α-1 : 4-*Glucosans. Part III.* The Molecular Structure of Brewer's Yeast Glycogen.*

By D. J. Manners and Khin Maung.

[Reprint Order No. 5858.]

Brewer's yeast (*Saccharomyces cerevisiae*) contains a glucosan which is stained reddish-brown by iodine, is degraded by salivary α-amylase, and has a molecular weight of $\sim 2 \times 10^6$. The polysaccharide has an average chain length of 13, a β-amylolysis limit of 44%, and a phosphorolysis limit of 23%. The molecular structure therefore resembles that of a typical glycogen. The properties of the glycogens from baker's yeast and brewer's yeast are compared.

Although the glycogen from baker's yeast has been studied several times (*e.g.*, Northcote, *Biochem. J.*, 1953, **53**, 348; Jeanloz, *Helv. Chim. Acta*, 1944, **27**, 1501), little attention has been paid to the corresponding polysaccharide from brewer's yeast. A sample of the latter, isolated by Daoud and Ling (*J. Soc. Chem. Ind.*, 1931, **50**, 365T), had $[\alpha]_D$ +179° in water and was stained brownish-red by iodine; Yokoyama (*Beitr. Physiol.*, 1925, **3**, 95) found $[\alpha]_D$ +192° in water. No structural examination appears to have been published. The present communication deals with an investigation of the molecular structure of brewer's yeast glycogen; this is being used as a substrate in studies on the carbohydrate-metabolising enzymes in this organism.

The glycogen was extracted from yeast cell-wall material (obtained by disruption of the whole yeast with hot dilute sodium hydroxide) with 0·5N acetic acid (Bell and Northcote, *J.*, 1950, 1944; Northcote and Horne, *Biochem. J.*, 1952, **51**, 232), and was purified by several precipitations with 80% acetic acid (cf. Bell and Young, *Biochem. J.*, 1934, **28**, 882) and with ethanol. The purified glycogen gave an opalescent solution in water ($[\alpha]_D$ +198°), which was stained reddish-brown by iodine. The iodine binding power of the glycogen was quantitatively determined by Mr. D. M. W. Anderson, using the potentiometric titration method described by Anderson and Greenwood (*Chem. and Ind.*, 1953, 642); it was similar to that of mammalian glycogens. Acid hydrolysis of the glycogen gave glucose (96%) and no other reducing sugar. The glycogen was readily attacked by salivary α-amylase, as shown by the rapid loss of iodine staining power, and the production of glucose, maltose, and α-dextrins; the glucosidic linkages in the glycogen are therefore predominantly of the α-1 : 4 type. Since maltulose was absent from the α-amylolytic digest, fructose is not a constituent of the glycogen (cf. Peat, Roberts, and Whelan, *Biochem. J.*, 1952, **51**, xvii). Oxidation of the glycogen by potassium periodate (cf. Halsall, Hirst, and Jones, *J.*, 1947, 1399; Bell and Manners, *J.*, 1952, 3641) and determination of the maximum amount of formic acid produced indicated a unit-chain length of thirteen glucose residues. In an attempt to detect 1 : 2 or 1 : 3 linkages, periodate-oxidised glycogen was hydrolysed with acid, and the hydrolysate analysed for glucose (cf. Hirst, Jones, and Roudier, *J.*, 1948, 1779; Bell and Manners, *J.*, 1954, 1891). Paper chromatography showed that glucose was absent; the glycogen must therefore contain only α-1 : 4 and 1 : 6 glucosidic linkages. Treatment of the glycogen with β-amylase gave 44% conversion into maltose, indicating that the exterior chains comprise 8 glucose residues; the interior chains, on the average, thus contain 4 glucose residues. Treatment of the glycogen with muscle phosphorylase in the presence of excess of inorganic phosphate resulted in a 23% conversion into glucose 1-phosphate; 12-unit and 13-unit glycogens from various animal tissues have phosphorolysis limits of 20—25% (Liddle and Manners, unpublished). Examination of the glycogen in an ultracentrifuge, by Dr. C. T. Greenwood, showed it to be multimolecular, the sedimentation constant (S_{20}) being 52×10^{-13} c.g.s. units [equivalent to a molecular weight of *ca.* 2×10^6, the diffusion constant being assumed to be of the same order as that of other glycogens (cf. Bell, Gutfreund, Cecil, and Ogston,

* Part II, *J.*, 1954, 3527.

Biochem. J., 1948, **42**, 405)]. An estimate of the shape of the molecule, and the degree of multiple branching in the molecule will be described in a later communication.

The available data indicate that brewer's yeast glycogen consists of *ca.* 10^3 unit-chains, each comprising, on the average, 13 α-1 : 4-linked glucose residues, and randomly interlinked by 1 : 6-glucosidic linkages. The molecular structure thus resembles that of the majority of the glycogens from mammalian, invertebrate, and protozoan tissues examined in our previous studies (Bell and Manners, *loc. cit.*; Manners and Ryley, *Biochem. J.*, 1952, **52**, 480); nevertheless, small but significant differences in degree and position of branching between the baker's yeast and the brewer's yeast glycogen are revealed (see Table).

A comparison of the properties of yeast glycogens with rabbit-liver glycogen.

Source :	Baker's yeast		Brewer's yeast	Rabbit-liver
	A	*B*		*C*
$[\alpha]_D$ in water	+184°	+187°	+198°	+196°
Unit-chain length	11—12	—	13	12—13
β-Amylolysis limit	50	46—48·5	44	43
Exterior chain length *	8	—	8	8
Interior chain length †	2—3	—	4	3—4

A, Data from Northcote (*loc. cit.*). *B*, Data from Jeanloz (*loc. cit.*). *C*, Data from Bell and Manners (*loc. cit.*).

* No. of glucose units removed on β-amylolysis plus 2·5. † Unit-chain length — Exterior chain length — 1.

In our previous papers (Bell and Manners, *loc. cit.*; Manners and Ryley, *loc. cit.*) exterior chain lengths were calculated on the assumption that the exterior " stubs " of β-limit dextrins contained 1·5 glucose residues (cf. Meyer, *Adv. Enzymology*, 1943, **3**, 109); Peat, Whelan, and Thomas (*J.*, 1952, 4546), however, have shown that a proportion of the " stubs " in a β-dextrin from waxy-maize starch contain two or three glucose residues. A mean of these latter figures has been used in the present calculation.

In view of the different conditions of growth and fermenting properties of baker's and brewer's yeast, and hence, enzymatic composition, it is not unexpected that the glycogens show small differences in molecular structure; the unit-chain length and the position of branching in the chains depend upon the " balance " between the activities of phosphorylase and the branching and debranching enzymes, *i.e.*, on the metabolic condition of the organism at the time of isolation of the glycogen.

Experimental

Analytical Methods.—(a) *Determination of reducing sugar.* Reducing sugars were determined by use of the Shaffer–Somogyi reagent **60** (*J. Biol. Chem.*, **1933**, **100**, 695) as modified by Hanes and Cattle (*Proc. Roy. Soc.*, **1938**, *B*, **125**, 387) or by the Somogyi reagent (*J. Biol. Chem.*, **1945**, **160**, 61) which had been calibrated against glucose and maltose.

(b) *Paper chromatography.* Descending chromatograms were carried out at room temperature with Whatman No. 1 paper and benzene–pyridine–butanol–water (1 : 3 : 5 : 3) as solvent. Development was by spraying with aniline oxalate or with a silver nitrate–sodium hydroxide reagent (Trevelyan, Procter, and Harrison, *Nature*, **1950**, **166**, 444).

(c) *Iodine stain.* Polysaccharide solution (2 ml.) was added to iodine solution (1 ml.; containing 1 mg. of iodine and 10 mg. of potassium iodide per ml.) and water (2 ml.), and the absorption value of the polysaccharide–iodine complex measured on a Spekker Photoelectric Absorptiometer (1 cm. cells), an Ilford filter No. 603 being used, against an iodine blank.

(d) *Determination of glucose 1-phosphate.* Glucose 1-phosphate was determined by a slight modification of Allen's method (*Biochem. J.*, **1940**, **34**, 858).

Preparation of Glycogen.—A dispersion of washed brewer's yeast (1·5 kg.) in 3% sodium hydroxide (1 l.) was heated at 95° for 6 hr. (cf. Northcote and Horne, *loc. cit.*). After the mixture had cooled, the cell-wall material was collected on the centrifuge and treated again with hot 3% sodium hydroxide. (The sodium hydroxide extracts did not contain any appreciable amount of glycogen.) The cell-wall material was extracted by three successive treatments with 0·5N-acetic acid (each 500 ml.) at 75° for 2 hr. The combined acetic acid extracts were concentrated under reduced pressure to about 500 ml., and ethanol (6 vols.) was added. The

crude precipitate of glycogen was purified by three precipitations from 80% acetic acid (Bell and Young, *loc. cit.*) and finally from ethanol. The yield was 17·7 g.

Properties of the Glycogen.—The glycogen had $[\alpha]_D^{18}$ +198° (*c*, 0·25 in H_2O); +175° (*ca.* 0·50 in N-NaOH) (Found: N, 0·05%; P, nil; Ash, 0·10%). An aqueous solution was opalescent, and was stained red-brown with iodine. Hydrolysis by 1·5N-sulphuric acid at 100° for 2 hr. gave glucose and no other sugar (paper chromatography). The glycogen had a glucose content of 96%, determined by quantitative acid hydrolysis (Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224).

Salivary α-Amylolysis of the Glycogen.—Salivary α-amylase solution was prepared by dissolving freeze-dried human saliva in distilled water, and removing insoluble material by centrifugation. The amylase solution showed no maltase activity, but was contaminated with maltotriase. During the digestion of waxy-maize starch, maltose, maltotriose, and α-dextrins were the initial products of the reaction, and glucose, maltose, and α-dextrins the end-products (cf. Whelan and Roberts, *Nature*, 1952, **170**, 748; *J.*, 1953, 1298).

An enzymic digest was set up containing glycogen (50·0 mg.), phosphate–citrate buffer (0·16M with respect to phosphate) of pH 7·0 (20 ml.), sodium chloride (25·0 mg.), salivary amylase solution (1 ml.), and water (29 ml.). Aliquot portions (2 ml.) were analysed at intervals (Shaffer–Somogyi reagent 60), after incubation at 35°.

The decrease in iodine-staining power was as follows:

Time of incubation (min.)	0	4	10	20
Absorption value	0·305	0·035	0·020	0·010

The apparent percentage conversion into maltose after 1, 2, and 48 hours' incubation was 62, 68, and 95, respectively.

Paper chromatography showed the presence of glucose ($R_G = 1$), maltose ($R_G = 0·55$), and a series of sugars of higher molecular weight ($R_G < 0·09$) in the digest. Maltulose was absent.

Potassium Periodate Oxidation of the Glycogen.—Glycogen (549·0 mg.) was dissolved in 5% potassium chloride solution (100 ml.); 10 ml. were withdrawn for a control determination. 8% (w/v) sodium metaperiodate (20 ml.) was added to the bulk, from which 10-ml. portions were withdrawn at intervals for determination of formic acid by titration in a carbon dioxide-free atmosphere against 0·01N-sodium hydroxide, methyl-red being used as indicator (cf. Halsall, Hirst, and Jones, *loc. cit.*; Bell and Manners, *loc. cit.*). The following results were obtained.

Time (hr.)	96	168	266	386
Total formic acid produced (mg.)	8·9	10·2	10·6	10·5
Apparent chain length * (glucose residues) ...	15·6	13·6	13·2	13·2

* Calculated from the weight of glycogen oxidised.

A 12-unit or 14-unit glycogen would yield 11·6 or 10·0 mg. of formic acid, respectively.

The remaining solution of periodate-oxidised glycogen was neutralised with ethylene glycol (5 ml.) and dialysed against running tap water for 36 hr., and the non-diffusible material collected by freeze-drying. 50 mg. of this were hydrolysed by 2N-sulphuric acid (2 ml.) at 100° for 3 hr. No glucose could be detected in the hydrolysate by paper chromatography; brewer's yeast glycogen does not therefore contain 1 : 2- or 1 : 3-glucosidic linkages.

β-Amylolysis of the Glycogen.—β-Amylase was prepared from soya beans by Bourne, Macey, and Peat's method (*J.*, 1945, 882). A solution of β-amylase was prepared by dissolving soya bean β-amylase (50 mg.) in 0·2M-acetate buffer (pH 4·6; 20 ml.), and removing insoluble material by centrifugation; the supernatant liquid had an activity of 125 units/ml., estimated by Hobson, Whelan, and Peat's method (*J.*, 1950, 3566). Control experiments with maltose and starch showed it to be free from maltase and α-amylase. Glycogen (48·4 mg.) was incubated with 0·2M-acetate buffer (pH 4·6; 6 ml.), water (21 ml.), and β-amylase solution (3 ml.; 375 units) at 37°. Samples (3 ml.) were withdrawn at intervals and analysed for maltose. The course of degradation was as follows:

Time of incubation (hr.)	1	2	20	44
% Conversion into maltose	30·7	38·4	43·5	44·0

In a duplicate experiment with 50·4 mg. of glycogen, the β-amylolysis limit was 43·8%.

Phosphorolysis of the Glycogen [with A. MARGARET LIDDLE].—Glycogen (52·4 mg.) was incubated at 35° with 0·5M-phosphate buffer (pH 6·8; 2·0 ml.), adenylic acid (1 mg.), muscle

phosphorylase solution (0·20 ml.), and water to a total volume of 10 ml. Crystalline rabbit-muscle phosphorylase was prepared by Green and Cori's method (*J. Biol. Chem.*, **1943**, **151**, 21).

Time of incubation (hr.)	5	24	48
% Conversion into glucose 1-phosphate	20·0	23·2	23·2

In a duplicate experiment with 53·4 mg. of glycogen, the phosphorolysis limit was 22·8%.

The authors are indebted to Professor E. L. Hirst, F.R.S., for his interest and advice, to Dr. C. T. Greenwood for determination of the sedimentation constant of the glycogen, and to Mr. D. M. W. Anderson for the potentiometric titration. They also thank the Government of the Union of Burma for the award of a State Scholarship (to K. M.) and Messrs. Wm. Younger and Company Ltd. for supplies of yeast.

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF EDINBURGH. [*Received, November 8th*, 1954.]