Biochimica et Biophysica Acta, 357 (1974) 370–379

BBA 46786

RATE OF ELECTRON TRANSFER BETWEEN PLASTOCYANIN, CYTOCHROME *f*, RELATED PROTEINS AND ARTIFICIAL REDOX REAGENTS IN SOLUTION

P. MUIR WOOD

Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW (U.K.)

(Received February 28th, 1974)

SUMMARY

The rate of electron transfer between reduced cytochrome *f* and plastocyanin (both purified from parsley) has been measured as $k = 3.6 \cdot 10^7\ M^{-1} \cdot s^{-1}$, at 298 °K and pH 7.0, with activation parameters $\Delta H^{\ddagger} = 44\ kJ \cdot mole^{-1}$ and $\Delta S^{\ddagger} = +46\ J \cdot mole^{-1} \cdot °K^{-1}$. Replacement of cytochrome *f* with red algal cytochrome *c*-553, *Pseudomonas* cytochrome *c*-551 and mammalian cytochrome *c* gave rates at least 30 times slower: $k = 5 \cdot 10^5$, $7.5 \cdot 10^5$ and $1.0 \cdot 10^6\ M^{-1} \cdot s^{-1}$, respectively.

Similar measurements made with azurin instead of plastocyanin gave $k = 6 \cdot 10^6$ and approx. $2 \cdot 10^7\ M^{-1} \cdot s^{-1}$ for reaction of reduced azurin with cytochrome *f* and algal cytochrome respectively.

Rate constants of 115 and 80 $M^{-1} \cdot s^{-1}$ were found for reduction of plastocyanin by ascorbate and hydroquinone at 298 °K and pH 7.0. The rate constants for the oxidation of plastocyanin, cytochrome *f*, *Pseudomonas* cytochrome *c*-551 and red algal cytochrome *c*-553 by ferricyanide were found to be between $3 \cdot 10^4$ and $8 \cdot 10^4\ M^{-1} \cdot s^{-1}$.

The results are discussed in relation to photosynthetic electron transport.

INTRODUCTION

There is general agreement that cytochrome *f* and plastocyanin are situated in the photosynthetic electron transport system between plastoquinone and P700, the primary donor of Photosystem I. Most of the evidence supports the view that they act in series [1–4], but some authors prefer schemes in which the two proteins act in parallel [5–7]. A further possibility is that plastocyanin facilitates the interaction between cytochrome *f* and P700 by a modification of the structure rather than by participation in electron transport. A mechanism with the two in series requires demonstration of fast cytochrome–plastocyanin electron transfer, and this has not previously been done, partly because it is difficult to monitor plastocyanin directly in vivo [8]. Malkin and Bearden [9] have recently succeeded in demonstrating

Abbreviations: DBMIB, 2,5-dibromomethylisopropyl-*p*-benzoquinone; cytochrome c_{mam}, horse heart cytochrome *c*.

photooxidation and photoreduction of plastocyanin in isolated chloroplasts by EPR spectroscopy at 25 °K, but kinetic studies are difficult by this technique.

This paper reports the results of experiments with the purified proteins which reveal a very fast electron transfer rate, at present the fastest reaction known for either protein. To see whether the interaction was specific the result was compared with rates for a number of *c* type cytochromes reacting with plastocyanin and azurin. Also included are some measurements with various redox reagents, partly for comparison, but also in the hope that data of this sort will help to clarify the function of plastocyanin in photosynthetic electron transport.

The cytochromes used for comparison with cytochrome *f* were cytochrome *c*-553 from the red alga *Plocamium coccineum*, cytochrome *c*-551 from the bacterium *Pseudomonas fluorescens* and mammalian cytochrome *c* (basic, unlike the others). Plastocyanin is absent from red algae [10], and cytochrome *c*-553 appears to combine the functions of cytochrome *f* and plastocyanin in higher plants.

Among the well characterized "blue" copper proteins azurin has the greatest resemblance to plastocyanin. They have similar isoelectric points (5.2 for azurin [11], 4.2 for plastocyanin [12]), broadly similar spectra, and redox potentials differing by approx. 70 mV. Ambler and co-workers [13, 14] have sequenced both; they state that "sequence similarities are not sufficient to provide convincing evidence for homology but are striking enough to suggest at least a functional similarity".

MATERIALS AND METHODS

Cytochrome *f* was prepared from parsley (*Petroselinum sativum*) by the method of Bendall et al. [15], based on the procedure developed by Hill and Scarisbrick [16]. Material with an absorption ratio $A_{422\,nm}/A_{278\,nm}$ of 2.5-3.0 was used in most cases since further purification was only possible at very low yield. Much less pure fractions, with an $A_{422\,nm}/A_{278\,nm}$ ratio of approx. 1, gave identical kinetics suggesting that purity was not critical.

Plastocyanin was prepared from parsley as described by Plesničar and Bendall [17]. This yielded material with an $A_{278\,nm}/A_{597\,nm}$ ratio of 1.6, unaltered by passage through a Sephadex G75 column. Ramshaw et al. [12] list $A_{278\,nm}/A_{597\,nm}$ ratios for plastocyanin from nine species of dicotyledon, not including parsley; the values show considerable variation being usually between 1.0 and 1.2, but up to 1.7 in one case.

Azurin and cytochrome *c*-551 were extracted from *Pseudomonas fluorescens*, Strain P6009/1. Two 801 batches were grown and harvested as described by Ambler [18]. The azurin and cytochrome *c*-551 were extracted together from an acetone powder following Ambler's procedure, with additional purification of the azurin based on Ambler and Brown [13]. No cytochrome absorption near 420 nm was detectable in the spectrum of the purified azurin.

Red algal cytochrome *c*-553 was prepared by the methods of Katoh [19] from *Plocamium coccineum* collected on the Norfolk coast. 5.8 kg of thalli yielded 0.2 μmole with an $A_{553\,nm}/A_{272\,nm}$ ratio of 0.66, a yield much lower than Katoh's from *Porphyra*.

Horse heart cytochrome *c* (cytochrome c_{mam}) was obtained from BDH Chemicals, Poole, and used without further purification.

Absorption spectra were recorded on a Beckman DK2 spectrophotometer.

The cytochrome *f*-plastocyanin reaction was studied with a Durrum–Gibson stopped-flow apparatus fitted with a 2-cm reaction chamber, monitoring absorption at 424 nm. The instrumental dead time was 4 ms. For all the other reactions a dual wavelength recording spectrophotometer was used, fitted with an efficient stirrer so that added reagents were completely mixed in less than 1 s. Suitable wavelengths were 409–423 nm for cytochrome *f*, 405–417 nm for other cytochromes, and 540–593 nm for plastocyanin reactions in the absence of cytochromes. The dual wavelength instrument was used whenever possible since measurements on the stopped-flow apparatus needed about 10 times as much protein. The much poorer time resolution of the dual wavelength instrument was to some extent compensated by its greater sensitivity, permitting the use of lower concentrations; for cytochrome reactions, rate constants up to $k = 5 \cdot 10^6\ M^{-1} \cdot s^{-1}$ could be measured on the dual wavelength machine.

Protein concentrations were determined by use of the following absorption coefficients: reduced cytochrome *f*, $\varepsilon_{554.5\ nm} = 2.6 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ [15]. Oxidised plastocyanin, $\varepsilon_{597\,nm} = 4.5 \cdot 10^3\ M^{-1} \cdot cm^{-1}$ [12]. Oxidised azurin, $\varepsilon_{625\ nm} = 4.8 \cdot 10^3\ M^{-1} \cdot cm^{-1}$ [13]. Reduced *Pseudomonas* cytochrome *c*-551, $\varepsilon_{551\ nm} = 3.0 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ [18]. Reduced horse heart cytochrome *c*, $\varepsilon_{550\ nm} = 2.76 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ [20]. For reduced *Plocamium* cytochrome *c*-553 $\varepsilon_{553\ nm} = 2.6 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ was assumed since no measurements have been made for *Plocamium* or similar species, and in all kinetic work an excess of the other component was maintained.

Cytochromes *f* and *c*-553 were prepared in the reduced state; the others were oxidized. When necessary reduction or oxidation was carried out by the addition of a 10-fold excess of ascorbate or ferricyanide, followed by passage of the solution through a Sephadex G-25 column equilibrated with pH-7.0 buffer.

Except where otherwise stated, measurements were made at 298 °K in a 10 mM phosphate buffer (pH 7.0), with 90 mM NaCl added to give an ionic strength of 0.1.

RESULTS

Cytochrome f + plastocyanin

This very fast reaction was studied using the stopped-flow apparatus. As shown in Fig. 1, oxidation of cytochrome *f* by an excess of plastocyanin followed a

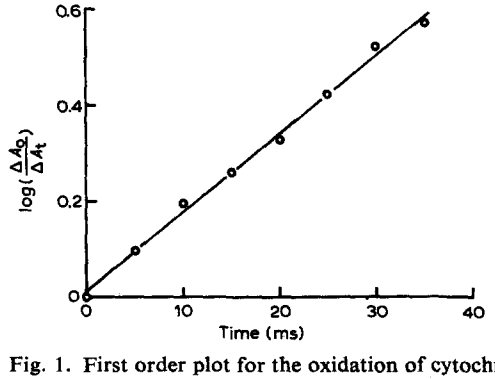

Fig. 1. First order plot for the oxidation of cytochrome *f* by plastocyanin. 10 mM phosphate buffer (pH 7.0) plus 90 mM NaCl. Wavelength 424 nm. Temperature 298 °K. The cytochrome concentration was 0.22 μM and the plastocyanin concentration 2.2 μM. Zero time corresponds to 10 ms after mixing.

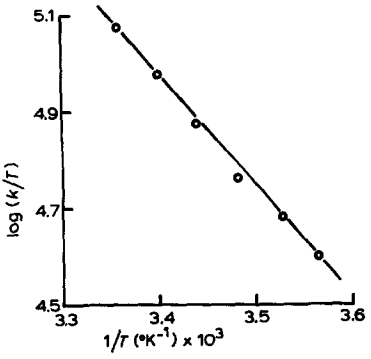

Fig. 2. Eyring plot of rate data for the reaction between reduced cytochrome *f* and plastocyanin. 10 mM phosphate buffer (pH 7.0) plus 90 mM NaCl. The cytochrome concentration was 0.22 μM and the plastocyanin concentration 2.2 μM.

pseudo first-order rate dependence, with no sign of any complications. The mean value for the rate coefficient at 298 °K and pH 7.0 was $k = 3.6 \cdot 10^7\ M^{-1} \cdot s^{-1}$.

The activation parameters were determined by measuring the rate coefficient at a series of temperatures from 276 to 298 °K. Fig. 2 shows a plot of $\log(k/T)$:$1/T$ which is a good straight line as expected from the Eyring equation

$$k = (k'T/h)\exp(\Delta S^{\ddagger}/R)\exp -(\Delta H^{\ddagger}/RT),$$

where k' is Boltzmann's constant, and $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are the enthalpy and entropy of activation. The values $\Delta H^{\ddagger} = 44$ kJ $\cdot$ mole^{-1} and $\Delta S^{\ddagger} = +46$ J $\cdot$ mole$^{-1} \cdot$ °K^{-1} were derived from the graph. The activation energy, E_A, can be defined by the Arrhenius formula

$$\ln(k) = -E_A/RT + \text{constant},$$

which gives $E_A = \Delta H^{\ddagger} + RT$, or 46 kJ $\cdot$ mole^{-1}.

A few observations with plastocyanin from spinach (*Spinacea oleracea*) gave similar kinetics; this result may be compared with the finding of Plesničar and Bendall [17] that plastocyanin from peas and parsley gave identical rates in their enzymatic assay.

Since the internal pH of an illuminated thylakoid is about 5, the rate was also measured at pH 5.0 (succinate buffer), but was not markedly different.

2,5-Dibromomethylisopropyl-*p*-benzoquinone (DBMIB), an inhibitor of photosynthetic electron transport [21] which probably acts close to the site of electron transfer from plastoquinone to cytochrome *f*, had no effect on the rate of cytochrome oxidation when present in a ratio of 1:1 with the cytochrome.

Other cytochrome–cuproprotein reactions

All the other reactions investigated were slower and measurements were made on the dual wavelength spectrophotometer. Except for reactions involving hydroquinone (see below) the kinetics were always pseudo first order. The results for cytochrome–cuproprotein reactions are shown in Table I, where for purposes of comparison all rates are given in the thermodynamically favourable direction; the relative redox potentials are needed in order that this may be defined. An approximate series of E'_0 was established as follows: 0.26 V for cytochrome c_{mam} and *Pseudomonas*

TABLE I

CYTOCHROME–CUPROPROTEIN ELECTRON TRANSFER RATES

Rate constants ($M^{-1} \cdot s^{-1}$) for pH 7.0, ionic strength 0.1, 298 °K, are given in the thermodynamically favourable direction. The relative redox potentials (see text) are: cytochrome c_{mam} ≈ *Pseudomonas* cytochrome *c*-551 < azurin < cytochrome *f* ≈ *Plocamium* cytochrome *c*-553 < plastocyanin.

	Cytochrome *f*	*Plocamium* Cytochrome *c*-553	*Pseudomonas* Cytochrome *c*-551	Cytochrome c_{mam}
Plastocyanin	$3.6 \cdot 10^7$	$5 \cdot 10^5$	$7.5 \cdot 10^5$	$1.0 \cdot 10^6$
Azurin	$6 \cdot 10^6$ *	$\approx 2 \cdot 10^7$ *	$5 \cdot 10^6$ **	$3 \cdot 10^3$ ***

* Measured in the reverse direction.

** Antonini et al. [22]. From $k_{293\,°K}$ and E_A.

*** Greenwood et al. [23] From $k = 1.1 \cdot 10^3$ $M^{-1} \cdot s^{-1}$ in the reverse direction, and equilibrium constant $K = 3$ [22, 24].

cytochrome *c*-551, 0.29 V for azurin, 0.34 V for cytochrome *f* and *Plocamium* cytochrome *c*-553, and 0.36 V for plastocyanin. This series was compiled by assuming $E'_0 = 0.259$ V for cytochrome c_{mam} [25], $K = 3$ for *Pseudomonas* cytochrome *c*-551 and azurin [22], $K = 1$ for cytochrome c_{mam} and *Pseudomonas* cytochrome *c*-551, and measuring three other equilibrium constants spectroscopically: $K = 6$ for azurin and cytochrome *f*, $K = 6$ for azurin and *Plocamium* cytochrome *c*-553, and $K = 2$ for cytochrome *f* and plastocyanin.

For the reactions of *Pseudomonas* cytochrome *c*-551 and cytochrome c_{mam} with plastocyanin the cytochromes were first reduced as described above. The reactions between azurin and cytochromes *f* and *c*-553 were measured in the thermodynamically unfavourable direction (oxidized azurin, reduced cytochrome), the results being multiplied by the equilibrium constants ($K = 6$ in each case) to give the values in Table I. Care was taken to use for the kinetic analysis only that part of the time course for which the rate of the back reaction was less than 10 % of the pseudo first-order rate being measured.

TABLE II

ELECTRON TRANSFER FROM CYTOCHROMES AND CUPROPROTEINS TO FERRICYANIDE

Rate constants ($M^{-1} \cdot s^{-1}$) are for pH 7.0, ionic strength 0.1 and 298 °K, unless otherwise stated.

Plastocyanin	$7 \cdot 10^4$
Azurin	$1.2 \cdot 10^4$ *
Cytochrome *f*	$8 \cdot 10^4$
Plocamium cytochrome *c*-553	$3.5 \cdot 10^4$
Pseudomonas cytochrome *c*-551	$8 \cdot 10^4$ *
Cytochrome c_{mam}	$6.7 \cdot 10^6$ **

* 293 °K. Antonini et al. [22].

** Morton et al. [24].

Rates of oxidation by ferricyanide

Table II gives the results of kinetic measurements of plastocyanin, cytochrome *f* and *Plocamium* cytochrome *c*-553 oxidation by ferricyanide, and also shows published data for the other proteins for comparison. The three reactions studied all gave simple pseudo first-order kinetics, and very similar rates.

Reduction of plastocyanin by ascorbate and hydroquinone

To extend the available kinetic data for plastocyanin (see Discussion) measurements were made for reduction by ascorbate and hydroquinone. For comparison the rate of reduction of cytochrome *f* by hydroquinone was also measured.

Reduction of plastocyanin by ascorbate was quite slow but followed simple pseudo first order kinetics, as found by Van Buuren et al. [26] for reduction of cytochrome c_{mam} by ascorbate. A plausible formulation is

$$AscH_2 + PC_{ox} \xrightarrow{k'} AscH + PC_{red}$$

$$AscH + PC_{ox} \xrightarrow{fast} Asc \quad + PC_{red},$$

where PC stands for plastocyanin. The measured rate constant for reduction of plastocyanin was $k = 2k' = 115\ M^{-1} \cdot s^{-1}$, at pH 7.0.

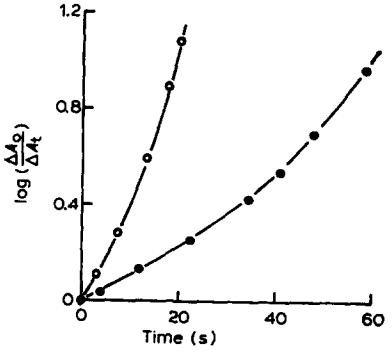

Fig. 3. First-order plots for the reduction of cytochrome *f* (○) and plastocyanin (●) by hydroquinone. 10 mM phosphate buffer (pH 7.0) plus 90 mM NaCl. Wavelengths 409 and 423 nm for cytochrome *f*, 540 and 593 nm for plastocyanin. Temperature 298 °K. The concentrations were: hydroquinone 390 μM, plastocyanin 2.6 μM, cytochrome *f* 120 nM. Zero time corresponds to 2 s after mixing.

Fig. 3 shows pseudo first-order plots for reduction of plastocyanin and cytochrome *f* by excess hydroquinone at pH 7.0. In both cases the apparent order of reaction was less than one, and the rate of reduction of plastocyanin increased by a factor of thirty on changing from pH 6.4 to 7.4; removal of O_2 from the solution had no effect. Approximate values for the rate constants at pH 7.0 were 80 $M^{-1} \cdot s^{-1}$ for plastocyanin and 270 $M^{-1} \cdot s^{-1}$ for cytochrome *f*. Comparison with Yamazaki and Ohnishi's results [27] for cytochrome c_{mam} reduction by hydroquinone suggests that the high pH dependence can be ascribed to the much greater reactivity of hydro-

quinone anions, while the departure from pseudo first order kinetics may be associated with equilibria involving the semiquinone anion; the reaction of reduced dichlorophenolindophenol with ferricyanide also shows some similar features [28].

DISCUSSION

In Table III the kinetic data for electron transfer reactions of plastocyanin is summarised, including earlier results for phenylenediamine [29], dithionite [30] and chromous ions [31]. The low rates with ascorbate and hydroquinone are attributable to a high activation energy for $1e^-$ reduction, and agree with the fact that these reagents are both poor Photosystem I donors, unlike phenylenediamine [32]. Preliminary experiments have also shown that reduced DBMIB is no more efficient than hydroquinone at reducing plastocyanin. This is as expected from the behaviour of DBMIB as an electron transport inhibitor [21]; besides blocking electron transfer from plastoquinone, DBMIB is itself reduced [33] but does not donate electrons to plastocyanin at a significant rate. Trebst and Reimer [34] have shown that inhibition of Photosystem I by DBMIB can be relieved by phenylenediamine, which shuttles electrons from reduced DBMIB to plastocyanin.

Ferricyanide shows very uniform rates of oxidation for all the acidic proteins listed in Table II, but the basic cytochrome c_{mam} reacts about a 100 times faster. The results suggest that ferrocyanide should be a reasonable donor to Photosystem I in preparations where membrane permeability is not a problem (e.g. with detergent or Photosystem I particles) provided exogenous plastocyanin is added to restore a reasonable concentration.

When Antonini et al. [22] compared the kinetics of azurin reduction by *Pseudomonas* cytochrome *c*-551 and cytochrome c_{mam} the results suggested a high

TABLE III

KINETIC DATA FOR PLASTOCYANIN

A summary of kinetic data for oxidation and reduction of plastocyanin. Unless otherwise stated, pH 7.0, ionic strength 0.1 and 298 °K.

	$M^{-1} \cdot s^{-1}$
Electron donors	
ascorbate	115
hydroquinone	80
phenylenediamine	$3.2 \cdot 10^4$ *
dithionite, $S_2O_4^{2-}$	$1.4 \cdot 10^5$ **
SO_2^-	$2.9 \cdot 10^7$ **
chromous (CrII)	$3.2 \cdot 10^4$ ***
cytochromes *c*-553, *c*-551, c_{mam}	$5 \cdot 10^5 - 1 \cdot 10^6$ (Table I)
cytochrome *f*	$3.6 \cdot 10^7$
Electron acceptor	
ferricyanide	$7 \cdot 10^4$

* Nagamura and Ogura [29].
** Lambeth and Palmer [30]; pH 8.0; ionic strength 0.4.
*** Dawson et al. [31], pH 4.2.

degree of biological specificity, since the unnatural reaction was more than a thousand times slower than the natural one. However both of the photosynthetic cytochromes tried here react with azurin at least as fast as cytochrome *c*-551, and on the basis of this admittedly small sample the only specificity of azurin seems to be for acidic cytochromes rather than basic ones (cf. ferricyanide above).

In vivo cytochrome *f* is bound firmly to the thylakoid membrane [16]. Isolation requires fairly drastic methods and on extraction from parsley it aggregates to a tetramer [15]. Consequently if the purified protein had shown only slow rates of electron transfer to plastocyanin it would have been unsafe to draw any conclusions about their roles in photosynthesis. This is not the case; the results in Table I show a degree of specificity in that the rate of cytochrome *f*–plastocyanin electron transfer is thirty times higher than that of any of the other cytochromes tried, as well as a very high rate coefficient, $k = 3.6 \cdot 10^7\ M^{-1} \cdot s^{-1}$, at present the fastest rate measured for either protein. There are not many cases known of protein–protein reactions in solution which are faster than this; examples are $k = 4 \cdot 10^7\ M^{-1} \cdot s^{-1}$ for cytochrome c_{mam} and purified cytochrome oxidase [35], and $k = 3 \cdot 10^8\ M^{-1} \cdot s^{-1}$ for interaction of horse or yeast cytochrome *c* with yeast cytochrome *c* peroxidase [36]. The diffusion limit for large molecules is about $10^9\ M^{-1} \cdot s^{-1}$.

The magnitudes of the activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the cytochrome *f* and plastocyanin reaction suggest that the high rate is attributable not to a low activation energy but to a positive entropy term. Evidently unfolding of the proteins or breakage of ordered water structure outweighs the negative entropy contribution expected from electrostatic interaction of two negatively charged species.

The high rate of cytochrome *f*–plastocyanin electron transfer provides indirect support for a series mechanism in photosynthesis. The question that remains is whether the rate in solution can be related quantitatively to the behaviour in vivo. All procedures which rupture the thylakoid membrane (detergents, sonication, French press, freezing) result in loss of much of the plastocyanin, although Knaff and Malkin [37] have recently shown that some plastocyanin is left in most Photosystem I preparations. The plastocyanin content in chloroplasts is also several times that of cytochrome *f* or P700 [17]. These facts, together with the hydrophilic nature of plastocyanin, which being an acidic protein cannot dissolve in anionic lipids as cytochrome c_{mam} can [38], suggest that much of the plastocyanin is either loosely adsorbed at the aqueous-lipid interface (probably with a low barrier to mobility), or is actually free in solution inside the thylakoid. In the latter case, assuming an internal volume of 10 μl/mg chlorophyll [39] and 6 nmoles plastocyanin per mg chlorophyll [17], the internal concentration could be 0.6 mM. The anchorage of cytochrome *f* to the membrane need not change the rate coefficient appreciably from the value in solution (see [40] for examples of bimolecular reactions with similar kinetics when both components are in solution and when one is confined to an aqueous–lipid interface), and a rate constant of $k = 3.6 \cdot 10^7\ M^{-1} \cdot s^{-1}$ would lead to a rate of oxidation of reduced cytochrome *f* equal to *k*. Plastocyanin concn. is $2 \cdot 10^4\ s^{-1}$, or $\tau = 50\ \mu s$. This is comparable with measurements of the rate of cytochrome *f* oxidation in chloroplasts following a flash of light [7, 41, 42], though if one accepts the series cytochrome *f* → plastocyanin → P700 such rates reflect predominately the slower of the two steps. Hildreth [41] found $\tau = 60$–$150\ \mu s$ for intact leaves and much slower rates for isolated chloroplasts, while Haehnel and Witt [42] found two components

in P700 reduction, $\tau = 20$ μs and $\tau = 200$ μs, with complex kinetics linking cytochrome *f* and P700 [7]. These results would not be surprising if part of the plastocyanin pool is adsorbed on the membrane while part is free in solution, with the relative proportions varying according to the experimental conditions and perhaps differing for grana and stroma lamellae.

ACKNOWLEDGEMENTS

I am most grateful to Dr D. S. Bendall for helpful discussions at all stages of the work. I should also like to thank Dr K. F. Tipton for use of his Durrum-Gibson stopped-flow apparatus, Dr M. Plesničar for purifying the plastocyanin, Mr P. G. Pilbeam for growing the *Pseudomonas* culture, and Dr R. P. Cox for assistance in the algal cytochrome preparation.

I am indebted to Peterhouse, Cambridge for a research fellowship.

REFERENCES

1 Levine, R. P. and Gorman, D. S. (1966) Plant Physiol. 41, 1293–1300
2 Hind, G. (1968) Biochim. Biophys. Acta 153, 235–240
3 Avron, M. and Schneyour, A. (1971) Biochim. Biophys. Acta 226, 498–500
4 Nelson, N. and Racker, E. (1972) J. Biol. Chem. 247, 3848–3853
5 Kok, B., Rurainski, H. J. and Harmon, E. A. (1964) Plant Physiol. 39, 513–520
6 Knaff, D. B. and Arnon, D. I. (1970) Biochim. Biophys. Acta 223, 201–204
7 Haehnel, W. (1973) Biochim. Biophys. Acta 305, 618–631
8 de Kouchkovsky, Y. and Fork, D. C. (1964) Proc. Natl. Acad. Sci. U.S. 52, 232–239
9 Malkin, R. and Bearden, E. P. (1973) Biochim. Biophys. Acta 292, 169–185
10 Amesz, J., Visser, J. W. M., Van den Engh, G. J. and Pulles, M. P. J. (1972) Physiol. Vég. 10, 319–328
11 Yamanaka, T. (1965) in Biochemistry of Copper, pp. 283–285, Academic Press, New York
12 Ramshaw, J. A. M., Brown, R. H., Scawen, M. D. and Boulter, D. (1973) Biochim. Biophys. Acta 303, 269–273
13 Ambler, R. P. and Brown, L. H. (1967) Biochem. J. 104, 784–825
14 Kelly, J. and Ambler, R. P. (1973) Biochem. Soc. Trans. 1, 164–166
15 Bendall, D. S., Davenport, H. E. and Hill, R. (1971) Methods Enzymol. 23, 327–344
16 Hill, R. and Scarisbrick, R. (1951) New Phytol. 50, 98–111
17 Plesničar, M. and Bendall, D. S. (1970) Biochim. Biophys. Acta 216, 192–199
18 Ambler, R. P. (1963) Biochem. J. 89, 341–349
19 Katoh, S. (1960) Nature 186, 138–139
20 Schejter, A., Glauser, S. C., George, P. and Margoliash, E. (1963) Biochim. Biophys. Acta 73, 641–643
21 Böhme, H., Reimer, S. and Trebst, A. (1971) Z. Naturforsch. B 26, 341–352
22 Antonini, E., Finazzi-Agrò, A., Avigliano, L., Guerrieri, P., Rotilio, G. and Mondovì, B. (1970) J. Biol. Chem. 245, 4847–4856
23 Greenwood, C., Finazzi-Agrò, A., Guerrieri, P., Avigliano, L., Mondovì, B. and Antonini, E. (1971) Eur. J. Biochem. 23, 321–327
24 Morton, R. A., Overnell, J. and Harbury, H. A. (1970) J. Biol. Chem. 245, 4653–4657
25 Watt, G. D. and Sturtevant, J. M. (1969) Biochemistry 8, 4567–4571
26 Van Buuren, K. J. H., Van Gelder, B. F. and Eggelte, T. A. (1971) Biochim. Biophys. Acta 234, 468–480
27 Yamazaki, I. and Ohnishi, T. (1966) Biochim. Biophys. Acta 112, 469–481
28 Diebler, H. (1963) Ber. Bunsenges. Physik. Chem. 67, 396–402
29 Nagamura, T. and Ogura, Y. (1968) J. Biochem. Tokyo 64, 267–270

30 Lambeth, D. O. and Palmer, G. (1973) J. Biol. Chem. 248, 6095–6103
31 Dawson, J. W., Gray, H. B., Holwerda, R. A. and Westhead, E. W. (1972) Proc. Natl. Acad. Sci. U.S. 69, 30–33
32 Trebst, A. (1972) Methods Enzymol. 24, 146–165
33 Lozier, R. H. and Butler, W. L. (1972) FEBS Lett. 26, 161–164
34 Trebst, A. and Reimer, S. (1973) Biochim. Biophys. Acta 325, 546–557
35 Gibson, Q. H., Greenwood, C., Wharton, D. C. and Palmer, G. (1965) J. Biol. Chem. 240, 888–894
36 Nicholls, P. and Mochan, E. (1971) Biochem. J. 121, 55–67
37 Knaff, D. B. and Malkin, R. (1973) Arch. Biochem. Biophys. 159, 555–562
38 Green, D. E. and Fleischer, S. (1963) in Metabolism and Physiological Significance of Lipids (Dawson, R. M. C. and Rhodes, D. N., eds), pp. 581–618, Wiley, London
39 Deamer, D. W. and Packer, L. (1969) Biochim. Biophys. Acta 172, 539–545
40 Davies, J. T. and Rideal, E. K. (1963) Interfacial Phenomena, Academic Press, New York
41 Hildreth, W. W. (1968) Biochim. Biophys. Acta 153, 197–202
42 Haehnel, W. and Witt, H. T. (1972) in Proc. 2nd Int. Congr. Photosynth. Res. (Forti, G., Avron, M. and Melandri, A., eds), pp. 469–476, Junk, The Hague