Metabolism of Inorganic Nitrogen Compounds in Plants and Micro-organisms

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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

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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

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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