2. Most dimercaptols do not react normally with bromine water. The methyl and ethyl derivatives react normally but the succeeding members of the homologous series, methyl through dodecyl, fail to react quantitatively.

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[CONTRIBUTION FROM THE DIVISION OF PLANT NUTRITION, COLLEGE OF AGRICULTURE, UNIVERSITY OF CALIFORNIA]

Starch. I. End-group Determination of Amylose and Amylopectin by Periodate Oxidation

BY A. L. POTTER AND W. Z. HASSID

Hirst, Jones and associates[1] showed that data obtained from periodate oxidation of starch can be utilized for determining the number of glucose residues per non-aldehydic end-group. The values they have obtained for the chain-lengths of a number of starches from different sources and for one sample of crude potato amylopectin were in fair agreement with those obtained by Haworth's methylation end-group method.

This procedure is based on the observation of Jackson and Hudson[2] that when methyl hexopyranosides are attacked by periodate, the ring is disrupted with the elimination of the third carbon atom of the hexose as formic acid. Reducing disaccharides, such as maltose or cellobiose, consisting of two 1,4-linked hexopyranose units, should yield on periodate oxidation one mole of formic acid due to the non-reducing glucose unit and two more moles of this acid due to the reducing glucose residue. Since amylopectin consists of a multitude of branched chains made up of 1,4-linked glucose units, the terminal glucose unit of each chain, like that in the non-reducing end of maltose, contains three hydroxyls on contiguous carbon atoms 2, 3 and 4, and should yield one mole of formic acid on oxidation with periodate. In this polysaccharide the proportion of reducing end-groups is small compared with that of the non-reducing end-groups. Theoretically, only one reducing glucose unit should be present in an undegraded amylopectin molecule, and the formic acid produced from this reducing end-group can thus be ignored. A quantitative determination of the formic acid produced under proper conditions by oxidation of amylopectin with periodate should give a measure of the average chain-length of the branches.

However, formic acid will be liberated from both ends on oxidation of the long straight-chain amylose molecule, the non-reducing glucose unit producing one mole of formic acid, while the reducing glucose unit at the other end will give rise to two moles of formic acid, as shown in Fig. 1. A total of three moles of formic acid should be produced from one mole of amylose.

As pointed out by Halsall, Hirst and Jones,[3] the difficulty of determining end-groups by this method is the fact that polysaccharides, like methylglycosides of reducing disaccharides, have a tendency to be oxidized by periodate beyond the theoretical stage. However, when there is no large excess of sodium periodate present, the rate of production of formic acid is much slower after the theoretical amount has been liberated. Using potassium periodate, which is only slightly soluble in water, and keeping the concentration of formic acid low Hirst, *et al.*,[1,4] obtained satisfactory end-group determinations for amylopectin and glycogen. However, the lowering of concentration of the periodate ion results in a considerable reduction of the rate of reaction. About one-hundred eighty hours at 15° was required to liberate quantitatively the formic acid from the terminal glucose residues in starch.

The purpose of this work was to find conditions which would overcome the difficulty due to over-oxidation and that would lead to an accurate method for estimating the end-groups in amylose and amylopectin. Since only the terminal non-reducing and the reducing glucose units are involved in the actual determination with periodate, the amylose (Fig. 1) can be regarded as a maltose molecule in which the reducing and non-reducing units are united by a long chain of 1,4-linked glucose residues. If conditions for oxidation of maltose to give three moles of formic acid could be established, it would be reasonable to assume that these conditions could also be applied to the determination of end-groups in amylose and amylopectin. Conditions that satisfy this requirement were found when maltose was oxidized with sodium metaperiodate at 2°. The formation of formic acid at first proceeds at a comparatively rapid rate and after three moles of this acid have been liberated, there is a marked decline in rate, as shown in Fig. 2. A similar rate of oxidation was obtained for various amyloses. The point of inflection, which occurs at about twenty-five hours at 2°, is taken as the end-point. However, it is observed that after the primary

(1) F. Brown, Sonia Dunstan, T. G. Halsall, E. L. Hirst and J. K. N. Jones, *Nature*, **156**, 785 (1945).

(2) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **58**, 378 (1936); **59**, 994 (1937).

(3) T. G. Halsall, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1427 (1947).

(4) T. G. Halsall, E. L. Hirst and J. K. N. Jones, *ibid.*, 1399 (1947).

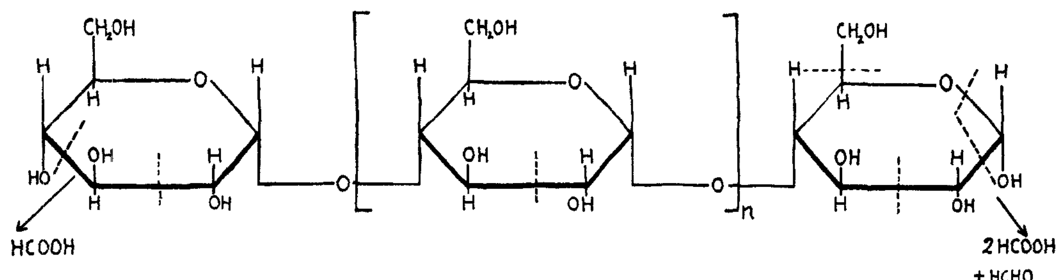


Fig. 1.—Amylose.

oxidation has taken place, the rate of oxidation of amylopectin proceeds at a much slower rate as compared with that of amylose (Fig. 3).

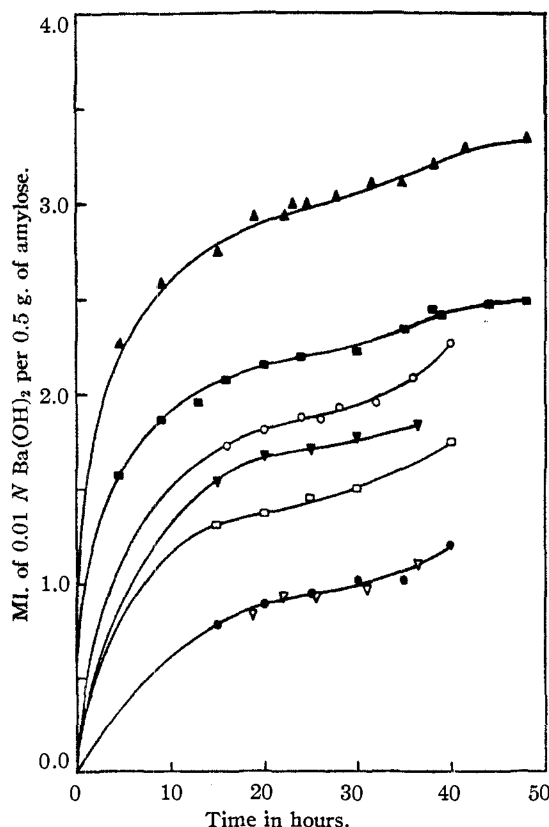
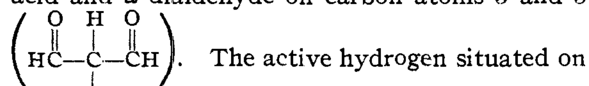


Fig. 2.—Periodate oxidation of amyloses: The uppermost curve shows the periodate oxidation of maltose in moles of formic acid produced per mole of maltose: ■, sago; O, corn; ▼, wheat; □, easter lily; ●, tapioca; ▽, potato.

The probable reason for this behavior (3) may be attributed to the fact that maltose and amylose (Fig. 1) contain a higher proportion of reducing glucose residues to non-reducing glucose residues than amylopectin. These reducing residues first undergo oxidation with the formation of formic acid and a dialdehyde on carbon atoms 3 and 5



(O H O / HC—C—CH). The active hydrogen situated on the fourth carbon atom between two carbonyl groups is subject to further oxidation forming a hydroxyl group. This structure (O OH O / HC—C—CH) is apparently readily oxidized with periodate producing formic acid. Since amylopectin is practically devoid of reducing glucose residues, secondary oxidation under these conditions occurs only to a slight extent.

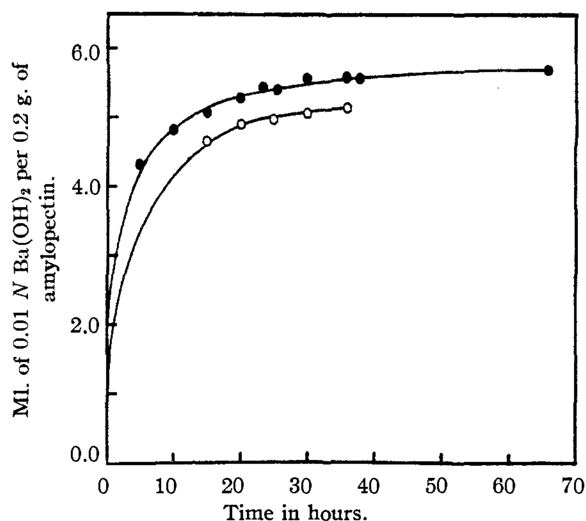


Fig. 3.—Periodate oxidation of amylopectins: ●, tapioca; O, C-109-B, corn.

The results in Tables I and II show that for six various starches the amylopectin components contain from 22 to 27 glucose units per end-group, while the amyloses consist of chains from 420 to 980 glucose units in length.

TABLE I

CHAIN-LENGTHS OF AMYLOSES FROM STARCHES OF DIFFERENT PLANT SOURCES

No.	Plant	Ml. 0.01 N $Ba(OH)_2$	Number of glucose residues per chain
S-1/2-A	Sago	2.21	420
C-107/111-A	Corn	1.88	490
W-1/2-A	Wheat	1.71	540
L-3-A	Easter lily	1.44	640
T-3/4-A	Tapioca	0.94	980
P-3/4-A	Potato	0.94	980

The periodate oxidation procedure is considerably less time-consuming than the standard methylation end-group assay method. Since by this procedure the material is directly oxidized and does not require preliminary methylation, the possibility of degradation of the starch molecule during preparation of the methylated derivative is eliminated. Only a few grams of material are required to make an end-group determination by this method.

Experimental

Starch Samples.—The starch fractions were supplied by Dr. Thomas J. Schoch of the Corn Products Refining Company. Primary separation was effected by selective precipitation of the linear components with "Pentasol."[5] The linear components were purified by two or more recrystallizations with *n*-butyl alcohol, until their iodine-binding capacity reached a maximum value. It is recognized that some subfractionation occurred during this purification. Other data on these identical samples (*viz.*, osmotic pressure, intrinsic viscosity, etc.) will be reported in future papers by the present authors and by Dr. Thomas J. Schoch.

Oxidation with Sodium Metaperiodate. (a) Maltose.—A 0.222-g. sample of maltose ($C_{12}H_{22}O_{11}\cdot H_2O$) was dissolved in 10 ml. of 3% sodium chloride solution, and the mixture cooled to 2°. Ten ml. of 0.37 *M* sodium metaperiodate was introduced and the solution was allowed to remain in a refrigerator at 2°. The total volume of the mixture was 19.8 ml. One-ml. samples were taken for analysis at intervals during a period of forty-eight hours. The excess periodate was decomposed by adding 0.3 ml. (about 6 drops) of ethylene glycol and the sample was allowed to remain in the dark at room temperature for one hour. The samples were titrated with 0.01 *N* barium hydroxide, using methyl red as an indicator. The maltose curve, Fig. 2, indicates that the termination of the primary oxidation reaction occurs at approximately twenty-five hours and coincides with the liberation of 3 moles of formic acid.

(b) Amylose.—A series of 0.5-g. amylose samples in 125-ml. erlenmeyer flasks was dispersed[6] with rapid stirring to prevent lump formation in 10-ml. aliquots of 3% sodium chloride. The flasks and contents were cooled to 2°, after which 10-ml. portions of 0.37 *M* sodium metaperiodate were added. An agate marble, 2 cm. in diameter, was placed in each flask to keep the amylose dispersed in solution during the reaction. The flasks and contents were then placed on a mechanical shaker[7] and put in a refrigerator at 2° in the dark. Because the sodium metaperiodate used was often found to give a slight acid reaction, especially after it had been in solution for a considerable length of time, it is necessary to run a blank on the reagents. For this purpose, a mixture of 10 ml. 3% sodium chloride solution and 10 ml. 0.37 *M* sodium metaperiodate solution treated with ethylene glycol was used. The samples (the whole flask contents) were analyzed at approximately five-hour intervals. They were treated with 1 ml. of ethylene glycol, allowed to remain at room temperature for one hour and titrated with 0.01 *N* barium hydroxide. The number of ml. of base taken from the titration-time curve (Fig. 2) at twenty-five hours was taken as the end-point. The results are given in Table I.

The chain length of amylose, in terms of anhydroglucose units, is obtained by calculating the grams of amylose per three moles of formic acid formed, and dividing the result by 162 (molecular weight of anhydroglucose). Thus, for example, a titration of 2.21 ml. of 0.01 *N* barium hydroxide obtained from oxidation of 0.5 g. of amylose gives a chain of 420 glucose units in length.

(c) Amylopectin.—A series of 0.2 g. of amylopectin samples was dissolved in 5 ml. of 3% sodium chloride and oxidized with 5-ml. portions of 0.37 *M* sodium periodate at 2°. Since amylopectin is soluble in water, shaking during the reaction is not essential. The analyses were carried out in the same manner as with amylose. Time curves were run only on tapioca and corn amylopectins. The other amylopectins were determined on duplicate samples after having been subjected to periodate oxidation for twenty-five hours. The results are tabulated in Table II.

TABLE II

CHAIN-LENGTHS OF AMYLOPECTINS FROM STARCHES OF DIFFERENT PLANT SOURCES

No.	Plant	Ml. 0.01 *N* $Ba(OH)_2$	Number of glucose residues per end-group
S-1-B	Sago	5.57	22
C-109-B	Corn	5.00	25
C-141-B	Corn	4.71	26
W-2-B	Wheat	5.33	23
L-3-B	Easter lily	4.52	27
T-3-B	Tapioca	5.41	23
P-3/4-B	Potato	4.59	27

Calculation of the amylopectin chain-length is made on the basis of one mole of formic acid produced per chain.

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Summary

A method for estimating the end-groups of amylose and amylopectin is described. This procedure is based on the quantitative determination of the formic acid produced when the starch fractions are subjected to oxidation with sodium metaperiodate at 2°.

The amylopectins from starches of six different plants ranged from 22 to 27 glucose residues per end-group. The chain lengths of the corresponding amyloses ranged from 420 to 980 glucose residues.

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(5) T. J. Schoch, "Advances in Carbohydrate Chemistry," edited by W. W. Pigman and M. L. Wolfrom, Academic Press, Inc., New York, N. Y., 1, 259 (1945).

(6) Addition of 3 to 4 ml. of petroleum ether to the amylose sample facilitates dispersion.

(7) A Fisher Gyrosolver shaker, obtained from Eimer and Amend Company, was used. In order to withstand prolonged and continuous shaking, its motor was replaced by a ball bearing Bodine gear motor, 1/50 H. P., 139 r. p. m. The roller bearings were also replaced with precision sealed-in roller bearings.