considered to be equivalent to oxidize whole linkage. Then, the solution was shaken by a reciprocal shaker in a refrigerator kept at 5–6°C for 24 hours. In the end of the period of standing, 10 ml. portion of the solution was pipetted and a 0.1 ml. part of conc. ethylenglycol was added in order to reduce all the remaining meta-periodate. After standing for 30 minutes, the solution was titrated with 0.01 N sodium hydroxide using phenol-red indicator under perfusion of nitrogen gas. The value of formic acid can be then obtained from the consumption of 0.01 N sodium hydroxide solution.

Periodate oxidation of polysaccharide

It has been admitted that one mole of formic acid should be formed from one mole

Fig. 1. Proposed reaction of periodate oxidation for dextran and amylose.

of anhydroglucose, which locates in dextran as three adjacent hydroxyl group with 1,6 glucosidic linkage, by oxidation process with periodic acid or periodate, while, in the case of polysaccharide likes amylose, which involves none of these adjacent hydroxyl group because of its 1,4 glucosidic linkage, thus periodate would not give formic acid by oxidation.

Further, for dextran, two moles of periodate are required to release each mole of formic acid (See Fig. 1).

Therefore, a possibility of formic acid liberation from polysaccharide with periodate would give whether or not there could be 1,6 glucosidic linkage in structure of the polymer.

Preparation of sodium periodate.

As any commercial reagent could not be available, sodium periodate used in this experiment was prepared according to LANGLOIS method by using sodium para-iodate as a starting material. The following reaction would proceed by giving sodium iodate together with sodium nitrite and water.

Quantitative analysis of sodium meta-iodate.

To a 10 ml. portion of the mixture, withdrawn from Erlenmeyer flask at the end of oxidation reaction, 5 ml. of saturated sodium bicarbonate solution, 5 ml. of 0.1 N sodium arsenite and 5 ml. of 0.15 N sodium iodide were added in this order, and after well shaken, the mixture was kept for about 30 minutes in room temperature. And then, sodium arsenite remained in the mixture was titrated with 0.02 N iodine solution. The concentration of iodine solution was carefully checked every day with 0.05 N solution of sodium thiosulphate.

Results and discussion

As shwn in Figures 2, 3, 4, it is obvious that the velocity of acid hydrolysis of 'Neto' was lower than that of amylose at 40°C, and clinical dextran was found most resistant to hydrolysis. The trend of susceptibility for hydrolysis among three samples is similar even under increased temperature, though amylose is very labile at 60°C. This implies that the number of 1,6 glucosidic linkage contained in 'Neto' should be

Table 1. The velocity constant of acid hydrolysis of 'Neto' dextran and amylose (60°C ± 0.1)

Materials	$k(\text{min}^{-1})$
Amylose	0.00756
Dextran	0.00132
'Neto'	0.00267

less than that of clinical pure dextran and also it coincides with the observation on viscosity of these two kind of dextran reported in the previous paper.

Table 1, in which the velocity constant of hydrolysis for three samples at 60°C is indicated, gives about 5.7 times

higher rate for amylose than dextran and nearly 2 times high rate for 'Neto' from the value for clinical pure dextran.

In Figure 5, rate of formic acid formation for each sample with treatment of

Fig. 2. Rate of acid hydrolysis of amylose, dextran and "Neto" at $40^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$.

Fig. 3. Rate of acid hydrolysis of amylose, dextran and "Neto" at $50^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$.

periodate is given. Most abundant production of formic acid can be traced in clinical dextran and the middle is 'Neto', while least amount of formic acid is liberated from amylose.

Fig. 4. Rate of acid hydrolysis of amylose, dextran and "Neto" at $60 \pm 0.1^\circ\text{C}$.

Fig. 5. Formic acid production from amylose, dextran and "Neto" by means of periodate oxidation. ($6-10^\circ\text{C}$)

Table 2. Periodate oxidation of 'Neto', dextran, amylopectin and α -methylglucoside

Materials	Formic acid produced in mole per mole of anhydroglucose	Periodate consumed in mole per mole of anhydroglucose
'Neto'	0.58	1.66
Dextran	0.78	1.88
Amylopectin	0.08	0.66
α -Methyl glucoside	1.00	2.03

As will be seen in Table 2, a control run for α -methylglucoside indicates that one mole of formic acid can be obtained from exactly one mole of anhydroglucose, where 2.03 moles of sodium periodate are required to oxidize one mole of the anhydroglucose. These values well agree with theoretical figures.

Meanwhile, amount of formic acid from clinical dextran is 0.78 moles per one mole anhydroglucose where the consumption of periodate is in a level of 1.88 moles. And, 0.58 mole of formic acid was produced from one mole of anhydroglucose by consuming 1.66 mole of periodate. The value is obviously lower than the case of clinical dextran, which indicates a smaller percentage of 1,6 glucosidic linkage in 'Neto' than in clinical dextran. This evidence presumably implies that the number of 1,3 or 1,4 linkage in 'Neto' should be higher than in dextran, though the major portion of the binding consists of 1,6 linkage.

However, higher amount of formic acid produced from 'Neto' as well as the higher rate in consumption of periodate involved in this sequence than the finding of amylopectin would suggest that 1,6 linkage still remains principal part in the structure of 'Neto' compound.

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Summary

1. The velocity constant of hydrolysis of 'Neto' showed about 2.8 times as higher than that of amylose at the temperature of 60°C, which suggests a majority of glucosidic linkage in 'Neto' should be composed of 1,6 form.

2. By oxidation test with periodate a comparatively large amount of formic acid was produced from a unit mole of anhydroglucose in 'Neto' compound, which could be another proof to indicate the presence of more 1,6 glucosidic linkage, a characteristic of dextran.

3. From above two finding, together with the evidences already mentioned in our other reports the 'Neto' obtained from spoiled sugared Kamaboko would be determined as a dextran.

References

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