Metabolism of Inorganic Nitrogen Compounds in Plants and Micro-organisms

By D. J. D. NICHOLAS (*Department of Biochemistry, Waite Agricultural Research Institute, University of Adelaide, Adelaide, S. Austral. 5064, Australia*)

In this paper biochemical aspects of the following pathways will be discussed: (a) the assimilation of nitrate via ammonia into cell nitrogen compounds by micro-organisms and green plants; (b) the dissimilation of nitrate when it serves as an alternative terminal respiratory acceptor in place of O_2 in facultative bacteria; this includes the important denitrification process resulting in the production of nitrite and nitrogen gases (N_2O , NO and N_2) from nitrate; (c) the nitrification of ammonia to nitrate by soil bacteria; thus ammonia is oxidized to nitrite by *Nitrosomonas* and nitrite to nitrate by *Nitrobacter*.

Comparisons will be made between the mode of action of the various oxidoreductase enzymes involved in the reductive routes (a) and (b) and those found in the oxidative sequence (c). In addition, evidence will be presented for the various intermediates formed, some of which are common to both the oxidation and reduction pathways.

Induction of Asparagine Synthetase in γ -Irradiated Potatoes

By P. M. NAIR (*Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-85, India*)

The asparagine content of γ -irradiated (10 krd) potato (*Solanum tuberosum*) increased by about 20% in 24 h. This increase was not due to release of the amino acid on protein breakdown, for there was also a sevenfold increase of asparagine synthetase activity. Potato asparagine synthetase was specific for L-aspartate and devoid of any transferase activity. The reaction showed a definite requirement for ATP, Mg^{2+} and NH_4^+ . ADP and P_i were products of the reaction.

The activation of this enzyme is due not to hydrolysis or release of bound protein from an inactive complex but to synthesis *de novo*. This synthesis took place mainly in the bud tissue. When buds excised from irradiated potatoes were incubated maximum asparagine synthetase activity was discernible in 5 h, after which enzyme activity declined rapidly. During this time irradiated buds exhibited increased protein-synthetic capacity, as assessed by [^{14}C]leucine incorporation into soluble protein and its inhibition by cycloheximide, puromycin and actinomycin D. These inhibitors were capable of inhibiting the synthesis of asparagine synthetase also. The formation of

asparagine synthetase was suppressed by L-asparagine and more potently by β -aspartylhydroxamate. Asparagine synthetase from the bud tissue was shown to be electrophoretically homogeneous. Most of the radioactivity incorporated into soluble protein from [^{14}C]leucine went into asparagine synthetase protein. Also, there was a good correlation between the incorporation of [^{14}C]leucine into the enzyme fraction and increase in the specific activity.

The function of radiation-induced asparagine synthetase in the nitrogen metabolism of potatoes will be discussed.

Tryptophan Metabolism in Micro-organisms

By MITSUHIRO NOZAKI and YUZURU ISHIMURA (*Department of Medical Chemistry, Kyoto University Faculty of Medicine, Yoshida, Sakyo-ku, Kyoto, Japan*)

In micro-organisms tryptophan is degraded by several different pathways. Three of these pathways are initiated by oxygenase-catalysed reactions: (1) cleavage of the indole ring to form formylkynurenine (Hayaishi & Stanier, 1951); (2) mono-oxygenation of tryptophan to 5-hydroxytryptophan (Mitoma *et al.*, 1956); (3) oxygenative decarboxylation of tryptophan to indol-3-ylacetamide (Kosuge *et al.*, 1966). The first two are also known to be major pathways in mammals. Previous studies in our own and other laboratories revealed that many other oxygenase-catalysed reactions besides the initial step of the degradation also play an important role in the metabolism of tryptophan (Hayaishi & Nozaki, 1969).

In this communication we review the role of oxygenases in tryptophan metabolism and discuss our current work on the reaction mechanism of tryptophan 2,3-dioxygenase purified from *Pseudomonas fluorescens*. Tryptophan 2,3-dioxygenase, commonly known as tryptophan pyrrolase, is a haem-containing dioxygenase (Hayaishi *et al.*, 1957; Tanaka & Knox, 1959) and catalyses the insertion of two oxygen atoms from molecular O_2 into the pyrrole moiety of tryptophan to formylkynurenine. A catalytically inactive ferric form of the enzyme shows a characteristic absorption of a high-spin haemoprotein (λ_{max} at 405, 500 and 635 nm). The ferric enzyme can be converted into an active ferrous form (λ_{max} at 432, 553 and 558 nm) by the addition of an appropriate amount of certain reductants such as $Na_2S_2O_4$. A new spectral species of the enzyme (λ_{max} at 418, 545 and 580 nm), distinct from those of the ferric or the ferrous enzyme or a mixture of both, was observed during the steady state of the reaction (Ishimura *et al.*, 1967). The formation of the new species was absolutely dependent on the simultaneous presence of both O_2 and tryptophan with the ferrous

enzyme. Further analysis of the detailed kinetics by the stopped-flow technique revealed that the observed species was a ternary complex of O_2 , the enzyme and tryptophan, an oxygenated form of the enzyme and an obligatory intermediate in the reaction (Ishimura *et al.*, 1971). The following reaction mechanism was therefore compatible with the above data: the ferrous form of the enzyme first combines with tryptophan and then reacts with O_2 to form an oxygenated intermediate, degradation of which is the rate-limiting step of the overall reaction. This reaction mechanism is consistent with the view that dioxygenase reactions in general involve a ternary complex of enzyme, O_2 and organic substrate (Nozaki *et al.*, 1968). A similar oxygenated intermediate has been demonstrated with protocatechuate 3,4-dioxygenase, a non-haem-iron-containing dioxygenase (Fujisawa *et al.*, 1971).

- Fujisawa, H., Hiromi, K., Uyeda, M., Nozaki, M. & Hayaishi, O. (1971) *J. Biol. Chem.* **246**, 2320
 Hayaishi, O. & Nozaki, M. (1969) *Science* **164**, 389
 Hayaishi, O. & Stainer, R. Y. (1951) *J. Bacteriol.* **62**, 691
 Hayaishi, O., Rothberg, S., Mehler, A. H. & Saito, Y. (1957) *J. Biol. Chem.* **229**, 889
 Ishimura, Y., Nozaki, M., Hayaishi, O., Tamura, M. & Yamazaki, I. (1967) *J. Biol. Chem.* **242**, 2574
 Ishimura, Y., Nozaki, M., Hayaishi, O., Nakamura, T., Tamura, M. & Yamazaki, I. (1971) *J. Biol. Chem.* **245**, 3593
 Kosuge, T., Heskett, M. G. & Wilson, E. E. (1966) *J. Biol. Chem.* **241**, 3788
 Mitoma, C., Weissbach, H. & Udenfriend, S. (1956) *Arch. Biochem. Biophys.* **63**, 122
 Nozaki, M., Nakazawa, T., Fujisawa, H., Kotani, S., Kojima, Y. & Hayaishi, O. (1968) *Advan. Chem. Ser.* **77**, 242
 Tanaka, T. & Knox, W. E. (1959) *J. Biol. Chem.* **234**, 1162

Biosynthesis of Flavonoid Compounds: Enzyme Activities in Relation to Cell Growth and Differentiation

By K. Hahlbrock (*Biologisches Institut II der Universität Freiburg, 78 Freiburg, Germany*)

Synthesis and degradation of secondary plant products can often be directly related to growth and development of certain organs or to processes of differentiation of special types of cells. One of the most widespread groups of secondary plant metabolites is that of flavonoid compounds. Most of the general aspects of their biosynthesis have been elucidated by feeding experiments with labelled precursors (Grisebach, 1968). Recently the relationship between organ development in young parsley plants and changes in the accumulation of flavone glycosides and in the activities of some of the enzymes related to their biosynthesis has been studied. As a result of these investigations, a high degree of correlation

between development of cotyledons and leaves and activities of enzymes involved in the formation of flavone glycosides was demonstrated (Hahlbrock *et al.*, 1971).

For experimental reasons more detailed studies on the relationship between cell differentiation, accumulation of flavonoid compounds and activities of the enzymes related to this biosynthetic pathway were carried out with cells grown in suspension culture. In contrast with parsley plants, cells grown in the dark in liquid medium (containing mineral salts, vitamins, sucrose and 2,4-dichlorophenoxyacetic acid) did not produce flavone glycosides. Induction of enzymes and subsequent formation of flavone glycosides were taken as an expression of cell differentiation and were initiated by treatment of dark-grown cultures with light (Hahlbrock & Wellmann, 1970).

All of the enzymes investigated exhibit a high degree of correlation with regard to changes in their activities during the initial stages of flavonoid biosynthesis. However, two groups of enzymes could be distinguished by the time-lag before reaching maximum activity. Group I consists of the three enzymes acting on substrates of the phenylpropanoid type (phenylalanine ammonia-lyase, cinnamate 4-hydroxylase and *p*-coumarate-CoA ligase), whereas group II comprises all those enzymes related exclusively to the formation of flavonoid compounds and their glycosides (chalcone-flavanone isomerase, UDP-glucose-flavone 7-*O*-glucosyltransferase, UDP-apirose, flavone-7-*O*-glucoside apiosyltransferase, UDP-apirose synthetase and *S*-adenosylmethionine-luteolin 3'-*O*-methyltransferase).

- Grisebach, H. (1968) in *Recent Advances in Phytochemistry* (Mabry, T. J., Alston, R. E. & Runeckles, V. C., eds.), vol. 1, p. 379, Appleton-Century-Crofts, New York
 Hahlbrock, K. & Wellmann, E. (1970) *Planta* **94**, 236
 Hahlbrock, K., Sutter, A., Wellmann, E., Ortmann, R. & Grisebach, H. (1971) *Phytochemistry* **10**, 109

Role of Indol-3-ylacetic Acid in the Induction of Enzymes during Seed Germination

By C. R. KRISHNA MURTI (*Central Drug Research Institute, Lucknow, India*)

Biochemical morphogenesis has not attracted the same degree of attention in plants as in animals or in unicellular micro-organisms, notwithstanding the dramatic changes in the growth of plant tissues that occur under the influence of plant hormones. Differentiated single cells cultured in chemically defined media would be the ideal system for the study of the regulation of biosynthesis in plants. However, investigations with whole germinating seeds can also be considered as a practical approach for elucidating the