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

This work was supported in part by U.S. Public Health Service Grant A1-04572 from the National Institute of Allergy and Infectious Disease.

- Ambler, R. P. & Meadway, R. J. (1969). *Nature, Lond.*, **222**, 24.
 Dubnau, D. A. & Pollock, M. R. (1965). *J. gen. Microbiol.* **41**, 7.
 Lampen, J. O. (1967). *J. gen. Microbiol.* **48**, 249.
 Sargent, M. G., Ghosh, B. K. & Lampen, J. O. (1968). *J. Bact.* **96**, 1231.
 Sargent, M. G., Ghosh, B. K. & Lampen, J. O. (1969). *J. Bact.* **97**, 820.

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

By R. H. WILSON. (*Botany School, University of Oxford, Oxford OX1 3RA, U.K.*)

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.

- Casselton, L. A. & Lewis, D. (1967). *Genet. Res.* **9**, 63.
 Lewis, D. (1961). *Genet. Res.* **2**, 141.
 Newsholme, E. A., Robinson, J. & Taylor, K. (1967). *Biochim. biophys. Acta*, **132**, 338.

Methylation of Homocysteine in *Coprinus lagopus*

By A. R. SALEM and M. A. FOSTER. (*Microbiology Unit, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, U.K.*) and R. H. WILSON. (*Department of Botany, University of Oxford, Oxford OX1 3RA, U.K.*)

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-

nine production from homocysteine and serine by extracts of *E. coli* 3/62, a methionine auxotroph specifically lacking the reductase (Guest, Foster & Woods, 1964). Either H_4PteGlu or $\text{H}_4\text{PteGlu}_3$ serves as cofactor for this process.

Similarly *Coprinus* extracts also stimulate methionine synthesis by extracts of *E. coli* 121/176, another methionine auxotroph, specifically lacking N^5 -methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (Guest, Friedman, Foster, Tejerina & Woods, 1964). Here $\text{H}_4\text{PteGlu}_3$, but not H_4PteGlu , is an effective coenzyme. Like *E. coli*, therefore, *Coprinus* has a methyltransferase specific for conjugated N^5 -methyltetrahydrofolates. This was confirmed by direct assay with $N^5[^{14}\text{C}]$ -methyl- $\text{H}_4\text{PteGlu}_3$ and $N^5[^{14}\text{C}]$ -methyl- H_4PteGlu . Only the former converts homocysteine into methionine by transmethylation.

However, extracts of *E. coli* grown with added vitamin B_{12} can use N^5 -methyl- H_4PteGlu as a methyl donor in a cobalamin-dependent transmethylation to homocysteine; the same enzyme will also use methylcobalamin as a methyl donor (Woods *et al.* 1964). Even when *Coprinus* is grown with added vitamin B_{12} neither of these activities can be detected in extracts. Further, *Coprinus* contains only traces of endogenous vitamin B_{12} .

Coprinus extracts do not use *S*-adenosylmethionine as a methyl donor to homocysteine, nor does *S*-adenosylmethionine dilute the transfer of radioactivity from $N^5[^{14}\text{C}]$ -methyl- $\text{H}_4\text{PteGlu}_3$ to homocysteine.

We conclude that in *Coprinus* the conversion of homocysteine and serine into methionine is similar to the cobalamin-independent pathway in *E. coli*.

Botsford, J. L. & Parks, L. W. (1967). *J. Bact.* **94**, 966.

Guest, J. R., Foster, M. A. & Woods, D. D. (1964). *Biochem. J.* **92**, 488.

Guest, J. R., Friedman, S., Foster, M. A., Tejerina, G. & Woods, D. D. (1964). *Biochem. J.* **92**, 497.

Guest, J. R. & Woods, D. D. (1962). 2. *Europäisches Symposium über Vitamin B₁₂ und Intrinsic Factor*, p. 686. Ed. by Heinrich, H. C. Stuttgart: Ferdinand Enke Verlag.

Woods, D. D., Foster, M. A. & Guest, J. R. (1964). In *Transmethylation and Methionine Biosynthesis*, p. 138. Ed. by Shapiro, S. K. & Schlenk, F. Chicago: University of Chicago Press.

A Reactive Histidine Residue at the Active Site of Pig Heart Mitochondrial Malate Dehydrogenase

By B. H. ANDERTON and B. R. RABIN. (*Department of Biochemistry, University College London, London W.C.1, U.K.*)

Pig heart mitochondrial malate dehydrogenase (EC 1.1.1.37) was inactivated by iodoacetamide (3mM). By using radioactive iodoacetamide it was found that 2mol of the reagent were incorporated/mol of enzyme (mol.wt. 70000). The reaction occurred with N-3 of a histidine residue, and from the pH-rate profile pK_a 7.1 was determined for this residue. The enzyme lost no activity on incubation with 50mM-iodoacetate at pH 7.80.

NADH gave essentially complete protection against inactivation by iodoacetamide; NAD^+ , ADP-ribose and *N*-methylnicotinamide in contrast gave only partial protection. The substrates, oxaloacetate and malate, did not protect the enzyme, but a mixture of NAD^+ and oxaloacetate protected completely, demonstrating the formation of the abortive ternary complex enzyme- NAD^+ -oxaloacetate. Oxaloacetate also enhanced protection by ADP-ribose and complete protection could be obtained with the mixture, again demonstrating formation of a ternary complex. Thus the ADP-ribose moiety of the coenzyme is important in promoting substrate binding, perhaps by a conformational change in the enzyme.

From the protection data and use of different alkylating agents, some tentative conclusions have been drawn about the nature of the active site of mitochondrial malate dehydrogenase.

Comparative Effects of Chemical Modification on Human Alkaline Phosphatases

By D. W. MOSS. (*Royal Postgraduate Medical School, Ducane Road, London W.12, U.K.*)

Human alkaline phosphatases (EC 3.1.3.1) can be divided into at least three classes (see Moss, 1969), but, with the exception of placental phosphatase for which amino acid analyses have been published (Ghosh & Fishman, 1968; Harkness 1968), little is known about the underlying structures of these enzymes. Acetylation of rat intestinal phosphatase (Fishman & Ghosh, 1967) and bovine brain phosphatase (Brunel, Cathala & Saintot, 1969), and treatment of kidney phosphatase with cyanate (Carey & Butterworth, 1969), increases the electrophoretic mobility of the enzymes while decreasing their activity. These treatments have been applied to human hepatic, intestinal and