MEETING REPORT

BIOPHYSICAL ASPECTS OF PHOTOSYNTHESIS A REPORT OF THE BRITISH BIOPHYSICAL SOCIETY MEETING HELD IN LONDON ON 17 AND 18 DECEMBER 1969

J.BARBER
Department of Botany and Plant Physiology, Imperial College, London, S.W.7.

Received 15 January 1970

1. Introduction

This British Biophysical Society meeting, organised by Dr. D.A.Walker, gave an opportunity to introduce many aspects photosynthesis to British scientists working in other fields of biophysics. The majority of the speakers came from Continental laboratories and there was also a large number of overseas visitors in the audience. The meeting was divided into three major sections each having an introductory speaker followed by several supporting speakers. The first dealt with primary photochemical events and was chaired by Professor C.P.Whittingham from Imperial College, London. Dr. R.Hill from Cambridge chaired the second session on electron transport while the third main session, concerned with photophosphorylation and ion transport, was chaired by Professor F.R.Whatley of King's College, London.

In addition to these formal sessions there were a number of informal colloquia and discussions which gave increased value to this very stimulating meeting.

2. Primary photochemical events

H.T.Witt (Max-Volmer-Inst., Berlin) in a well balanced introductory lecture began by outlining many of the basic concepts associated with the study of primary mechanisms in photosynthesis. It was inevitable that he should to discuss aspects of photosynthesis which were listed as topics for later sessions. This problem of trying to separate the early events of photosynthesis into three distinct sections became even more evident as the meeting proceeded.

From the very beginning Witt introduced the audience to the idea that two photochemical reactions with different pigment systems (S1 and S2) act in series to drive electrons from water to a redox level capable of reducing CO_2. He also explained the suggestion of Mitchell that such a charge transfer may bring about a proton gradient across the thylakoid membranes able to drive photophosphorylation.

Witt went on to describe how the use of very sensitive and fast spectrophotometry can be used to study the kinetics and magnitude of light induced optical density changes of photosynthetic pigments and electron transporting intermediates. In particular he and his colleagues have used these techniques and have been able to interpret the spectral changes in terms of several different molecular events. These he called, valve reactions, light reactions, electron transfer, electric field formation and field driven ion fluxes, primary pH formation and diffusion driven ion fluxes. The valve reaction seems to involve the carotenoids and may function as a means of tapping off excess light energy. The light reactions are associated with excitation of S1 and S2 chlorophylls and have rise times of about 10^{-8} sec, while the electron transfer reactions represent the reduction of plastoquinone. By com-

Abbreviations used:

FCCP,	carbonylcyanide *p*-trifluoromethoxyphenylhydrazone
CCCP,	carbonylcyanide *m*-chlorophenylhydrazone
DCMU,	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DNP,	2,4-dinitrophenol
BCP	bromocresolpurple
BChl,	bacteriochlorophyll

operation of four photoreactions occurring successively on the same photochemical centre. Although Joliot has some difficulty in repeating the observations of Kok he does think that this latter model is probably a better approximation of the truth.

In the final paper of this session A. Trebst (Inst. für Biochemie der Pflanzen, Ruhr Universität Bochum, W. Germany) presented some interesting information about a protein complex which may possibly be the primary electron acceptor in S1. This complex has been detected by using two antibodies from rabbits immunised against lamellar systems of chloroplasts. One of these antibodies inhibits photosynthetic NADP and ferredoxin dependent cytochrome *c* reduction while the other inhibits not only these two reactions but also photosynthetic anthraquinone reduction. The antigen associated with these antibodies has been isolated as a water-soluble factor released from lyophilised chloroplasts after treatment with diethylether. Although the exact nature of this compound is not known it does seem to consist of two fractions one of which is heat stable and similar to the compound FRS (Ferredoxin Reducing Substance) isolated independently from chloroplasts by Yocum and San Pitero. Trebst suggests that the heat unstable component of the complex is the prosthetic group and that it is this part which may act as the primary electron acceptor of S1.

3. Electron transport

The second session on electron transport was introduced by L.N.M.Duysens (Biophysical Lab., Leiden, The Netherlands) who explained how sensitive fluorescence and difference absorption spectrophotometry have been powerful tools for elucidating the sequence of electron carriers in photosynthetic systems. As examples he outlined what are now classical experiments conducted in his laboratory concerning the effect of S1 and S2 light on the redox levels of the quencher Q and cytochrome *f*. He described how these electron carriers are reduced by S2 light but additional light principally absorbed by S1 can partially releave this photoreduction. He also talked about the possible mechanism of energy transfer between the bulk photosynthetic pigments. In so doing he introduced a new idea, based on measurement of chlorophyll *a* fluorescence and oxygen evolution, that some light harvesting cholorophyll molecules may be able to shift in such a way that light energy normally channelled to the S2 reaction centres can be transferred to the S1 trapping centres. He speculated that such a process could regulate the flow of electrons through S1 and S2 in order to obtain a maximum rate and, indeed, had some evidence for this based on rates of cytochrome oxidation in *Anacystis*. Calculations assuming energy is transferred by inductive resonance and that the chlorophylls of S1 and S2 are adjacent suggested that a shift of only 6 Å would be sufficient to fit the experimental observations. He reported that S2 light caused a shift towards the S1 reaction centre (state II) while S1 light produced the opposite situation (State I), a shift towards S2.

The last lecture on Thursday was given by J.S.C. Wessels (Philips Research Lab., Eindhoven, The Netherlands) who described the preparation and properties of subchloroplast particles prepared by the action of digitonin. Using concentrations of this non-ionic detergent of about 0.5% he has obtained particles which were capable of carrying out many photosynthetic reactions including cyclic phosphorylation. With higher concentrations of digitonin other particles were produced which could not carry out photophosphorylation or show Ca^{2+}-dependent ATPase activity. These particles were separated on a sucrose gradient and gave rise to a yellowish green and a blue green band. Detailed investigation of these has suggested that the blue green band is mainly S1 particles being cylindrical vesicles of 40–45 Å diameter and 120 Å length. On the other hand the yellowish green band showed many S2 properties. An additional intermediate green band was also obtained in low yield which showed both S1 and S2 activity but Wessels was not certain whether these vesicles were derived from unseparated or recombined S1 and S2 fractions.

The second supporting paper of this session given by J.Amesz (Biophysical Lab., Leiden, The Netherlands) described some experiments designed to measure the difference in redox potential between Q and P700 *in vivo*. He estimated the concentration of reduced and oxidised P700 and cytochrome *f* by absorption difference spectrophotometry while the fluorescence yield of chlorophyll *a* was taken as a measure for the oxidation-reduction level of Q. From these measurements on algae he has concluded a difference in redox potential of about 60 mV between P700 and cyto-

made clear that both an influx and efflux of H^+ will be occurring and that the magnitude of the recorded pH change will alter if one of these fluxes is inhibited or stimulated. Experiments designed to measure H^+/e ratios on valinomycin and DNP treated chloroplasts supported this concept. Moreover he discussed the effect of having phosphorylating and non-phosphorylating conditions both upon the light induced 515 nm absorbance and pH changes. The dark decay of the 515 nm shift, thought to represent the p.d. across the thylakoids, was speeded up under phosphorylating conditions and the amplitude of the reversible pH change reduced. Because he did not increase the rate of decay of the pH shift in the dark under coupled conditions Rumberg concluded that this decay is independent of phosphorylation.

In the third supporting paper of this session by B. Chance, A.R.Crofts, N.Nishimura and B.Price (Johnson Research Foundation, Pennsylvania) experiments were reported on preparations isolated from *Chromatium*. Using sub-chromatophore particles with equiv. weights of about 500,000, having no membrane structure but a high concentration of reaction centres Chance and his colleagues have measured light induced electron transfer from cytochromes *C*553 and *C*555 to aceptor Y with little or no associated H^+ ion shift. With chromatophores however light induced H^+ ion binding has been measured with BCP but again the kinetics of this process ($t_{1/2}$ = 400 μsec) did not correspond to the very fast electron transfer time ($t_{1/2}$ = 24 μsec). Furthermore the pH profile and the activation energy were also inconsistent with a direct coupling mechanism. Nevertheless, the rate of H^+ movement is relatively large being about 50 H^+/BChl. sec and probably reflects a structural change in the membrane induced by electron flow. Chance compared this with the Bohr effect associated with haemoglobin and designated the shift in membrane structure by M1 and M2. This he explained was probably brought about by a change in the *p*K values of the membrane resulting in a corresponding increase in its volume. To check this, measurements of H^+ binding at various pressures have been made and indeed was decreased with increasing pressure being completely inhibited 2,400 atmospheres.

The following paper by A.R.Crofts (Biochemistry Dept., Medical School, Bristol) dealt with studies on chromatophores isolated from *R. rubrum* and *R. spheroides*. He presented evidence that the light induced H^+ pump associated with these preparations is electrogenic rather than neutral. By using the antibiotics nigericin and valinomycin Crofts argued, from the way K^+ was distributed, that a pH gradient of about 1 unit and an electrical gradient in the region of 200 mV exists across the chromatophore membrane during steady illumination and under non-phosphorylating conditions. He also explained that a light induced shift in the carotenoid absorption spectrum seems to reflect the magnitude of the membrane potential. This change was calibrated on dark suspensions of valinomycin treated chromatophores using various K^+ gradients. When this was correlated with changes induced by illumination a value of 420 mV for the initial p.d. developed and, in agreement with above, a value of 210 mV for steady illumination were obtained. Finally it was reported that a comparison of the rate of H^+ uptake and electron flow suggested that 2 protons were pumped per electron transferred.

This paper was followed by a short and related contribution on studies with *R. rubrum* chromatophores by Margareta Baltscheffsky (Bioenergetics Group, Plant Physiology Dept., Stockholm). Using 0.5–1 msec Xenon flashes she reported that the carotenoid shift was very fast and its magnitude resistant to electron blocking and uncoupling agents. Its decay however was accelerated by valinomycin, FCCP, phosphorylating conditions and also by ATP and PP_i. Other measurements using BCP for following light induced H^+ binding indicated biphasic kinetics. The slower secondary phase, unlike the initial rapid phase, was sensitive to low concentrations of uncoupling and electron blocking agents. When uncouplers are present the decay of the H^+ binding also shows rapid and slow phases. The rapid is abolished by arsenate, ATP and phosphates while the slower phase is speeded up by the addition of valinomycin.

5. Informal colloquia and discussions

The programme had been designed to make available some time for informal colloquia and discussions. In addition to the short discussion time at the end of each paper there was a further half hour at the end of each main session. These were very successful and gave an opportunity both for specialists and non-specialists to ask questions or make comments.

paring slow and flash excitation Witt suggested that there is a pool of plastoquinone able to regulate the flow of electrons from S2 to S1. Much of the work he described involved the use of repetitive pulse technique and using this he and his coworkers have been able to interpret a fast absorbancy change at 515 nm, believed to be partly due to a chlorophyll *b* shift, as an indicator for the development of an electric field. This signal had a rise time of 20 nsec and its decay gave a measure of the field driven ion movements and was speeded up by compounds known to increase the permeability of lipid membranes to ions, such as valinomycin. He correlated these absorbancy changes at 515 nm with experiments using UBF (Umbelliferone) as a means of estimating the magnitude of the primary pH change. Overall he concluded that the work of his laboratory was in agreement with Mitchell's chemiosmotic scheme and estimated that under steady illumination a pH gradient of 3 units (inside pH = 5) exists across the thylakoid membrane together with an electrical potential of 100 mV (inside positive).

The supporting speakers in this first session dealt with topics which ranged from chlorophyll fluorescence to oxygen evolution from artificial systems.

J.Lavorel (Laboratoire de Photosynthèse, Gif-sur-Yvette, France) gave a paper on light induced chlorophyll fluorescence changes measured on algal cells. He explained the Kautsky effect and outlined how these transients in fluorescence yield after onset of illumination have been partly accounted for by a reduction of a quencher Q and the interaction of a second quencher S or Q'. However, comparing flash and long illummination experiments in a flow apparatus Lavorel has found many features of the induction curves which do not fit the general scheme of the Q hypothesis. For example, he has detected a slow decay component of fluorescence yield induced by a light flash which has too long a life time to meet the requirements of the minimum turnover rate of the photosynthetic electron chain. Moreover this slow decay is not sensitive to S2 inhibitors and has a negative temperature coefficient. This together with other studies has led Lavorel to suggest that the Q hypothesis is in need of supplementary theory to give it a broader scope.

The third contribution in this session was given by J.B.Thomas (Fysisch Laboratorium, Rijksuniversiteit, Utrecht, The Netherlands) who presented some recent studies from his laboratory on chlorophyll *b*. He has been able to obtain an absorption spectrum of this pigment *in vivo* by comparing the difference in the spectra obtained from a chlorophyll *b* containing chloroplast preparation and chlorophyll *b* free algae. The spectra obtained were unaffected by temperature suggesting, that unlike chlorophyll *a*, only one type of chlorophyll *b* complex exists *in vivo*. Also there was no evidence that this pigment complex is preferentially associated with either S1 or S2.

This was followed by a paper given by H.Metzner (Inst. für Chemische Pflanzenphysiologie der universität, Tubingen) in which he explained some very interesting experiments designed to study the possible mechanism by which a strong oxidant initially produced in photosynthesis is capable of decomposing water. He postulated that this oxidant initially gives rise to an unstable H_2O^+ radical ion which then reacts with another water molecule to produce a hydronium ion (H_3O^+) and a OH· radical. It is then the OH· radical which is converted by the photosynthetic machinery into molecular oxygen. He and his colleagues have attempted to bring about this same type of conversion under completely artificial conditions. They chose the chloride electrode (E_o = +1.36 V) as a possible oxidising source. This was accomplished by suspending AgCl crystals, covered in a layer of chlorophyll *a*, in water such that on illumination with red light they observed a release of molecular oxygen. To maintain a steady O_2 evolution they continuously reoxidised the Ag by adding the azo dye Janus green.

The fifth paper in this first session, given by P. Joliot (Inst. de Biologie Physico-Chimique, Paris), also dealt with O_2 evolution but this time the experiments were carried out on photosynthetic systems. He posed the question of how many photochemical reaction centres are necessary for producing a molecule of oxygen. He outlined some elegant experiments involving measurement of O_2 evolution in flashing light. The amount of O_2 evolved followed a damped oscillation with a period of 4 flashes which he has interpreted as due to two primary photoxidants per reaction centre capable of giving rise to one atom of oxygen. The damping, he explained, was probably due to the general disorder in the system or possibly to flash times of too long a duration. He also talked of similar experiments by Kok and coworkers who have obtained higher yields with 3 flashes. From this observation Kok has suggested that one molecule of O_2 requires the co-

chrome *f* and ⩾ 120 mV betwen cytochrome *f* and Q. Assuming that the reaction is also coupled to phosphorylation with a P/2e ratio of 1 he estimated that the total redox potential difference between P700 and Q for the uncoupled reaction to be approximately 340 mV.

In the second half of this paper Amesz talked about recent experiments involving the stimulation of delayed light emission from chloroplasts. He explained how pH and various ionic treatments can stimulate delayed light emission and that compounds known to interact with lipid membranes, such as Triton X-100 and valinomycin, can modify or inhibit these effects.

The final contribution to the second session was given by G.Forti (Inst. e Orto Botanico Della Università, Napoli, Italy) who outlined the evidence for cyclic electron flow and photophosphorylation both in intact and isolated systems. He presented data that suggested the chloroplast flavoprotein cytochrome *f* reductase is required for cyclic phosphorylation in the isolated system and rates of 20 to 50 μmoles ATP/mg Chl.hr are normally obtained. Although high rates of cyclic phosphorylation are possible when additional ferredoxin is added as a catalyst, Forti stated that in his opinion such a situation did not represent the true physiological reaction. He also presented evidence that non-cyclic phosphorylation could produce 2 moles of ATP per mole of NADP reduced. From these observations he pointed out that sufficient ATP and reducing power is produced from non-cyclic electron flow to fix CO_2 and questioned the significance of cyclic phosphorylation.

4. Photophosphorylation and ion transport

M.Avron (Biochemistry Dept., Weizmann Inst., Rehovoth, Israel) introducing the third session discussed possible mechanisms for photophosphorylation. In so doing he stated that any hypothesis should explain the four basic observations of coupling, uncoupling, electron transfer inhibition and energy transfer inhibition. He went on to describe how both the chemical and chemiosmotic hypotheses can explain these four phenomena and outlined a number of observations for and against these theories. In particular he presented experiments carried out in his own laboratory on chloroplasts which suggest that light induced pH gradients do not represent the primary high energy state resonsible for phosphorylation. Comparison of the H^+/e ratio as a function of pH, effect of phosphorylation and non-phosphorylating conditions on the light induced pH changes and experiments with uncouplers including a comparison of the effect of ammonium chloride and citrate gave results which, in his opinion, were difficult to reconcile with the chemiosmotic scheme. He concluded by suggesting that an unknown intermediate represents the initial high energy product due to electron transport. He argued that the light induced uptake of protons is a secondary process utilizing this energy rich compound and that its function may be to help drive the negatively charged phosphorylating agents across the thylakoid membranes.

The following paper was given by H.Baltscheffsky, (Bioenergetics Group, Plant Physiology, Stockholm, Sweden) who concentrated on the mechanism and possible function of light induced inorganic pyrophosphate (PP_i) formation in chromatophores isolated from *R. rubrum*. The synthesis of PP_i from orthophosphate is accomplished by cyclic electron flow and seems to be unique to photosynthetic bacteria. Its formation, unlike ATP synthesis, is not inhibited by oligomycin. It can however, like ATP, bring about the reduction of cytochrome *b* when added to the chromatophore suspension. The PPase activity is stimulated by K^+ ions especially when valinomycin or nigericin are present but as yet no PPase component has been isolated. These observations seem to be consistent with the possibility that an unknown obligatory intermediate exists between electron flow and phosphorylation. Finally Baltscheffsky suggested that the PPase activity may have resulted from a primitive energy conserving system.

B.Rumberg (Max-Volmer-Inst., Berlin) presented the second supporting paper in this session. He outlined some recent work on chloroplasts which seems to agree with the chemiosmotic scheme. Using ion specific electrodes he has shown that under the experimental conditions employed a net movement of Na^+ and Cl^- ions balance the light induced H^+ movement. A calculation of the equilibrium potentials from the distribution of these ions (about 5 mV) did not agree with the estimated true membrane potential of 100 mV suggesting that some active transport process was operating. He argued that cation and anion exchange mechanisms were utilizing the pH gradient in a similar way to that already proposed by Mitchell. It was also

There were also two informal colloquia with the first being on the evening of the 17th and the second the following morning. The evening session was largely due to the efforts of A.R.Crofts and was concerned with energy coupling associated with light reactions. Crofts discussed current problems of the energetics and stoichiometry of phosphorylation. This was followed by a communication by W.Junge (Max-Volmer-Inst., Berlin) who outlined experiments, particularly involving the use of valinomycin, on the phosphorylating ability of chloroplasts under short flash and steady illumination. He reported that this antibiotic only inhibited phosphorylation under flash excitation. A third contribution by J.Barber (Botany Dept., Imperial College, London) discussed the possibility that the stimulation of delayed light emission by subjecting chloroplasts to sudden shifts in external salt concentrations may be due to the development of a diffusion potential across the thylakoid membranes.

On the following day G.Forti chaired the second informal colloquium. The initial talk was given by D. Bendall (Biochemistry Dept., Cambrdige) who discussed the properties of light induced absorbancy changes in the region of 550 nm measured on a variety of green photosynthetic material both at room and liquid nitrogen temperatures. Of particular interest at low temperature was the occurrence of a peak at 557 nm due to the high potential cytochrome *b* component which could be removed with ferricyanide and an additional DCMU resistant peak at 546 nm. The 546 nm peak is apparently the same as *C*550 reported by Arnon and seems to have the properties of a primary electron acceptor possibly associated of cytochrome *f*.

This paper was followed by a short contribution by W.Vredenberg (Centrum voor Plantenfysiologisch Onderzoek, Wageningen, The Netherlands) who described recent experiments on the vacuolated coenocytic algae *Nitella* and *Nitellopsis*. He has used microelectrodes to measure changes in electrical potential between the vacuole and the cell wall. Light induced changes of a few millivolts were recorded which were sensitive to DCMU and CCCP. Correlation of these potential changes with changes in fluorescence yield of chlorophyll has led Vredenberg to suggest that these two phenomena reflect the energised state across the chloroplast membranes. He proposed that the decline of fluorescence yield in continuous illumination was dependent on the integrity of the whole cell and was closely associated with changes in ionic balance within the cytoplasm.