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THE CYTOCHROMES OF HIGHER PLANTS AND ALGAE

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CONTENTS

A. INTRODUCTION

The purpose of this article is to summarize the current state of our knowledge concerning the role that one class of heme-containing proteins, the cytochromes, play in the membrane-bound electron transport chains of oxygen-evolving photosynthetic organisms. The earliest studies in this field were confined to simply identifying and cataloging the cytochromes present in these organisms. Subsequent research concentrated on attempting to delineate the positions, relative to other electron carriers, in which the cytochromes functioned in various photosynthetic electron transfer pathways. These approaches are far from being exhausted — new cytochromes continue to be discovered and the sequences of electron carriers in several photosynthetic organisms

have not yet been worked out. In recent years, these approaches have been supplemented by investigations into other areas such as the effects of membrane binding on the oxidation—reduction properties of the cytochromes, the location of cytochromes in specific regions of membranes and the possible role of cytochromes in carrying protons as well as electrons.

Photosynthetic organisms often contain cytochromes that are involved in non-photosynthetic pathways. Because the major focus of this article will be the role of cytochromes in light-driven electron transport, such cytochromes will be excluded from consideration. In so far as the photosynthetically active cytochromes are concerned, this article will not attempt to be exhaustively comprehensive but rather will summarize representative data and point out some of the more recent developments. Also, because a comprehensive recent monograph on bacterial photosynthesis covers the bacterial cytochromes [1], this article will deal only with plant photosynthesis.

B. PHOTOSYNTHETIC ELECTRON TRANSPORT

It has been known for some time that three cytochromes are associated with the chlorophyll-containing membranes of higher plant chloroplasts. These cytochromes include a *c*-type cytochrome (heme *c* is the prosthetic group) called cytochrome *f* and two *b*-type cytochromes (protoheme is the prosthetic group) called cytochrome b_6 (or cytochrome b_{563}) and cytochrome b_{559}. All three cytochromes have been shown to undergo light-induced oxidation—reduction reactions and thus presumably play a role in photosynthetic electron transport.

For those readers not familiar with the details of photosynthetic electron transfer pathways in higher plants, Fig. 1 summarizes a widely-accepted scheme for those pathways. P680 and P700 are the specialized chlorophyll *a* molecules (probably dimers) of Photosystems II and I, respectively, that undergo photooxidation; A_{II} and A_I are initial acceptors of electrons from P680 and P700 respectively; PQ is plastoquinone, a hydrophobic, substituted *p*-benzoquinone; PC is the copper-containing protein, plastocyanin; and Fd is ferredoxin, a soluble iron—sulfur protein. Non-cyclic electron flow accomplishes the reduction of a pyridine nucleotide $NADP^+$, which in turn supplies reducing equivalents for biosynthetic reactions. The ultimate electron donor for $NADP^+$ reduction is H_2O, which is oxidized to O_2 by a complicated series of reactions linked to the strong oxidant, $P680^+$ ($E_m > +0.82$ V), produced in the light by Photosystem II. $NADP^+$ is reduced by Photosystem I in a series of reactions initiated by the light-induced transfer of an electron from P700 to A_I. The electron lost by P700 is replaced by the electron removed from H_2O via a chain of electron carriers (including plastoquinone, plastocyanin and probably cytochrome *f*) linking the two photosystems. Energy released during exergonic electron flow between the photosystems is utilized to drive the endergonic formation of a pyrophosphate bond between ADP and inorganic phosphate, resulting in production of "the universal biological energy

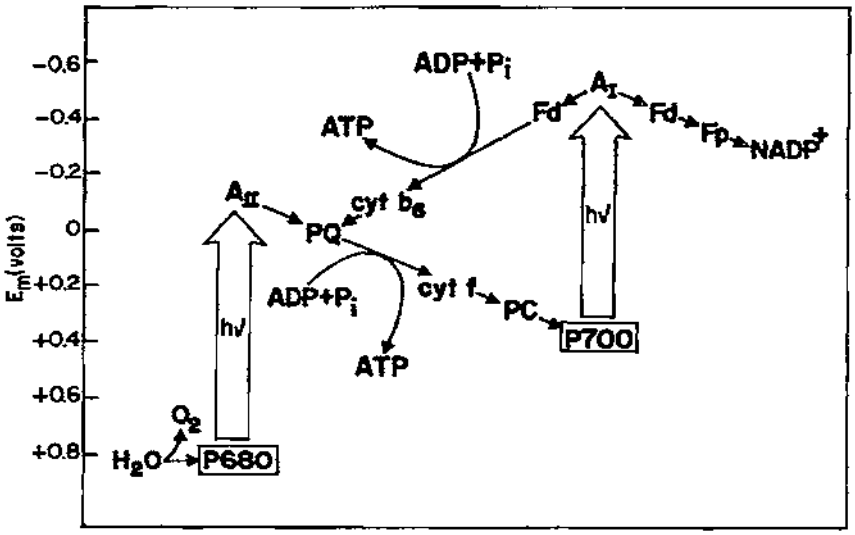

Fig. 1. Electron transport pathways in higher plants.

carrier", ATP. Actually, there appear to be two sites of ATP synthesis associated with non-cyclic electron flow, but only one has been indicated for the sake of simplicity.

In addition to non-cyclic electron flow from H_2O to $NADP^+$, driven by Photosystems II and I operating in series, Fig. 1 shows a cyclic electron flow involving only Photosystem I. In this pathway electrons lost by P700 during photooxidation are returned to $P700^+$ through a series of electron transfer reactions that probably involve two of the three chloroplasto cytochromes, cytochromes *f* and b_6. As electrons return from the primary electron acceptor of Photosystem I ($E_m < -530$ mV) to $P700^+$ ($E_m = +375$ mV), sufficient energy is released for the synthesis of at least 2 ATP molecules per pair of electrons and Fig. 1 shows two likely sites for such synthesis. Figure 1 depicts the cyclic and non-cyclic pathways sharing a common number of electron carriers. It has also been argued that the cyclic pathway is completely separate from the non-cyclic. Conclusive evidence concerning this point remains elusive.

C. ISOLATION AND PURIFICATION OF HIGHER PLANT CYTOCHROMES

(i) Cytochrome f

This cytochrome was the first cytochrome to be described in chloroplasts and the first to be solubilized from the membrane. It has been purified from several plants in several laboratories. Extraction of leaves from plants such as spinach or parsley with an alkaline ethanol—water mixture in the presence of

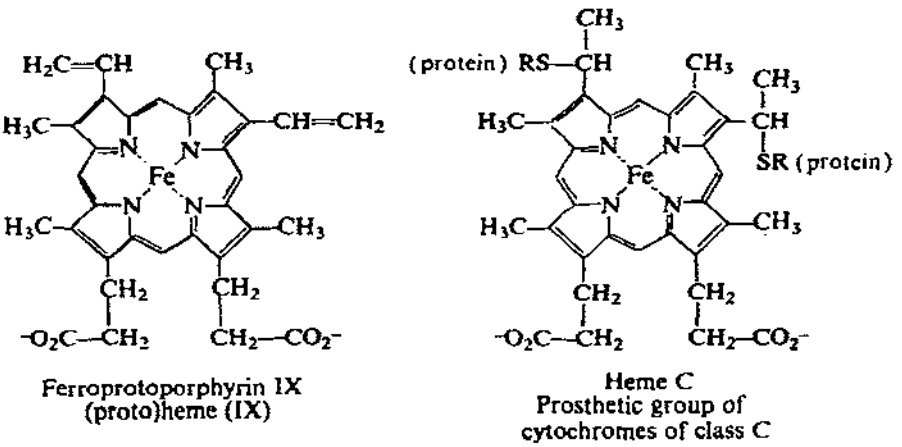

Fig. 2. Molecular formulae for protoheme and heme *c*.

the detergent Triton X-100 and subsequent purification yields an essentially pure protein. The reduced (ferrous) protein has an asymmetric α-band at 554—555 nm, a complicated β-band with maxima at 524 and 532 nm and a γ-band at 422 nm, while the oxidized (ferric) protein has a γ-band at 410 nm [2—7]. These absorbance features are identical to those of the membrane-bound cytochrome in vivo. At liquid nitrogen temperature, the α-band splits into two bands at 548 and 552 nm [8]. The cytochrome is clearly of the *c*-type containing heme *c* on the basis of its pyridine hemochromogen [2]. Figure 2 shows the structure for heme *c*. The only uncertainty in its physical properties concerns the molecular weights of the cytochrome. Determinations in the presence of detergent SDS indicate a molecular weight near 34,000 per heme [4] but the isolated preparations are oligomeric and there are reports of molecular weights of 110,000 [2] for a dimer and 250,000 [7] and 206,000 [3] for a tetramer. There are also reports of a 31,000 molecular weight subunit and one heme per 62,000 molecular weight [9] implying the association of one heme-containing and one non heme-containing subunit. Apparently cytochrome *f* from certain *Cruciferae* (such as Japanese radish) is monomeric in solution, with molecular weights of 32,000 to 33,000 per heme [5,6].

(ii) Cytochrome b_6

The only purification reported to date for this cytochrome involves extraction of spinach chloroplasts with urea and Triton X-100 [10]. The purified cytochrome has a molecular weight of 40,000 per heme and its *b*-cytochrome nature was confirmed when the prosthetic group was shown to he protoheme. (Figure 2 shows the structure for protoheme.) The purified ferrous cytochrome, like the membrane-bound cytochrome in vivo, is characterized by an α-band at 563 nm, a β-band at 536 nm and a γ-band at 434 nm, while the oxidized

cytochrome has a γ-band at 413 nm. Stuart and Wasserman [10] report that the α-band splits into two bands at 561 and 557 nm at liquid nitrogen temperature. This report is in contrast to data from the laboratory of Boardman and Anderson that cytochrome b_6 in Photosystem I-enriched subchloroplast preparations exhibits a single 561 nm α-band at liquid nitrogen temperature [11]. This apparent contradiction remains to be reconciled. The situation is further complicated by reports that some isolation procedures can result in a shift of the cytochrome b_6 α-band from 563 to 561 nm [12,13].

(iii) Cytochrome b_{559}

The other *b*-type cytochrome of chloroplasts has also been isolated in Wasserman's laboratory using urea—Triton X-100 extraction [14]. Like cytochrome b_6 cytochrome b_{559} contains protoheme and does not react with CO. Cytochrome b_{559} purified in this manner has a molecular weight of 110,000 per heme, with 46,000 of this coming from protein and the rest from polar lipids. The protein portion appears to consist of 8 short polypeptides with molecular weights near 6000. The preparation contains 3 tightly-bound β-carotene and 4 tightly-bound chlorophyll *a* molecules per heme. At this point, it is not clear whether the pigments and lipids found to be associated with cytochrome b_{559} in this preparation represent a physiologically significant complex or a preparative artifact. If this cytochrome b_{559} pigment complex is not an artifact, it may be related to some of the unusual properties of the cytochrome discussed below.

(iv) Cytochrome b_6: cytochrome f complex

As mentioned above, cytochromes b_6 and *f* are thought to function as electron carriers in a cyclic electron pathway around Photosystem I. The idea that these two cytochromes are associated is supported by the observation that a chlorophyll-free complex of the cytochromes can be isolated from chloroplasts. The initial preparation of such a complex by Nelson and Neumann [12] had a cytochrome b_6: cytochrome *f* ratio near 2 (which is similar to the ratio of the two cytochromes in vivo). More recent measurements, using a wide range of detergents to prepare the complex have shown that the ratio of the two cytochromes in the complex is variable [13]. This variation in stoichiometry raises some doubts as to whether the complex is a physiologically significant entity. However, the idea of such a complex is intriguing because of the existence of a similar complex between *b*-type and *c*-type cytochromes in the mitochondrial respiratory chain [15—17]. An additional similarity between the photosynthetic and respiratory systems [18,19] is the association of non-heme iron, in the form of an iron—sulfur center, with the cytochrome b_6 - *f* complex. Work in Ke's laboratory, while less than conclusive in presenting evidence for the existence of a true complex, has provided EPR evidence for iron—sulfur center(s) associated with the cytochromes [20]. Recent work

in San Pietro's laboratory has also produced evidence for a cytochrome $b_6 \cdot f$ particle (the cytochrome ratio is 1 : 1) probably containing an iron—sulfur center, that can be isolated from Photosystem I [21]. Ke's work has also confirmed the association of the two cytochromes with Photosystem I and raised the possibility of the additional association of the copper-containing protein plastocyanin with the cytochrome $b_6 \cdot f$ complex.

D. OXIDATION—REDUCTION PROPERTIES

(i) Allotopic properties of membrane proteins

Racker [22] has introduced the term allotopic to describe the phenomenon that many membrane-bound proteins behave quite differently in soluble form compared to their behavior when associated with other components of the membrane. One such well-known allotopic property of membrane-bound electron carriers is oxidation—reduction midpoint potential. The earliest example of this phenomenon was the observation that a respiratory *b*-type cytochrome, solubilized from mitochondrial membranes, had a midpoint oxidation—reduction potential near −340 mV [23,24]. This value is some 350 mV more electronegative than the value observed for cytochrome *b* in the mitochondria. Addition of a mitochondrial hydrophobic protein fraction to the solubilized cytochrome caused the midpoint potential of the cytochrome to revert to a value similar to that observed in situ [24]. Similar results have been obtained with cytochrome *b*, from the bacterium *E. coli* [25]. A similar (although considerably smaller in magnitude) shift in midpoint potential has been observed for a *c*-type cytochrome from the purple photosynthetic bacterium *Rps. sphaeroides* [1] and a large positive shift on membrane binding has been observed for cytochrome c' in the photosynthetic bacterium *Chromatium vinosum* [26]. No completely analogous results have been observed for plant cytochromes, although the midpoint potential of the two *b*-type cytochromes depends on the "state" of the membrane in a manner that suggests possible allotopic effects.

(ii) Cytochrome f

The midpoint potential of cytochrome *f* from several plants has been measured in a number of laboratories. Cytochrome *f* behaves as a one-electron carrier and values for its in situ midpoint potential have been reported ranging from +340 mV to +380 mV [27—29]. Values of +330 mV [12] and +345 mV [20] have been reported for the cytochrome in cytochrome $b_6 \cdot f$ complexes and +375 mV in Photosystem I-enriched fragments [30]. A midpoint potential of +365 mV has been reported for the purified cytochrome [2]. Considering the experimental errors included in these measurements, these values can probably be considered identical. There thus does not appear to be any allotopy involved in the redox properties of this cytochrome.

(iii) Cytochrome b_6

The original investigation of the midpoint potential of this cytochrome indicated that cytochrome b_6 was a one-electron carrier with a midpoint potential near 0.0 V [31]. A later reinvestigation in Cramer's laboratory yielded an $n \approx 1$ titration curve with a midpoint of —180 mV for cytochrome b_6 [28]. Investigations in our laboratory suggested that the midpoint potential of cytochrome b_6 in chloroplasts was close to the originally reported value of 0.0 V [32]. In an attempt to reconcile these differences, Böhme and Cramer [33] reinvestigated the oxidation—reduction properties of cytochrome b_6 and found a midpoint potential for the cytochrome in fresh, well-coupled chloroplasts, near 0.0 V. After some time, the midpoint potential shifted to a value near —20 mV, suggesting that the long times involved in insuring rigorously anaerobic conditions might have caused the potential to shift to the —180 mV value observed previously. More interesting than this purely methodological consideration, was the observation that the addition of uncoupling agents (reagents that cause a loss of ability to form ATP and other high energy species but do not inhibit electron transfer reactions) also causes a shift in the midpoint potential of cytochrome b_6 from 0.0 V to values near —150 mV. Because uncoupling agents are known to alter the properties of the chloroplast membrane (making the membrane more permeable to protons), this observation raises the possibility of an allotopic interaction of cytochrome b_6 with other membrane components. Perhaps the finding of greatest interest in this study was the observation that cytochrome b_6 in fresh well-coupled chloroplasts gave an $n = 2$ titration curve. Aging or addition of uncouplers not only shifted the midpoint to more negative values but resulted in $n = 1$ titration curves. These results suggest the possibility of an interaction, perhaps between two cytochrome b_6 molecules, in coupled chloroplasts that is disrupted in uncoupled chloroplasts.

No oxidation—reduction measurements have been reported for purified cytochrome b_6 so it is not known whether its midpoint potential is dramatically shifted when freed from the membrane as with respiratory *b*-type cytochromes. Measurements of the midpoint potential of cytochrome b_6 in subchloroplast fragments enriched in Photosystem I and in the cytochrome b_6 · cytochrome *f* complex have also been made. Values of +5 mV [34] and values near —100 mV [12,20,30] have been reported, probably reflecting the same phenomena that caused similarly divergent values to be reported for the cytochrome in chloroplasts.

(iv) Cytochrome b_{559}

The midpoint potential of this cytochrome was an item of controversy for a considerable period of time. The earliest determinations of the midpoint potential of cytochrome b_{559} gave values of +370 mV [27] and +330 mV [35]. This value is quite electropositive for *b*-type cytochromes in ATP synthesizing

membrane systems. Measurements in Cramer's laboratory gave a much lower value, +80 mV (pH 7.0) [28], and measurements by Hind and Nakatani showed a value of +55 mV in sub-chloroplast fragments prepared by treatment with the detergent Triton X-100 [36]. An extensive series of investigations in several laboratories have succeeded in clarifying the situation to a considerable extent. It appears that most (although perhaps not all) of the cytochrome b_{559} in freshly isolated chloroplasts is in the high potential ($E_m \simeq +350$ mV) form. A variety of treatments (e.g., aging, treatment with detergent) convert cytochrome b_{559} from this high potential form to the low potential ($E_m \simeq +80$ mV) form (see ref. 37 for a recent review). In some cases, the process is reversible and the low potential form can be reconverted to the high potential form [38].

It thus appears likely that there is but a single cytochrome b_{559} in chloroplasts and it can exist in either of two forms. The question is however far from definitely settled. Even freshly isolated chloroplasts contain some low potential cytochrome b_{559} [37,39]. It is not clear whether this is a result of some unavoidable "damage" to the membranes during preparation of the chloroplasts or if the low potential form plays some physiological role. There have also been reports that while the high potential form of cytochrome b_{559} is associated with Photosystem II, some low potential cytochrome b_{559} is associated with the cytochrome $b_6 \cdot f$ complex in Photosystem I [30,40]. The situation is further complicated by studies with algal mutants that suggest two different pools of high potential cytochrome b_{559} in Photosystem II [41]. Finally, there has even been a report that cytochrome b_{559} can exist in three different potential forms [39]. However this report does not include documentation for three different midpoint potentials and the observation of three apparent forms probably resulted from the kinetic slowness of the reductants used in this study.

(v) Effect of pH

The pH-dependence of midpoint oxidation—reduction potentials of photosynthetic electron carriers was often measured in the past more for the sake of completeness than for any great intrinsic interest. More recently, the widespread interest in the chemiosmotic theory of energy conservation (ATP production) has caused pH effects to assume a much more prominent place in the thoughts of biochemists interested in bioenergetics. A detailed description of the chemiosmotic hypothesis [42] is far beyond the scope of this article. Briefly, the hypothesis pictures a specific arrangement in the membrane of electron carriers, alternating between carriers of electrons only and carriers of both electrons plus protons. Electron flow along such a chain results in the uptake of protons from solution on one side of the proton-impermeable membrane and the deposition of protons on the other side of the membrane. This trans-membrane pH gradient (and any associated membrane potential) represents a form of energy that can be utilized by appropriate enzymes to drive ATP synthesis.

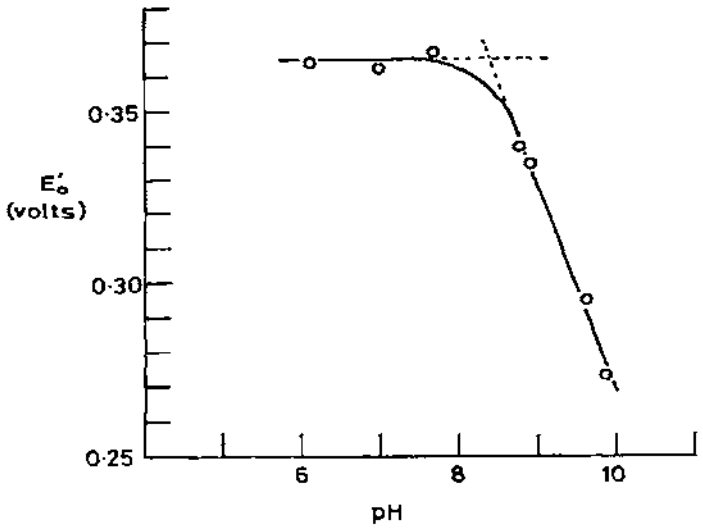

Fig. 3. The effect of pH on the oxidation—reduction midpoint potential of isolated cytochrome cytochrome *f* (D.S. Bendall, H.E. Davenport and R. Hill, in A. San Pietro (Ed.), Methods in Enzymology, Vol. 23, Part A, Academic Press, New York, 1971, p. 333).

Electron carriers that can also function as proton carriers will have pH-dependent midpoint potentials. Thus the search for electron carriers that could serve as trans-membrane proton carriers within the chemiosmotic context caused renewed interest in the pH-dependence of chloroplast cytochrome midpoint potentials. Figure 3 shows the pH-dependence of the midpoint potential for purified, soluble cytochrome *f*. The data show a slope of -60 mV pH^{-1} in the pH region above pH 8.4, indicating that reduction of cytochrome *f* is accompanied by the uptake of one proton per electron in this region. These data can be explained in terms of a pK = 8.4 for the oxidized form of the cytochrome. Thus for pH < 8.4, both oxidized and reduced cytochrome *f* are protonated and reduction of the cytochrome is not accompanied by proton uptake. At pH > 8.4 only the reduced cytochrome is protonated and the reduction of cytochrome *f*, which can be represented by the equation

$$\text{cyt. } f^{+3} + 1\ e^- + H^+ \rightarrow \text{H-cyt. } f^{+2} \qquad (1)$$

will result in a 60 mV decrease in midpoint potential for each unit increase in pH. Unfortunately, data are not available for the pH-dependence of the midpoint potential of cytochrome *f* in situ. Cases of allotopy are known where a soluble cytochrome shows a pH-dependent midpoint potential while the membrane-bound cytochrome has a pH-independent midpoint potential [43].

As was mentioned above, quantitative oxidation—reduction measurements have not yet been made on the solubilized chloroplast *b*-type cytochromes. Investigations of the cytochromes in situ have included measurements of the pH-dependence of their midpoint potentials. Fan and Cramer have reported

that cytochrome b_6 has a pH-dependent midpoint potential over the range from pH 6.0 to pH 8.0 [28]. Unfortunately, these measurements were made on the "damaged" (E_m = —180 mV) form of cytochrome b_6 and no information is available on the pH-dependence of cytochrome b_6 in fresh, coupled chloroplasts (E_m = +5 mV). The possibility thus exists that both cytochromes b_6 and f could act as proton carriers contributing to ATP synthesis during cyclic electron flow.

Perhaps the most interesting pH effects have been reported for cytochrome b_{559}. Fan and Cramer [28] found that the low potential form showed a —40 mV pH^{-1} dependence for E_m in the range from pH 6.0 to pH 8.0. Work in our laboratory [44] and in Bendall's laboratory [27] showed that the high potential form of cytochrome b_{559} had a midpoint potential that was pH-independent over the range from pH 6.0 to pH 9.0. Measurements of E_m below pH 6.0 were consistent with a —60 mV pH^{-1} dependence, suggesting a pK = 6.0 for the reduced cytochrome [44]. Thus

$$\text{pH} < 6.0 \qquad \text{cyt. } b_{559}^{+3} + H^+ + e^- \rightarrow \text{H-cyt. } b_{559}^{+2} \tag{2}$$

$$\text{pH} > 6.0 \qquad \text{cyt. } b_{559}^{+3} + e^- \rightarrow \text{cyt. } b_{559}^{+2} \tag{3}$$

Because of the difficulty in obtaining data with chloroplasts at pH <5.5, the conclusion that oxidized cytochrome b_{559} has a pK near 6.0 must be considered tentative.

There has been a report from Cramer's laboratory that lowering the pH below 6.0 converts most of the high potential cytochrome b_{559} to a lower potential [45,46]. Since it is known that light-induced electron flow is accompanied by large trans-membrane proton movements [47], this work suggested that the functional form of the cytochrome might be the low potential form. The high potential form found in isolated chloroplasts would be rapidly converted to the low potential form as light produces an acidification of the space inside the membrane. Changes in chloroplast membrane structure are known to result from these proton movements and it is possible that these alterations in membrane structure are responsible for the change in midpoint potential of this membrane-bound cytochrome. It was also found [46] that low concentrations of DCMU (an inhibitor of Photosystem II known to block the oxidation of the photoreduced primary acceptor) prevent the conversion of cytochrome b_{559} to its low potential form from occurring at low pH. The possible physiological effects of these phenomena will be discussed below.

E. ALGAL CYTOCHROMES

The pattern of light-induced electron flow in the several different types of algae appear to be essentially identical to that described above for higher plants. Cytochrome contents have been observed in many algae that are similar to that described above for higher plants. Certain cytochromes appear to be unique to algae, such as the low-potential, soluble *c*-type cytochrome

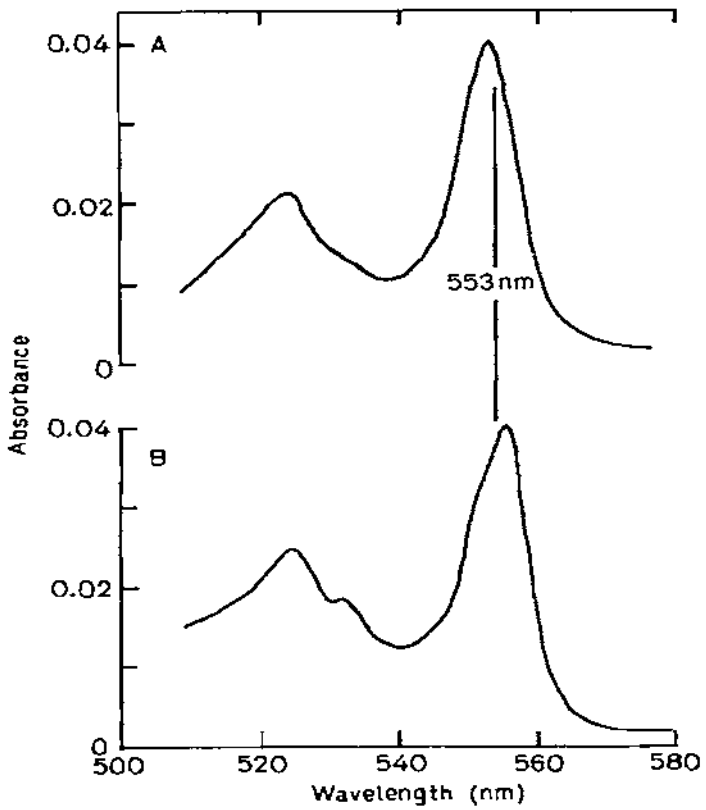

Fig. 4. The spectra of soluble cytochrome c_{552} and membrane-bound cytochrome *f* from *Euglena gracilis* (P.M. Wood, *Eur. J. Biochem.*, 72 (1977) 605).

found in blue–green algae [48,49]. As yet, no evidence has appeared favoring a role in photosynthetic electron transport for this cytochrome and so it will not be discussed further.

One unique aspect of algal cytochromes does appear to be worthwhile considering and that is the relationship of the soluble high potential *c*-type cytochromes found in these organisms to cytochrome *f*. A soluble *c*-type cytochrome with a midpoint potential similar to that of higher plant cytochrome *f* appears to be ubiquitous in the algae but absent in higher plants (see ref. 50 for a recent summary). These soluble *c*-type cytochromes differ spectrally from plant cytochrome *f*. The soluble cytochromes have relatively symmetric α-bands at 552–553 nm while cytochrome *f* has an asymmetric α-band at 554–555 nm. The soluble cytochromes have β-bands near 522 nm, while cytochrome *f* has a more complex β-band with the major peak at 523–524 nm and a second peak in the 530–532 nm region. Finally, the reduced soluble cytochromes have γ-bands near 416 nm, while cytochrome *f* has a γ-band near 422 nm. Figure 4 shows the different spectral characteristics of the soluble

cytochrome *c* and membrane-bound cytochrome *f* from the same organism. The soluble *c* cytochromes have molecular weights of 11,000—12,000 [51] compared to the 34,000 per heme observed for spinach cytochrome *f* [4], and also show different kinetic properties from cytochrome *f* in model systems (see below). Recent work by Wood has provided evidence that algae, in addition to containing the soluble *c*-type cytochrome, contains a membrane-bound *c*-type cytochrome that has properties essentially identical to cytochrome *f* [50]. Wood's results strongly suggest that this algal cytochrome *f* is not simply a membrane-bound form of the soluble cytochrome. In the case of one of the algae investigated, *Euglena gracilis*, the membrane-bound cytochrome *f* and the soluble cytochrome c_{552} have been shown to be immunologically distinct [50]. The possible role of the soluble *c*-type cytochrome in photosynthesis will be discussed below.

F. ROLE OF CYTOCHROMES IN PHOTOSYNTHESIS

(i) c-type cytochromes

1. Cytochrome f

(a) Non-cyclic electron flow. The first experimental evidence for the non-cyclic electron transport pathway shown in Fig. 1 (the "Z-scheme") came from experiments by Duysens and co-workers [52,53]. Using *Porphyridium cruentum*, a red alga in which the pigments associated with the two photosystems were different enough to allow a choice of monochromatic light that would activate primarily one photosystem, a series of "antagonistic effects" were found. Light absorbed primarily by Photosystem I resulted in the oxidation of certain electron carriers while light absorbed by Photosystem II caused a reduction of these same electron carriers. It was proposed that electron carriers exhibiting these antagonistic effects served as members of the chain linking the two photosystems. The first electron carrier to be found by Duysens and co-workers that satisfied this criterion was cytochrome *f*. Subsequent experiments with higher plants and other algae in many laboratories confirmed these initial findings (see ref. 54 for a typical example.). These later experiments, which examined the effects of specific inhibitors, non-cyclic electron acceptors ($NADP^+$ and $NADP^+$ substitutes), ADP and uncouplers, all provided support for the hypothesis that cytochrome *f* functioned as an electron carrier between the two light reactions in non-cyclic electron flow. Perhaps most convincing was the observation that algal mutants lacking cytochrome *f* have lost the ability to perform non-cyclic electron flow from H_2O to $NADP^+$ [55].

Although occasional challenges to the validity of the Z-scheme have been made, it is overwhelmingly accepted by workers in the field. Similarly, the position of cytochrome *f* in the Z-scheme described above seemed equally well established. Recently, Haehnel, in an elegant series of experiments, has provided evidence that suggests that plastoquinone and plastocyanin may be

the only carriers in the non-cyclic chain and that cytochrome *f* may play no role in this process [56,57]. Haehnel's initial experiments involved oxidizing P700 and all the carriers between the two photosystems by pre-illuminating chloroplasts with light absorbed only by Photosystem I. A subsequent series of μsec flashes reduced the plastoquinone pool completely (PQ can be reduced in 0.6 ms but takes 20 ms to reoxidize). Observations of electron flow as the reduced plastoquinone became reoxidized suggested that a total of only 3 electrons can be accommodated by electron carriers after plastoquinone (including $P700^+$) in the non-cyclic transfer chain. Of these 3 electrons lost by reduced plastoquinone, only 0.4 electrons are accepted by cytochrome *f*. More important, the kinetics of cytochrome *f* reduction do not show the lag expected if it were passing the electrons received from reduced plastoquinone on to $P700^+$. Plastocyanin, on the other hand, satisfies the criteria for a carrier between $P700^+$ and plastoquinone. It receives 1.8 electrons from reduced plastoquinone ($P700^+$ receives 0.8 electrons) and its reduction kinetics show the lag expected for a carrier that passes electrons on to $P700^+$ before accumulating in the reduced form. The oxidation time for plastocyanin, $t_{1/2} = 200$ μs, also corresponds to a known reduction time for $P700^+$ and quantitative measurements show an equivalence between the number of electrons lost by plastocyanin and accepted by $P700^+$. Haehnel concluded that at least 85% of the electrons placed in the plastoquinone pool by Photosystem II are transferred to P700 by plastocyanin without participation by cytochrome *f*. The role of cytochrome *f*, according to Haehnel, remains to be defined. Oxidation kinetics for cytochrome *f* showed $t_{1/2} < 40$ μs, presumably corresponding to the 20 μs component for $P700^+$ reduction (Haehnel feels that earlier reports of slower cytochrome *f* oxidation times were in error because of interfering absorbance changes). These kinetics may reflect the reaction of cytochrome *f* in the cyclic electron flow pathway.

It should be pointed out that the conclusions of Haehnel, while raising serious questions about the role of cytochrome *f* in non-cyclic electron transport, have not yet been accepted as conclusive. Because of the large amount of data accumulated over the past 15 years, consistent with a role for cytochrome *f* in this pathway, workers in photosynthesis have been reluctant to abandon the previously accepted formulation. It seems clear that a considerable amount of additional work will be necessary to settle this question. New experimental approaches may prove helpful and one that can briefly be mentioned is the use of fluid media at sub-zero temperatures. At these temperatures, the reactions occur at such a slow rate that the fast time response methods required at room temperature are no longer needed. Cox has recently demonstrated that P700 and cytochrome *f* could be photooxidized and photoreduced at −35° in 50% ethylene glycol [58]. These reactions showed many of the responses to inhibitors and uncoupling agents observed at physiological temperatures. Activation energies were measured for P700 and cytochrome *f* reduction and the value of 75 kJ mol^{-1} obtained is similar to the activation energy for overall electron transport, as would be expected from kinetic

measurements showing that electron transfer from plastoquinone on to $P700^+$ represents the rate limiting step in non-cyclic electron transport. Cox has argued that his data can best be interpreted in terms of cytochrome *f* participating in the main stream of non-cyclic electron flow [58].

(b) Cyclic electron flow. The physiological role of cyclic electron flow is outside the scope of this article. Accepting the fact that cyclic flow can be demonstrated in the laboratory, we can briefly examine the role of cytochromes in this flow. Some of the evidence for the participation of cytochrome *f* in cyclic electron flow is circumstantial and has been discussed above (its association with Photosystem I, the cytochrome $b_6 \cdot f$ complex, etc.). Somewhat more direct evidence for the participation of cytochrome *f* comes from the observation that soluble ferredoxin (presumably the physiological co-factor for cyclic electron flow) results in a more reduced steady-state oxidation level of cytochrome *f* [59], as would be anticipated from the scheme in Fig. 1. Experiments with spinach chloroplasts in Cramer's laboratory [60] and recent work in our laboratory using a cell-free preparation from blue-green alga has shown that under conditions favoring cyclic electron flow, cytochrome *f* responds to the addition of ADP in a manner consistent with the scheme in Fig. 1 [61].

2. Soluble algal cytochrome c

In higher plants and certain algae, the photooxidation of cytochrome *f* by Photosystem I appears to require the copper-containing protein, plastocyanin while the reduction of the cytochrome by Photosystem II does not require plastocyanin (see refs. 29 and 62 for a different view). Studies utilizing mutants lacking plastocyanin [55,63], the removal of plastocyanin by treatments such as sonication [64] or reagents such as KCN [65] or $HgCl_2$ [66] that inactivate plastocyanin, support the sequence of electron carriers shown below.

$$H_2O \rightarrow PSII \rightarrow PQ \rightarrow \text{cyt. } f \rightarrow PC \rightarrow PSI \rightarrow NADP^+ \qquad (4)$$

Certain algae, such as *Euglena gracilis* [67] and *Bumilleropsis filiformis* [68], appear to lack plastocyanin. The question naturally arises as to the identity of the immediate electron donor $P700^+$ in these algae. As discussed above, all algae appear to have a soluble *c*-type cytochrome in addition to the membrane-bound cytochrome *f* [50]. It has been known for some time that the soluble *c*-type cytochrome from *Euglena* is an excellent electron donor to $P700^+$ in *Euglena* chloroplasts [67,69,70] and this soluble cytochrome behaves in many ways analogously to plastocyanin in higher plants. The soluble *c*-type cytochromes in other organisms (*Bumilleropsis* [68] and the red alga *Porphyridium*) [71] also can serve as efficient donors to $P700^+$ in these organisms. Considering these results in conjunction with the newer findings that all algae appear to contain a membrane-bound cytochrome *f*, Wood has proposed the following sequence

$$H_2O \rightarrow PSII \rightarrow PQ \rightarrow \text{cyt. } f \rightarrow \text{cyt. } c_{552} \rightarrow PSI \rightarrow NADP^+ \qquad (5)$$

for those algae that lack plastocyanin. (The role that the soluble *c*-type cytochrome would play in algae such as the green algae *Chlamydomonas*, *Scenedesmus* and the blue—green alga *Anabaena*, that also have plastocyanin is not clear.)

The idea that two very different types of electron carriers — the copper-containing protein plastocyanin and the heme-containing soluble *c*-type cytochromes — could serve the same function in different organisms (and perhaps be interchangeable in organisms containing both) is remarkable. The scheme shown above with two *c*-type cytochromes functioning sequentially raises an interesting analogy to the respiratory electron transport chain in which cytochrome c_1 serves as the immediate electron donor to cytochrome *c* [22]. In this context, it is interesting to note that mitochondrial cytochrome *c* and the algal soluble *c*-type cytochromes have related amino acid sequences [51,72] which suggest an evolutionary connection between the respiratory and photosynthetic cytochromes. No data are available on the amino acid sequence of cytochrome *f* so it is not possible to comment on possible homologous amino acid sequences between it and mitochondrial cytochrome c_1. However, recent work indicates that the heme peptide of cytochrome c_1 has a molecular weight of 31,000—32,000 [73—75], very similar to the molecular weight reported for monomeric cytochrome *f* [4]. Also, *Euglena* cytochrome c_{552} has an absorbance band at 695 nm like that found in mammalian cytochrome *c* [76]. This absorbance band appears to be related to the sulfur from methionine serving as the sixth ligand to the heme iron.

(ii) Cytochrome b_6

There is substantial agreement that cytochrome b_6 functions as an electron carrier in Photosystem I-mediated cyclic electron flow. There is no evidence to suggest any participation by this cytochrome in non-cyclic electron flow in higher plants. The cytochrome can be both photooxidized and photoreduced by light absorbed only by Photosystem I [32,34,60,61,77] as would be expected for a carrier in the cyclic chain. Cytochrome b_6 becomes more reduced on the addition of ferredoxin [34,61,78] as would be expected and its oxidation level is affected by ADP and uncouplers [60,61] in a manner consistent with Fig. 1. Based on studies with algal mutants [77] and specific inhibitors [34,60], the oxidation of cytochrome b_6 appears to require at least plastoquinone, cytochrome *f* and plastocyanin, suggesting that these carriers are also part of the cyclic chain, functioning (as expected from their midpoint potentials) on the oxidizing side of cytochrome b_6. Kinetic experiments of Dolan and Hind show that cytochrome b_6 is photoreduced with $t_{1/2}$ = 1.3 ms and then oxidized with $t_{1/2}$ = 83 ms [79]. Thus electrons from cytochrome b_6 could serve as the source for cytochrome *f* reduction via the cyclic system.

(iii) Cytochrome b_{559}

The possible role of this cytochrome in photosynthetic electron transport is quite unclear and has elicited much controversy. Because this subject has

recently been reviewed [37,80], I will attempt to present only a brief summary of the current status of ideas about cytochrome b_{559}.

As discussed above, cytochrome b_{559} can exist in two different potential forms. The suggestion has been made that some low potential cytochrome b_{559} occurs naturally in chloroplasts and that this cytochrome b_{559} may function as an electron carrier associated with Photosystem I [30,40]. However, at this time, no evidence is available for light-induced oxidation—reduction reactions of cytochrome b_{559} during cyclic electron flow.

The major question in regard to cytochrome b_{559} is its role (if any) in non-cyclic electron transport. It is clear that most of the cytochrome b_{559} in chloroplasts is high-potential and fractionates [81,82] with Photosystem II during the preparation of subchloroplast fragments (some of the treatments used to fractionate the chloroplasts convert the cytochrome to the low potential form). A close association of cytochrome b_{559} with the Photosystem II reaction center can also be predicted on the basis of the observation that cytochrome b_{559} can be photooxidized by $P680^+$ at 77 K [83,84]. This low temperature photooxidation of cytochrome b_{559} has resulted in proposals that the cytochrome may be involved in water oxidation. Thermodynamic considerations make it highly unlikely that a component with $E_m = +350$ mV can be involved in oxidizing water to oxygen when the H_2O/O_2 couple has $E_m =$ +820 mV (pH 7). It appears more likely that $P680^+$ is such a strong oxidant that it reacts non-specifically with cytochrome b_{559} when reduction of $P680^+$ by the normal physiological donor is blocked at low temperature. There have been reports that etioplasts (chloroplast precursors found in the non-green leaves of dark grown plants) contain no high potential cytochrome b_{559} and that the capacity for oxygen evolution develops before any high potential cytochrome b_{559} appears during the greening process [85]. On the other hand, there exists a mutant of a green alga that has a functional Photosystem II reaction center but cannot oxidize water. This mutant has exactly half the amount of high potential cytochrome b_{559} as the wild type. These data have been interpreted in terms of two pools of cytochrome b_{559}, one required for H_2O oxidation [41]. As can be seen from this very brief summary, the possible role of cytochrome b_{559} in oxygen evolution is still a matter of considerable uncertainty.

Although cytochrome b_{559} in spinach chloroplasts can be photooxidized readily at low temperature, little or no photooxidation can be observed at physiological temperatures unless the chloroplasts are treated in one of a number of special ways [35,37,86]. An extensive series of experiments in Cramer's laboratory [87—89] showed that the low potential form of cytochrome b_{559} (presumably formed from the high potential cytochrome as a consequence of a light-induced pH gradient) could be photooxidized by Photosystem I and that plastoquinone functioned on the oxidizing side of cytochrome b_{559}. The following sequence of electron carriers for non-cyclic flow was proposed:

$$H_2O \rightarrow PSII \rightarrow \text{cyt. } b_{559} \rightarrow PQ \rightarrow \text{cyt. } f \rightarrow PC \rightarrow PSI \rightarrow NADP^+ \qquad (6)$$

Some studies with green algal mutants also supported such a sequence [90].

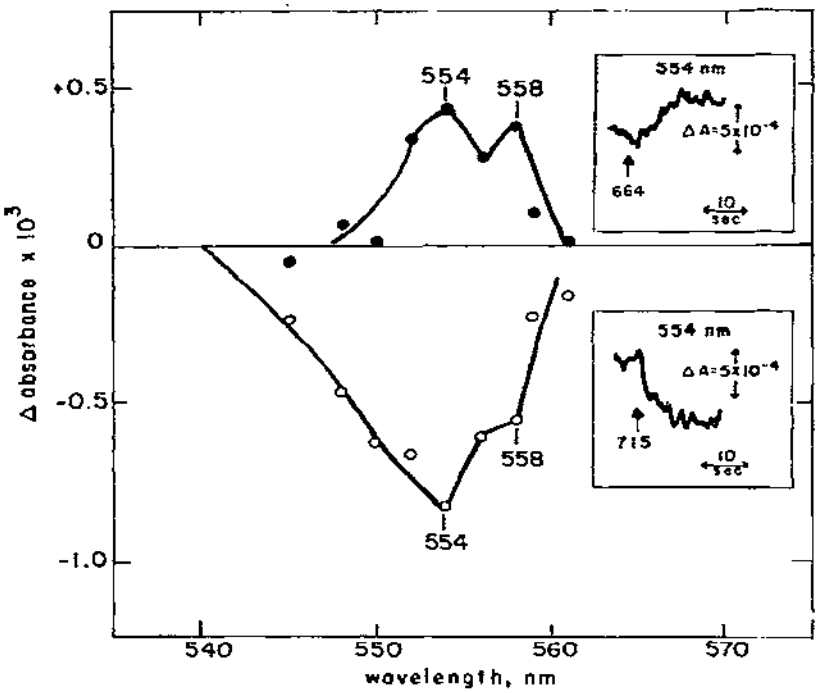

Fig. 5. Photosystem II cytochrome photoreduction and Photosystem I cytochrome photooxidation in the blue—green alga *Nostoc muscorum* (D.B. Knaff, Biochim. Biophys. Acta, 325 (1973) 284).

More recent experiments have shown that while the plastoquinone pool can be reduced in 6—10 ms, $t_{1/2}$ for cytochrome b_{559} reduction is 100 ms. Furthermore, uncouplers known to accelerate electron flow through the non-cyclic chain slow the rate of cytochrome b_{559} reduction [91]. These results are not compatible with cytochrome b_{559} functioning in the main chain during non-cyclic electron flow in higher plants.

In contrast to the absence of light-induced oxidation—reduction reactions of high potential cytochrome b_{559} under physiological conditions in spinach chloroplasts, the cytochrome does turn over in the light in several algal preparations. (There also has been a report of cytochrome b_{559} reactions in lettuce chloroplasts [92].) For example, as can be seen in Fig. 5, experiments in our laboratory with cell-free preparations from the blue—green alga *Nostoc muscorum* showed that cytochrome b_{559}, like cytochrome *f*, was oxidized by Photosystem I and reduced by Photosystem II [93]. The effects of Photosystem II inhibitors and non-cyclic electron acceptors on the steady-state oxidation level of the cytochrome are also consistent with it functioning in the main non-cyclic chain. Oxidation—reduction midpoint determinations and experiments with inhibitors known to be plastoquinone antagonists [93] can be best interpreted in terms of the cytochrome acting in its high potential

form. More rigorous kinetic experiments have yet to be performed in this algal system to see whether cytochrome b_{559} satisfies all the criteria expected for a component of the main non-cyclic chain. In the light of numerous observations indicating that electron flow in algae and plants is essentially identical, it appears unlikely that cytochrome b_{559} plays one role in higher plants and another in algae. However, such differences are not impossible (e.g., the substitution of a soluble *c*-type cytochrome in certain algae for the role filled by the copper-containing protein plastocyanin in higher plants) and it seems safest to say at this time that the physiological function of cytochrome b_{559} remains to be elucidated.

G. MODEL STUDIES

For those workers who favor the idea that cytochrome *f* and plastocyanin both participate in a linear non-cyclic electron transport chain, a question of importance has been the order of the two electron carriers. Isolated cytochrome *f* (+365 mV) and plastocyanin (+370 to +390 mV, refs. 29, 94 and 95) have midpoint potentials that are so close to each other as to make prediction of a sequence impossible. The midpoint of plastocyanin shifts to a less electropositive value when bound to the chloroplast membrane (we have measured values of +340 mV [29] in chloroplasts and +320 mV [30] in Photosystem I-enriched subchloroplast particles compared to +370 mV for soluble plastocyanin) while that of cytochrome *f* remains essentially the same (we have measured values of +380 mV [29] in chloroplasts and +375 mV [30] in Photosystem I-enriched fragments). Although the in situ midpoint potentials (as well as some data obtained in our laboratory using sonicated chloroplasts [62]) suggest a sequence

$$H_2O \rightarrow PSII \rightarrow PC \rightarrow cyt.\ f \rightarrow PSI \rightarrow NADP^+ \qquad (7)$$

rather than the more widely accepted sequence shown in eqn. 4, a wide variety of experiments with mutants and inhibitors that inactivate plastocyanin support the sequence shown in eqn. 1 with plastocyanin serving as the oxidant for cytochrome *f* (see discussion in Section D(i)).

A different approach to this problem has been taken by Wood and Bendall who have studied the kinetics of oxidation—reduction reactions of several cytochromes and copper-containing proteins. Wood found that the rate constant for the reduction of soluble plastocyanin by purified cytochrome *f* ($1.8 \times 10^7\ M^{-1}\ s^{-1}$) was much faster than the reduction of plastocyanin by other cytochromes, consistent with the sequence in eqn. 1 [96]. A continuation of this approach by Wood and Bendall examined the rate of reduction of photooxidized $P700^+$ by a number of cytochromes and copper-containing proteins using chloroplasts in which a detergent treatment made $P700^+$ accessible to the added electron donors [97]. The rate constant for $P700^+$ reduction by plastocyanin ($8 \times 10^7\ M^{-1}\ s^{-1}$) is at least 3 orders of magnitude higher than that for reduction of $P700^+$ by cytochrome *f* ($< 2 \times 10^4\ M^{-1}\ s^{-1}$). Wood and

Bendall argue that the observed rapid reductions of plastocyanin by cytochrome *f* and of $P700^+$ by plastocyanin support the sequence shown in eqn. 4, while the extremely slow reduction of $P700^+$ by purified cytochrome *f* is incompatible with the sequence shown in eqn. 4. The danger of extrapolating these results with purified, soluble components to the situation in vivo must be kept in mind. The data of Haehnel [57], showing that in chloroplasts $P700^+$ oxidizes plastocyanin with $t_{1/2} = 200$ μs and cytochrome *f* with $t_{1/2} < 40$ μs should be recalled.

Despite the above warning, the work of Wood and Bendall is extremely interesting. They have extended their work by examining electron transport in the dark along a portion of the electron transport chain and finding further suggestive evidence for eqn. 4 [98]. Electrons are fed into the chain of detergent-treated chloroplasts by plastoquinol-1 (a reduced plastoquinone similar in structure to the naturally occurring plastoquinol-9) and removed by soluble plastocyanin. The reduction of plastocyanin by plastoquinol-1, catalyzed by the chloroplasts in the dark, is sensitive to DBMIB, a well-known inhibitor of non-cyclic electron flow. Of interest is the observation that etioplasts, completely lacking chlorophyll, also catalyzed the reaction. Kinetic studies of the reaction at high plastoquinol concentrations, allowed calculation of a rate constant for plastocyanin reduction of 2.6×10^7 M^{-1} s^{-1} on the assumption that the cytochrome *f* in the chloroplasts was the species reducing plastocyanin. This value is in very good agreement with the rate constant measured directly for the reduction of plastocyanin by purified cytochrome *f* (see above). Furthermore, at low plastoquinol-1 concentrations (where the rate of plastocyanin reduction was proportional to chloroplast concentration and independent of plastocyanin concentration) the rate of plastocyanin reduction showed good agreement with the initial rate measured for the reduction of cytochrome *f* in the chloroplasts by plastoquinol-1. At high concentrations of both plastoquinol-1 and plastocyanin, the rate of the dark plastocyanin reduction saturated giving a rate of 70 s^{-1} (per cytochrome *f*). This limiting rate is remarkably similar to that for the maximal rate of light-driven non-cyclic electron transport, a process that has as its rate-limiting step, the oxidation of plastoquinol [99].

Wood has recently extended this work to algae and found a similar oxidation of plastoquinol-1 in the dark catalyzed by chloroplasts from *Euglena gracilis* [100]. The electron acceptor used in these studies was the soluble *Euglena* cytochrome c_{552}, in keeping with the idea that the soluble, low-molecular weight *c*-type cytochromes replace plastocyanin in organisms (such as *Euglena*) that appear to contain no plastocyanin (see eqn. 5). Reduction of the soluble *Euglena* cytochrome c_{552} by purified *Euglena* cytochrome *f* gave a rate constant $>10^7$ M^{-1} s^{-1}, similar to that measured for the reduction of higher plant plastocyanin by cytochrome *f* [50]. This observation, coupled with the earlier data of Wood and Bendall [97] showing that the soluble algal *c*-type cytochromes could reduce $P700^+$ almost as rapidly as plastocyanin ($K = 1.9 \times 10^7$ M^{-1} s^{-1} for cytochrome c_{553} from the red alga *Plocamium*

coccineum) supports the sequence shown in eqn. 5 for organisms lacking plastocyanin.

H. STRUCTURAL ASPECTS

The photosynthetic electron transport apparatus in higher plants and algae is embedded in a complex lipoprotein membrane. One eventual aim of research in photosynthesis is to map these membranes and establish the spatial location of the electron carriers in the membrane. The membranes can be fractionated into several major pigment–protein complexes and fractionation studies combined with techniques that label surface proteins have begun to give some idea of the architecture of photosynthetic membranes [101]. Similarly, comparative electron microscopy of membranes from normal mutant and etiolated systems has provided important information about the possible locations of certain proteins [102,103]. Other less direct approaches have also provided structural information. For example, the light reactions appear to produce a potential across the membrane (see the discussion of the chemiosmotic hypothesis above) and a determination of the polarity of this potential makes it likely that P700 and P680 are located near the inner side of the membrane while the primary acceptors are located near the outer side [104]. As an example of yet an additional approach, antibody studies have established the location of the flavoprotein that catalyzes $NADP^+$ reduction [105] and the enzyme that catalyzes the phosphorylation of ADP [106] on the membrane surface.

Unfortunately, no information is currently available concerning the exact location of any of the electron carriers. Experiments with antibodies and membrane-impermeable labelling compounds have produced conflicting results as to whether plastocyanin is on the surface of the membrane or buried in the membrane (see ref. 107 for a recent discussion). Antibodies against purified cytochrome *f* have no effect on light-induced electron transfer reactions in chloroplasts [108]. Thus, cytochrome *f* is probably buried in the membrane rendering it inaccessible to the antibodies. Interesting results have been obtained by Wildner and Hauska on the location of the soluble cytochrome c_{552} in the alga *Euglena gracilis* [67,109]. Antibodies against the purified cytochrome did not inhibit light-driven electron transfer, indicating that cytochrome c_{552} is located in the soluble space inside the membrane and is thus not accessible to the antibody. If *Euglena* chloroplasts were depleted of cytochrome c_{552} by treatments such as sonication, non-cyclic electron flow from H_2O to $NADP^+$ and cyclic ATP formation both stopped. While $NADP^+$ photoreduction could be reactivated by adding purified cytochrome c_{552}, cyclic ATP formation was not reactivated by the simple addition of the cytochrome. However addition of cytochrome c_{552} during sonication restores cyclic ATP formation. This implies that the cytochrome must be present at a specific site on the inside of the membrane for ATP-producing cyclic electron flow to occur. Sonication in the presence of the cytochrome allows it to cross

the temporarily open membrane. These results are strikingly similar to those previously obtained for plastocyanin in spinach chloroplasts [110], re-enforcing the idea that in organisms lacking plastocyanin, low molecular weight cytochromes fulfil the role played by plastocyanin in higher plants.

Antibodies have not yet been prepared against any of the photosynthetic *b*-type cytochromes. However, some indirect information concerning the location of cytochrome b_{559} comes from observations that cytochrome b_{559} in situ is oxidized by the highly charged oxidant ferricyanide at a considerably more rapid rate than is cytochrome *f*. Because charged species are unable to penetrate the hydrophobic core of the membrane, it has been argued that cytochrome b_{559} is probably located considerably closer to the aqueous interface of the membrane than is cytochrome *f* [111]. Illumination in the presence of the uncoupling agent CCCP, slows the rate of subsequent reaction of the cytochromes with chemical oxidants and reductants. Cramer and coworkers suggest the uncoupler may cause an increase in membrane microviscosity, inhibiting access to the cytochrome in the hydrophobic membrane's interior.

I. CONCLUSION

It is surprising that despite the enormous amount of work done on the cytochromes of higher plants and algae, the exact position of these electron carriers in the cyclic and non-cyclic chains is still not known with complete certainty. This uncertainty is in contrast to the reasonably well-established sequences available for the electron carriers of the mitochondrial electron transport chain [22] and the electron transport chains of certain photosynthetic bacteria [112]. Hopefully, the new techniques (some of which have been outlined above) being brought to bear on this problem will prove fruitful in the near future. Despite this major area of uncertainty, a great deal has been learned about plant and algal cytochromes in recent years (as I have tried to show) and it is anticipated that this progress will continue in the immediate future.

NOTES ADDED IN PROOF

1. R.P. Cox (Abstracts 4th Int. Congr. Photosynthesis, 1977, p. 76) has reported that the α-band of reduced purified cytochrome b_6 does not split at 77K, in contrast to the report [10] of such splitting discussed in Section C. (ii).
2. A recent investigation (B. Bouges-Bocquet, Biochim. Biophys. Acta, 462 (1977) 362) of cytochrome *f* and plastocyanin kinetics in the green alga *Chlorella pyrenoidosa* is consistent with the reaction sequence shown in Fig. 1 and in eqn. 4. In contrast to the results of Haehnel (see Section F.(i). 1.(a)), Bouges-Bocquet did observe a sigmoidal time-course for cytochrome *f* oxidation and the data can be fitted using rate constants of $4.6 \times 10^3\ s^{-1}$ for $P700^+ + PC_{Red} \rightarrow P700 + PC_{ox}$ and $1.4 \times 10^4\ s^{-1}$ for $PC_{ox} + cyt.f_{Red} \rightarrow PC_{Red} + cyt.f_{ox}$.

3. Recent data (H. Bohner and P. Böger, FEBS Lett., 85 (1978) 337) show that plastocyanin and the soluble cytochrome c_{553} serve as alternative electron donor to P700 in the green alga *Scenedesmus acutus*, with cytochrome c_{553} being present only when the copper content of the medium is too low for plastocyanin to be formed.

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