Hexose Monophosphate Metabolism During Basidiospore Germination in *Schizophyllum commune*

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Studies concerning the regulation of hexose monophosphate metabolism in the basidiomycetes have lagged considerably behind work on other fungi, animal systems, and bacteria. The existence of cellular control mechanisms which regulate carbohydrate utilization in

basidiomycetous fungi may be inferred from radiorespirometric data indicating disparate carbon flow via the pentose phosphate pathway in spores of *Tilletia contraversa* (Newburgh and Cheldelin, Can. J. Microbiol. **5**:415, 1959) and mycelium of *T. caries* (Newburgh and Cheldelin, J. Bacteriol. **76**:308, 1958). Work dealing with basidiospore germination in the wood-rotting mushroom *Schizophyllum commune*, on the other hand, indicates that the ratio of glucose-6-phosphate dehydrogenase to 6-phosphogluconate dehydrogenase changes significantly during cell differentiation. The additional finding that phosphoglucose isomerase activity is inhibited by 6-phosphogluconate in *S. commune* suggests that this combination of events could provide a possible mechanism for the control of alternate pathways of glucose utilization during morphogenesis.

The specific enzyme activities for glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were compared during basidiospore germination and subsequent growth of vegetative mycelium of *S. commune* 699 (Table 1). The ratio of dehydrogenase activities for glucose-6-phosphate and 6-phosphogluconate was considerably greater than unity in extracts of spores and germlings, whereas extracts of mycelium showed a significant increase in 6-phosphogluconate dehydrogenase activity. A similar increase in 6-phosphogluconate dehydrogenase activity was shown in extracts of mycelium of *S. commune* 845. These data suggest that 6-phosphogluconate can accumulate to some degree during basidiospore germination, provided that the observed level of 6-phosphogluconate dehydrogenase in extracts of basidiospores is rate-limiting in vivo.

Work by Parr (Nature **178**:1501, 1956; Biochem. J. **65**:34P, 1957) showed that phosphoglucose isomerase activity in extracts of liver, *Escherichia coli*, and potato is subject to inhibition by 6-phosphogluconate. Incubation of basidiospore extracts of *S. commune* with glucose-

TABLE 1. Specific activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase during development of *Schizophyllum commune**

Enzyme activity	Basidiospores	Germlings (12 hr)	Mycelium
Glucose-6-phosphate dehydrogenase	2.066 2.432 2.720	3.740	2.972 1.980
6-Phosphogluconate dehydrogenase	0.333 0.168 0.435	0.584	1.621 1.980 1.620

* Assays were performed on cultures harvested and extracted on the day of enzyme preparation. Results are expressed as the change in optical density at 340 m μ per min (times milligrams of protein). Replicate values indicate repetition of experiment from different culture lots. Assay conditions: Glucose-6-phosphate or 6-phosphogluconate, 5 μ moles; nicotinamide adenine dinucleotide phosphate, 0.35 μ mole; tris(hydroxymethyl)aminomethane-HCl buffer (pH 7.3), 40 μ moles; enzyme extract and water (glass-distilled) to 1.0 ml. Temperature, 30 C. Enzyme extract prepared by French pressure cell extraction (24,000 psi) of cells suspended in phosphate buffer (pH 6.8, 0.08 M), followed by differential centrifugation at 2,000 $\times g$ (10 min) and 105,000 $\times g$ (120 min) at 4 C in a Spinco (model L) preparative centrifuge. The final supernatant liquid served as the source of enzyme. Dehydrogenase activities for glucose-6-phosphate and 6-phosphogluconate were assayed by the increase in absorption of reduced nicotinamide adenine dinucleotide phosphate at 340 m μ in a Zeiss PMQII spectrophotometer.

FIG. 1. Effect of 6-phosphogluconate on phosphoglucose isomerase activity in basidiospore extracts of *Schizophyllum commune*. Reaction mixture: glucose-6-phosphate, 5 μ moles; 6-phosphogluconate, 5 μ moles, where indicated; tris(hydroxymethyl)-aminomethane-HCl buffer (pH 7.3), 40 μ moles; dialyzed enzyme extract, 0.07 mg of protein; water (glass-distilled), to 1.0 ml. Incubation time, 30 min. Temperature, 30 C. Ketohexose formation determined by cysteine-carbazole procedure.

6-phosphate in the absence of nicotinamide adenine dinucleotide phosphate yielded a product which reacted with cysteine-carbazole (Dische and Borenfreund, J. Biol. Chem. **192**:583, 1951) to give a derivative with maximal absorbance at 560 m μ , presumably because of the formation of ketohexose by phosphoglucose isomerase. The inhibitory effect of 6-phosphogluconate on ketohexose formation from glucose-6-phosphate is shown in Fig. 1. No significant inhibition was evident with gluconic acid, D-glucose, or L-sorbitol. Importantly, phosphoglucose isomerase activity in extracts of vegetative mycelium of *S. commune* was also sensitive to 6-phosphogluconate. Since only extracts of basidiospores and germlings of this mushroom have low levels of 6-phosphogluconate dehydrogenase relative to glucose-6-phosphate dehydrogenase, inhibition of phosphoglucose isomerase by 6-phosphogluconate could provide a mechanism whereby the pentose phosphate pathway would be favored during cell differentiation in *S. commune*.

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