THE MOLECULAR STRUCTURE OF LIVER GLYCOGEN OF THE DOG*

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Haworth and Percival (1) showed that the glycogen molecule consists of a chain of 12 anhydroglucose units joined by α-glucosidic linkages between the 1st and 4th contiguous carbon atoms. There is therefore a close structural relation between this polysaccharide and starch, the essential difference being that the latter possesses a longer chain; namely, 24 to 30 glucose units (2-5). Recently Haworth and Isherwood (6) obtained from rabbits liver glycogen with a chain length of 18 glucose units. Bell (7, 8), applying Haworth's "end-group" assay method, examined glycogen from various sources and found it all to be of the same chain length, namely 12 glucose units, except in the case of glycogen formed in the rabbit liver after the ingestion of galactose, in which case it corresponded to a chain length of 18 glucose units. It has been shown that the glycogens with these two different chain lengths possess similar structures, exhibiting no essential differences in their general properties. In view of these variations in chain length, a study has been made here of liver glycogen obtained from another species; namely, the dog, an animal not hitherto investigated in this respect. The results obtained show that liver glycogen in this animal contains a chain length of 12 anhydro-glucose units.

EXPERIMENTAL

Preparation of Glycogen—The glycogen was obtained from dog liver by the following procedure. After removal from the animal,

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the liver was rapidly cut into thin slices and put into boiling water. After the tissue had been boiled for about 15 minutes, the slices were finely ground and the extraction with boiling water repeated. The aqueous extracts were combined and the proteins precipitated by the addition of an equal volume of 10 per cent trichloroacetic acid. The glycogen in the filtrate was then precipitated in the presence of 40 per cent alcohol. This glycogen was redissolved and reprecipitated twice from the same concentration of alcohol. The glycogen was dried at 70° in vacuo, extracted with ether in an all-glass Soxhlet apparatus for a period of 12 hours, and then again dried at the same temperature to constant weight. Various lots of glycogen prepared in this manner contained between 0.01 and 0.001 per cent phosphorus. The properties of the dog glycogen were similar to those of glycogens obtained from rabbits and other sources. It was a white powder, which did not reduce Fehling's solution and gave a deep brown color with iodine. It was soluble in water, forming an opalescent solution. The specific rotation, \([\alpha]_b\), of an anhydrous sample was +194° (in water, \(c = 0.25\)).

Acetylation—The glycogen was acetylated by Haworth, Hirst, and Webb’s modification of Barnett’s method (9). 2 gm. of well powdered glycogen were soaked for 30 minutes in 12 cc. of acetic acid through which chlorine gas had been previously bubbled for 1 minute. 10 cc. of acetic anhydride, through which sulfur dioxide was passed for 1 minute, were then added, and the mixture was maintained at 80°, with occasional shaking, for 1.5 hours. The clear solution was poured into a large excess of cold water, and the resulting precipitate was washed with water, alcohol, and ether and then dried in vacuo at 40°. The yield of the triacetate was 85.3 per cent of the theoretical and had a P content of 0.004 per cent.

Specific Rotation—\([\alpha]_b = +170^\circ\) (in chloroform, \(c = 1\))

Analysis—\((C_6H_{10}O_5(CH_3CO)_{3})_n\). Calculated. CH_3CO 44.8

Found. “ 45.0

The specific viscosity of the glycogen acetate, when 0.04 gm. was dissolved in 5 cc. of \(m\)-cresol, was 0.111. This corresponds to an apparent molecular weight of 4000, determined by Staudinger’s formula with \(K_m = 10^{-3}\) (10).

Methylation—The method of direct methylation, as described by Hassid and Dore (5) for methylation of canna starch, was employed. In this procedure preliminary acetylation is avoided.
and the first stage of the process is carried out in a medium of carbon tetrachloride. The method was carried out thus: 15 gm. of finely ground glycogen were treated with a mixture of 125 cc. of carbon tetrachloride and 90 cc. of methyl sulfate and vigorously stirred with a mechanical stirrer for 15 minutes. 200 cc. of 30 per cent sodium hydroxide were then slowly added over a period of half an hour. 460 cc. of 30 per cent sodium hydroxide and 200 cc. of methyl sulfate were then simultaneously admitted into the reaction flask. The methylating reagents were added from two dropping funnels in portions of 3.3 cc. of methyl sulfate and 7.5 cc. of sodium hydroxide every 10 minutes. At the end of this process the carbon tetrachloride was evaporated, and the mixture cooled and almost neutralized with sulfuric acid, the reaction mixture being left, however, slightly basic. 400 cc. of water were then added, the mixture heated to 100°, and the partially methylated glycogen separated and dried. The methoxy content, OCH₃, of this product was 32 per cent. This partially methylated glycogen was then methylated in a medium of acetone by the method of Haworth and Percival (1). It separated as a solid and was remethylated. After nine such treatments it was dissolved in chloroform, evaporated to dryness, and extracted with boiling ether. The product thus obtained had a methoxy content of 44.8 per cent; further methylation did not raise this value. The specific rotation, [α]ᵡ, of the methylated glycogen was +208° (in chloroform, c = 1).

Hydrolysis of Methylated Glycogen and Separation of Cleavage Products The methylated glycogen was hydrolyzed and the products of hydrolysis were separated according to the procedure of Bell (7). From 9.2 gm. of methylated glycogen, 0.805 gm. of 2,3,4,6-tetramethylglucose, 6.36 gm. of 2,3,6-trimethylglucose, and 1.1 gm. of dimethylglucose were obtained. These products were identified by their specific rotation and methoxy content.

<table>
<thead>
<tr>
<th>Product</th>
<th>[α]ᵡ</th>
<th>OCH₃ found</th>
<th>OCH₃ calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-Tetramethylglucose</td>
<td>+83</td>
<td>52.1</td>
<td>52.5</td>
</tr>
<tr>
<td>2,3,6-Trimethylglucose</td>
<td>+70</td>
<td>41.3</td>
<td>41.9</td>
</tr>
<tr>
<td>Dimethylglucose</td>
<td></td>
<td>31.2</td>
<td>29.8</td>
</tr>
</tbody>
</table>

* In water, c = 1.
The yield of 0.805 gm. of tetramethylglucose from 9.2 gm. of methylated glycogen corresponds to an estimated chain length of about 12 glucose units.

**DISCUSSION**

It has been shown that the extent to which glycogen can be methylated closely approaches the theoretical value for the methoxy content of trimethyl glycogen; namely, 45.6 per cent (1, 7, 8). This agreement, however, is purely accidental, since it has been demonstrated here as well as elsewhere (6–8) that the cleavage products of methylated glycogen are a mixture of di-, tri-, and tetramethylglucose, and it is the average methoxy content of these three products that closely approximates the theoretical methoxy content of trimethyl glycogen.

The occurrence of dimethylglucose among the cleavage products of methylated glycogen is somewhat difficult to reconcile with the current views on the structure of glycogen (1). The uniform 1,4 linkage of a continuous chain of 12 or 18 glucose units should yield trimethyl- and tetramethylglucose as the products of hydrolysis of methylated glycogen. The fact that dimethylglucose is present in considerable quantities indicates that some hydroxyl groups are "blocked" and therefore not subject to methylation. As an explanation of the occurrence of the dimethylglucose, Haworth and Isherwood (6) offer the possibility of 1,6 "cross-linkages" between individual glycogen chains. The assumption of such "cross-linkages" would leave occasional glucose units with only two exposed hydroxyl groups, thus accounting for the dimethylglucose in the methylated glycogen. In this connection it is interesting to note the recent observations of Karrer and Escher (11) on the structure of cellulose, which is closely related to that of glycogen. These workers point to the fact that certain hydroxyl groups in the cellulose molecule cannot be methylated; they suggest that the "blocking" may be due to the formation of anhydrides by the elimination of 1 molecule of water for every 8 or 10 glucose units. If this occurred, it could hardly be detected by elementary analyses. Such a hypothesis might also explain the occurrence of dimethylglucose in the hydrolysis of methylated glycogen. This point, however, requires further investigation.
SUMMARY

1. The molecular structure of glycogen from dog liver has been investigated by the "end-group" assay method. The methylated glycogen was hydrolyzed and quantitatively separated into 2,3,4,6-tetramethylglucose, 2,3,6-trimethylglucose, and dimethylglucose. The amount of tetramethylglucose obtained showed the glycogen from dog liver to be made up of chains of about 12 anhydroglucose units.

2. The significance of dimethylglucose among the products of hydrolysis of methylated glycogen is discussed.

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BIBLIOGRAPHY

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