α-1 : 4-*Glucosans. Part II.* The Molecular Structure of the Liver Glycogen from a Case of von Gierke's Disease.*

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The liver glycogen isolated from a case of von Gierke's disease has a chain length of 6 glucose residues, a molecular weight of $\sim 10^6$, and a β-amylolysis limit of 14%. Its $[\alpha]_D$ is +201° and it gives no colour with iodine. The glycogen is degraded by salivary α-amylase and is not attacked by muscle phosphorylase. The molecular structure resembles that of a phosphorylase limit-dextrin of a normal glycogen, but differs in having shorter exterior chains.

VON GIERKE'S or glycogen-storage disease is a metabolic disorder which is accompanied by the deposition of unusually large amounts of glycogen in the liver, skeletal muscle, and other tissues. Illingworth and Cori (*J. Biol. Chem.*, 1952, **199**, 653) isolated glycogen from the livers of ten cases of von Gierke's disease. Structural studies (by enzymic methods; cf. Cori and Larner, *ibid.*, 1951, **188**, 17; Larner, Illingworth, Cori, and Cori, *ibid.*, 1952, **199**, 641) showed that eight of these glycogens had structures similar to those of other mammalian glycogens, *viz.*, unit chains of 11—13 glucose residues, phosphorolysis limits of 33—42%, and absorption maxima of the iodine complexes at 470 mμ. In the ninth case, glycogen was obtained from both liver and muscle tissues which had shorter unit chains and was only slightly degraded by muscle phosphorylase (see Table 1). The tenth glycogen superficially resembled an amylopectin since the unit chains comprised 21 glucose residues, and were degraded 51% by phosphorylase, whilst the "glycogen"–iodine complex had an absorption maximum at 530 mμ. No estimate of the degree of multiple branching in these glycogens was recorded (cf. Hirst and Manners, *Chem. and Ind.*, 1954, 224). Polglase, Smith, and Tyler (*J. Biol. Chem.*, 1952, **199**, 97) examined glycogen obtained from two cases of von Gierke's desease: in one case the liver glycogen had a normal chain length; in the second the unit chains of the liver glycogen were *ca.* 25% shorter, whilst the muscle glycogen appeared to be normal. Sedimentation studies were also carried out on these glycogens (Polglase, Brown, and Smith, *ibid.*, p. 105; see Table 2). The present paper is concerned with the molecular structure of a von Gierke liver glycogen which was found to be different from those described above.

The liver, obtained at autopsy, was kindly placed at our disposal by Professor H. A. Krebs, F.R.S. Glycogen was isolated from the tissue by repeated extraction with hot water; the aqueous extracts were freed from protein by 4% trichloroacetic acid, and the glycogen was precipitated with ethanol and further purified by precipitation with 80% acetic acid (Bell and Young, *Biochem. J.*, 1934, **28**, 882). The purified glycogen dissolved in water ($[\alpha]_D$ +201°) to give an opalescent solution which, unlike that of other glycogens, was not stained brown with iodine. Quantitative measurements of the iodine-binding power by potentiometric titration (Anderson and Greenwood, *Chem. and Ind.*, 1953, 642) showed a much lower binding power than that of normal glycogens (Greenwood and Manners, unpublished work). Acid hydrolysis of the glycogen gave glucose and no other reducing sugar. Salivary amylolysis of the glycogen caused rapid breakdown to reducing sugars (maltose and higher oligosaccharides), showing the presence of α-1 : 4-glucosidic linkages. The absence of maltulose in the α-amylolytic digest indicates that fructose is absent in the original polysaccharide (cf. Peat, Roberts, and Whelan, *Biochem. J.*, 1952, **51**, xvii). End-group assay by potassium periodate (Bell and Manners, *J.*, 1952, 3641; Halsall, Hirst, and Jones, *J.*, 1947, 1399) showed that the unit chains contain, on the average, 6 glucose residues. This low value was not due to impurities of low molecular weight or to degradation products since the chain length was unaltered after prolonged dialysis of the glycogen. In an attempt to detect the presence of glucosidic linkages

* Part I, *J.*, 1954, 1891.

other than 1 : 4 or 1 : 6, a sample of periodate-oxidised glycogen was hydrolysed with acid, and the hydrolysate examined for glucose (cf. Hirst, Jones, and Roudier, *J.*, 1948, 1779; Part I, *loc. cit.*). No glucose could be detected (by paper chromatography) in the hydrolysate; 1 : 2- and 1 : 3-glucosidic linkages are therefore not present in the von Gierke glycogen. Treatment of the glycogen with a solution of crystalline sweet potato β-amylase resulted in a 14% conversion into maltose. The glycogen was not appreciably attacked by muscle phosphorylase in the presence of an excess of inorganic phosphate. Ultra-centrifugal examination of the glycogen, by Dr. C. T. Greenwood, showed that the glycogen was polydisperse and contained two components; the major component sedimented at 48 *S* * and the minor component at 200 *S* [equivalent to molecular weights of *ca.* 2 and 9 × 10^6 respectively, on the assumption that the diffusion constant is similar to that of rabbit-liver glycogen (see Bell, Gutfreund, Cecil, and Ogston, *Biochem. J.*, 1948, **42**, 405)]. The von Gierke glycogen is therefore a highly branched α-1 : 4-glucosan, composed of *ca.* 10^3 unit chains—each comprising, on the average, 6 glucose residues—which are interlinked only by 1 : 6-glucosidic linkages.

These results suggest that the von Gierke glycogen *qualitatively* resembles the phosphorylase limit-dextrin of a normal glycogen. Such a dextrin would have short unit chains and a low β-amylolysis limit [that of a normal glycogen is *ca.* 45% (Bell and Manners, *loc. cit.*)], and be resistant to attack by muscle phosphorylase. However, from a consideration of the action pattern of β-amylase and muscle phosphorylase, it will be shown that the von Gierke glycogen is not *identical* with a normal phosphorylase limit-dextrin.

Branched α-1 : 4-glucosans contain three types of unit chain; all three are linear chains of α-1 : 4-linked glucose residues, but they differ in the position of the linkage(s) to adjacent chains (Peat, Whelan, and Thomas, *J.*, 1952, 4546), *viz.*: A-chain, joined to the rest of the molecule only by a single glucosidic linkage from the reducing group; B-chain, joined to the molecule by its reducing group and one or more linkages involving $C_{(6)}$ of constituent glucose residues; C-chain, the sole chain in the molecule which is terminated by a free reducing group. An A-chain corresponds to a "side-chain," and a B-chain to a "main-chain" in the terminology of Cori and Larner (*loc. cit.*). Muscle phosphorylase has a different affinity for A- and B-chains; Cori and Larner (*loc. cit.*) have shown that in a phosphorylase limit-dextrin, the A-chain contains only one glucose residue whilst the exterior portion of the B-chain probably comprises 5 or 6 glucose residues (assuming that the dextrin contains equal numbers of A- and B-chains). This dextrin has a β-amylolysis limit of 24% (Hestrin, *J. Biol. Chem.*, 1949, **179**, 943), equivalent to the loss of one maltose residue per unit chain, enzyme action being limited to the B-chains from which two maltose residues are removed. The von Gierke glycogen thus differs from a phosphorylase limit-dextrin, since the β-amylolysis limit is only 14%. This figure represents the loss of *ca.* one glucose residue per chain, equivalent to the loss of one maltose residue from only half of the chains. The average exterior chain length of the von Gierke glycogen is therefore approximately half that of a normal phosphorylase limit-dextrin, *i.e.*, two glucose units. The interior chains of the von Gierke glycogen thus comprise three glucose residues. The low β-amylolysis limit obtained in the present study suggests either that the glycogen contains a relatively small proportion of B-chains (*i.e.*, a high degree of multiple branching) or that, *in vivo*, muscle phosphorylase has a higher affinity for the glucosidic linkages in B-chains than *in vitro*. The former possibility is now being investigated.

The occurrence of a glycogen with short unit chains of *ca.* 6 glucose residues is unusual, but not unique. Certain samples of glycogen from *Mytilus edulis* and *Helix pomatia* have chain lengths of *ca.* 5 and 7 glucose residues, respectively (Bell and Manners, *loc. cit.*), whilst Illingworth, Larner, and Cori (*J. Biol. Chem.*, 1952, **199**, 631) reported that a specimen of tubercle bacillus glycogen had an apparent end-group value of 16% (equivalent to a chain length of *ca.* 6). The last glycogen was not attacked by phosphorylase, and appears to be a phosphorylase limit-dextrin, unlike the short-chain invertebrate glycogens

* Sedimentation constants (S_{20}) are given in Svedberg units where $S = 1 \times 10^{-13}$ c.g.s. units.

which are partially degraded by phosphorylase and have β-amylolysis limits of 41 and 37% respectively. These invertebrate glycogens therefore differ from the von Gierke glycogen in having longer exterior chains and shorter interior chains, even though the constituent unit chains, on the average, are of approximately similar lengths.

The present sample of von Gierke glycogen differs from those examined by Illingworth and Cori and by Polglase *et al.* (*locc. cit.*) as shown in Tables 1 and 2.

TABLE 1. *Action of muscle phosphorylase on von Gierke glycogens.*

Sample	Source	Chain length	Conversion into glucose-1 phosphate (%)
Illingworth and Cori (*loc. cit.*)			
Case no. 1	Liver	12—13	36
Case no. 9	Liver	9	12
	Muscle	7—8	3
Case no. 10	Liver	21	51
Present study	Liver	6	1

When a glycogen is found to have a structure different from that in the " normal organism " one must postulate enzymic imbalance, *i.e.*, an abnormally greater or smaller activity in one or more of the several enzymes concerned in the equilibrium, glycogen ⇌ glucose. The above data suggest that there are different types of glycogen storage disease. Cori and Cori (*ibid.*, p. 661) observed that, in two fatal cases of the disease,

TABLE 2. *Sedimentation constants of von Gierke glycogens.*

Sample	Source	Sedimentation constant (S_{20}) of major component	Sedimentation constant (S_{20}) of minor component
Polglase, Brown, and Smith (*loc. cit.*)	Liver (patient N.D.)	72—74	220
	Liver (patient P.N.)	75	—
	Skeletal muscle (N.D.)	59—67	29—36
	Cardiac muscle (N.D.)	75	38
Present study	Liver	48	200

glucose-6 phosphatase was " practically absent " from the liver tissue, whilst the formation of the amylopectin-like glycogen may have been due to a deficiency in branching enzyme. Although in the present study no biopsy samples were available for estimation of enzymic activity, it seems probable that the abnormal structure was caused, in part, by a deficiency in the debranching enzyme, amylo-1 : 6-glucosidase, thereby preventing complete phosphorolysis of the glycogen.

EXPERIMENTAL

Isolation of Glycogen.—Liver tissue (500 g.; from a 12-year-old female) which had been boiled in water (500 ml.) and stored under toluene (10 ml.) and octyl alcohol (3 ml.) was received from Professor H. A. Krebs. The solid material was strained off, ground with sand, and extracted four times with boiling water (600 ml.). To the combined, cooled, aqueous extracts and washings (3350 ml.), 40% trichloroacetic acid (380 ml.) was added and the mixture stored at 0° for 12 hr. The precipitated protein was removed by filtration through kieselguhr, and 2 vols. of ethanol were added to the filtrate. The precipitated glycogen was further purified by two precipitations from 80% acetic acid (Bell and Young, *loc. cit.*) and six precipitations from ethanol, and then dried *in vacuo* over phosphoric oxide for 4 hr. at 100°. The yield was 5·2 g.

Properties of the Glycogen.—The glycogen had $[\alpha]_D^{18}$ +201° (*c*, 0·68 in H_2O) (Found: N, 0·026%). Hydrolysis with 1·5N-sulphuric acid at 100° for 2 hr. gave glucose and no other sugar (paper chromatography).

Salivary α-Amylolysis of the Glycogen.—Glycogen (169·8 mg.) was incubated with 0·2M-phosphate buffer (pH 7·0; 10 ml.), 3% aqueous sodium chloride (2 ml.), distilled water (30 ml.), and salivary amylase solution (2 ml.) at 37°. Aliquot portions were removed at intervals for examination on a paper chromatogram and for determination of reducing power (as maltose)

by means of the Shaffer–Somogyi reagent (cf. Bell and Manners, *loc. cit.*). The course of hydrolysis was as follows :

Time of incubation (hr.)	0·08	0·33	0·67	2·50	4·00	6·00	24·00
Apparent conversion into maltose (%)	7·2	13·4	17·8	21·7	23·1	24·6	31·0

No further increase in reducing power was observed after 24 hr. The paper chromatograms showed the presence of maltose and higher maltosaccharides. Glucose and maltulose were absent.

Periodate Oxidation of the Glycogen.—Glycogen (235 mg.) was oxidised by a saturated aqueous solution of potassium periodate, as previously described (Bell and Manners, *loc. cit.*) :

Time (hr.)	168	265	432	576
Formic acid production (mg.)	8·2	9·8	10·6	10·9

The maximum formic acid titre is equivalent to a chain length of six glucose residues.

Glycogen (3·0 g.) was dialysed against successive changes of distilled water at room temperature for one week, and then isolated by freeze-drying. The dialysate was concentrated *in vacuo* at 45° ; no reducing carbohydrates were present (aniline oxalate spray on paper ; heating for 5 min. at 110°).

A sample of the dialysed glycogen (165 mg.) was oxidised with potassium periodate solution as above. The maximum yield of formic acid (obtained after *ca.* 400 hr.) was 7·8 mg., again equivalent to a chain length of six glucose residues.

The remaining solutions of periodate-oxidised glycogen from the assays were combined, dialysed, and freeze-dried. Periodate-oxidised glycogen (64 mg.) was hydrolysed with 2N-sulphuric acid (15 ml.) at 100° for 2 hr. The hydrolysate was cooled, neutralised to pH 5·0 (barium hydroxide) and, after removal of barium sulphate, was freeze-dried. This material was suspended in distilled water (1 ml.) and examined on a paper chromatogram No glucose was present, indicating the absence of 1 : 2- and 1 : 3-linkages in the original glycogen.

β-*Amylolysis of the Glycogen.*—Glycogen (14·0 mg.) was incubated with 0·2M-acetate buffer (pH 4·64; 5 ml.), distilled water (10 ml.), and crystalline sweet-potato β-amylase solution (1 ml.). The course of hydrolysis was :

Time of incubation (hr.)	2	24	48
Conversion into maltose (%)	7·7	14·1	14·3

Phosphorolysis of the Glycogen [with A. M. Liddle].—Glycogen (34·3 mg.) was incubated at 35° with 0·4M-phosphate buffer (pH 6·8; 2·5 ml.), adenylic acid (0·5 mg.), phosphorylase solution (0·25 ml.), and distilled water to a total volume of 10 ml. The phosphorylase was prepared in crystalline form from rabbit muscle by Green and Cori's method (*J. Biol. Chem.*, 1943, **151**, 21). Samples (2 ml.) were withdrawn at intervals for estimation of glucose-1 phosphate, a slight modification of Allen's method being used (*Biochem. J.*, 1940, **34**, 858). Enzyme action gave *ca.* 1% conversion into glucose-1 phosphate after 45 hr. Under these conditions, 13-unit fœtal sheep-liver glycogen was degraded as follows :

Time (hr.)	1·3	4·0	25·5	49·5
Conversion into glucose-1 phosphate (%)	19	21	23	23

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