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.

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Structure and Possible Regulatory Properties of Plant Aldolases

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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

By A. SAN PIETRO (*Department of Botany, University of Indiana, Bloomington, Ind. 47401, U.S.A.*)

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

carried out in two stages: the photoreduction of ferredoxin-reducing substance followed by the dark reduction of NADP (or ferredoxin) by photo-reduced ferredoxin-reducing substance.

The reaction partner for the photoreduction of ferredoxin-reducing substance is the specialized form of chlorophyll *a*, chlorophyll *P*-700. After a 1 ns flash to oxidize chlorophyll *P*-700 to chlorophyll *P*-700*, the dark kinetics of the reduction of chlorophyll *P*-700* are consistent with this reaction scheme.

Additional information on the characteristic potential, amino acid composition and other properties of ferredoxin-reducing substance will be presented.

Biosynthesis of Fatty Acids by Isolated Chloroplasts

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Because of the importance of lipids in the structural components of chloroplasts in higher plants, studies on the biosynthesis of these compounds will provide a better understanding of the functional properties of the organelles.

Since the discovery by Smirnov (1960) of the capacity of chloroplasts to synthesize fatty acids from acetate, a number of investigators have extended our knowledge in this area. At present, isolated class I spinach chloroplasts (with intact outer envelope) readily incorporate [¹⁴C]acetate into palmitate and oleate in the presence of ATP, CoA, Mg²⁺, CO₂ and light (Stumpf *et al.*, 1967). In the absence of light, little if any synthesis occurs. When Triton X-100 or one of a number of other detergents is added to the reaction at very low concentrations, a large increase in fatty acid synthesis occurs as well as a marked increase in the formation of oleate. This effect is reversed specifically by increasing the concentration of ATP, which lowers the rate of synthesis as well as the extent of desaturation (Stumpf & Boardman, 1970). Further studies revealed that the site of regulation of Triton X-100 and ATP is at the C₁₆ → C₁₈ elongation step (Givan & Stumpf, 1971) and is not directly related to the desaturation reaction.

Very recently we have detected rather significant accumulation of acyl thiol esters in intact chloroplasts formed *de novo* from [¹⁴C]acetate. These acyl thiol esters have a fivefold higher amount of unsaturated acyl components than do the oxygen esters, suggesting that desaturation takes place on an acyl thiol ester level. Also present in chloroplasts are acyl-CoA synthetases, acyl-CoA hydrolase and transacylases (C. G. Kannagara & P. K. Stumpf, unpublished work). The interaction of these and other

enzymes in the biosynthetic capacity of isolated chloroplasts will be further described.

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The Sulphoglycolytic Pathway in Plants

By A. A. BENSON and RICHARD F. LEE (*Scripps Institution of Oceanography, University of California, San Diego, La Jolla, Calif. 92037, U.S.A.*)

The ubiquity of sulpholipid in green plants and of sulphopropanediol in all diatoms implies the existence of biodegradative pathways for such compounds. Martelli & Benson (1964) have outlined the terminal portions of the pathway for bacterial degradation of sulphoquinovose (6-deoxyglucose-6-sulphonate). Martelli & Souza (1970) established the ultimate conversion into glyoxylate-sulphite addition product followed by production of sulphate ion.

In experiments with intact leaves of alfalfa (*lucerne*; *Medicago sativa*) and the coral tree (*Erythrina cristagalli*) we have observed that glycerol sulphoquinovoside is readily degraded whereas sulphoquinovose is relatively resistant. Sulpholactaldehyde, sulpholactate, sulphopropanediol, analogues of glyceraldehyde 3-phosphate, 3-phosphoglycerate and glycerol 1-phosphate were identified among the intermediates. The first step appears to be a phosphorolysis of glycerol sulphoquinovoside, since free sulphoquinovose was only slowly metabolized by any system yet examined. Hydrolysis of glycerol sulphoquinovoside would be expected to yield appreciable concentrations of sulphoquinovose since the observed rate of degradation of the latter was relatively low.

A striking difference was observed between the metabolism of glycerol [³⁵S]sulphoquinovoside by leaves of alfalfa and the Indian shrub *Erythrina*. The major compound produced by *Erythrina* was sulphoacetate, analogue of phosphoglycollate. Alfalfa and other plants produced first sulpholactaldehyde and then, after about 24 h, sulpholactate. Sulphate ion appeared slowly thereafter. We presume that *Erythrina* leaves utilize the sulphoglycolytic pathway but possess a more active system for decarboxylating sulpholactate than do most plants. The alkaloids of *Erythrina* bark are esters of sulphoacetate (Folkers, 1944; Mondon, 1970). Massive accumulation of sulpholactate in spores of *Bacillus subtilis* was observed by Bensen *et al.* (1969). Relative activities of enzymes in the sulphoglycolytic pathway determine