885. α-1,4-*Glucosans. Part XIV.*[1] *The Interaction of Concanavalin-A with Glycogens.*

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Forty-seven samples of glycogen, amylopectin, and related polysaccharides have been treated with concanavalin-A. An approximately linear relation was found between the degree of branching of a glycogen-type polysaccharide and the resultant turbidity.

Amylopectin and amylopectin β-dextrin gave no reaction with concanavalin-A, indicating that the internal branching characteristics of the polysaccharide may be a controlling factor in the interaction.

The relation between the molecular structures of Floridean starches and *Zea mays* polysaccharides and their interaction with concanavalin-A is discussed.

THE precipitation of glycogen by concanavalin-A, a globulin from jack-bean meal, was first described by Sumner and Howell.[2] Later studies by F. Smith and his co-workers [3] showed that glycogens from a variety of biological sources gave a precipitin reaction, but that the "glycogen value" (G.V.; defined as the optical density of a glycogen–concanavalin-A solution relative to that obtained with the protein and a standard sample of purified rabbit-liver glycogen which by definition is 1·00) was not constant, the results ranging from ox-liver glycogen G.V. 0·95 to baker's yeast glycogen G.V. 2·85. By contrast, amylose, amylopectin, laminarin, and dextran failed to react. β-Amylolysis of a glycogen caused an increase in the glycogen value and it was suggested [3] that concanavalin-A interacted with intact interior chains in the polysaccharide. Many of these results were obtained with glycogens of unreported average chain length ($\overline{CL}$). With the availability from our previous studies [4–7] of a large number of glycogen-type polysaccharides of known $\overline{CL}$, it was of interest to investigate the relation between glycogen value and degree of branching.

The polysaccharides listed in the Table were treated with concanavalin-A, under the conditions described by Smith and his co-workers,[3] and the optical densities at 420 mμ are recorded. Results obtained in a previous study [5] are also included. The majority of glycogens had glycogen values in the range 0·8—1·3, and an approximate relation between the degree of branching and the G.V. was observed (Fig. 1). Several samples of mammalian muscle glycogen [5] with $\overline{CL}$ *ca.* 17 had a lower glycogen value than any reported by

[1] Part XIII, Manners and Wright, *J.*, 1962, 1597.
[2] Sumner and Howell, *J. Biol. Chem.*, 1936, **115**, 583.
[3] Cifonelli, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1956, **78**, 2485.
[4] Liddle and Manners, *J.*, 1957, 3432.
[5] Lawrie, Manners, and Wright, *Biochem. J.*, 1959, **73**, 485.
[6] Archibald, Fleming, Liddle, Manners, Mercer, and Wright, *J.*, 1961, 1183.
[7] Manners and Wright, *J.*, 1961, 2681.

The " glycogen values " (G.V.) of some glycogen and starch-type polysaccharides.

Polysaccharide	G.V.	$\overline{CL}$ *	$\overline{ECL}$ †	$\overline{ICL}$ †	Ref.†
Glycogens					
Rabbit liver	1·00	12	—	—	3
Horse psoas (post-rigor)	0·76	17	10	6	5
Ox sternocephalicus (pre-rigor)	0·78	19	12	6	5
Horse diaphragm (pre-rigor)	0·81	17	11—12	4—5	5
Ram liver	0·82	14	9	4	*a*
Horse l. dorsi (post-rigor)	0·82	17	10	6	5
Horse heart (post-rigor)	0·83	16—17	10—11	5	5
Horse diaphragm (post-rigor)	0·85	17	11	5	5
Ox psoas (pre-rigor)	0·86	16—17	11	4—5	5
Horse l. dorsi (pre-rigor)	0·87	17	11—12	4—5	5
Pig liver	0·87	15	10	4	*a*
Ox sternocephalicus (post-rigor)	0·89	15	9	5	5
Rabbit liver VII	0·97	14	9—10	3—4	7
Glycogen isodextrin	1·07	12	8—9	2—3	*b*
Trichomonas foetus	1·10 ‡	15	11—12	2—3	6
Fœtal pig liver	1·13	11	8	2	6
Brewer's yeast	1·14	13	8	4	6
Rabbit liver IX	1·16	13	9	3	6
Tetrahymena pyriformis	1·19	13	8—9	3—4	6
Helix pomatia	1·28	7	4	2	6
Baker's yeast	1·36	12	8—9	2—3	*c*
Glycogen β-dextrins					
Rabbit liver L.D.1	0·99	9	5	3	*d*
Rabbit muscle III	1·14	7	2—3	3—4	*a*
Rabbit liver VII	1·38	7	2—3	3—4	*a*
Fœtal sheep liver	1·39	6—7	2—3	3	*a*
Ram liver	1·41	7—8	2—3	4	*a*
Mytilus edulis XI	1·62	6—7	2—3	3	*a*
Oyster	1·76	6	2—3	2—3	*a*
Other polysaccharides					
Glycogen α-dextrin (L.D.2)	0·0	—	—	—	*d*
Phytoglycogen B	0·96	7	3	3	*e*
Fraction 55—60	0·78	10	—	—	*e*
60—65	0·75	11	—	—	*e*
65—70	0·74	10	—	—	*e*
Floridean starch I	0·08	10	7	2	*a*, *f*
II	1·40	12	—	—	*f*
IV	0·17	15	9	5	*g*
Starch-type polysaccharides					
Soluble starch	0·0	—	—	—	—
Nitella translucens	0·0	19	13—14	4—5	*h*
Caulerpa filiformis	0·0	24	16	7	*i*
Potato amylose	0·0	2,700	—	—	*a*
Potato amylopectin	0·0	22	16	5	6
Potato amylopectin β-dextrin	0·0	9	2—3	5—6	*a*
Rice amylopectin	0·0	21	15	5	*j*
Maize amylopectin	0·0	19	13	5	*a*
Waxy maize starch IV	0·0	22	15	6	6
Dunaliella bioculata amylopectin	0·0	15—16	12	2—3	*k*
" Synthetic " amylopectin	0·0	—	—	—	*l*

* Average chain length, determined by periodate oxidation.

† $\overline{ECL}$, exterior chain length, *i.e.*, no. of glucose residues removed by β-amylase + 2·5; $\overline{ICL}$, interior chain length, *i.e.*, $\overline{CL} - \overline{ECL} - 1$. Letters refer to the following results: *a*, Kjølberg, Manners, and Wright, unpublished work; *b*, Gunja, Manners, and Khin Maung, *Biochem. J.*, 1961, **81**, 392; *c*, Northcote, *Biochem. J.*, 1953, **53**, 348; *d*, Bell and Manners, *Biochem. J.*, 1951, **49**, lxxvii; *e*, Peat, Whelan, and Turvey, *J.*, 1956, 2317; *f*, Fleming, Hirst, and Manners, *J.*, 1956, 2831; *g*, sample supplied by Dr. J. R. Turvey; *h*, Anderson and King, *J.*, 1961, 2914; *i*, Mackie and Percival, *J.*, 1960, 2381; *j*, Mercer, unpublished work; *k*, Eddy, Fleming, and Manners, *J.*, 1958, 2827; *l*, this polysaccharide was synthesized by the action of yeast branching enzyme on potato amylose; see Gunja, Manners, and Khin Maung, *Biochem. J.*, 1960, **75**, 441.

‡ Professor F. Smith (personal communication) reports G.V. 1·04 for this glycogen.

Smith and his co-workers, although the latter have apparently not encountered glycogens with $\overline{CL}$ values outside the range of 10—14 glucose residues.[8] The highest glycogen value (1·76) was given by oyster-glycogen β-limit dextrin, whereas Smith and his co-workers reported values of 2·25 and 4·5 for the β-limit dextrins of rabbit hair and baker's yeast glycogen. A sample of baker's yeast glycogen, kindly supplied by Dr. D H. Northcote,[9] had G.V. 1·36 whilst two different samples examined by Smith and his co-workers had G.V. 2·85 and 3·0, respectively.[3]

A major factor in controlling the precipitin reaction is the average interior chain length, about 3—5 glucose residues in glycogens, and about 5—9 in amylopectins. Thus, amylopectin β-dextrin which has the same degree of branching as a normal glycogen, and presumably the same exterior chain length (2—3 glucose residues) as glycogen β-dextrin,

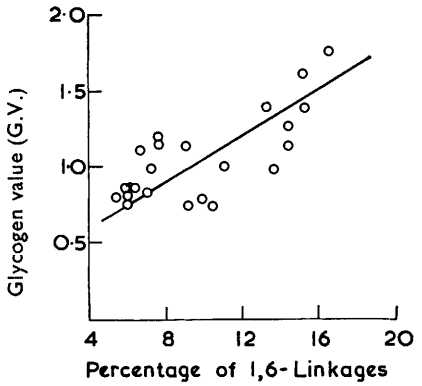

FIG. 1. Relation between the percentage of 1,6-linkages in a glycogen-type polysaccharide and the glycogen value.

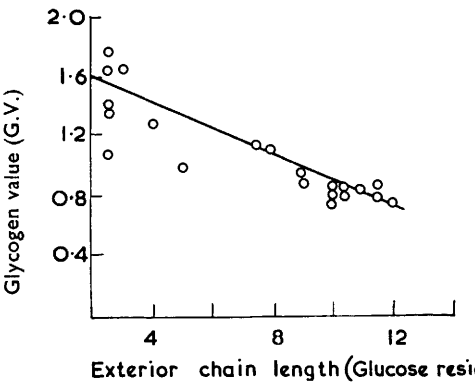

FIG. 2. Relation between the exterior chain length of a glycogen-type polysaccharide and the glycogen value.

does not react. The nature of the interaction between the concanavalin-A protein and the interior chains of a glycogen molecule is not yet known, but we have confirmed the previous observation [3] that the hydroxyl groups in the α-glucopyranose residues play some part, since periodate oxidation of glycogen destroys the precipitating ability.

With glycogens, the glycogen value also appears to be controlled to some extent by the exterior chain length ($\overline{ECL}$), since a decrease in this property results in an increase in glycogen value (Fig. 2). However, since the exterior chain length is indirectly related to the degree of branching, the relative importance of this property cannot be stated.

Molecular size is unlikely to be a controlling factor in the interaction. The glycogens examined have molecular weights [10] in the range 10^6—10^7 whereas an amylopectin-type polysaccharide from *Caulerpa filiformis* with molecular weight 15,000 [11] and plant amylopectins of molecular weight $\sim 10^7$ failed to react.

A number of results merit special comment: (*a*) That the amylopectin components of the starches from malted barley [12] ($\overline{CL}$, 18; $\overline{ECL}$, 11; $\overline{ICL}$, 6) and the alga *Dunaliella bioculata* [13] ($\overline{CL}$, 15—16; $\overline{ECL}$, 12; $\overline{ICL}$, 2—3) have G.V. 0·0, whilst horse muscle glycogens ($\overline{CL}$, 17; $\overline{ECL}$, 11—12; $\overline{ICL}$, 5—6) have G.V. 0·81—0·85, provides further evidence for the view that there are fundamental structural differences between " amylopectin " and " glycogen " in addition to the degree of branching. These differences are not revealed by

[8] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 994.
[9] Northcote, *Biochem. J.*, 1953, **53**, 348.
[10] Bryce, Greenwood, Jones, and Manners, *J.*, 1958, 711.
[11] Mackie and Percival, *J.*, 1960, 2381.
[12] Aspinall, Hirst, and McArthur, *J.*, 1955, 3075.
[13] Fleming, Hirst, and Manners, *J.*, 1956, 2831.

end-group assay or β-amylolysis, but are also shown by measurement of iodine-staining power,[6] iodine-binding power,[14] limiting viscosity number,[14] and variation of sedimentation constant with concentration,[14] and in reaction with yeast branching enzyme [15] and potato phosphorylase.[16] (*b*) The glycogen value of the sweet-corn (*Zea mays*) polysaccharides (in the range 0·74—0·96) confirms their close structural relation to the animal glycogens.[17] (*c*) The extremely weak reaction of two samples of purified Floridean starch indicates a structural resemblance to amylopectin, in agreement with other recent results.[14] The reaction of a Floridean starch sample (G.V. 1·40) contaminated with galactan sulphate is of interest in view of the observed reaction of concanavalin-A with certain mucopolysaccharides;[18] this protein might, therefore, be used as a sensitive test for the presence of galactan sulphate impurities in Floridean starches. (*d*) The low glycogen value (0·14) of the polysaccharide synthesized[15] by the action of yeast branching enzyme on potato amylopectin suggests that, although this synthetic " glycogen " has similar $\bar{C}L$, β-amylolysis limit, and iodine staining power to yeast glycogen, it still retains some amylopectin-type characteristics which are not revealed by other methods of analysis.

Experimental

Concanavalin-A.—A solution of concanavalin-A was prepared by the method of Cifonelli and Smith,[19] stabilized by the addition of polyvinyl alcohol, and stored at 0°. The solution tended to become turbid after several days, and was centrifuged before use.

Measurement of Glycogen Value (G.V).—Glycogen solution (1 ml., containing 150—1000 μg.) was added to concanavalin-A solution (9 ml.). After 10—15 min. at room temperature (18—20°) the optical density at 420 mμ was measured on a Unicam S.P. 600 spectrophotometer against a concanavalin-A–water control. Optical densities were measured at three different glycogen concentrations, and the optical density for 1·000 mg. was calculated from the linear graph of optical density against concentration. This value was compared with that of a standard sample of rabbit-liver glycogen (G.V. 1·00) kindly provided by Professor F. Smith. Individual G.V.'s are considered to be accurate to within ±0·05.

A standard glycogen was used as a control in each series of determinations. The optical density reached a maximum after 10 min. and remained constant for a further 20 min. Individual optical-density readings ranged from 0·06 to 0·40 for the glycogen, and the corresponding values per mg. of glycogen from 0·25 to 0·45.

Potato amylopectin (10 mg.) or its β-dextrin (5 mg.) gave a turbid solution with concanavalin-A which quickly cleared. At 420 mμ, the optical density for amylopectin was 0·98 (3 min.), 0·41 (6 min.), 0·35 (9 min.), 0·22 (11 min.), 0·10 (15 min.), 0·00 (20 min.), and for the β-dextrin, 1·65 (1 min.), 1·10 (3 min.), 0·41 (5 min.), 0·30 (8 min.), 0·20 (10 min.), 0·11 (20 min.), 0·03 (30 min.). With 1 mg. of these polysaccharides, the optical density did not exceed 0·0 during 30 min.

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[14] For example, see Greenwood and Thomson, *J.*, 1961, 1534.
[15] Gunja, Manners, and Khin Maung, *Biochem. J.*, 1960, **75**, 441.
[16] Liddle, Manners, and Wright, *Biochem. J.*, 1961, **80**, 304.
[17] Peat, Whelan, and Turvey, *J.*, 1956, 2317.
[18] Cifonelli, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1956, **78**, 2488.
[19] Cifonelli and Smith, *Analyt. Chem.*, 1955, **27**, 1639.