cellular multiplication by maintaining a normal balance between the two types of nucleic acid. Further speculation might lead one to suppose that in tumor cells this balance is disturbed.

Summary

Lactobacillus bifidus (ATCC 4963) has been found to grow in response to increasing amounts of desoxyribonucleic acid (DNA) over a range of from 5 to 50 micrograms per tube. The ability of the organism to utilize intact DNA has been used to search for a substance that specifically interferes with the utilization of DNA. Ribonucleic acid (RNA) has been found specifically to inhibit the utilization of DNA by *L. bifidus*. The application of this finding to cellular metabolism has been discussed briefly.

GLENOLDEN, PA. RECEIVED JUNE 24, 1949

[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[1]]

Gelation Properties of Partially Acetylated Pectins[2]

BY E. L. PIPPEN, R. M. MCCREADY AND H. S. OWENS

The presence of acetic acid in pectins from various sources has been reported by many investigators.[3-11] In some cases the acetic acid seems to be an artifact, but there is agreement that sugar beet pectin contains at least four per cent.[3,4,6,9,10] The lack of jelling power of sugar beet pectin has been attributed to the presence of acetyl groups.[12b] Roboz and van Hook[12a,b] have also suggested that low molecular weight of sugar beet pectin as found by Schneider and Bock[13] is a possible explanation. There is also the possibility that its high non-uronide content might be a factor.

To resolve this problem and to obtain further information on the mechanism of jelly formation, several partially acetylated pectins now have been prepared. Their jelly power has been measured before and after deacetylation. This resulting knowledge has been applied to the preparation of a jellying sugar beet pectin.

Materials and Methods

Preparation of Pectin Acetates.—A commercial citrus pectin (sample 444-P-42, 9.8% methoxyl; 1.03% ash, m. f. b.) was used throughout for the preparation of the acetates according to the procedure of Carson and Maclay.[14] The amount of acetyl in the resulting pectin was controlled by the amount of acetic anhydride used. Isolation of the pectin acetates was accomplished by pouring the reaction mixture into four volumes of alcoholic hydrochloric acid (47.9 ml. of concd. hydrochloric acid/1180 ml. of 95% comm. ethanol). The collected precipitates were washed with three portions of the alcoholic hydrochloric acid, washed free of chloride with 95% ethanol, and finally washed free of ethanol with acetone. The samples were then air dried, ground, dried overnight *in vacuo* at 60° and stored over calcium chloride. The analyses for these samples are given in Table I.

Viscosity Determinations.—For viscosity measurements, 0.6 g. of sodium chloride, 1.0 g. of sodium polymetaphosphate and the sample being investigated were dissolved in 75 ml. of distilled water. The *p*H of the solution was then adjusted to 6.0 with 0.1 *N* sodium hydroxide and the resulting solution, after dilution to 100.0 ml. was used for viscosity measurements in an Ostwald-Cannon-Fenske viscometer.[15] All measurements were made in a water-bath at 25 ± 0.05°. For each sample a plot of log η_{sp}/c *vs.* C was made and the antilog of intercept at $c = 0$ was taken as the intrinsic viscosity.

Preparation of Jellies.—Pectin-sucrose-acid jellies (65% solids) were prepared according to the method of Cox and Higby[16] except that a citric acid solution containing 600 g. of citric acid monohydrate per liter of solution was used instead of a solution of tartaric acid.

Testing of Jellies.—Jellies were tested within forty-eight hours after their preparation. The shear moduli were determined by the method of Owens, Porter and Maclay.[17] Breaking strengths were determined by measuring the force required to break the surface of the jelly with a plunger having an area of 3.14 sq. cm.

Acetyl Determinations.—The percentage of acetyl in the pectin samples was determined by a modification of Clark's method[18] and will be discussed in greater detail elsewhere. The sample was dispersed in and permitted to stand overnight at room temperature in *N*/8 aqueous sodium hydroxide (50 ml. alkali/g. of pectin). The solution was then diluted to 100.0 ml., a 20.0-ml. aliquot withdrawn and acidified with 20 ml. of Clark's sulfuric acid-magnesium sulfate solution, and the mixture steam distilled until 100 ml. of distillate was collected. The acetic acid in the distillate was determined by titration with 0.05 *N* sodium hydroxide using phenol red as the indicator.

Methoxyl Determinations.—Methoxyl analyses were performed by the Zeisel method as described by Shriner.[19]

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

(2) Presented before the Sugar Division, American Chemical Society, April 7, 1949, San Francisco, California.

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(18) E. P. Clark, "Semimicro Quantitative Organic Analysis," Academic Press, Inc., New York, N. Y., 1943, p. 73.

(19) R. L. Shriner, "Quantitative Analysis of Organic Compounds," Edwards Brothers, Inc., Ann Arbor, Michigan, 1940, p. 35.

Samples were humidified to remove alcohol before analysis.

Isolation of Pectin from Jellies Prepared from Sample No. PAc 7.—The jelly was dispersed by adjusting its *p*H to 7.0 by the addition of 0.5 *N* sodium hydroxide with vigorous stirring. The pectin was precipitated as described under preparation of the pectin acetates.

Acid Hydrolysis of Acetyl from Sample No. PAc 7.—Thirty-six grams (dry wt. basis) of sample No. PAc 7 was dispersed in somewhat less than 1800 ml. of distilled water. After the *p*H was adjusted to 1.0 with concd. hydrochloric acid, the mixture was made up to a total volume of 1800 ml. Completion of the adjustment of the *p*H was taken as the starting time of the hydrolysis. The mixture was then covered and permitted to stand at room temperature which averaged 23 ± 1°. At the intervals listed in Table III 300-ml. aliquots were withdrawn, and the pectin therein precipitated by pouring the aliquot into 900 ml. of 95% ethanol. In each case, the collected precipitate was washed free of chloride with 95% ethanol and then washed free of ethanol with acetone. After air drying overnight, the samples were ground and dried.

Preparation of Sugar Beet Pectin.—Sugar beets were cut into small pieces and dropped into boiling 95% ethanol to denature the pectic enzymes. The drained pulp was pressed and resuspended, with stirring, in 80% ethanol. The pulp was pressed and dried at room temperature. One kilogram of this air-dried pulp which was ground to pass a 1/8" screen, was soaked overnight in 30 liters of water. The *p*H of the mixture was then brought to 1.9 with concd. hydrochloric acid and the mixture was boiled for one hour. After adding 300 g. each of paper pulp and filter aid, the suspension was filtered through a screen and the residue pressed free of liquor on a hydraulic press. The resulting liquors were refiltered and the pectin in the filtrate was precipitated with two volumes of acetone. The pectin was pressed on muslin, redissolved in 10 liters of water, the *p*H of the solution adjusted to 4.5 by adding 0.5 *N* sodium hydroxide with vigorous stirring and the resulting solution filtered through filter paper and filter aid. To the filtered pectin solution there was added over a period of three hours, with stirring, 2 liters of 2.5% copper sulfate solution. The precipitated pectin was collected by centrifugation and suspended in and permitted to stand overnight in 6 liters of 0.5% copper sulfate solution. Next the copper in the pectin, which had been collected by centrifugation, was washed out by repeated washing with 95% ethanol which had been adjusted to *p*H 1 with concd. hydrochloric acid. Chloride ion was next washed out with 95% ethanol which was followed by acetone washes to remove the ethanol. After grinding and thorough air drying a yield of 50.9 g. of pectin was obtained. To effect a further separation of the pectin from accompanying solids the pectin was dissolved in 5 liters of water and the solution centrifuged for fifteen minutes. The pectin from the supernatant liquid was obtained by pouring the combined supernatant liquid into 10 liters of acetone. The yield of the ground, humidified sample, designated as S. B. P. No. 1, was 38.8 g.

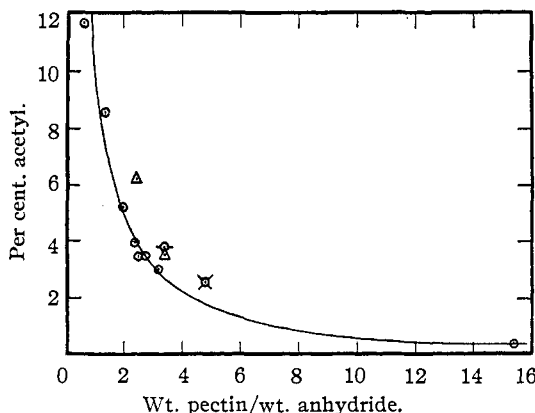

Fig. 1.—Influence of ratio of pectin to acetic anhydride to amount of acetyl introduced: ⊙ pectin 444-P-42, 9.8% MeO; ▵ pectin 804, 8.5% MeO; -o- pectin 1412-1, 8.2% MeO; ¤ pectin 1412-2, 7.2% MeO.

Acid Hydrolysis of Sugar Beet Pectin.—Eighteen grams (ash and moisture free basis) of S. B. P. No. 1 was dissolved in about 950 ml. of water. The *p*H of the solution was adjusted to 1.0 with concd. hydrochloric acid and the solution diluted to a total volume of 923 ml. Completion of the adjustment of the *p*H to 1 was taken as the starting time of the reaction. The mixture was then covered and permitted to stand at room temperature which averaged 25 ± 1°. At the intervals listed in Table IV aliquots were withdrawn and the pectin therein isolated and worked up as described under acid hydrolysis of acetyl from sample No. Pac-8. The data obtained for these samples are given in Table IV.

Results

Introduction of acetyl into pectin proceeded smoothly. The percentage of acetyl introduced into the pectin is a function of the ratio of pectin to acetic anhydride as shown in Fig. 1.

The results of a study of the jelling ability of partial acetates prepared from citrus pectin 444-P-42 are summarized in Table I. Attempts to prepare jellies from samples containing about 5% acetyl resulted in sirups which did not set in several weeks time. Samples containing 3.5 to 4% acetyl resulted in sirups which formed weak gels after standing from one to four weeks. Analysis of the pectin isolated from one of these jellies showed a decrease in acetyl content from 3.5 to 2.3%, a decrease in methoxyl from 9.7 to 9.6 and change in intrinsic viscosity from 3.3 to 2.9. Samples containing greater than 8.6% acetyl were so insoluble in the jelly medium that they were not studied further.

Table I

Description of Pectin No. 444-P-42 and Acetates Prepared from It

Sample no.	Acetyl, %	Methoxyl, %	[η] dl/g.	Jelly data: Pectin, %	Shear modulus, g./sq. cm.	Breaking strength, g./sq. cm.
444-P-42	0.3	9.8	3.9	..	...	..
Control pectin[a]	0.3	10.0	3.9	0.38	4.3	61.3
PAc-2	0.5	9.7	3.7	.38	4.4	47.7
PAc-3	3.5	9.5	3.6	.38	0	0[b]
PAc-7	3.5	9.7	3.3	.38	0	0[c]
PAc-8	4.0	9.6	3.5	.38	0	0
PAc-6	5.2	9.6	3.0	.38	0	0
PAc-6	5.2	9.6	3.0	.46	0	0
PAc-4	8.6	9.1	2.9	.38	...	..[d]

[a] This sample consists of a sample of pectin 444-P-42 which had been carried through the acetylating procedure but with omission of the acetylating agent. [b] One week after preparation gelation had occurred and the gel had a shear modulus of 1.2 g./sq. cm., breaking strength of 36.6 g./sq. cm. [c] After standing for twenty-seven days, the mixture set to a weak gel having a shear modulus of 0.5 g./sq. cm. [d] Precipitation of pectin occurred during preparation of the jelly.

The effect of degree of esterification of the carboxyl groups on the jelling properties of

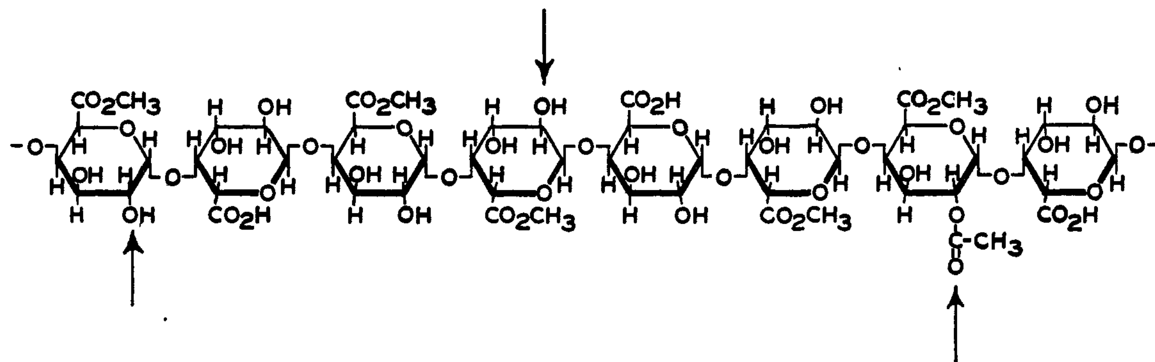

Fig. 2.—Hypothetical segment of pectin molecule corresponding to 9.4% methoxyl, 2.6% acetyl and 8% non-uronide. (The non-uronide is not shown in the drawing.)

pectin acetates was studied. The percentage of acetyl required to prevent jelly formation was little influenced by the degree of esterification of the uronic acid groups as shown in Table II.

TABLE II

DESCRIPTION OF LOWER METHOXYL PECTINS AND THEIR ACETATES

Sample no.	Acetyl, %	Methoxyl, %	[η], dl/g.	Jelly data: Pectin, %	Jelly data: Shear modulus, g./sq. cm.	Jelly data: Breaking strength, g./sq. cm.
1412-1	...	8.2	5.8	0.31	6.1	...
1412-2	...	7.2	4.6	0.31	5.4	121
PAc-11[a]	3.7	7.7	5.3	0.45, 0.38	0	0
PAc-13[b]	2.6	6.9	4.7	0.31	0	0

[a] Prepared from 1412-1. [b] Prepared from 1412-2.

The above results indicate that the presence of acetyl inhibits jelly formation, but more conclusive evidence was sought. For example, removal of acetyl groups should restore jellying ability. Deacetylation by means of barium methylate, citrus acetylesterase[20] and hydrolysis at *p*H 8 were unsuccessful either because of degradation of the galacturonide or of the methyl ester linkage. By means of acid hydrolysis, the acetyl groups were selectively removed from one of the pectin acetate samples with very little degradation. As shown in Table III, when the percentage acetyl dropped to 2.4 or less the jellying ability of the pectin was restored. From data obtained in this experiment a plot of the log of the concentration of unhydrolyzed acetyl *vs.* time was prepared. A straight line relationship was obtained and the specific rate constant for the hydrolysis of acetyl was calculated to be 8.9×10^{-5} min.$^{-1}$. The constant was the same throughout the course of the reaction assuming it to be first-order. The value for it is close to the initial values obtained by Henglein and Vollmert if an Arrhenius energy of activation of 19 kcal. is assumed for the reaction.

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TABLE III

DATA FOR PECTIN SAMPLES OBTAINED BY ACID HYDROLYSIS AT 23 ± 1°

Sample no.	Time of hydrolysis, hr.	Acetyl, %	Methoxyl, %	[η], dl/g.	Jelly data: Pectin (wt. basis) %	Jelly data: Shear modulus, g./sq. cm.	Jelly data: Breaking strength, g./sq. cm.
PAc-8	0	4.0	9.6	3.5	0.38	0	0
PAc-8-1	2	3.7	9.7	3.7	..	...	..
PAc-8-2	6	3.6	9.3	3.7	.38	0	0
PAc-8-3	78	2.4	9.0	3.9	.38	1.6	31.8
PAc-8-4	143.8	1.7	8.7	3.8	.38	3.5	75.7
PAc-8-5	334.8	0.6	7.7	3.7	..	...	..
PAc-8-6	409.3	0.4	7.3	3.6	..	...	..

Sugar beet pectin was studied to determine if removal of its acetyl groups would result in a jellying pectin. Acid hydrolysis was employed and the results, which are in Table IV, are similar to those reported in the previous table. They show

TABLE IV

DATA FOR PECTIN SAMPLES OBTAINED BY ACID HYDROLYSIS OF SUGAR BEET PECTIN AT 25 ± 1°

Sample no.	Time of hydrolysis, hr.	Acetyl, %	Methoxyl, %	Uronic anhydride, %	[η] dl/g.	Jelly data: Pectin, %	Jelly data: Shear modulus, g./sq. cm.
S. B. P. 1	0.0	4.5	6.3	62.8	2.5	0.91	0
S. B. P. 1 A1	47.8	3.3	6.0	64.2	2.5	.64	0
S. B. P. 1 A2	146.0	1.9	6.0	64.2	2.4	.75	0
S. B. P. 1 A3	382.9	0.6	5.5	69.0	2.5	.91	3.5

that despite the relatively low intrinsic viscosity and low uronic anhydride content, jellying sugar beet pectin is produced when the percentage acetyl drops below a value of one. In this experiment the specific rate constant for the hydrolysis of acetyl was calculated to be 8.7×10^{-5} min.$^{-1}$.

Discussion

The results of this work have an important bearing on the theory of mechanism of formation of pectin gels. Although discussion of this aspect of the work is beyond the scope of this paper, two points should be mentioned. First, the amount of

acetyl introduced which prevents gel formation represents only one acetyl group for about eight uronide groups. Second, this quantity seems practically independent of the methoxyl content between 7 and 10%. At the higher percentage of methoxyl one of about three free carboxyl groups could be hindered in hydrogen bond formation if the acetyl group should enter positions indicated by arrows in Fig. 2. At the lower percentage only one free carboxyl group out of about four could be hindered. The fact that this increase in available carboxyl groups is not reflected in the percentage of acetyl required to prevent gelation appears to deëmphasize the role of carboxyl to carboxyl bridges in formation of high solids pectin gels. Further discussion will require more data on pectin gels than presently available.

Acknowledgment.—The authors thank A. D. Shepherd and A. L. Smith for extraction of the sugar beet pectin and E. F. Jansen for the sample of acetylesterase.

Summary

A series of pectin acetates were prepared. When 2.6 or more per cent. acetyl is present in the molecule, the ability of the pectin to form high-solids gels is markedly reduced if not eliminated. Jellying ability was restored by partial acid hydrolysis of the pectin acetate.

Acid hydrolysis of sugar beet pectin produced a jellying pectin.

RECEIVED MAY 31, 1949

[CONTRIBUTION FROM THE GEORGE M. MOFFETT RESEARCH LABORATORIES, CORN PRODUCTS REFINING COMPANY]

The Action of Nitrogen Dioxide on Corn Starch and its Fractions

BY RALPH W. KERR

Since the early reports of Unruh and Kenyon[1] and of Yackel and Kenyon[2] showing the specific action of nitrogen dioxide on cellulose, some thought has been given to the possible use of the reaction in a study of structure in a related carbohydrate, starch. It is now generally believed that the common starches, such as corn, consist of a mixture of polymer types, one of which is linear, the α-glucose units being joined only by 1–4 glucoside linkages, and the other is a branched polymer, the branches being formed through 1–6 glucoside linkages. Although chemical data to support this viewpoint are convincing within the experimental limitations of the general methods which have been employed, such as exhaustive methylation and periodate oxidation, these methods applied to starch involve certain considerations which would make confirmation by an independent approach very desirable.

The work of Unruh and Kenyon would tend to show that when nitrogen dioxide acts on a polyglucoside such as cellulose, the action is primarily directed to the oxidation of the primary alcohol groups to carboxyls. These carboxyl groups may of course be readily determined by appropriate methods and distinguished from carbonyl groups formed in the primary phase of possible side reactions between the nitrogen dioxide and any hydroxyl group other than the primary alcohol group on carbons number six in the polyglucoside chain. Moreover, the work of Unruh and Kenyon would tend to show that the action of nitrogen dioxide on cellulose in forming uronic acid carboxyl groups could be extended, virtually to completion. Structurewise, amylose is thought to be quite similar to cellulose and should, accordingly, give a theoretical yield also of glucuronic acid groups. On the other hand, since amylopectin is thought to be branched through hydroxyl groups on carbons number 6, then for every branch in the structure there should be one less than the theoretical number of uronic acid carboxyl groups when oxidation with nitrogen dioxide is complete. Consequently, oxidation with nitrogen dioxide should provide a method to determine quantitatively the difference in structure between the two major fractions in starch.

In our early preliminary studies of the action of nitrogen dioxide on unfractionated, whole corn starch, using procedures essentially as described for obtaining a theoretical yield of polyglucuronide from cellulose, it was readily apparent that the results fell somewhat short of the theoretical yield. Mench and Degering[3] have already reported that when starch is treated in chloroform solution of nitrogen dioxide, the carboxyl content was somewhat less than that of an anhydropolyglucuronic acid chain. These results may be expected on the basis of analytical determinations which have shown[4] that the starch contains very nearly 70% of the branched type of polymer and only 30% of linear, or cellulose-like polymer. Therefore, qualitatively, at least, the method appeared to show promise and investigations were begun on the action of nitrogen dioxide on starch fractions. The following is a preliminary report of these studies.

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