the cell-bound forms that may result from conformational differences that enable the enzyme to be cell-bound.

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The Regulation by Methionine of Three Inducible Enzymes in *Coprinus lagopus*

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Coprinus lagopus is a basidiomycete fungus with many genetic advantages over other fungi in the elucidation of systems regulating the synthesis of specific enzymes (Lewis, 1961; Casselton & Lewis, 1967). Wild-type strains of *Coprinus* may be grown aerobically in a simple defined medium.

The synthesis of three enzymes has been found to be regulated by methionine: *S*-adenosylmethionine synthetase (ATP-methionine *S*-adenosyltransferase, EC 2.5.1.6) and two methionine decarboxylases (L-methionine carboxy-lyase, EC 4.1.1.-), with pH optima at pH 7.8 and pH 4.6. These enzymes were assayed by using ¹⁴C-labelled methionine as substrate and trapping any labelled cationic product (*S*-adenosylmethionine or 3-methylthiopropylamine) on CM-cellulose paper discs.

For all assays samples were spotted on to CM-cellulose paper discs impregnated with 50 mM-L-methionine. The large molar excess of unlabelled substrate effectively stops the radioactive reaction while the discs are drying. The discs are then washed with distilled water to remove unchanged substrate, dried and their radioactivities measured in a liquid-scintillation counter as described by Newsholme, Robinson & Taylor (1967).

The methionine decarboxylases were assayed with L-[Me-¹⁴C]methionine as substrate. Because the decarboxylases also produce a labelled cation from L-[Me-¹⁴C]methionine, *S*-adenosylmethionine synthetase cannot be measured easily with this as a substrate. Instead, L-[carboxy-¹⁴C]methionine was used as substrate, any decarboxylation resulted in unlabelled product, the label thus being incorporated into *S*-adenosylmethionine only.

It was found that the two methionine decarboxy-

lases were repressed by adding methionine to the growth medium, whereas the *S*-adenosylmethionine synthetase was induced. Ethionine, a toxic analogue of methionine, could induce *S*-adenosylmethionine synthetase to only 60% of the methionine-induced activity, but could repress the decarboxylases much more effectively. Ethionine is a substrate for the decarboxylases but has a *K_m* 100-fold greater than that of methionine for *S*-adenosylmethionine synthetase. All the enzymes have been purified.

A possible mechanism is that the methionine decarboxylases are repressed by their product, and that charged methionine tRNA acts as the inducer for *S*-adenosylmethionine synthetase.

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Methylation of Homocysteine in *Coprinus lagopus*

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The basidiomycete *Coprinus lagopus* may be grown aerobically in simple defined media. Cell-free extracts form L-methionine from homocysteine when L-serine is used as ultimate source of the methyl group. The individual reactions involved have been studied in the light of the pathways established in, for example, *Escherichia coli* (Woods, Foster & Guest, 1964) and *Saccharomyces cerevisiae* (Botsford & Parks, 1967).

With extracts of *Coprinus* either tetrahydropteroyltriglutamate (H₄PteGlu₃) or a boiled extract of *E. coli*, containing natural polyglutamyl folates (Guest & Woods, 1962), can serve as the folate coenzyme carrying the C₁ unit. Tetrahydropteroylmonoglutamate (H₄PteGlu) is inactive.

An isotopic method has been developed for the determination of serine transhydroxymethylase (serine-tetrahydrofolate 5,10-hydroxymethyltransferase, EC 2.1.2.1). *Coprinus* extracts transfer C₁ fragments from L-serine to tetrahydrofolate at a rate sufficient to satisfy the overall production of methionine measured under similar conditions. Both H₄PteGlu and H₄PteGlu₃ were effective C₁ acceptors. *Coprinus* extracts contain N⁵N¹⁰-methylene tetrahydrofolate reductase activity (N⁵-methyltetrahydrofolate-FAD oxidoreductase, EC 1.1.1.68), forming N⁵-methyltetrahydrofolates. Small quantities of these extracts promote methio-