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THE ACCESSIBILITY OF THE CHLOROPLAST CYTOCHROMES *f* AND *b*-559 TO FERRICYANIDE

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SUMMARY

(1) (a) A concentration range of ferricyanide (~ 0 125–0 5 mM) can be found which in the dark causes oxidation of cytochrome *f* with two distinct kinetic components of comparable amplitude The slow oxidation has a half time of 1–2 min (b) The oxidation of cytochrome *f* by ferricyanide is rapid and monophasic after the chloroplasts are frozen and thawed (c) The oxidation of cytochrome *b*-559 by ferricyanide in the dark is mostly monophasic with a time course similar to that of the fast component in the cytochrome *f* oxidation (d) Ascorbate reduction of cytochromes *f* and *b*-559 appears monophasic Reduction of cytochrome *b*-559 by ascorbate is somewhat faster, and that by hydroquinone somewhat slower, than the corresponding reduction of cytochrome *f*

(2) (a) The kinetics of dark ferricyanide oxidation of cytochrome *f* after actinic preillumination in the presence of an electron acceptor are approximately monophasic with a half time of about 30 s and do not show the presence of the slowly oxidized component observed after prolonged dark incubation (b) The effect of actinic preillumination in altering the time course of ferricyanide oxidation appears to persist for several minutes in the dark (c) Preillumination causes an increase in the extent of cytochrome *b*-559 oxidation by low concentrations of ferricyanide The increase is inhibited if 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea is present during the preillumination (d) The presence of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea during preillumination does not inhibit the amplitude or rate of ferricyanide oxidation of cytochrome *f*, although the presence of the inhibitor KCN does cause such inhibition

(3) It is proposed that a significant fraction of the cytochrome *f* population resides at a position in the membrane relatively inaccessible to the aqueous interface compared to high potential cytochrome *b*-559 Actinic illumination would cause a structural or conformational change in the cytochrome *f* and/or the membrane resulting in an increase in accessibility to this fraction of the cytochrome *f* population

Abbreviations DCMU, 3-(3′ 4′-dichlorophenyl)-1 1-dimethylurea, DBMIB 2 5-dibromo-3-methyl-6-isopropylbenzoquinone

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INTRODUCTION

There is reason to believe that the components of the chloroplast electron transport chain differ with respect to their accessibility and distance from the outer membrane surface Evidence for such heterogeneity in the locations of some of the electron transport components, electron donation and acceptor sites, and different chloroplast functions has been clearly summarized by Trebst [1] The data on the relative position of the electron transport components themselves is thus far limited to reactivity with antibody, from which it has been inferred that ferredoxin [2] and ferredoxin–$NADP^+$ reductase [3] are accessible to antibody Plastocyanin and cytochrome *f* in chloroplasts do not react with antibody and are considered to be relatively inaccessible by these criteria [4] Information on the position of other electron transport components might be obtained from the use of small charged chemical oxidants and reductants, which could also be used to probe the accessibility of components for which antibody is not available Establishment of the relative positions of the electron transport components, as well as changes in these positions upon illumination, is of considerable importance to consideration of pathways of electron transport and mechanisms of energy coupling

It will be assumed in this work that the charged anion ferricyanide $Fe(CN)_6^{3-}$ can be used as a selective oxidant of electron transport components whose oxidation sites are close to the aqueous interface of the thylakoid membrane The assumption is based upon the theoretical consideration that energy is required to move a water soluble ion into a hydrophobic membrane (dielectric constant $\approx$ 2) in the absence of any special ionophore or carrier mechanism The energy requirement is a consequence of the increase in "self-energy" of the electrostatic field in the presence of the lower dielectric constant [5] This energy barrier has been calculated to be approximately 40 Kcal/mole for a single electronic charge with an ionic radius of 2 Å at the center of a 70 Å thick membrane [6] The energy barrier would be approximately 360 Kcal/mole for an ion of the same radius with three uncompensated charges It is found in practice that small inorganic ions do not penetrate continuous phospholipid biomolecular leaflet membranes in the absence of a carrier (e g , ref 7) and it has been shown that ascorbate as a charged water soluble reductant does not penetrate liposome membranes [8] The assumption that ferricyanide is a selective oxidant neglects the possibility of special ferricyanide channels extending into the chloroplast membrane

The general purpose of the experiments presented here is to obtain qualitative data on the relative accessibility and on accessibility changes of electron transport components with similar oxidation–reduction potential Ferricyanide has been used previously as a probe for the localization of cytochromes in intact and sonicated mitochondrial membranes [9] Charged reagents which carry radioactive label have been used to define membrane protein components of red cells (e g , ref 10) and chloroplasts [11, 12] which are located relatively close to the aqueous interface

In this work we compare the accessibility to oxidant and reductants of cytochrome *f* and high potential *b*-559, both of which have a midpoint potential of approximately +0 35 volts [13–16] Positional or conformational changes of the cytochromes considered in terms of changes in accessibility to ferricyanide are investigated after illumination and treatment with inhibitors

METHODS

(*1*) *Chloroplast preparation and media*

The procedure for rapid isolation of coupled chloroplasts from spinach leaves grown in a controlled climate facility (20 °C, 8-h light cycle) has been described previously [17] Chloroplasts at a concentration of 80 μg/ml of chlorophyll were resuspended in a hypotonic medium containing 25 mM Tricine–KOH, pH 7 8, 5 mM K_2HPO_4, 5 mM NaCl, 2 mM $MgCl_2$ and 0 1 mM methyl viologen at 22–23 °C unless otherwise noted

(*2*) *Dual wavelength spectrophotometry*

The rate and amplitude of chemically-induced absorbance changes were studied using a dual wavelength spectrophotometer with a reference wavelength of 540 nm Additions to the sample were made in the dark with a microsyringe to a magnetically stirred (mixing time $\approx$ 1 s) thermostatted cuvette without interrupting the measurement The measuring beam was chopped at 120 cycles/s and had a half-band width of 3 nm The actinic light had an intensity of 6 5 10^4 ergs/cm^2 per s at 645 nm (red) and 713 nm (far-red)

(*3*) *Low temperature measurements*

The sample compartment for the measurements at liquid nitrogen temperature was very similar in design to that described by Butler [18] It was found to be necessary to cover the dewar with a plastic disc to avoid oxygen condensation in the sample The sample volume and thickness were 1 0 ml and 0 35 cm, respectively Samples were frozen in the hypotonic reaction medium immediately after addition of 0 3 M sucrose to the hypotonic medium in which chloroplasts were incubated with 250 μM ferricyanide for specified time intervals

(*4*) *Single beam spectra*

Continuous measurements of cytochrome difference spectra at 77 °K were obtained by using a stepping motor under computer (Data General 1220 with 16K memory) control to drive one monochromator of the Aminco-Chance duochromator in 1/8 nm steps with the band width of the monochromator set at 1 4 nm The wavelength drive and gear train for the stepping motor were built by Mr Barrett Robinson and Dr Gerald Birth Normalized spectra and appropriate difference spectra were stored in memory, plotted on an X-Y recorder and stored on tape The interface between spectrophotometer and computer utilized an operational amplifier (Analog Devices 42J), an Analogic MP 2814 analog to digital converter, MP 255 sample and hold amplifier, MP 4108 multiplexer, and two Datel VR12B3D digital-to-analog converters The computer program was written in assembly language by Mr Michael Squires

Fourth order finite difference spectra were calculated using successive increments of 16, 14, 12, and 10 times the 1/8 nm wavelength step, or 2, 1 75, 1 50, and 1 25 nm, respectively, basically using the approach of Butler and Hopkins [19]

RESULTS

I Chemical oxidation and reduction of cytochromes f and high potential b-559

Cytochromes *f* and *b*-559$_{HP}$ are initially fully reduced in the dark in coupled chloroplasts incubated in the dark in the hypotonic medium utilized in these experiments Absorbance changes of cytochromes *f* and *b*-559 are measured at 551–552 nm and 561–562 nm, respectively, to minimize interference There is no interference from C-550 and cytochrome *b*-563 in the chemically induced absorbance changes because both of these components are initially oxidized and neither is ascorbate reducible The mutual interference of the absorbance changes of cytochrome *f* and *b*-559 on the long wavelength side of cytochrome *f* was approximately calculated assuming a gaussian absorption curve with a half-band width at half maximum of 4 nm for both cytochromes *f* and *b*-559 The contribution of the cytochrome *f* absorbance change at 561 nm would be 0 14 of a pure cytochrome *f* change measured at 552 nm By symmetry, 0 14 of a pure cytochrome *b*-559 absorbance change measured at 561 nm would be seen at 552 nm

Addition of ferricyanide causes a decrease in absorbance which is biphasic with a slow ($\tau_{\frac{1}{2}} \approx 1$ min) component of appreciable amplitude when measured at 552 nm (Fig 1A) The fast component can be seen with better resolution (Fig 2A) The slow component in the time course of the ferricyanide-induced absorbance decrease is not present at 552 nm if the chloroplasts are first frozen and thawed before ferricyanide is added (Fig 1B), nor is it present in the absorbance decrease measured at 561 nm (Fig 2B) The main effect of the mutual interference discussed above on the patterns of the different kinetic changes seen in the cytochrome *f* and *b*-559 regions is that approximately one-third and one-fourth of the fast component seen in the cytochrome *f* oxidation traces of Figs 1A and 2A is due to high potential cytochrome *b*-559

The slow component in Fig 1A arises mostly from cytochrome *f* oxidation as shown by the spectrum for the slow component measured at room temperature (Fig 3), and as well in a comparison of low temperature absorption and fourth-order

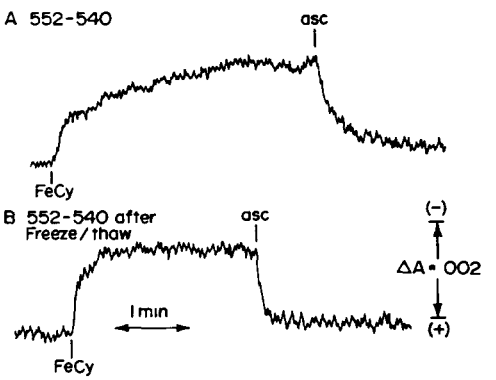

Fig 1 Chemical oxidation and reduction of cytochrome *f* measured in unilluminated chloroplasts Concentration of ferricyanide 125 μM, and ascorbate, 2 mM Cytochrome *f* oxidation without (A) and with (B) treatment by freezing to 77 °K and thawing before addition of ferricyanide Amplifier time constant, 1 s Oxidative negative absorbance change is shown as upward deflection

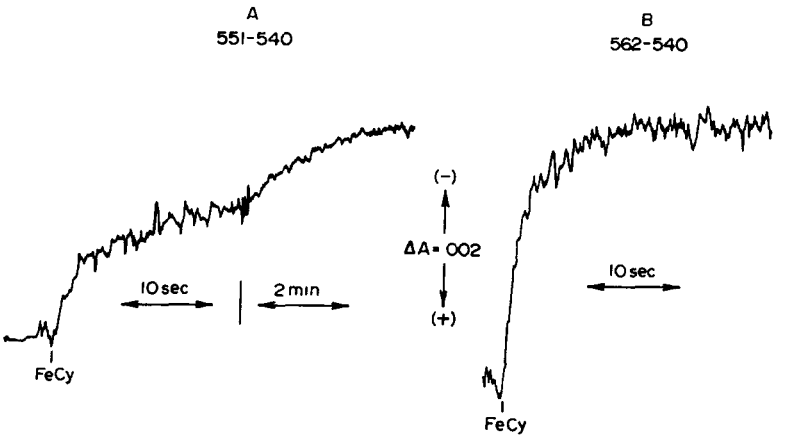

Fig 2 Ferricyanide oxidation of cytochrome *f* (A) and *b*-559 (B) with expanded time scale Ferricyanide concentration, 500 μM Amplifier time constant 0 7 s

difference spectra measured in samples frozen 2 min (Fig 4A) and 20 s (Fig 4B) after addition of ferricyanide The spectrum of Fig 3 is weighted toward the long wavelength side indicating that there is also a small amount of cytochrome *b*-559 oxidized slowly at this ferricyanide concentration It should be noted that the amplitudes of the absorbance changes shown in Fig 1 associated with the oxidation of cytochrome *f* are relatively small The reason is that the ferricyanide concentration used in Fig 1 is 125 μM and the total absorbance change of cytochromes *f* and *b*-559 is dependent upon ferricyanide concentration as shown in Fig 5 The biphasic oxidation of cytochrome *f* is not consistently observed with high ($\geq$ 0 5 mM) ferricyanide concentrations In contrast to the biphasic oxidation of cytochrome *f* by ferricyanide, the reduction of cytochrome *f* by ascorbate (Fig 1A, B) and hydroquinone (Fig 6A) does not seem to involve more than one kinetic phase The reduction of ferricyanide-oxidized cytochrome *b*-559 by hydroquinone (Fig 6B) and ascorbate (data not shown) also appears to be monophasic Ascorbate reduction of cytochrome *b*-559 is more rapid than cytochrome *f* (Fig 1A, 7A vs 8B) Of the three chemical redox reagents (ferricyanide, ascorbate, hydroquinone) employed here, the only one which reacts

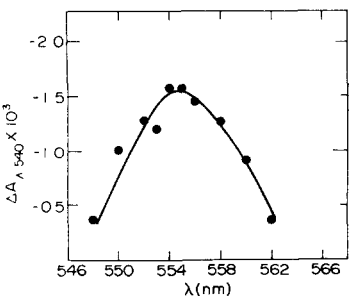

Fig 3 Difference spectrum for the slow component in the time course of the ferricyanide-induced oxidation change shown in Fig 2A

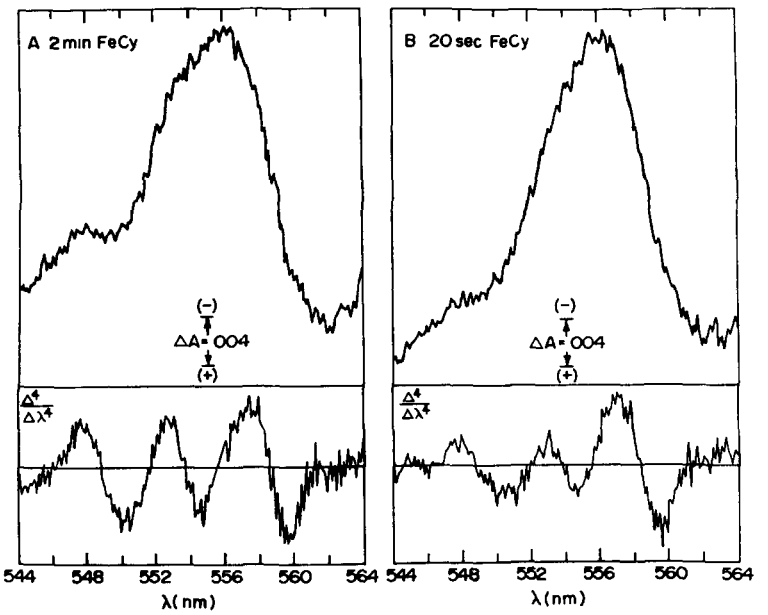

Fig 4 Absorption and fourth order finite difference spectra at liquid nitrogen temperature for the computer-corrected difference between samples incubated with 250 μM ferricyanide (FeCy) for 2 min (A) or 20 s (B) and a reduced sample not treated with ferricyanide

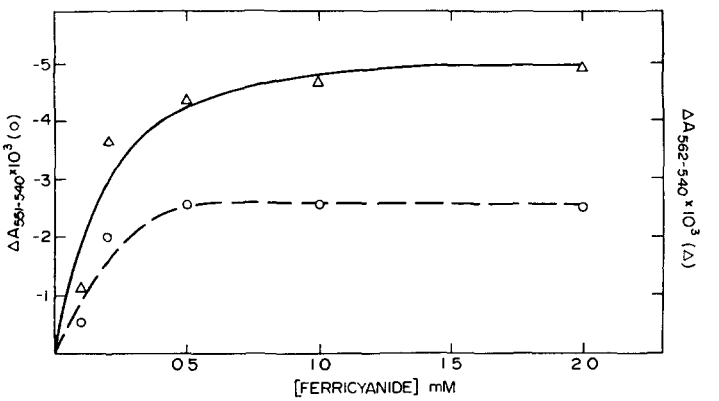

Fig 5 Total absorbance decrease measured at 551 nm (○) and 562 nm (△) as a function of the concentration of added ferricyanide

more rapidly in the dark with cytochrome *f* than with *b*-559 is the more lipophilic compound hydroquinone, which reduces cytochrome *f* with a half time $\leqslant$ 1–2 s (Fig 6A) compared to a half-time of 4–5 s for the reduction of cytochrome *b*-559 (Fig 6B)

II The effect of actinic preillumination on the time course of chemical oxidation and reduction of cytochrome f and b-559

The biphasic oxidation of cytochrome *f* by ferricyanide in chloroplasts which

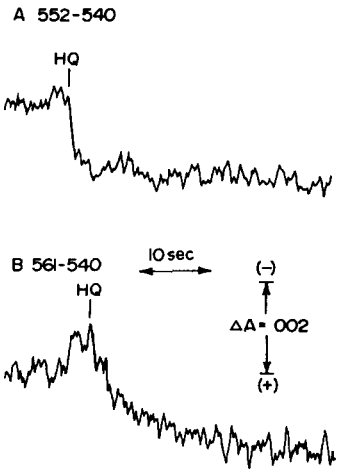

Fig 6 Time course of hydroquinone (1 mM) reduction of ferricyanide (125 μM)-oxidized cytochrome *f* (A) and *b*-559 (B) without prior illumination Amplifier time constant 0 3 s

have been transferred from a stock suspension to the cuvette in darkness is not apparent after the chloroplasts are first exposed to actinic light (Fig 7) The ferricyanide oxidation of cytochrome *f* after preillumination appears monophasic and has a half-time of 20–30 s (Fig 7B), approximately midway between the half-times of the fast and slow components observed in the absence of actinic illumination There is no obvious change in the pattern or kinetics of the ascorbate reduction of ferricyanide-oxidized cytochrome *f* following actinic illumination Actinic illumination does not cause a significant change in the rate of ferricyanide oxidation of cytochrome *b*-559, but does increase the total amplitude through an additional slowly oxidized com-

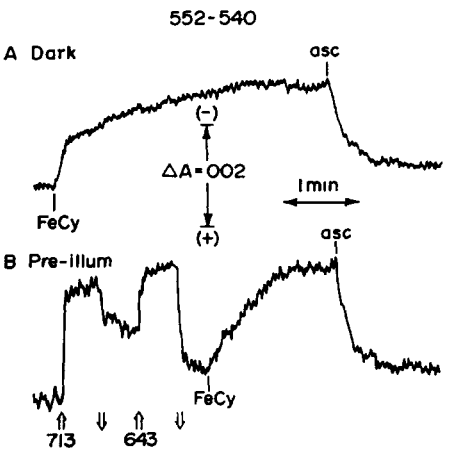

Fig 7 Comparison of the kinetics of ferricyanide oxidation of cytochrome *f* without (A) and with (B) prior actinic illumination Ferricyanide and ascorbate concentrations, 250 μM and 2 mM, respectively Amplitude time constant, 1 s Upward arrows actinic light on, downward arrows, light off

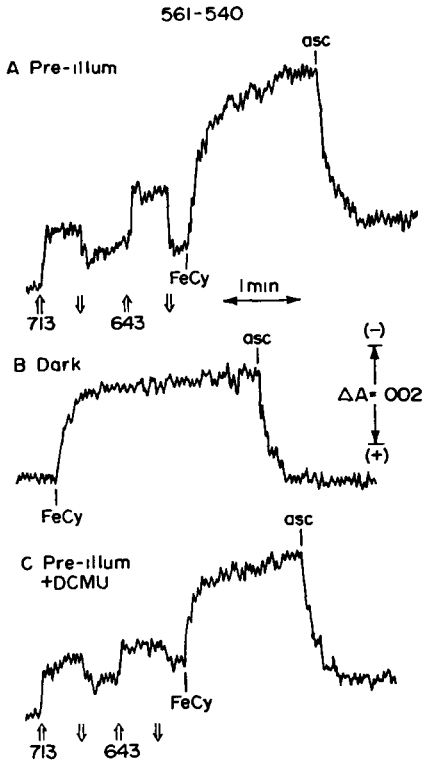

Fig 8 Comparison of the kinetics of ferricyanide oxidation of cytochrome *b*-559 with (A) and without (B) prior actinic illumination and with prior illumination in the presence of 5 μM DCMU (C) Ferricyanide and ascorbate concentrations are 125 μM and 1 mM, respectively Amplifier time constant, 1 s

ponent (Fig 8A, B) when low concentrations of ferricyanide are used No effects of actinic illumination on chemical oxidation of *b*-559 are detectable when the ferricyanide concentration is 0 5 mM or greater The absence of an effect of pre-illumination on the kinetics of *b*-559 oxidation by ferricyanide provides evidence for the monophasic absorbance change measured in Fig 7B arising mostly from cytochrome *f*, since the rate of the ferricyanide oxidation of cytochrome *b*-559 after illumination is much faster than the monophasic oxidation of cytochrome *f* The preillumination regime includes successive applications of far-red and red actinic light The kinetic changes in cytochrome *f* oxidation can be achieved with far-red light alone (data not shown) The red light is used just to reduce cytochrome *f* more rapidly

Actinic illumination evidently causes an unidentified change in the state or properties of the cytochrome or the membrane, which is obviously expressed in the modification of the post-illumination kinetics of cytochrome *f* oxidation by ferricyanide Measurements have been made of the lifetime of this altered state in terms of the time course of the preillumination ferricyanide oxidation (data not shown) It is difficult to calculate a half-time for the reversion of the post-illumination state to the dark state, as the changes in fast and slow components do not correlate in time

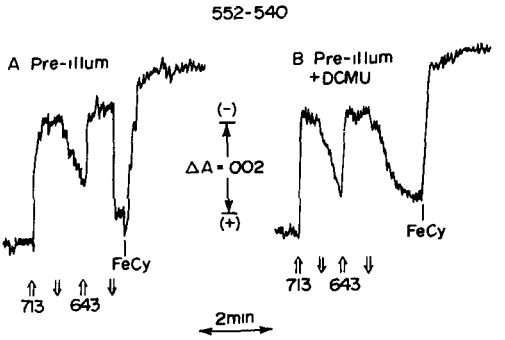

Fig 9 Comparison of the time course of ferricyanide (0 5 mM) oxidation of cytochrome *f* after prior illumination in the absence (A) and presence (B) of 20 μM DCMU

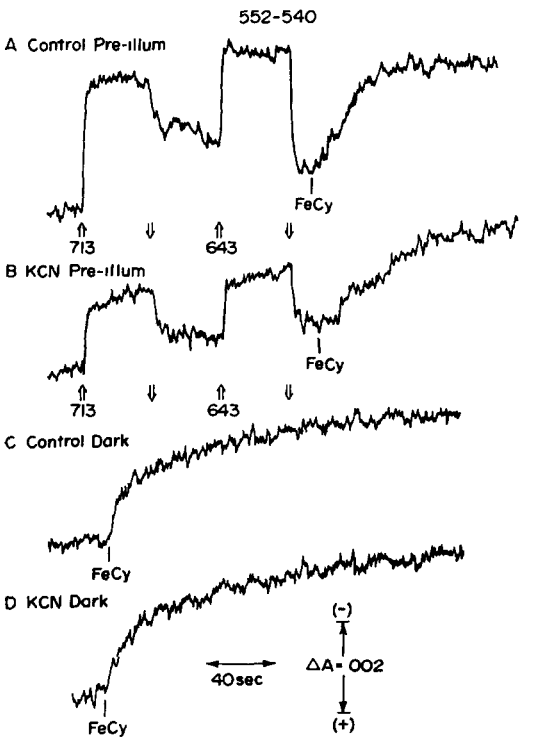

Fig 10 Comparison of the time course of ferricyanide oxidation of cytochrome *f* after prior illumination in the absence (A) and presence (B) of potassium cyanide Control additions of ferricyanide were made in samples kept in the dark without (C) and with prior incubation in KCN (D) The KCN incubation was performed essentially as in ref 20, chloroplasts were resuspended in 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-NaOH buffer, pH 7 4, 0 1 M sorbitol 2 mM $MgCl_2$ at 2 mg chlorophyll/ml The chloroplasts were incubated at 1 2 mg chlorophyll/ml in 60 mM tricine–KOH, pH 8 1, 50 μM ferricyanide, and 30 mM KCN for 60 min The chloroplasts were then diluted to 80 μg/ml chlorophyll in reaction medium for spectral measurements

We would estimate that the half-time of an altered post-illumination state is between 2 and 5 min, with observable differences in the time course of ferricyanide oxidation persisting for 5–10 min after illumination

III Effect of electron transport inhibitors on the post-illumination state

The electron transport inhibitors DCMU and KCN affect the post-illumination ferricyanide oxidation of cytochrome *f* in different ways The presence of DCMU (20 μM) during illumination does not inhibit the amplitude or rate of the cytochrome *f* oxidation by ferricyanide, and the rate of ferricyanide oxidation may occur even faster with DCMU present during preillumination (Fig 9) The ferricyanide oxidation rates are faster in Fig 9 with 0 5 mM ferricyanide than in Fig 7B where 0 25 mM ferricyanide was used DCMU, which acts close to Photosystem II and cytochrome *b*-559, does inhibit the increase in amplitude of cytochrome *b*-559 oxidation by low concentrations of ferricyanide (Fig 8C) When chloroplasts are incubated in KCN, photooxidation of cytochrome *f* is inhibited during the preillumination [20] as is the rate and amplitude of the subsequent ferricyanide oxidation of cytochrome *f* (Fig 10B) The amplitude of dark controls (Figs 10C and 10D) measured after 60 min is normal, although the fast component of cytochrome *f* oxidation is absent in Fig 10D The kinetics of the latter control were somewhat variable The effect of DBMIB present during illumination on the ferricyanide oxidation of *f* is ambiguous (data not shown) because DBMIB increases the rate of dark reduction after illumination [21]

DISCUSSION

The time course of cytochrome *f* oxidation by ferricyanide shows two distinct kinetic phases (Figs 1A, 2A) The slow component ($\tau_{\frac{1}{2}} \approx 1$ min) has a rate constant which is orders of magnitude smaller than that for ferricyanide oxidation of cytochrome *c* in solution [22] and is also at least one order of magnitude slower than the oxidation of the cytochrome *f* after freeze-thaw treatment (Fig 1B) and the bulk of the high potential cytochrome *b*-559 (Fig 2B) The rate constants for the fast component of the cytochrome *f* oxidation and the oxidation of cytochrome *b*-559 cannot be determined in this experiment because of the extended mixing time Part of the cytochrome *f* might be oxidized slowly (a) because of hindrance in the protein structure or (b) a membrane barrier preventing penetration of the ferricyanide In either case, the biphasic ferricyanide oxidation of cytochrome *f* argues for two distinct species of cytochrome *f* in the membrane in the dark There is no evidence for a heterogeneity of cytochrome *f* with respect to midpoint potential which could account for the biphasic response

One cannot totally exclude the possibility that the slow component of the cytochrome *f* oxidation by ferricyanide is due to steric effects of the cytochrome protein structure, but the monophasic oxidation of cytochrome *f* after freeze–thaw treatment would rather suggest the existence of an appreciable membrane barrier as the cause of the sluggish oxidation That the total cytochrome *f* complement is at least somewhat removed from the membrane surface is shown by the inability of cytochrome *f* antibody to react with cytochrome *f* in chloroplasts [4] These data together with the relatively rapid oxidation of cytochrome *b*-559 by ferricyanide lead

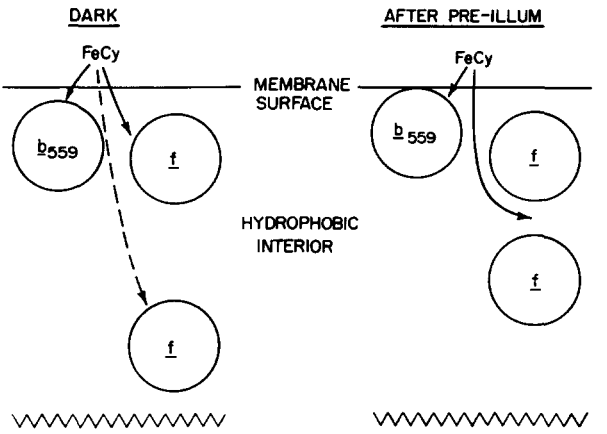

Fig 11 Interpretation of the effect of chemical oxidant and reductant on cytochromes *f* and *b*-559 before and after pre-illumination in terms of the position of these cytochromes relative to the aqueous interface of a hydrophobic membrane

us to propose a qualitative model for the distribution of cytochrome *f* and cytochrome *b*-559 in relation to the membrane surface (Fig 11) We would place cytochrome *b*-559, or at least its site of chemical oxidation, close to the aqueous interface, since most of the *b*-559 is rapidly oxidized even at low ferricyanide concentrations The biphasic cytochrome *f* oxidation leads to the suggestion that part of the cytochrome *f* complement in the dark is positioned relatively far from the membrane surface No statement can be made about the absolute distances of the components from the membrane surface The biphasic oxidation curve for cytochrome *f* implies, as noted above, that the cytochrome *f* population is heterogeneous and the rapidly oxidized component should be closer to the membrane surface The discussion of the position of the cytochrome components in the membrane, based on the ferricyanide oxidation experiments, really concerns the oxidation site of the proteins, since the oxidation site of a cytochrome may be distinct from the site of reduction [23] With respect to reduction, the cytochrome *b*-559 is also somewhat more accessible in the dark to the polar reductant ascorbate than is cytochrome *f*, which is consistent with the model for cytochrome positioning shown in Fig 11 The approximately monophasic kinetics for cytochrome *f* reduction by ascorbate in the dark might suggest that the sites of reduction are more homogeneously distributed than those for oxidation, but the ascorbate concentrations used for reduction are necessarily high

Actinic illumination of the chloroplasts causes a large change in the kinetics of the subsequent dark oxidation of cytochrome *f* by ferricyanide (Fig 7) The main effect is the disappearance of the slowly oxidized component after preillumination This is most simply described by an increase in the effective accessibility of the cytochrome *f* population (Fig 11) The increase in accessibility of part of the cytochrome *f* population shown in Fig 11 is meant to be symbolic, since the two cytochrome *f* populations could be (a) different subunits or (b) intact cytochromes at different positions in the membrane In addition, the increase in accessibility of part of the *f* population after illumination could be caused by (a) actual transmembrane motion

of the cytochrome relative to the membrane, or (b) light-induced activation of a carrier shuttling between the inaccessible *f* component and the membrane surface If ferricyanide, in fact, can truly only react at the membrane surface, then a carrier is absolutely required to reach a buried cytochrome

Whatever model one uses to describe the change in the time course of ferricyanide oxidation of cytochrome *f* after illumination, it would appear that the actinic light causes a structural or conformational change in the cytochrome and/or the membrane In what way does the actinic light trigger the structural change? The slow component in the ferricyanide oxidation of cytochrome *f* disappears as well if the actinic illumination is provided in the presence of DCMU The effect of the actinic light is then either exerted through the pigment system or through limited electron transport involving photosystem I alone Cyclic electron transport should be negligible in the presence of methyl viologen and aerobic conditions Since it is known that cytochrome *c* undergoes large conformational changes in going from the reduced to the oxidized state (see ref 24, for example), a simple mechanism for triggering a conformational change of cytochrome *f* would be through its photooxidation This hypothesis is consistent with the ability of far-red light alone to achieve the actinic effect, but would require that the structural change not be immediately reversed by red light reduction of cytochrome *f* One might test the role of photooxidation in causing the accessibility change by illuminating the chloroplasts under conditions where cytochrome *f* photooxidation is inhibited This can be accomplished through incubation of the chloroplasts in high concentrations of KCN for an extended period [20] The result of the KCN experiment is consistent with the hypothesis that cytochrome *f* photooxidation triggers a conformational change responsible for the alteration in kinetics of cytochrome *f* chemical oxidation However, the KCN result can be interpreted in addition to mean that plastocyanin is a carrier mediating the dark chemical oxidation of cytochrome *f*

Having discussed the evidence based on the use of a charged oxidant and reductant which leads to the model of Fig 11, it is necessary to explain why the more lipophilic hydroquinone will reduce cytochrome *f* more rapidly than cytochrome *b*-559 which we infer to be closer to the membrane surface An ad hoc explanation is that a channel for hydroquinone reduction exists in the membrane, possibly through the plastoquinone pool, and that intrinsically the reduction of oxidized cytochrome *f* by hydroquinone is more rapid than reduction of cytochrome *b*-559

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REFERENCES

1 Trebst, A (1974) Annu Rev Plant Physiol 25, 423–458
2 Hiedemann-Van Wyk, D and Kannangara, C G (1971) Z Naturforsch 26b 46–50
3 Berzborn R J (1968) Z Naturforsch 23b, 1096–1104
4 Racker E , Hauska, G A , Lien, S , Berzborn R T and Nelson, N (1971) Proc 2nd Int Cong Photosyn Res , Stresa, (Forti G , Avron, M and Melandri, A , eds), pp 1097–1113
5 Abraham, M and Becker, R (1950) Electricity and Magnetism, p 83, Blackie and Sons, Ltd, London
6 Parsegian, A (1969) Nature 221 844–846
7 Bangham, A D (1972) Annu Rev Biochem p 765
8 McConnell, H (1971) Biochemistry 10, 1111–1120
9 Chance, B (1970) Proc Natl Acad Sci U S 66, 928–935
10 Berg, H C (1969) Biochim Biophys Acta 183, 65–78
11 Dilley, R A , Peters G A and Shaw E R (1972) J Memb Biol 8, 163–180
12 Giaquinta, R T , Dilley, R A and Anderson, B J (1973) Biochem Biophys Res Commun 52, 1410–1417
13 Davenport, H E and Hill R (1952) Proc R Soc Lond Ser B 139, 327–345
14 Bendall D S (1968) Biochem J 109, 46p
15 Knaff D B and Arnon, D I (1971) Biochim Biophys Acta 226, 400–408
16 Boardman, N K Anderson, J M and Hiller, R G (1971) Biochim Biophys Acta 234, 126–136
17 Cramer, W A and Bohme, H (1972) Biochim Biophys Acta 256, 358–369
18 Butler, W L (1972) Methods in Enzymology (San Pietro A , ed), Vol XXIV, Part B p 5, Academic Press New York
19 Butler, W L and Hopkins, D (1970) Photochem Photobiol 12, 451–456
20 Izawa, S , Kraayenhof R , Ruuge E K and DeVault D (1973) Biochim Biophys Acta 314 328–339
21 Bohme, H and Cramer W A (1971) FEBS Lett 15, 349–351
22 Brandt K G Parks, P C , Czerlinski G H and Hess G P (1966) J Biol Chem 241, 4180–4185
23 Margoliash, E Ferguson-Miller, S , Tulloss, J , Kang, C H Feinberg, B A , Brautigan, D L and Morrison M (1973) Proc Natl Acad Sci U S 70 3245–3249
24 Takano, T Kallai, O B , Swanson, R and Dickerson, R E (1973) J Biol Chem 248, 5234–5255