DEVELOPMENTAL CONTROL OF ENZYME MODIFICATION DURING FRUITING OF THE BASIDIOMYCETE SCHIZOPHYLLUM COMMUNE

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SUMMARY: The basidiomycete Schizophyllum commune produces a modifier of its own phosphoglucomutase. The enzyme-like modifier is found in mature fruit bodies but not in the vegetative mycelium. The modifier causes an increase in phosphoglucomutase cold lability.

INTRODUCTION

The basidiomycete Schizophyllum commune produces fruit bodies on a simple, defined medium in a temporally synchronized manner (1). Utilizing this organism as a model system in studies of the control of development, a number of biochemical markers of development have been observed, including a 10-fold loss in the specific activity of phosphoglucomutase (2). As part of our study of the mechanism controlling this change in enzyme level we observed that mixing of extracts from different morphogenetic states, the vegetative dikaryotic mycelium and the mature fruit body, resulted in alterations in phosphoglucomutase stability.

In recent years, several types of enzymatic activities have been observed which modify the activity or function of other enzymes. These include group specific proteases in mammalian cells (3) and yeast (4) as well as the enzyme-activating proteins in yeast (5, 6). This communication will present evidence that modification of enzymes occur in S. commune and is found in specific morphogenetic states.

Table 1. Effect of heat and cold treatments on phosphoglucomutase activity.

Source of phosphoglucomutase	Activity (ΔOD/min/ml) zero time	37°, 1hr.	2°, 2hr.	37°, 1hr.-2°, 2hr.
Vegetative mycelium	4.0	2.8	4.0	3.8
Mature fruit body	1.3	0.6	0.5	<0.1
Vegetative mycelium-mature fruit body mixture	2.7	1.5	2.3	0.0

MATERIALS AND METHODS

Cultures were grown as previously described (1). Material was suspended in 0.1M tricine buffer, pH 7.5 and homogenized in a VirTis model 60K homogenizer. The extracts used are the supernatants from 8,000 x g centrifugations. The assay for phosphoglucomutase has also been described (2). Dialysis was performed in tricine buffer overnight. Protein content of the extracts as measured by the Lowry procedure (7) were in the range of 1.5 to 2.5 mg./ml. Mixtures were prepared in equal proportions unless otherwise noted.

RESULTS

Table I demonstrates effect of heat, cold and heat-cold treatments on S. commune phosphoglucomutase. After treatment at 37° for 1 hr., the mutase from both the vegetative mycelium and the mature fruit body shows a decrease in activity of 29 and 55% respectively. The loss in activity of a 1:1 mixture of the two extracts is equal to the average of the loss for the individual extracts. When extracts are placed at 2° for 2 hr. the vegetative mycelium shows no loss in mutase activity. Fruit body mutase is more cold sensitive, losing 40% of its activity after 2 hr. A mixture of the two extracts again shows the average of the individual extracts. When the extracts are treated at 37° for 1 hr. and then placed at 2°, a different pat-

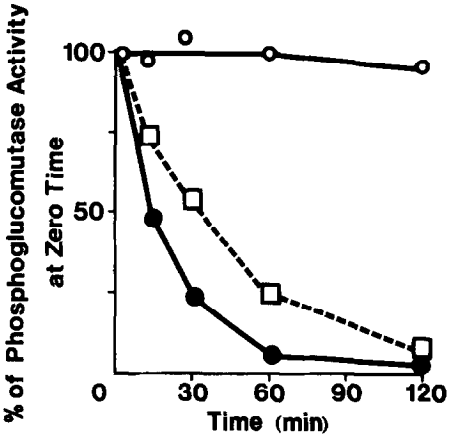

Fig. 1. Change in phosphoglucomutase activity at 2° after treatment at 37° for 30 min.

Vegetative mycelium (○), mature fruit bodies (□), mixture of vegetative mycelium and mature fruit bodies (●). The zero time level of activities (ΔOD/min/ml) are 4.2 for the vegetative mycelium, 0.7 for the mature fruit bodies and 2.5 for the mixture.

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tern is obtained. While the vegetative mycelial extract has only a slight loss in phosphoglucomutase activity, the mature fruit body extract has lost 95% of its phosphoglucomutase activity. When extracts from both morphological stages are mixed prior to treatment at 37°, the mixture shows an almost total loss in phosphoglucomutase activity after two hr. at 2° C. Figure 1. shows the kinetics of the changes in activity in the cold after heat treatment.

Several properties of the modifying factor were examined. Placing mature fruit body extracts in a boiling water bath for three minutes destroys the modifying activity. Boiling of vegetative extracts does not effect the loss of fruit body mutase. The activity survives dialysis, is recovered in the 40-60% ammonium sulfate fraction and appears near the void volume on a G-100 Sephadex column.

The Q10 (24-34°) for the modifying activity is 2.2. The activity is proportional to the concentration of fruit body extract and is also proportional to the time of incubation at 37°. When the heat-cold treatment is performed in the presence of 3.5×10^{-3} M glucose-1-phosphate there is a 50% reduction of modifier effect.

DISCUSSION

The results demonstrate that S. commune contains an enzyme-like modifier of its own phosphoglucomutase. The modifier causes an increase in the cold lability of phosphoglucomutase from both the vegetative and fruit body stages, although the modifier is found only in the fruit body stage. Since the fruit body mutase is affected by cold treatment without preincubation, some of the fruit body enzyme may be modified in vivo. However, preincubation at 37° increases the sensitivity of the fruit body phosphoglucomutase. While the properties of the modifier are indicative of an enzymatic activity, more definitive kinetic studies will have to await preparation of suitable quantities of substrate (S. commune phosphoglucomutase).

The results indicate that modifications of enzyme structure can occur which are not observable as a change in specific activity under physiological conditions. What effects this modification might have on the kinetic properties, allosteric sensitivities, etc. has yet to be determined. The modifier activity is found in one differentiated state and not another. This suggests that enzyme modifiers could play a role in development, such as altering enzyme functions as part of the biochemical organization of a new morphology.

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