in the range 30000–120000). In 0.1% sodium dodecyl sulphate–2-mercaptoethanol the enzyme behaves as a homogeneous species whose molecular weight, after correction for a large amount of sodium dodecyl sulphate binding, is 31000.

By the use of dansylation procedures equal amounts of *N*-terminal lysine and valine are detected for both the phospho- and the dephospho-enzyme. The latter, when subjected to the carbamoylation technique of Stark & Smythe (1963), gives 0.90–1.09 mol of valine/62000 g of phosphoglucomutase. The evidence indicates that phosphoglucomutase is a dimer of subunits that are about the same size but have different *N*-terminal residues.

Stark, G. R. & Smythe, D. G. (1963) *J. Biol. Chem.* **238**, 214

Studies on Tissue Cultures of Maize, Wheat, Rice and Sorghum

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Tissues of monocotyledonous plants have been more difficult to grow *in vitro* than dicotyledonous tissues. Callus cultures of maize (*Zea mays*), wheat (*Triticum vulgare*), rice (*Oryza sativa*) and sorghum (*Sorghum vulgare*) were obtained and have been shown to be viable on prolonged subculture *in vitro* for 3–6 years (Mascarenhas *et al.*, 1969). This is the first report of viable callus and root cultures of maize and sorghum. The nutrition and differentiation of these tissues were studied. Good growth was obtained on chemically defined media consisting of inorganic salts, sucrose, an amino acid mixture, vitamins and auxin. No requirement for a cytokinin, gibberellin or coconut milk could be demonstrated. Naphth-1-ylacetic acid (5–10 p.p.m.) gave maximum growth. The growth curves of maize and wheat in liquid culture and the utilization of sucrose, N, P and Fe were determined.

Maize, wheat and sorghum showed the unusual property of growing mostly as callus on agar, whereas on the same medium in shake flasks without agar they grew only as a mass of roots. The roots grew again as callus when transferred back to agar. This differentiation and de-differentiation of maize, wheat and sorghum tissues are unique, since they require no change in the composition of the medium and are readily reversible.

Mascarenhas, A. F., Hendre, R. R., Seetarama Rao, B. & Jagannathan, V. (1969) *Indian J. Exp. Biol.* **7**, 65

Role of δ -Aminolaevulinate Dehydratase in the Regulation of Haem Synthesis

By G. PADMANABAN and K. MALATHI (*Department of Biochemistry, Indian Institute of Science, Bangalore-12, India*)

It is believed that δ -aminolaevulinate dehydratase is not a rate-limiting enzyme in the pathway of haem biosynthesis, since it has been found to be present in considerable excess of δ -aminolaevulinate synthetase, the first enzyme of the pathway, in animal and bacterial systems (Lascelles, 1964). However, detailed investigations with *Neurospora crassa* have shown that the enzyme is rate-limiting in this organism, and that it can be induced by iron and repressed by protoporphyrin (Muthu Krishnan *et al.*, 1968, 1969). *Neurospora* extracts contain a protein inhibitor for the enzyme and an unidentified low-molecular-weight activator. The enzyme is also subject to feedback inhibition by coproporphyrinogen III. The existence of so many controls on the enzyme indicates its regulatory nature, which is also supported by the rapid turnover of this protein in the organism, the half-life for the induced enzyme being 4.5 min. The possible regulation of this enzyme at the translational level and transcriptional level will be discussed. δ -Aminolaevulinate dehydratase activity has also been found to be low in normal yeast, where it appears to have a role in haem synthesis and mitochondriogenesis (Jayaraman *et al.*, 1971). However, the enzyme is present at very high activities in photosynthetic bacteria, higher plants and animal systems. Yeast and fungi appear to constitute a separate class where the enzyme is rate-limiting, and the protozoan *Euglena gracilis* exhibits an intermediate behaviour.

Jayaraman, J., Padmanaban, G., Malathi, K. & Sarma, P. S. (1971) *Biochem. J.* **121**, 531

Lascelles, J. (1964) *Tetrapyrrole Biosynthesis and its Regulation*, W. A. Benjamin, New York

Muthu Krishnan, S., Padmanaban, G. & Sarma, P. S. (1968) *Biochem. Biophys. Res. Commun.* **31**, 333

Muthu Krishnan, S., Padmanaban, G. & Sarma, P. S. (1969) *J. Biol. Chem.* **244**, 4241

Regulation of Coenzyme Nucleotide Metabolism in Plants

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The concentrations of coenzyme nucleotides could be controlled by environmental conditions, by regulation of the enzymes involved in their biosynthesis

and degradation and by the availability of apoenzymes (Kaplan, 1966). The evidence in support of any one of these hypotheses is meagre.

Flavokinase is the first enzyme in the biosynthesis of flavin coenzyme from riboflavin. This reaction is essentially irreversible and specific for the substrates ATP and riboflavin (Giri *et al.*, 1958). The second enzyme, FAD synthetase, catalyses the reversible reaction yielding FAD and PP_i in the forward reaction (Giri *et al.*, 1960).

The enzymes cleaving FAD function optimally at pH 3.8 (Ravindranath & Appaji Rao, 1971), pH 7.4 (Kumar *et al.*, 1965) and pH 9.4 (Ravindranath & Appaji Rao, 1968). The enzyme cleaving FAD at pH 9.4 is inhibited uncompetitively by AMP and non-competitively (linear) by ATP. The partially inactivated enzyme is activated by ADP (Ravindranath & Appaji Rao, 1969). Reversible denaturation with urea causes decreased affinity and increased activity. This enzyme contains zinc, which can be replaced by cobalt without significantly altering the properties of the enzyme. The enzyme functioning optimally at pH 7.4 changes its locus of attack on treatment with thiol-group reagents. This change appeared to be due to the reduction of a disulphide bond in the protein. The V_{max} , K_m and temperature optima were unaltered. The non-specific acid pyrophosphatase probably has a scavenging function and is significantly activated by AMP.

These results suggest that AMP formed during the initial stages of hydrolysis slows down the rapid degradation of coenzyme nucleotides. On the other hand ATP, a substrate for both the biosynthetic reactions, inhibits in a non-competitive manner, presumably by affecting the rate-limiting release of AMP. This control would permit the biosynthesis to proceed uninterrupted. Compartmentation of these enzymes could be an additional parameter in the control of coenzyme nucleotides.

Giri, K. V., Krishnaswamy, P. R. & Appaji Rao, N. (1958) *Biochem. J.* **70**, 66

Giri, K. V., Appaji Rao, N., Cama, H. R. & Kumar, S. A. (1960) *Biochem. J.* **75**, 381

Kaplan, N. O. (1966) in *Current Aspects of Biochemical Energetics* (Kaplan, N. O. & Kennedy, E. P., eds.), p. 447, Academic Press, New York

Kumar, S. A., Appaji Rao, N. & Vaidyanathan, C. S. (1965) *Arch. Biochem. Biophys.* **111**, 646

Ravindranath, S. D. & Appaji Rao, N. (1968) *Indian J. Biochem.* **5**, 137

Ravindranath, S. D. & Appaji Rao, N. (1969) *Arch. Biochem. Biophys.* **133**, 54

Ravindranath, S. D. & Appaji Rao, N. (1971) *Indian J. Biochem. Biophys.* in the press

Structure and Possible Regulatory Properties of Plant Aldolases

By B. L. HORECKER (*Department of Molecular Biology, Division of Biological Sciences, Albert Einstein College of Medicine, Bronx, N.Y. 10461, U.S.A.*)

Fructose diphosphate aldolase has been purified from a variety of plant and animal sources. Spinach leaf aldolase has been isolated in homogeneous form in our laboratory and shown to resemble mammalian aldolases, although the molecular weight is smaller. The enzyme is a tetramer with molecular weight 120000, compared with 160000 for mammalian aldolases. The fructose 1,6-diphosphate/fructose 1-phosphate activity ratio is approx. 20:1, and both activities are decreased during removal of the C-terminal tyrosine residues with carboxypeptidase. However, the four tyrosine residues are not equally sensitive, and loss of activity towards fructose 1,6-diphosphate is associated with removal of the one or two most sensitive residues. This suggests a co-operative interaction between the subunits. Aldolase is found in both chloroplasts and cytosol, and two forms can be separated by chromatography.

A pentapeptide isolated from the active site has been shown to have the sequence Leu-Leu-Lys-Pro-Ser. This sequence is similar to that found in all aldolases from animals, both invertebrate and vertebrate, in which the corresponding sequence is Leu-Leu-Lys-Pro-Asn. The possible significance of this homology will be discussed.

Generation of Reducing Potential in Photosynthesis

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The utilization of light energy for assimilation of CO₂ into cellular material requires that the available (and absorbed) electromagnetic energy be converted into chemical free energy. It is generally accepted that the end products of this conversion process are reduced ferredoxin and ATP plus molecular O₂.

During the past 2 years we have isolated and purified a new carrier that functions to accept electrons from photosystem I and in the reduced form serves as the reductant for ferredoxin reduction. This new factor, termed 'ferredoxin-reducing substance', is a peptide of low molecular weight (about 4600) and appears to contain an isoflavone chromophore.

Initially we demonstrated that the photoreduction of NADP⁺ by sonicated chloroplast fragments required the presence of ferredoxin-reducing substance. To demonstrate unequivocally that ferredoxin-reducing substance functions on the reducing side of photosystem I, the reduction of NADP⁺ was