GLUCOSE OXIDATION IN MYCELIA AND SPORES OF THE WHEAT SMUT FUNGUS TILLETIA CARIES

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Received for publication May 8, 1958

Previous studies with the wheat smut fungus, *Tilletia caries*, showed that this organism contains all the enzymes necessary for the operation of the pentose cycle and apparently of glycolysis as well (Newburgh et al., 1955). Since the occurrence of a different pathway of carbohydrate catabolism or the predominance of a known pathway in the smut organism may be of importance for the control of smut, it was felt of value to assess the relative contribution to carbohydrate dissimilation of the several known pathways, using specifically labeled glucose as substrate. In addition, a comparison was made of glucose catabolism by field-grown spores and by mycelia grown in the laboratory.

METHODS

The organism was grown on a rotary shaker as previously described (Newburgh et al., 1955) at 18°C for 4 days. The cells were collected on a Buchner funnel, washed, and resuspended for the experiments with the mycelium. The spores (race T-10) were from smutted wheat collected by C. S. Holton. They were removed from the florets of the wheat plant, washed, filtered, and dried and then stored in the refrigerator. For the radioactive studies, the spores were resuspended in 0.2 M phosphate buffer at the desired pH.

For the original experiments with mycelium the macroradiorespirometer described by Wang et al. (1956) was used. In later experiments it was found economical to employ a micro adaptation developed by Wang et al. (1958) which can be used on a standard Warburg apparatus. This consists of a Warburg flask and a gas scrubber containing 0.5 N CO₂-free NaOH for the collection of respiratory CO₂. Up to 10 flasks may be operated simultaneously. The air flow is adjusted so that it is identical in all flasks as measured by a calibrated flowmeter. Samples can be removed at various times, the CO₂ precipitated as BaCO₃, and then counted. Since the results with either the micro or macro apparatus were the same when mycelial tissue was studied, the micro apparatus was used more extensively primarily for reasons of cell and substrate economy.

Into each macro flask was added 100 ml of cell suspension, 75 ml of 0.02 M phosphate buffer, pH 6.5, and 25 ml of water containing 250 μmoles of glucose (with 0.5 μc of glucose-1-C¹⁴, glucose-2-C¹⁴, glucose-6-C¹⁴, or 3 μc of glucose-u-C¹⁴, respectively). Into the micro flasks, to each Warburg flask used for kinetic studies, was added 1.0 ml cell suspension, 0.5 ml of 0.2 M phosphate buffer, pH 6.5, 0.5 ml H₂O containing 13 μmoles of glucose (with 0.5 μc of glucose-1-C¹⁴, glucose-2-C¹⁴, glucose-6-C¹⁴, or 3 μc of glucose-u-C¹⁴) or, where indicated, 13 μmoles of Δ-gluconolactone containing 0.5 μc of Δ-gluconolactone-1-C¹⁴, or 13 μmoles of acetate containing 0.5 μc of acetate-2-C¹⁴.

Glucose-1-C¹⁴, -2-C¹⁴, and -6-C¹⁴ were purchased from the National Bureau of Standards; glucose-u-C¹⁴ and acetate-2-C¹⁴ from Tracerlabs, Inc.; and α-glucuronolactone-1-C¹⁴ from Nuclear Instruments, Inc.

RESULTS

The results with mycelia using the macro apparatus are shown in figure 1. It can be seen that after 5 hr nearly all of the glucose was utilized and much of the radioactive CO₂ had been
Figure 1. Recovery of $\text{C}^{14}\text{O}_2$ from labeled glucose by *Tilletia caries* mycelium of race T-10. 1 = glucose-1-C$^{14}$, 2 = glucose-2-C$^{14}$, 3,4 = glucose-3,4-C$^{14}$, 6 = glucose-6-C$^{14}$.

Figure 2. Recovery of $\text{C}^{14}\text{O}_2$ from labeled glucose by *Tilletia caries* spores, race T-10. 1 = glucose-1-C$^{14}$, 2 = glucose-2-C$^{14}$, 3,4 = glucose-3,4-C$^{14}$, 6 = glucose-6-C$^{14}$.
collected. Over 70 per cent of the radioactivity from glucose-1-C⁴ was recovered as C⁴O₂.

The presence of glycolysis is evident from the rapid appearance of C⁴O₂ from the 3 and 4 carbons of glucose and since the recovery of these two carbon atoms is over 80 per cent, it is evident that little incorporation of these carbons into cellular material occurred. Evidence of a cleavage to a C₁-C₃ compound by way of phosphogluconic decarboxylation stems from the rapid appearance of C⁴O₂ from glucose-1-C⁴ in addition to the higher recovery of C⁴O₂ from this carbon than carbon 6 of glucose. Figure 2 illustrates that a difference in glucose catabolism occurs when teliospores of race T-10 are used in place of mycelium. The appearance of C⁴O₂ from glucose-1-C¹⁴ is more rapid and extensive than that from glucose-3,4-C¹⁴. In addition the radiochemical recovery is higher from all of the glucose carbons. Several other labeled compounds were tested and the accumulative radiochemical recovery from these were glutamic-1-C⁴, 24 per cent; glucuronolactone-1-C⁴, 80 per cent; ribose-1-C⁴, 23 per cent.

**DISCUSSION**

From the results it is apparent that a difference exists between the pathways of glucose catabolism in spores and mycelium of *T. caries*, race T-10. At present, three main routes for glucose catabolism are known, namely glycolysis, pentose cycle and Entner-Doudoroff pathway. If we make the assumptions and use the methods of calculation of Wang et al. (1956, 1958) certain possibilities exist for glucose oxidation by *T. caries*. These methods of calculation can be used only if two pathways are operative in a particular organism, namely glycolysis and pentose cycle (use equation 1, table 1), or Entner-Doudoroff and pentose cycle (use equation 2, table 1). Using these assumptions then if the Entner-Doudoroff pathway is operative, the radiochemical recovery from glucose-1-C¹⁴ should be equal to or greater than that from carbon 4. It can be seen from table 1 that the results negate Entner-Doudoroff activity in the mycelium but in the spores it is possible that glucose catabolism is 100 per cent by way of the Entner-Doudoroff pathway.

It is realized that certain assumptions are made for these calculations whose validity requires further experimental proof but the patterns obtained demonstrate that a difference in glucose catabolism occurs between mycelium and spores. This is particularly true of the behavior of glucose-1-C¹⁴ and glucose-3,4-C¹⁴.

**ACKNOWLEDGMENTS**

The authors wish to acknowledge the advice of Dr. C. H. Wang and the technical assistance of Miss Kay Black.

**SUMMARY**

A comparison of glucose catabolism in mycelia and spores of *Tilletia caries* has been made. The pattern of glucose catabolism differs in these two forms particularly in the behavior of glucose-1-C¹⁴ and glucose-3,4-C¹⁴. The implications of this finding are discussed as well as the amount of glucose which may be catabolized by various pathways.

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**TABLE 1**

Pathways of glucose catabolism in mycelium and teliospores of *Tilletia caries*

<table>
<thead>
<tr>
<th>Recovery of Substrate Activity in CO₂</th>
<th>% Pathway Participation</th>
<th>Equation Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycelium</td>
<td>G₁', G₁', G₁, G₁</td>
<td></td>
</tr>
<tr>
<td>Spores</td>
<td>G₂', G₂', G₂, G₂</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G₁' - G₁'</td>
<td>G₁ = 1 - G₂ and</td>
</tr>
<tr>
<td></td>
<td>G₂' - G₂'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G₁' = Fraction glucose catabolized by direct oxidative pathway</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G₂' = Fraction glucose catabolized by glycolysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G₁t = Fraction glucose catabolized by Entner-Doudoroff</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G₁', G₁', G₁t', G₁t = total activity recovered in metabolic CO₂ from cells using equal amounts (chemical + radiochemical) of glucose-1', 2', 3(4), 6-C¹⁴</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G₁ = 100.</td>
<td></td>
</tr>
</tbody>
</table>

G₁ = 2 G₂, G₂ = G₁ - G₂ * Assume G₁ = G₂
REFERENCES

