1968). The most important of these ammonia-assimilating enzymes appeared to be glutamate dehydrogenase and aspartate aminotransferase, and the present communication describes studies on the mechanism involved in the induction of these enzymes in *Rhizobium japonicum*.

When *R. japonicum* was transferred from a medium containing glutamate or aspartate to one containing 2-oxoglutarate plus ammonium chloride, the glutamate dehydrogenase and aspartate aminotransferase activity increased with time. Glutamate dehydrogenase activity in the latter medium was maximal after about 27 hr., after which the activity declined and reached its former value after about 90 hr. Aspartate aminotransferase, on the other hand, reached maximum activity after about 90 hr., after which it also began to decrease and reached its former value after about 120 hr.

Active protein and RNA synthesis was necessary for the induction of both enzymes. When actinomycin D (1.0 µg./ml.) was included in the growth medium, no increase in the activity of either glutamate dehydrogenase or aspartate aminotransferase was detected. Likewise, puromycin (2.5 µg./ml.) completely inhibited the induction of both enzymes. When cycloheximide was included in the growth medium at concentrations of 2 µg./ml., 3 µg./ml. and 4 µg./ml., the induction of aspartate aminotransferase was inhibited by 60%, 88% and 90% respectively.

The induction of both the aforementioned enzymes was completely reversible. For example, when the cells were transferred to a medium containing glutamate or aspartate, the glutamate dehydrogenase activity was completely repressed. Likewise, the activity of aspartate aminotransferase was completely repressed by aspartate in the growth medium. Considerable though not complete repression of glutamate dehydrogenase took place when the bacteria were transferred to a medium containing glutamine, ornithine or alanine.

These results show that enzymes active in ammonia assimilation in *R. japonicum* are induced or repressed by different nitrogen sources in the growth medium.

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Dissociation of the Prosthetic Group of Aspartate Aminotransferase

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Aspartate aminotransferase (EC 2.6.1.1) contains pyridoxal 5-phosphate (PLP), whose removal from the apoprotein is facilitated by conversion into pyridoxamine 5-phosphate (PMP), by acid pH, and by either phosphate or precipitation with ammonium sulphate (Banks & Vernon, 1961; Wada & Snell, 1962; Scardi, Scotto, Iaccarino & Scarano, 1963). Jenkins & D'Ari (1966) converted the enzyme into its PMP form, overcoming the unfavourable equilibrium constant, by gel filtration through a band of amino acid, so that the enzyme constantly encountered fresh reagent. We present a similar method for obtaining the apoenzyme.

Pig heart enzyme was obtained by extraction, heat treatment and ammonium sulphate fractionation modified from the method of Jenkins, Yphantis & Sizer (1959), followed by chromatography on microgranular CM-cellulose in 64 mM-sodium acetate-30 mM-acetic acid. The enzyme was then gel-filtered into water and freeze-dried.

For removal of the prosthetic group a column (70 cm. × 1 cm.) of dextran particles (Sephadex G-25, fine grade) was washed with water. Buffer (2 ml. of 50 mM-tris-25 mM-HCl) was added, then 1 ml. of water, and then 10 ml. of m-NaH₂PO₄-50 mM-glutamic acid-0.2 M-sodium glutamate. The enzyme (up to 50 mg.) dissolved in 1 ml. of 0.2 M-sodium glutamate was added, and the column was eluted with water at 40 ml.cm.⁻²hr.⁻¹. The protein peak was detected by its extinction at 280 mµ. It proved to have only about 2% of its potential activity until PLP was added, when 60% of the initial activity appeared. Activity was measured by rate of increase of extinction at 265 mµ in 10 mM-aspartic acid-4 mM-α-oxoglutaric acid-0.1 M-tris-50 mM-HCl. The apoenzyme was freeze-dried.

The glutamate added did not merely convert the PLP prosthetic group into PMP, but assisted the dissociation of the PMP. This was shown by gel-filtering samples (5 mg.) first through 2 ml. of 0.1 M-glutamic acid in 0.2 M-tris, which completely converted the enzyme into the PMP form, and then through 1 ml. of water followed by 5 ml. of a reaction mixture. Loss of coenzyme was complete in m-NaH₂PO₄-50 mM-sodium glutamate-50 mM-glutamic acid, but was only partial when the glutamate was replaced by acetate, glutarate or aspartate. The phosphate, however, could be replaced by sulphate.

Thus the method of Scardi *et al.* (1963) for removing the prosthetic group by treating the PMP form

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of the enzyme with phosphate at acid pH can be simplified, if glutamate is also added, by gel-filtering the enzyme through the reaction mixture.

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Regeneration of Aspartate Aminotransferase from its Apoenzyme

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Churchich & Farrelly (1968) found that the apoenzyme of pig heart aspartate aminotransferase (EC 2.6.1.1) recovered activity only slowly on incubation with 1 mM-pyridoxal 5-phosphate (PLP) in 0.1 M-phosphate buffer, pH 6.8, at 30°, although the aldimine bond with a specific lysine residue was rapidly formed. We found, however, that reactivation of the apoenzyme was almost complete in 10 min. at 20° in 2.0 ml. of 40 μ M-PLP-100 mM-tris-62.5 mM-HCl, which was made into the assay solution by addition of 0.5 ml. of 50 mM-aspartic acid-20 mM- α -oxoglutaric acid-0.1 M-tris. Comparison of these conditions, together with the known facilitation of the dissociation of pyridoxamine 5-phosphate (PMP) in acid solution by phosphate (Scardi, Scotto, Iaccarino & Scarano, 1963), suggested that phosphate might slow the recombination of PLP, and so this was investigated.

The apoenzyme was treated at 20° with PLP or PMP (10 μ M) in either 50 mM-tris-37.5 mM-HCl-100 mM - Na₂HPO₄ or 50 mM - tris - 25 mM - HCl - 287.5 mM-NaCl. These two solutions have the same pH and ionic strength. Samples were taken at various times and introduced into the assay mixture. Phosphate greatly slowed the rate of recovery of activity.

When phosphate accelerates the dissociation of PMP in acid solution (Scardi *et al.* 1963), it does not act merely by inhibiting recombination, since the rate of dissociation increases with phosphate concentration even under conditions in which the position of equilibrium overwhelmingly favours dissociation. It is therefore likely that there is an intermediate stage in which the phosphate ion has displaced the phosphate group of PMP, but in which the rest of the molecule is still bound. Com-

parison of the present results with those of Churchich & Farrelly (1968) points to the formation of a somewhat analogous intermediate during the reassociation of PLP. The aldimine bond is formed in this intermediate, but a phosphate ion prevents the prosthetic group from adopting the position in which it can function.

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Adaptation of Baker's Yeast to the Oxidation of Acetate

By J. P. GOSLING and P. F. DUGGAN. (*Department of Biochemistry, University College, Dublin, Irish Republic*)

After a short lag baker's yeast oxidizes acetate linearly (Weinhouse & Millington, 1947; Stoppani, Conches, de Flavelukes & Sacerdote, 1958; Duggan, 1964). The final rate attained is pH-dependent, being greater at lower pH values. However, when the concentration is sufficiently high the readily diffusible undissociated acetic acid interferes with its own oxidation (Duggan, 1964).

The lag in O₂-uptake rate decreases and disappears on storage at 4°. The lag may be extended by starvation of the yeast and this extension is proportional to oxygenation time whether the yeast is shaken in air or bubbled with O₂.

When 1 mM-ammonium chloride is added with 9 mM-acetate (final concentrations), it delays further the attainment of a linear O₂-uptake rate by starved yeast. However, if acetate or acetate and ammonium are added to yeast that is already oxidizing acetate linearly, there is no divergence in the subsequent O₂-uptake rates. Ammonium therefore does not affect the oxidation of acetate by adapted yeast. Ammonium has a stimulatory effect on the oxidation of glucose, an effect also found by Holzer & Witt (1964).

O₂-uptake rates and the specific activities of isocitrate lyase and malate dehydrogenase were measured in yeast oxidizing acetate. Starved and unstarved yeast was used at pH values 7.5 and 4.3. Glucose 6-phosphate dehydrogenase activity was also measured, as this enzyme is neither induced nor repressed in *Saccharomyces cerevisiae* under these conditions (Witt, Kronau & Holzer, 1966). In all cases there was close correlation between increases in O₂-uptake rate and increases in the specific activities of isocitrate lyase and malate dehydrogenase.

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