Differential Glucose Incorporation into Glycogen by Hymenolepis diminuta*

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SUMMARY

1. A differential incorporation of 14C-glucose into two distinct molecular weight fractions of glycogen from the tapeworm Hymenolepis diminuta has been observed. Changes in these labeling patterns as a function of the glycogen level have been examined.

2. After incubation of Hymenolepis diminuta in media containing 14C-glucose under steady state conditions of glycogen metabolism, the specific activity of the high molecular weight fraction was significantly greater than that of the low molecular weight fraction. This difference was due to an almost 3-fold greater specific activity in the phosphorylase limit dextrin of the high molecular weight fraction.

3. By contrast, upon reduction of the glycogen level from 8% to approximately 1.5% and subsequent resynthesis of glycogen from 14C-glucose, the specific activity of the high molecular weight fraction was much lower than that of the low molecular weight fraction.

4. The relative rates of incorporation of 14C-glucose into glycogen as a function of the initial glycogen level were determined.

5. In any given glycogen sample, regardless of the glycogen level, the high and low molecular weight fractions had identical outer chain lengths and the same degree of branching.

6. The results obtained establish significant metabolic differences between the high and low molecular weight glycogen fractions.

When glycogen is isolated by the use of a mild and quantitative cold water procedure (3), the product exhibits sedimentation coefficient distributions highly characteristic for a given tissue and its physiological state. These distinguishing characteristics cannot be observed if glycogen is degraded by more drastic extraction procedures involving the use of hot alkali, cold trichloracetic acid, or heat, or if preferential losses occur during the isolation (4). In the cold water-extracted glycogen of the tapeworm Hymenolepis diminuta, 30% of the polysaccharide is present in a very high molecular weight component with an average molecular weight of 900 million. This component is separated distinctly from a lower molecular weight component with an average molecular weight of 60 million (5). Reduction in the glycogen concentration of the parasite from 8% to approximately 1.5% (wet weight), produced by depriving the host of food, results in a progressive decrease in the average molecular weights of the two molecular weight components, while there is no change in their relative concentrations. On subsequent incubation of these worms in a medium containing glycogen, glycogen is resynthesized, and as the glycogen level increases, the average molecular weights increase progressively, thus indicating the complete reversibility of these changes (5). In the present study, the differential incorporation of 14C-glucose into the two molecular weight fractions has been examined.

EXPERIMENTAL PROCEDURE

Glycogen was isolated by the cold water extraction procedure described previously (3), except that 0.2 M glycine buffer (pH 10.7) was used throughout. Glycogen concentrations were determined by the phenolsulfuric acid procedure (6) or by a more specific microenzymatic method (7, 8). Sedimentation coefficient distribution diagrams were determined as described previously (4).

High and low molecular weight fractions of glycogen were separated by differential zone centrifugation in a density gradient of aqueous lithium bromide solution varying in density from 1.05 to 1.20 at a temperature of 20°C. Samples (0.7 ml at a glycogen concentration of 2%) were layered on each gradient and centrifuged at 40,000 x g in the Spinco bucket rotor, model SW 25.1, for from 15 to 45 min, depending on the sedimentation coefficients of the components to be separated.

Fractions were recovered by removal of an upper portion of the gradient column (containing the low molecular weight fraction) and subsequent isolation from the lithium bromide solutions by precipitation with ethanol. The purity of each fraction obtained was verified by ultracentrifugal analysis in the model E Spinco analytical ultracentrifuge.

After a single fractionation, the lower molecular weight fraction contained no high molecular weight contaminant detectable by ultracentrifugal analysis. However, in the case of the high molecular weight fraction, the amount of low molecular weight contaminants varied between 10 and 20% after one fractions-

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tion. The contaminants were removed by a second and, occasionally, even a third refractionation until no more than 3% of the low molecular weight material remained in the high molecular weight fraction.

Outer branch length and percentage of branch points of each glycogen fraction were determined as described previously (7, 8). The phosphorylase limit dextrins were prepared by incubating a solution of 2 to 6 mg of glycogen in a total volume of 1 ml containing 1 μ mole of AMP, 800 μ moles of phosphate buffer (pH 7.0), EDTA (final concentration, 7×10⁻⁵ m), and 300 units of phosphorylase b at 20° (7, 8). Incubation was continued until no further increase in glucose 1-phosphate was observed.

Glucose 1-phosphate from the outer tiers was separated from the phosphorylase limit dextrin by centrifugation of the above reaction mixture at 0–4° for 16 hours at 100,000 × g. The resulting supernatant contained the glucose 1-phosphate, while the limit dextrin was present in the pellet at the bottom of the tube. The two fractions were separated by decantation, and the limit dextrin pellet was homogenized in water.

In order to minimize quenching during scintillation counting, inorganic phosphate was removed prior to determination of the radioactivity of the supernatant layer containing glucose 1-phosphate. This was accomplished by using a specific magnesium reagent (9). This reagent removed 270 μ moles of inorganic phosphate per ml of added reagent. A small excess of the reagent was used to ensure precipitation of all inorganic phosphate; samples were stored at 0–4° overnight. The fine precipitate was then removed by filtration, and the glucose 1-phosphate in the inorganic phosphate-free supernatant layer was determined by the use of a specific microenzymatic method (7, 8). Specific activities were measured by suspending aliquots of known concentration. The contaminants were removed by a second and, occasionally, even a third refractionation until no more than 3% of the low molecular weight material remained in the high molecular weight fraction.

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reversal of the pattern observed with worms whose hosts had not been fasted or deprived of food for shorter periods of time. Furthermore, the patterns of the increases in glycogen and of glucose utilization in the worms whose hosts had been fasted for 48 hours were different. In these organisms, a much greater percentage of the glucose taken up from the medium was incorporated into glycogen (Table VII).

Table VIII illustrates the ratios of the specific activities of the two molecular weight fractions after incubation in 14C-glucose as a function of the initial glycogen level. As the initial glycogen concentration of the worm decreased, the ratio of the specific activities of the high to those of the low molecular weight limit dextrans decreased, until, at severe depletion, the ratio was

<table>
<thead>
<tr>
<th>Incubation with 14C-glucose</th>
<th>Undegraded fraction</th>
<th>Limit dextrin</th>
<th>Outer branches</th>
</tr>
</thead>
<tbody>
<tr>
<td>High mol wt</td>
<td>Low mol wt</td>
<td>High mol wt</td>
<td>Low mol wt</td>
</tr>
<tr>
<td>min</td>
<td>dpm/μ mole</td>
<td>% mol wt</td>
<td>% mol wt</td>
</tr>
<tr>
<td>30</td>
<td>480</td>
<td>350</td>
<td>350</td>
</tr>
<tr>
<td>60</td>
<td>750</td>
<td>550</td>
<td>500</td>
</tr>
</tbody>
</table>

**Table VII**

Rates of glucose incorporation and glycogen synthesis in severely depleted *H. diminuta*

<table>
<thead>
<tr>
<th>Incubation in 14C-glucose</th>
<th>Glucose</th>
<th>Increase in glycogen</th>
<th>Glucose taken up</th>
<th>Glucose incorporated into glycogen</th>
<th>Glucose taken up converted to glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>% wet wt</td>
<td>%</td>
<td>μmole/g wet wt</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>3.05</td>
<td>1.56</td>
<td>50</td>
<td>4.0</td>
<td>40.0</td>
</tr>
<tr>
<td>15</td>
<td>2.20</td>
<td>9.0</td>
<td>8.4</td>
<td>3.4</td>
<td>47.0</td>
</tr>
<tr>
<td>60</td>
<td>2.49</td>
<td>60</td>
<td>54.2</td>
<td>25.5</td>
<td>75.0</td>
</tr>
<tr>
<td>120</td>
<td>3.45</td>
<td>120</td>
<td>89.0</td>
<td>65.5</td>
<td>75.0</td>
</tr>
<tr>
<td>480</td>
<td>6.50</td>
<td>350</td>
<td>270.0</td>
<td>270.0</td>
<td>90.0</td>
</tr>
</tbody>
</table>

**Table VIII**

Relationship between initial glycogen levels and 14C-glucose incorporation into high and low molecular weight fractions of *H. diminuta* glycogen

<table>
<thead>
<tr>
<th>Initial glycogen</th>
<th>Ratio of specific activities of high to low mol wt components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Limit dextrin</td>
</tr>
<tr>
<td>% wet wt</td>
<td>% mol wt</td>
</tr>
<tr>
<td>8.0</td>
<td>2.85</td>
</tr>
<tr>
<td>3.05</td>
<td>1.87</td>
</tr>
<tr>
<td>1.56</td>
<td>0.37</td>
</tr>
</tbody>
</table>

**Table IX**

Comparison of branching structure of *H. diminuta* glycogen at various glycogen concentrations

<table>
<thead>
<tr>
<th>Glycogen</th>
<th>Molecular weight fraction</th>
<th>Glucose units removed by phosphorylase above</th>
<th>Branch points</th>
</tr>
</thead>
<tbody>
<tr>
<td>% wet wt</td>
<td>% total</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1.56*</td>
<td>Low</td>
<td>14.2</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>13.3</td>
<td>11.5</td>
</tr>
<tr>
<td>3.05†</td>
<td>Low</td>
<td>39.5</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>31.0</td>
<td>8.7</td>
</tr>
<tr>
<td>8.00‡</td>
<td>Low</td>
<td>35.0</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>35.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

* Average of six experiments.
† Average of five experiments.
‡ Average of eight experiments.
inverted. In addition, after severe depletion, the specific activities of the outer branches of the low molecular weight fraction also increased relative to the outer branches of the high molecular weight fraction.

The results of partial degradation by phosphorylase and of total degradation by the combined action of phosphorylase and α-1,6-amyloglucosidase of the various fractions are shown in Table IX. For every given glycogen sample analyzed in this manner, the outer chain length and number of branch points were identical in the high and low molecular weight fraction. As the level of glycogen of the worms decreased, the percentage of glucose units removed by phosphorylase alone decreased and the number of branch points increased.

**DISCUSSION**

A differential incorporation of glucose into liver and muscle glycogens and Escherichia coli glycogen as a function of molecular weight has been reported by Stetten and Stetten (13), and by Holme, Laurent, and Palmstierna (14), respectively. However, the methods of isolation of glycogen used (cold trichloroacetic acid or hot alkali) result in a severe reduction of the molecular weight of the glycogen to between 1% and 10% of its original size (4). In addition, the fractions obtained were not subjected to sedimentation analysis. Furthermore, light scattering was used for estimating the average molecular weights of the glycogen fractions (13, 14). Determination of the molecular size of the polydispersity of the method gives results that are quite ambiguous in the presence of even small amounts of higher molecular weight material. Moreover, light scattering yields only a single average value and supplies no information as to whether a separation into distinct molecular weight fractions has been accomplished. Therefore, it is difficult to compare these data with those reported in this paper, where all fractions were checked by ultracentrifugal analysis.

In contrast to other methods, the milder quantitative isolation procedure used in this study results in a product with a much broader molecular weight spectrum that has two distinct regions of molecular weight (4, 5). The low molecular weight component (approximately 70% of the total) has an average molecular weight of 60 million; the high molecular weight component (30% of the total) has an average molecular weight of 900 million. The observed differences in the specific activities of the two molecular weight fractions indicate a metabolic distinction between these two fractions. It should be emphasized that these differences can be found only when glycogen is isolated by a mild and quantitative procedure. If preferential losses occur at any time during the isolation, no meaningful interpretation of such differences can be made. In the present study, great care has been taken to avoid degradation of the glycogen or losses preferential to any region of molecular weight.

As in the case of cold water-extracted glycogen from other sources (3–5), cold water-extracted H. diminuta glycogen is converted completely to glucose 1-phosphate and glucose by the action of phosphorylase and α-1,6-amyloglucosidase, indicating that this material consists entirely of glucose units joined by α-1,4- and α-1,6-glucosyl linkages. Recently, this conclusion has been confirmed by the observations of Mordoh, Leloir, and Krisman (15), who have reported the synthesis of high molecular weight glycogen by the action of phosphorylase and amylo-(1,4 → 1,6)-transglucosidase.

Since the greater specific activity of the high molecular weight fraction is principally due to a greater incorporation of 14C-glucose into its phosphorylase limit dextrin, it would appear that a differential affinity of a common enzyme, possibly the branching enzyme, for the two molecular weight components exists in the tissue. As previously reported by Stetten and Stetten (16) for glycogen extracted with cold trichloroacetic acid, the outer branches of cold water-extracted glycogens always have a greater specific activity than the limit dextrins.

When worms severely depleted of glycogen are incubated with 14C-glucose, the specific activities both of the phosphorylase limit dextrin and of the outer branches of the low molecular weight glycogen fraction are greater than those of the high molecular weight fraction. Since the relative amount (70% and 30%) of the low and high molecular weight components is constant, the low molecular weight material must be both degraded and synthesized at a more rapid rate than the high molecular weight material under these conditions.

The similarities in the labeling patterns and in the percentages of glucose taken up from the medium and converted to glycogen in moderately depleted, as compared to undepleted worms, indicates that moderate depletion does not produce the marked alteration in glycogen metabolism which is observed in severely depleted worms. On the other hand, the moderately depleted worms differ from the undepleted organisms by a significant increase in glycogen concentration during incubation with glucose.

While there are large differences in the labeling patterns of the high and low molecular weight fractions as a function of the glycogen level, it is remarkable that there are no differences in the outer branch lengths and the degree of branching between the high and low molecular weight components of any given sample.

Another factor that must be borne in mind is the enormous size of the glycogen substrate compared to that of the enzymes presently known to be involved in its metabolism. The low molecular weight component ranges in size from several hundred thousand to over 100 million, while the high molecular weight component varies from 100 million to over 1000 million.

The natural division of the molecular weight spectrum of glycogen into two distinct components in itself suggests that there are differences in the mechanisms by which these two components arise. A consideration of the nature of these differences offers at least two possibilities: the two components may arise by separate pathways or a common pathway involving some mechanism having different affinities toward substrates of different molecular weight. The present study excludes neither of these possibilities.

**REFERENCES**


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