Light-Driven Synthesis of the Large Subunit of Fraction I Protein by Isolated Chloroplasts

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Treatment of greening cells with 70S ribosomal inhibitors such as lincomycin results in the inhibition of the synthesis of fraction I protein, but not that of other photosynthetic enzymes or chloroplast RNA polymerase (Ellis & Hartley, 1971). Fraction I protein is the major protein found in chloroplasts, and consists of large and small subunits (Rutner & Lane, 1967). The differential labelling of the large and small subunits *in vivo* (Kawashima, 1970) and the sensitivity of such labelling to chloramphenicol and cycloheximide suggests that only the large subunit is made by chloroplast ribosomes (Criddle *et al.*, 1970). However, conclusive evidence can only come from studies with isolated chloroplasts.

Isolated chloroplasts are known to carry out the incorporation of labelled amino acids into protein by means of a 70S ribosomal system, but there has been no convincing identification of any specific protein that these ribosomes synthesize *in vitro* (Kirk, 1970). We believe that the reason for this is that precautions were not taken to ensure that incorporation takes place only in intact chloroplasts in which conditions for correct termination and release of polypeptide chains are likely to be optimal. We now report that isolated intact pea chloroplasts synthesize the large subunit of fraction I protein but not the small subunit.

Chloroplasts were isolated by the rapid method of Ramirez *et al.* (1967) from 7–10-day-old pea plants (*Pisum sativum*) grown at 2000 lx on a 12h photo-period. Incorporation of [¹⁴C]leucine into protein is stimulated 20-fold by red light in the absence of either added ATP or catalysts of photophosphorylation; rates of incorporation are in the range 0.5–1.0 nmol of [¹⁴C]leucine/h per mg of chlorophyll at 20°C. Incorporation is inhibited by chloramphenicol, by lincomycin and by lysis of the chloroplasts, but not by ribonuclease. Lysed chloroplasts supplemented with ATP and GTP show very low incorporation. We believe therefore that protein synthesis is proceeding only in intact chloroplasts.

Analysis of the chloroplast soluble protein by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis and gel filtration revealed only one labelled product, which migrates exactly with the large subunit of fraction I protein. This product is not found at zero time, in chloroplasts incubated in the dark or in the presence of chloramphenicol.

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Pathways of Malate Oxidation in Isolated Plant Mitochondria

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Plant mitochondria differ from animal mitochondria in that they will readily oxidize malate in the absence of an oxaloacetate-removing system (Palmer, 1967). Further, it has been reported that rotenone only partially inhibited this oxidation and markedly decreased the ADP/O ratio; the latter feature was not observed when succinate was used as the substrate (Ikuma & Bonner, 1967). It has been reported (Palmer & Passam, 1971; Coleman & Palmer, 1971) that plant mitochondria contain two NADH dehydrogenases localized on opposite sides of the inner membrane: the dehydrogenase on the inside of the membrane is inhibited by rotenone and oxidizes endogenous NADH, and the dehydrogenase on the outside of the membrane is inhibited by ethyleneglycol-bis-(β -amino-ethyl-ether)N,N'-tetra acetic acid, is activated by Ca²⁺ and is responsible for the oxidation of exogenous NADH.

We now report that there are also two pathways for oxidation of malate in plant mitochondria. By using ferricyanide as a non-penetrating electron acceptor and butylmalonate as an inhibitor of malate uptake it has been possible to show that they are located on opposite sides of the inner membrane. Determination of the products formed as a result of malate oxidation suggested that malate dehydrogenase was responsible for the oxidation in the inner compartment and that an NAD-linked 'malic' enzyme was responsible for the oxidation in the outer compartment. With the use of rotenone or piericidin A to inhibit the internal dehydrogenase and ethyleneglycol-bis-(β -amino-ethyl-ether)N,N'-tetra acetic acid to inhibit the external dehydrogenase it was possible to show that the malate dehydrogenase was linked to electron transport by the internal NADH dehydrogenase and coupled to phosphorylation at three sites, whereas the 'malic' enzyme utilized the external NADH dehydrogenase and was coupled to phosphorylation at only two sites. The isolation and partial purification of the 'malic' enzyme from Jerusalem-artichoke mitochondria has been carried

out and the enzyme shows properties similar to that already obtained from several other plant tissues (McCrae, 1971), suggesting that the two pathways for malate oxidation may be a general property of higher-plant mitochondria. The operation of these two pathways may be of importance in controlling the redox equilibrium of NAD in the mitochondrial matrix and in the cytosol.

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The Role of Component C₁ in Cellulolytic Systems

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Over the years, biological attack on cellulose has been variously attributed to a single enzyme, CM-cellulase (Whitaker, 1953), or to a prime component called C₁ thought to produce shorter chains for a more conventional hydrolysis by CM-cellulase (Reese *et al.*, 1950). Other workers support a modified version of the last scheme in which the combined action of both CM-cellulase and component C₁ is believed to be essential, since no specific function has so far been assigned to component C₁ (Selby, 1968).

Recent evidence from this laboratory showed that component C₁ hydrolysed native cellulose to cellobiose in a solubilizing reaction promoted more effectively by cellobiase than by CM-cellulase (Halliwell & Riaz, 1971). Later preparations of component C₁ indicate that the stimulation of cellulolysis so achieved can be increased even further by up to 80%. Prolonging the incubation beyond the usual period of 7 days had little effect on cellulolysis by component C₁ alone. The additional presence of cellobiase, however, proved beneficial in enabling up to 70% solubilization of cotton to be attained in 21 days. The only products recognized chromatographically in the two systems were cellobiose and glucose respectively. The ability of cellobiase, which itself had no action on cellulose, to promote saccharification of the substrate by component C₁ suggested that this component was inhibited by its product cellobiose. Inhibition was competitive, being dependent on the relative concentrations of cellulose

and cellobiose and overcome completely by increasing the substrate concentration. CM-cellulase behaved similarly. Component C₁ exhibits maximum activity and stability at pH 5 and 37°C with K_m approx. 0.05 mg/ml. With cellulose and its derivatives component C₁ apparently acts as a cellobiohydrolase with a preference for the ends of chains and in the vicinity of terminal non-reducing groups.

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The Kinetics of Respiratory Degeneration and Regeneration During Drug Treatment of Two *petite*-Negative Yeasts

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We have studied the respiration rates of the *petite*-negative yeasts (Bulder, 1963) *Kluyveromyces (Saccharomyces) lactis* and *Saccharomyces fragilis* during treatment with drugs known to interfere selectively with mitochondrial macromolecular synthesis (Kellerman *et al.*, 1969), and similarly during respiratory adaptation after pre-growth of the cells in a drug-containing medium. Methods for the measurement of respiration rates and selection of suitable drug concentrations have been described (Luha *et al.*, 1971). The mitochondrial protein-synthesis inhibitors chloramphenicol and erythromycin and the mitochondrial nucleic acid-synthesis inhibitors acriflavine and ethidium bromide appear to have similar effects on the respiration rates of *K. lactis*. No significant inhibition of respiration rate occurs until between 60 and 90 min after addition of the drug. Thereafter the respiration rate falls off exponentially while the cells continue to grow in exponential phase.

Regeneration of respiratory activity has been studied in *K. lactis* after growth in the presence of chloramphenicol at 2 and 4 mg/ml and of ethidium bromide at 5 and 10 µg/ml. In contrast with the repression experiments there is a significant difference. De-repression from chloramphenicol proceeds much more rapidly, the Q_{O_2} (µl of O₂ taken up/h per mg dry wt.) rising from 10 to about 150 in 2½ h. A similar rise after ethidium bromide treatment takes between 6 and 7 h. Ethidium bromide exerts a similar effect on repression of the respiratory system of *S. fragilis*. De-repression from this drug is also observed in this system. In this case, however, a distinct lag