These results imply that the binding sites for oligomycin and carbonyl cyanide trifluoromethoxyphenylhydrazone are on the ATP synthetase. *p*-Chloromercuribenzenesulphonate also inhibits the exchange reaction, which points to the involvement of a thiol group in the exchange reaction. The energy-coupling factor D resolved from the complex is also inhibited by *p*-chloromercuribenzenesulphonate in its activity (stimulating ATP-dependent NAD⁺ reduction by succinate on addition to the urea-depleted submitochondrial particle).

The ATP-P_i exchange activity and 95% of the protein remain in the supernatant fraction when the synthetase is centrifuged at 105000g for 60min in 60mM or higher concentrations of phosphate buffer. In 10mM-phosphate buffer a significant fraction of the protein sediments and the activity in the supernatant liquid is considerably diminished. This

suggests aggregation of the ATP synthetase in solutions of low salt concentration.

The ATP synthetase stimulates the ATP-P_i exchange activity of urea-depleted submitochondrial particles. The specific activity is increased more than tenfold in the presence of urea-depleted submitochondrial particles. This may be due to allosteric activation of the ATP synthetase when it is bound to the membrane or to some necessary component that is provided by the membrane.

The ATP-ADP exchange rate is at least twice the ATP-P_i exchange rate, and this would indicate the formation of X~P as an intermediate in ATP synthesis. These data suggest that the soluble ATP synthetase complex contains X~C. This represents the first report of a soluble ATP-P_i exchange activity related to mitochondrial oxidative phosphorylation.

SYMPOSIUM ON PLANT SCIENCES

The Ecological Significance of 'Unusual' Amino Acids in Plants

By E. A. BELL (*Department of Botany, University of Texas at Austin, Austin, Tex. 78712, U.S.A.*)

Twenty amino acids are found universally distributed as protein constituents in all living organisms. In addition to these a few others occur frequently as metabolic intermediates. The great majority of natural amino acids are more limited in their distribution, however, and relatively little is known of their metabolism or significance in the organisms that synthesize them.

The higher plants are a rich source of such 'unusual' amino acids: 60 for example have been isolated from or identified in species of one single family, the Leguminosae (Bell, 1971). Certain of the 'unusual' amino acids that have been isolated from this and other plant families are known to be toxic to other species of plants, to micro-organisms, to insects or to animals. Bell & Janzen (1971) have suggested that the high concentrations of L-3,4-dihydroxyphenylalanine (L-dopa) and 5-hydroxy-L-tryptophan that occur in species of *Mucuna* and *Griffonia* may serve to protect the seeds of these species from insect attack. This and other possible roles of 'unusual' amino acids will be discussed.

Bell, E. A. (1971) in *Chemotaxonomy of the Leguminosae* (Harborne, J. B., Boulter, D. & Turner, B. L., eds.), p. 179, Academic Press, London and New York

Bell, E. A. & Janzen, D. H. (1971) *Nature (London)* **229**, 136

Studies on the Metabolism of *sym*-Homospermidine in Sandal (*Santalum album* L.)

By R. KUTTAN and A. N. RADHAKRISHNAN (*Wellcome Research Unit, Christian Medical College Hospital, Vellore-4, Tamil Nadu, India*)

Although the occurrence of polyamines, spermine and spermidine, in the animal kingdom has been established for a long time (Tabor & Tabor, 1964), only limited reports are available on their occurrence in plants (Smith, 1970). A new polyamine, *sym*-homospermidine, has been isolated from sandal leaves and it is present in a free state in high concentrations: 0.5-1.5% of the dry leaves (Kuttan *et al.*, 1971). The present communication deals with the study of the biosynthesis of this polyamine in the sandal plant by using radioactive amino acids as precursors. The procedure for the infiltration of amino acids into the leaves under vacuum has been described previously (Kuttan & Radhakrishnan, 1970). For determination of radioactivity homospermidine was isolated by using a small Dowex 50 (X8; H⁺ form) ion-exchange column and eluting with different concentrations of HCl. With DL-[¹⁴C]ornithine as the precursor nearly 7.5% of the initial radioactivity (assuming only the L-isomer was active) was incorporated into homospermidine. When L-arginine was used nearly 5.5% of the initial radioactivity was in the homospermidine. However, considering the pool sizes of the respective amino acids, arginine was nearly ten times as effective as ornithine. Proline and glutamic acid also served as precursors. With L-[¹⁴C]arginine as the precursor the radioactivity in homospermidine was significantly

diminished by unlabelled agmatine, putrescine and ornithine. Similarly with DL-[^{14}C]ornithine as the precursor labelling of homospermidine was decreased by unlabelled putrescine and arginine. From the results of these experiments it was considered that putrescine, for which multiple pathways exist, was the main intermediate in the synthesis of homospermidine.

In view of the symmetrical nature of the homospermidine molecule it was further considered that the other half of the molecule was also derived from putrescine. The biosynthesis may then involve a Schiff-base formation between putrescine and γ -aminobutyraldehyde, a metabolite of putrescine. Further reduction of the Schiff base would yield homospermidine. Such a mechanism is supported by the observed incorporation of ^3H from the medium into homospermidine, suggesting a nicotinamide nucleotide-dependent reduction. Also, in model systems containing γ -aminobutyraldehyde and putrescine, followed by reduction with NaBH_4 or Pt/H_2 , the formation of homospermidine was demonstrated. On the basis of these results a scheme for the biosynthesis of homospermidine in sandal is presented.

Kuttan, R. & Radhakrishnan, A. N. (1970) *Biochem. J.* **117**, 1015

Kuttan, R., Radhakrishnan, A. N., Spande, T. & Witkop, B. (1971) *Biochemistry* **10**, 361

Smith, T. A. (1970) *Ann. N.Y. Acad. Sci.* **171**, 988

Tabor, H. & Tabor, C. W. (1964) *Pharmacol. Rev.* **16**, 245

The Regulation of Nitrate Reductase in *Neurospora crassa*

By K. N. SUBRAMANIAN and G. J. SORGER (*Department of Biology, McMaster University, Hamilton, Ont., Canada*)

Nitrate is known to be necessary for the synthesis of nitrate reductase, and ammonium ion is known to inhibit the formation of this enzyme. The sites of action of nitrate and ammonium ions are unknown.

Neurospora mycelia were grown on ammonium ions as nitrogen source and subsequently induced by transfer to a medium containing nitrate and cycloheximide. Under these conditions nitrate reductase mRNA accumulated in the absence of protein synthesis and was measured by again transferring the mycelia to a medium containing nitrate and actinomycin D for 120 min, to allow the formation of nitrate reductase by translation of the existing mRNA. Nitrate was essential for the accumulation of nitrate reductase mRNA, and ammonium ions prevented this accumulation. Ammonium ions had no effect on the translation of the accumulated nitrate reductase mRNA. Ammonium ion is obviously acting as co-repressor and nitrate as co-inducer.

Mycelia in which nitrate reductase had been maximally induced by nitrate were transferred to fresh media containing different nitrogen sources. Nitrate stabilized the enzyme *in vivo*, as shown by contrast with the rapid loss of enzyme activity on transfer of mycelia to a medium containing no nitrogen source. Ammonium ions decreased the stability of nitrate reductase *in vivo*. Nitrate did not antagonize this effect of ammonium ions, which suggests that nitrate and ammonia might act at two different sites. Cycloheximide partially protected nitrate reductase from loss of activity after the removal of nitrate or the addition of ammonium ions.

In contrast with the tobacco cell-culture system of Heimer & Filner (1971) we have found no evidence for inhibition of nitrate uptake by ammonium ions in *Neurospora*.

Heimer, Y. M. & Filner, P. (1971) *Biochim. Biophys. Acta* **230**, 362

Role of Light in the Induction of Nitrate Reductase and Nitrite Reductase in Rice Seedlings

By S. K. SAWHNEY and M. S. NAIK (*Division of Biochemistry, Indian Agricultural Research Institute, New Delhi-12, India*)

Assimilatory nitrate reductase is a substrate-induced enzyme both in higher plants and micro-organisms, but in green tissues light is also essential for the synthesis of the enzyme (Travis *et al.*, 1970a). The precise role of light is obscure at present (Beever & Hageman, 1969; Travis *et al.*, 1970b).

In germinating rice (*Oryza sativa*, var. TN-1) seeds light was not found to be necessary for the synthesis of nitrate reductase and nitrite reductase up to 7 days. After this period light substantially stimulated the synthesis of the former enzyme but was without effect on the latter. Subsequent transfer of seedlings to the dark resulted in a continuous decline in the activity of nitrate reductase. Lower activity in the absence of light could not be attributed to the production of inhibitors or to a depressed uptake of the inducer. NADH oxidase activity was higher in dark-grown seedlings, but this also did not account for the lower NADH-nitrate reductase activity.

Beever, L. & Hageman, R. H. (1969) *Annu. Rev. Plant Physiol.* **20**, 495

Travis, R. L., Jordan, W. R. & Huffaker, R. C. (1970a) *Physiol. Plant.* **23**, 678

Travis, R. L., Huffaker, R. C. & Key, J. L. (1970b) *Plant Physiol.* **46**, 800