Meeting report

CHLOROPLAST STRUCTURE AND FUNCTION
A report of the British Photobiology Society Meeting held in London on 19 October 1973

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Received 2 November 1973

1. Introduction

This one-day British Photobiology Society meeting dealt mainly with biochemical properties of isolated intact chloroplasts and with some aspects of the biochemical relationships which exist between chloroplast and cytoplasmic compartments. The meeting was held at Imperial College and was very well attended both by British and overseas visitors, and useful discussions were generated between papers. The morning session was chaired by *Professor F.R. Whatley* from Oxford while *Professor D.A. Walker* from Sheffield took the chair in the afternoon.

2. Chloroplast compartmentation and control of carbon fixation

The first paper of the day was presented by *D.A. Walker* (Sheffield) and was concerned with the light activation of ribulose diphosphate carboxylase. *Walker* described how he and his colleagues have been comparing the activity of this carboxylase in osmotically ruptured and intact isolated chloroplasts. This enzyme converts ribulose diphosphate* into 3-phosphoglycerate and both in leaves and intact chloroplasts has a high affinity for CO_2 (K_m about 0.4 M HCO_3^-). However, simply by removing the outer chloroplast membranes by osmotic rupture it is found that this enzyme greatly decreases its affinity for CO_2 so that the K_m is comparable with that found with the isolated enzyme (K_m about 20 mM HCO_3^-). *Walker* described experiments which clearly demonstrate that the affinity of this carboxylase for CO_2 is a function of Mg^{2+} concentration. In his laboratory they have found that reconstituted chloroplast systems consisting of envelope-free chloroplasts, ATP, NADP, ferredoxin and 'chloroplast extract' obtained from the supernatant during isolation, will fix CO_2 at rates comparable with that found with intact chloroplasts as long as the Mg^{2+} level was 3 mM or more in the suspending medium. He concluded his paper by outlining a model for the control of the Benson–Calvin cycle. He visualises a reversible light induced net movement of Mg^{2+} from the thylakoid interiors to the stroma acting as a switch for RuDP carboxylase activity. Net Mg^{2+} movement could be powered by exchange with H^+ driven into the granal spaces by photosynthetic electron flow. This process could be partly responsible for the lag in O_2 evolution upon the initial illumination of intact chloroplasts and would also explain why intact chloroplasts are unable to fix CO_2 in the dark even in the presence of ample substrate (i.e. when the stromal Mg^{2+} level falls to a low value).

Later in the morning *H.W. Heldt* (Munich) also presented a paper which contained evidence that the net movement of ions between the granal and stromal compartments could control carbon fixation. In a clear presentation *Heldt* described experiments in which he used the weak acid dimethyloxazolidinedione (DMO) and the weak base methylamine to demonstrate that substantial pH gradients exist across the thylakoids of illuminated intact isolated chloroplasts.

* *Abbreviations* used: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p* benzoquinone; DHAP, dihydroxyacetone phosphate; DMO, dimethyloxazolidinedione; OAA, oxaloacetate; PEP, phosphoenol pyruvate; PGA, 3-phosphoglycerate; RuDP, ribulose diphosphate.

From his experiments he calculates that under saturating light conditions the granal spaces have a pH of about 5.5 while the stroma is about 8.0. Calculation of the buffer capacities gave values of pK = 5.5 for the granal space and 6.8 for the stroma. On darkening the chloroplasts or adding uncouplers such as nigericin or CCCP the pH gradient is abolished. By using very low concentrations of uncouplers, such as CCCP and NH_4Cl, *Heldt* has found the ability of chloroplasts to fix CO_2 to be more sensitive to changes in stromal pH than in PGA reduction. He explained that unlike PGA reduction CO_2 fixation has a very sharp pH optimum at 7.8. It seems that the pH sensitive step is between the utilisation of dihydroxyacetone phosphate and the formation of PGA, possibly the reaction catalysed by fructosediphosphatase and suggests that the rate of CO_2 fixation is regulated by the pH of the stroma. This he suggested would explain why chloroplasts cannot fix CO_2 in the dark when the stromal pH is about 6.8. With this model in mind *Heldt* outlined a preliminary and striking experiment which indicated that when chloroplasts are incubated in the dark with DHAP and oxaloacetate (OAA) they will fix CO_2 as long as the pH of the medium is adjusted to give a stromal pH similar to that normally generated in the light (i.e. about 8.0).

In a meeting which was dominated by biochemistry it was refreshing to listen to *D. Greenwood's* (Imperial College) paper on ultrastructural aspects of chloroplast compartmentation. Drawing on electron microscopy studies of many different types of photosynthetic systems *Greenwood* was able to present a scheme which classified chloroplasts into categories primarily based on variations in the membrane bounded compartments emphasising those which he thought may serve as useful tools for future physiological and biochemical studies. In particular he drew the attention of the audience to the possibility of using those algae which have extremely regular patterns of compartmentation and lamellar structure such as the *Rhodophycean* type with separate thylakoids and the 'banded thylakoid' types which occur in both *Chromophyta* and *Chlorophyta*. He also pointed out that care should be taken when selecting a particular system for study, explaining for example that organisms like *Euglena* occupy a strikingly anomalous position, being closer to the *Chromophyta* than to other *Chlorophyta* or Higher Plants.

3. Electron transport and photophosphorylation

The nature of the primary electron acceptor in photosystem one (S1) and the stoichiometry between electron transport and photophosphorylation were the subjects of three papers presented at this meeting. *M.C.W. Evans* (University College, London) described how low temperature electron paramagnetic resonance (EPR) measurements give good support to the possibility that iron sulphur proteins (ferredoxins) are intimately involved in the primary photochemical reactions of photosynthesis. In O_2-evolving organisms the EPR spectrum is complex having at least four different light induced g values (2.04, 1.95, 1.92 and 1.86) corresponding to photoreductions. Redox potential titrations of these signals suggest that all the changes occur below −530 mV and Evans has evidence that one of these active centres may have a midpoint potential below −580 mV which would represent the most reduced compound ever detected in chloroplasts. He concluded his paper by outlining the properties of various light induced EPR signals obtained with chromatophores of purple photosynthetic bacteria. From his studies he suggested that iron sulphur proteins not only act on the reducing side of the bacterial reaction centre but also on the oxidising side. In particular he thought the high potential iron protein (HIPIP) with a midpoint potential of +350 mV acted as an electron doning agent to the primary oxidant C555 while a low potential iron-sulphur centre with a g value of 1.92 (E_m = −50 mV) is a secondary electron acceptor to a low iron containing protein with a g value of 1.82 (E_m = −150 mV) which is generally thought to be the primary electron acceptor in bacterial reaction centres.

The stoichiometry of electron transport and photophosphorylation was covered in some depth in papers given by *U. Heber* (Dusseldorf) and *D.O. Hall* (King's College, London). *Heber* has tackled the problem of obtaining the ATP/2e ratio in intact isolated chloroplasts in a very elegant way. He has studied four reduction reactions which require different amounts of ATP for every two electrons extracted from water. For CO_2 reduction 1.5 ATP molecules are required, for PGA reduction 1 ATP, for glycerate reduction 2 ATP while OAA reduction is not dependent on ATP. He has found that when the substrate is CO_2 or glycerate the quantum requirement for 674 nm light at

low O_2 pressure is in the region of 12 to 20. He also reported that with these substrates the sudden lowering of the light intensity affected the ATP levels in the chloroplasts to a much greater extent than the NADPH levels. On the other hand when PGA was added to the intact chloroplast suspension the quantum requirement was 4 to 5 and sudden lowering of the light intensity caused both the ATP and NADPH level to fall simultaneously. From these observations *Heber* concluded that two molecules of ATP are not made for every two electrons transferred to NADP but that the ATP/2e ratio must be near to unity. However, he did not think that the stoichiometry is fixed since coupling of electron transport to ATP production does not seem to be as tight in chloroplasts as in mitochondria. He based this conclusion on experiments where OAA was used as a substrate. As expected OAA reduction was like PGA reduction in that the quantal efficiency at a limited light intensity was 4 to 5. At higher light intensities the quantal efficiency of the OAA reduction increased but could be reduced when uncouplers were added. This suggests that there is some restraint placed on OAA reduction by the high energy state but as *Heber* pointed out even under coupled conditions the rate of electron flow to OAA was comparable to rates found when the substrate was PGA. This would seem to indicate that the coupling of electron flow to ATP synthesis in intact chloroplasts may be flexible rather than tight.

In contrast to this *D.O. Hall* argued the case for a strict ATP/2e ratio of 2 for non-cyclic electron flow in chloroplasts. He and his colleagues have used osmotically broken chloroplasts prepared in such a way as to show good photosynthetic control with potassium ferricyanide as the electron acceptor. Under these conditions, and making allowances for basal electron flow (state 2) and its sensitivity to ATP as well as taking into account contributions from cyclic phosphorylation, the ATP/2e ratio is close to 2. Addition of DBMIB to the chloroplast suspension reduced the ratio to unity and from this *Hall* concludes that there may be two sites in the electron transport chain which give rise to ATP production, one on the photosystem two (S2) side of the DBMIB block and one on the S1 side of the block.

4. Chloroplast–cytoplasm interactions

Chloroplasts are not completely autonomous organelles and must undergo a series of complex biochemical interactions with the cytoplasm. The synthesis and turn over of many compounds require both chloroplast and cytoplasmic enzymes and it was the nature of some of these interrelationships which dominated the last three papers of this meeting. The division of labour between chloroplasts and cytoplasm and its overall effect on cellular energetics and growth was the topic of a paper given by *J. Raven* (Dundee). Initially he outlined many energy requiring processes in the cell, such as active ion transport, which are stimulated by light and presumably are powered by high energy compounds exported from the chloroplast. He pointed out that these photosynthetically derived high energy compounds could be in a primary form as ATP and NADPH or in a secondary form as reduced carbon. On the other hand the chloroplast is always dependent on the cytoplasm for certain compounds and under some conditions such as during its development it is totally dependent on the cytoplasm for its energy. In order to assess the cost to the cell of synthesis and maintenance of chloroplasts *Raven* presented data obtained from the literature which compared the difference in the specific growth rates, measured as carbon incorporated to carbon respired, for algal cells grown autotrophically and heterotrophically. With *Euglena*, which does not synthesise chloroplasts in the dark, there was no difference between the light and dark growth rates while organisms which always synthesise chloroplasts were found to grow slower when cultured heterotrophically. Apparently heterotrophic growth can never be greater than autotrophic growth for any particular photosynthetic organism and from this *Raven* concluded that overall these organisms benefit from the manufacture of their chloroplasts although he did consider whether his analyses should include specialised heterotrophs such as yeasts and bacteria.

The cooperation between cytoplasm and chloroplast in biosynthesis was considered more specifically in a paper by *Rachel Leech* (York). She dealt with photosynthetically driven synthesis of amino acids and chloroplast lipids. When $^{14}CO_2$ is fixed by photosynthetic organisms the primary amination takes place in the chloroplast, is specifically into glutamate

and is dependent on the availability of NADPH. However, in whole cells, unlike isolated chloroplasts, the ^{14}C is rapidly passed on from glutamate to aspartate. *Leech* presented evidence obtained with intact chloroplasts that the formation of [^{14}C]aspartate is a collaborative process involving both chloroplast and non-chloroplast compartments. By conducting reconstitution experiments using a 'cell supernatant' fraction (144 000 *g*) she suggested that the OAA required for the glutamate-OAA aminotransferase chloroplast reaction comes from the cytoplasm. This reaction gives rise to aspartate which was labelled in such a way to indicate that the OAA must be derived from photosynthetically produced PGA which had been transported out of the chloroplast and converted to OAA via the glycolytic pathway. By carrying out similar reconstitution experiments with intact isolated chloroplasts *Leech* also has evidence that the synthesis of their polyunsaturated fatty acids rely on certain cytoplasmic components which as yet have not been chemically identified.

In the final and perhaps most controversial paper of the meeting *J. Coombs* (Tate and Lyle) outlined his ideas concerning the location of the partial reactions of the C4 dicarboxylic acid pathway. Although he accepts the general scheme of chemical reactions of the C4 pathway he doubts whether its strict stoichiometry with the Benson–Calvin cycle and the requirement for long distance intercellular transport of intermediates, as suggested by the Hatch–Slack scheme, is correct. The main reason for his doubts is that it is thought that the initial carboxylation of the C4 pathway involving phospoenol pyruvate (PEP) carboxylase takes place in the cytoplasm of the mesophyll cells. In his opinion it would make more sense if the mesophyll chloroplasts, like the bundle sheaf chloroplasts, were able to carry out the conventional Benson–Calvin reduction cycle so that they could directly utilise cytoplasmic carboxylation products of the C4 pathway. In this way long distance and complex metabolic transfer between the mesophyll and bundle sheaf cells would be avoided and the mesophyll chloroplasts would be given more employment. Thus *Coombs* visualises the possibility that the C4 pathway only acts as a supplementary mechanism for assimulating CO_2. He suggested the activity of the C4 pathway could be controlled in some way by environmental factors such as changes in cytoplasmic ion or sugar phosphate levels. Although his ideas make good sense they will be more acceptable when it is generally shown experimentally that mesophyll chloroplasts have reasonable RuDP carboxylase activity.