Title

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LIGHT-INDUCED CHANGES OF BOUND CHLOROPLAST PLASTOCYANIN AS STUDIED BY EPR SPECTROSCOPY: THE ROLE OF PLASTOCYANIN IN NONCYCLIC PHOTOSYNTHETIC ELECTRON TRANSPORT

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Running Title: Light-induced changes of bound chloroplast plastocyanin

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DISCLAIMER

SUMMARY

A low-temperature electron paramagnetic resonance (EPR) signal in spinach chloroplasts has been identified as originating from oxidized bound plastocyanin. The EPR parameters of the bound plastocyanin, which are the same as those of soluble plastocyanin, are: $g_{\perp} = 2.04$, $g_{//} = 2.23$, and $A_{//} = .006$ cm^{-1}. An estimation of the amount of bound plastocyanin based on the *in situ* EPR signal indicates a concentration of approximately 3 nmoles per mg chlorophyll, a value which agrees with the amount of plastocyanin determined by chemical assay after release from the chloroplast lamellae by sonication.

Light-induced changes of plastocyanin *in situ* have been examined after illumination with monochromatic light at physiological temperatures. The bound plastocyanin undergoes a photoreduction in Photosystem II light which is sensitive to inhibitors of noncyclic electron transport. The presence of the NADP acceptor system in Photosystem II light causes a shift in the steady-state level of plastocyanin providing direct evidence for the role of plastocyanin in noncyclic electron transport from water to NADP. Phosphorylation cofactors and the uncoupler, NH_4Cl, also cause shifts in the plastocyanin steady-state level in red light.

Photosystem I light is able to photooxidize reduced plastocyanin, while Photosystem II light is only capable of photooxidation in the presence of inhibitors of the photoreduction. These results of studies on plastocyanin

photoreactions *in situ* are discussed in terms of different proposed roles for plastocyanin in the chloroplast noncyclic electron transport chain.

INTRODUCTION

Plastocyanin, a copper-containing protein, was discovered and characterized by Katoh and co-workers[1-3]. The protein was shown to be localized in chloroplasts[4], a finding which suggested an involvement in the photosynthetic process of the green plant.

The role of plastocyanin in photosynthetic electron transport has been studied extensively and there is widespread agreement that plastocyanin is required for noncyclic electron transport from water to NADP (ref. 5-9) but the site of action of this carrier in the chain and its relation to photochemical reactions associated with Photosystem I are still not fully understood (see ref. 8,9). Although most investigators maintain that plastocyanin functions in the electron transport chain between a Photosystem I light reaction and a Photosystem II light reaction[8,9], a recent proposal[10-12] suggests that plastocyanin functions solely between two Photosystem II light reactions in noncyclic electron transport and is not involved in the cyclic photophosphorylation pathway associated with Photosystem I. This newer, three-light-reaction scheme also places plastocyanin in a different electron transfer chain from cytochrome *f* and P700 (ref. 10-12), which are confined solely to the Photosystem I electron transport chain.

Most of the evidence for the role of plastocyanin in chloroplast photosynthesis has been obtained from studies of the effect of added soluble plastocyanin on photochemical reactions in plastocyanin-depleted chloroplast fragments[5-7,13-19]. These fragments are usually prepared by sonication, although Levine and co-workers have studied photochemical reactions in chloroplast fragments prepared from an algal mutant deficient in plastocyanin[20]. The only studies of changes attributed to plastocyanin in situ have been those of Fork and co-workers[21,22] on a light-induced absorbance change in the 600-nm region in whole cells of a marine alga. Because it has been impossible to detect absorbance changes of plastocyanin in chloroplasts by optical methods, light-induced changes of plastocyanin in situ have not been reported for chloroplasts.

In this communication we report the identification in isolated spinach chloroplasts of a low-temperature electron paramagnetic resonance (EPR) signal which is associated with the Cu^{2+} ion of oxidized plastocyanin bound to the chloroplast lamellae. Results of studies on light-induced changes in chloroplasts after illumination at physiological temperature also indicate that this EPR-detectable component is involved in noncyclic electron transport from water to NADP, a finding consistent with the assignment of this EPR component to plastocyanin. The role of plastocyanin in the photosynthetic electron chain has been studied through examination of

light-induced changes, and the results are considered in the context of the different models for noncyclic electron transport cited above.

METHODS AND MATERIALS

Whole spinach chloroplasts[23] were resuspended in 50 mM Tricine (pH 8.2) + 10 mM NaCl and centrifuged at 35 000 x g for 5 min. The pellet, which was resuspended in 50 mM Tricine (pH 8.2) + 10 mM NaCl, was used for light-induced studies. In some experiments 1 mM EDTA (pH 8.0) was added to the final chloroplast suspension. Chlorophyll concentrations were determined as described by Arnon[24].

For removal of plastocyanin, chloroplasts were sonicated at 4° (chlorophyll concentration, 0.5 mg/ml) for 2 min at power setting 3 (2.5-3 amps) with a Branson sonifier (Model S125) and then centrifuged at 144 000 x g for 1 hr to reisolate the fragments. The plastocyanin released by sonication was assayed by determining the oxidized minus reduced (ferricyanide minus ascorbate) difference spectrum of the 144 000 x g supernatant solution on a Cary Model 14 spectrophotometer after concentration on a small DEAE-cellulose column. The amount of plastocyanin released was calculated per 1 mg chlorophyll in the starting material using a differential extinction coefficient at $597^{nm}_{minus\ 500\ nm}$ ($\varepsilon_{ox - red}$) = 7.2 $mM^{-1} \cdot cm^{-1}$ (ref. 2). Soluble plastocyanin, isolated from spinach leaves, was a gift of Mr. R. K. Chain. The absorbance ratio (A_{280}/A_{597}) of the sample was 1.2, and the protein was homogeneous on polyacrylamide gel electrophoresis.

Plastocyanin concentrations were calculated on the basis of an extinction coefficient of 9.8 $mM^{-1} \cdot cm^{-1}$ at 597 nm (ref. 2).

ADP, NADP, and Tricine were purchased from the Sigma Chemical Company.

EPR techniques

EPR spectra were recorded at X-band in a Jeol ME-1X spectrometer with a TE_{012} cylindrical cavity operating at a frequency of 9.26 GHz. First-derivative EPR spectra were obtained with modulation of the magnetic field and phase detection at 100 KHz. Spectra were recorded at 25°K with a power to the cavity of 10 mW and a modulation amplitude of 10 gauss. Signal averaging on all samples was by an "on-line" PDP 8/L digital computer and a program written by Mr. H. Holmes. Five to 10 spectra were averaged on a sample prior to the plotting of the spectrum on an X-Y recorder[25,26].

Light-induced studies

Since the EPR signal of plastocyanin could only be observed at a temperature lower than 77°K, the following procedures were used to study light-induced changes. In the initial experiments, samples were placed in quartz EPR tubes (3-mm inner diameter) and illuminated at room temperature. These samples were then frozen in liquid nitrogen in an unsilvered dewar flask which was placed in the illumination beam. This procedure required an illumination time of approximately 10 sec. In later experiments a different freezing procedure was used which

allowed for a more rapid freezing of the samples and hence a shorter illumination time. Samples in EPR tubes were cooled to 4° in the dark and then illuminated for 1-2 sec. The tube was next immersed in a cold isopentane solution (approximately 110°K) placed adjacent to the light source. With this procedure, samples could be frozen in approximately 1 sec without tube breakage. The two freezing procedures gave similar results with regard to light-induced changes. Because of the shorter illumination time possible with the isopentane freezing technique, this method was used in most experiments.

Because of the requirement for the low-temperature determination of the plastocyanin EPR signal, each EPR tube represented a different experiment and each sample was used only once. Thus it was not possible to make additions of reagents in the light; rather, a comparison between different samples had to be made. Calibrated EPR tubes were used to minimize any differences arising from the use of different samples. Duplicate estimations agreed to within 10%. In addition, kinetic studies of the light-induced changes were not made, although it was found that the illumination time used (1-2 sec) was sufficient for reactions to go to completion.

The light source was a General Electric Quartzline lamp (type EJL: 200 watts, 24 volts). The light beam passed through a heat-absorbing filter and then a Baird-Atomic interference filter (715 or 645 nm) of half-band width 10 nm. The incident light intensity

on the sample was 16.1 nEinsteins·cm^{-2}·sec^{-1} with 645-nm light and 6.0 nEinsteins·cm^{-2}·sec^{-1} with 715-nm light.

RESULTS

Identification of a plastocyanin EPR signal in chloroplasts

We previously reported an EPR signal in washed, broken chloroplast fragments which was attributed to the Cu^{2+} ion on the basis of its g-value and line shape[25]. Since the EPR spectra of Cu^{2+} complexes are commonly characterized by two different g-values ($g_{//}$ and $g_{\perp}$) and a hyperfine splitting constant ($A_{//}$) (see ref. 27 for a more detailed discussion of the EPR properties of Cu^{2+} complexes in biological systems), it was of interest to compare the EPR parameters of spinach plastocyanin with those of the chloroplast copper component.

As shown in Fig. 1A and 1B, the EPR spectra in the high-field region ($g_{\perp}$) for spinach plastocyanin and the chloroplast component are the same, although free-radical signals in chloroplasts at $g = 2.00$ overlap some of the chloroplast Cu^{2+} signal. A more positive identification of this chloroplast component as a typical "blue" copper protein[27] comes from a comparison of the low-field region ($g_{//}$) of the two EPR spectra. The EPR spectrum of all copper complexes has four equally spaced lines in this field region. The distance between the lines is the hyperfine splitting constant, $A_{//}$. Plastocyanin, in common with other members of the class of "blue" copper proteins, is characterized by an unusually small hyperfine splitting constant when compared with other Cu^{2+} complexes

Fig. 1

(see ref. 27). As shown in Fig. 1C and 1D, the low-field portions of the EPR spectra of soluble plastocyanin and the chloroplast component are similar, although there is some line narrowing in the chloroplast spectrum. This absorption is much less than in the high-field portion of the spectrum, so that the hyperfine splitting in the chloroplast sample could be measured only at high power and gain and with signal-averaging techniques.

The EPR properties of the soluble spinach plastocyanin and the chloroplast component and previously reported data for soluble Chenopodium album plastocyanin[28] are summarized in Table I. The agreement between the EPR parameters of the bound component and those of the soluble plastocyanins is a first indication that the $g = 2.05$ signal may be due to plastocyanin bound to the chloroplast lamellae. However, since other "blue" copper proteins, such as ascorbate oxidase, have similar EPR parameters[27], a conclusion based solely on the EPR spectrum may be premature. Evidence based on the quantitative estimation of this component, and on the light-induced changes to be discussed below, strongly supports our contention that this EPR component is plastocyanin.

Table I

The chloroplast component is bound to the chloroplast lamellae, as evidenced by the observation that repeated washing of the chloroplast fragments had no effect on the intensity of the EPR signal at $g = 2.05$. In addition, repeated washing with EDTA (1 mM) did not

affect the $g_{\perp}$ = 2.05 signal, indicating that the signal did not arise from loosely bound Cu^{2+} ions.

The effect of sonication, a technique known to solubilize chloroplast plastocyanin[5-7], on the component with the $g_{\perp}$ = 2.05 signal is shown in Fig. 2. After the addition of ferricyanide sonicated chloroplasts show only a small residual signal in the high-field region of the EPR spectrum (Fig. 2B) as compared with untreated chloroplasts (Fig. 2A). If one assumes this small signal is a residual of the original $g_{\perp}$ = 2.05 component, a 95% removal has been achieved by mild sonication.

Fig. 2

The amount of the bound chloroplast component in unfractionated chloroplasts has been estimated by comparing the amplitude of the high-field ($g_{\perp}$) absorption in the presence of ferricyanide with that of soluble plastocyanin. The two samples were run under the same EPR instrumental conditions (microwave power, modulation amplitude, and temperature) in order to minimize possible errors in the quantitation. The soluble plastocyanin concentration was determined on the basis of the absorbance at 597 nm. With the data presented in Fig. 1, 5.6 natoms of copper per mg of chlorophyll were found by this method (equivalent to 2.8 nmoles of plastocyanin per mg chlorophyll). The plastocyanin content of another aliquot of the same chloroplast sample was also determined by the chemical method described in Materials and Methods after the plastocyanin was released by sonication; a value of 6.0 natoms of copper per mg of

chlorophyll (equivalent to 3.0 nmoles of plastocyanin per mg chlorophyll) was determined. The values determined by these two independent methods are similar to those reported by other investigators for the plastocyanin content of spinach chloroplasts[4,19,29]. The finding that the chloroplast plastocyanin content can be accurately estimated from the g = 2.05 signal strongly supports the contention that the observed EPR signal is caused by bound plastocyanin.

The oxidation-reduction properties of chloroplast-bound plastocyanin are similar to those of the soluble protein. In washed, broken chloroplast fragments, plastocyanin has been found to be predominantly in the oxidized form, although the addition of ferricyanide usually produces a somewhat larger signal. The addition of ascorbate or hydroquinone eliminates the chloroplast EPR signal, indicating the reduction of bound plastocyanin, but the reaction is slower than with the soluble protein. The addition of a catalytic amount of 2,6-dichlorophenol indophenol greatly increases the rate of chemical reduction of the bound plastocyanin by ascorbate.

Photoreduction of plastocyanin in situ

As previously stated, in washed, broken chloroplast fragments bound plastocyanin is predominantly in the oxidized (paramagnetic) form. The amount of oxidized plastocyanin in such preparations is usually 75-100% of the amount detected in the presence of an added oxidant (1 mM ferricyanide or persulfate). Thus, the photoreduction

of the bound plastocyanin may be studied in untreated chloroplasts without the addition of reagents.

As shown in Fig. 3, bound plastocyanin in chloroplasts undergoes a change after illumination with red light (645 nm). The disappearance of the signal at $g = 2.05$ (compare Fig. 3A and 3B) indicates that a photoreduction has occurred since the signal is only observed when plastocyanin is in the oxidized form (Cu^{2+} ion is paramagnetic). The small signals appearing after illumination with red light ($g = 2.05$ and 1.94) are due to the photoreduction of the bound ferredoxin of chloroplasts, a newly discovered electron transfer component[25,26]. No reduction of plastocyanin occurs after illumination with far-red light (715 nm) (Fig. 3C); some photooxidation was actually observed in this particular sample, as indicated by the increase in amplitude of the $g = 2.05$ signal in Fig. 3C as compared with the dark signal (Fig. 3A). Fig. 3

The effect of inhibitors of noncyclic electron transport on the photoreduction of plastocyanin in red light was examined next. As shown in Fig. 4, 3-(3',4'-dichlorophenyl)-1,1-dimethyl urea (DCMU) at a concentration of 10 uM (Fig. 4C) inhibits the photoreduction of plastocyanin. In addition, the plastoquinone antagonist 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB) which has been introduced by Trebst and co-workers[30,31] also inhibits the plastocyanin photoreduction (Fig. 4D), although complete inhibition with this reagent requires Fig. 4

a slightly higher concentration (35 uM) than is required with DCMU. It should be noted that the concentrations of inhibitor in these measurements are much higher than those commonly used--because of the high chlorophyll concentration (0.5 mg/ml) required in the EPR experiments. The ratio of inhibitor to chlorophyll in these studies is approximately the same as that used by other investigators (see ref. 16,30-35).

The results presented in Figs. 3 and 4 indicate that water serves as the source of electrons in the photoreduction of bound plastocyanin. In order to study the relation of plastocyanin to noncyclic electron transport, the effect of the physiological noncyclic electron acceptor NADP (in the presence of soluble ferredoxin) on the steady-state level of plastocyanin in red light was examined; the results are shown in Fig. 5. Fig. 5 As previously observed (Fig. 5B), plastocyanin is fully reduced in red light in the absence of an acceptor but in the presence of ferredoxin-NADP (Fig. 5C) the steady-state level is shifted to a more oxidized level, although the final steady-state level is still predominantly reduced. The steady-state level in the presence of ferredoxin-NADP was approximately 50% reduced, as shown in Fig. 5C as compared with Fig. 5A. The addition of methyl viologen (0.1 mM) in place of ferredoxin-NADP as the electron acceptor had a similar effect on the steady-state level of the bound plastocyanin in red light.

The relationship of the bound plastocyanin to photosynthetic phosphorylation was also examined. As shown in Fig. 6B, the addition of ADP to a reaction mixture (in the presence of potassium phosphate and Mg^{2+}) causes a shift in the steady-state level of plastocyanin to a more reduced level (compare with Fig. 6A). This shift was dependent on the cofactors of phosphorylation and the light-induced change was sensitive to DCMU. The addition of an uncoupler of phosphorylation, NH_4Cl, also produced a change in the steady-state level to a more reduced state, as shown in Fig. 6C. These results indicate that under conditions of maximum noncyclic electron flow (phosphorylating or uncoupled state) the steady-state level of plastocyanin is predominantly reduced.

Fig. 6

Plastocyanin photooxidation in situ

For study of the photooxidation of plastocyanin, ascorbate was added to the chloroplasts prior to illumination. As shown in Fig. 7A, after incubation with ascorbate (5 mM) for approximately 5 min, the bound plastocyanin was fully reduced in the dark. Illumination with red light (645 nm) (Fig. 7B) produced no change, while far-red illumination (715 nm) (Fig. 7C) resulted in a large photooxidation of plastocyanin, as measured by the increase of the $g = 2.05$ signal. The amount of plastocyanin photooxidized in far-red light was 70-100% of the amount detected in the presence of a chemical oxidant.

Fig. 7

The effect of DCMU and DBMIB on plastocyanin photooxidation was examined next. Since both of these reagents were found to inhibit the photoreduction of plastocyanin, it was anticipated that they would have no effect on plastocyanin photooxidation. DCMU, at a concentration of 20 uM, did not affect the far-red-light photooxidation of plastocyanin (compare Fig. 7C and 8C). In red light (645 nm), plastocyanin can also be photooxidized in the presence of DCMU since red light can activate Photosystem I as well as Photosystem II (Fig. 8B). It is important to note that the amount of plastocyanin photooxidized in the presence of DCMU is the same in either red or far-red light (compare Fig. 8B and 8C). Fig. 8

DBMIB showed results similar to those observed with DCMU, as shown in Fig. 9. Both red and far-red light photooxidized plastocyanin in the presence of a DBMIB concentration which inhibited photoreduction (35 uM) (Fig. 9B and 9C). In contrast to the result in the presence of DCMU, in the presence of DBMIB a large amount of reduced bound ferredoxin accumulated (signal at $g = 1.94$), a finding which may be related to the observation that DBMIB, in addition to inhibiting noncyclic electron transport in chloroplasts, inhibits the cyclic electron transport pathway[31]. Fig. 9

DISCUSSION

Studies of the role of plastocyanin in photosynthetic electron transport have been confined primarily to effects of externally added plastocyanin on reactions in plastocyanin-depleted chloroplast fragments. There have been no reports of light-induced changes attributable to plastocyanin in spinach chloroplasts, and the only *in situ* studies of plastocyanin are in whole algal cells[21,22]. In this work we have identified a low-temperature EPR signal in spinach chloroplasts which we assign to the bound form of plastocyanin; we have then studied light-induced changes at physiological temperatures of this EPR-detectable component in order to evaluate the role of plastocyanin in the noncyclic photosynthetic electron transfer chain.

The low-temperature EPR signal detected in chloroplasts has EPR parameters and oxidation-reduction characteristics similar to those of soluble plastocyanin isolated from spinach. A more definitive assignment of this component as the bound plastocyanin comes from quantitative estimation of its concentration. Approximately 3 nmoles of bound plastocyanin per mg chlorophyll were calculated on the basis of the $g_{\perp}$ region of the EPR spectrum, in excellent agreement with the amount of plastocyanin assayed by a chemical method after release from the chloroplast lamellae by sonication. The agreement between these two values makes it unlikely that the EPR component having a $g = 2.05$ signal represents

non-specifically bound Cu^{2+} ions or a different chloroplast copper protein. In addition, the g = 2.05 signal is absent from fragments after sonication, a treatment known to release plastocyanin from the lamellae of the chloroplasts.

The conclusion that the g = 2.05 component behaves as a carrier in the photosynthetic electron transport chain is based on low-temperature EPR studies of light-induced changes produced at physiological temperatures. The component responsible for the g = 2.05 signal undergoes photoreduction and photooxidation in monochromatic light of different wavelengths and, in addition, undergoes a shift in its steady-state level in the presence of ferredoxin-NADP, the physiological acceptor system of the noncyclic electron transport chain, after illumination with red light. These results, which will be discussed in more detail below, indicate that the bound component with a g = 2.05 EPR signal is involved in the electron transfer chain from water to NADP, a photochemical system known to require plastocyanin[5-7].

Plastocyanin as an electron carrier in the noncyclic electron transport chain

There is widespread agreement that in chloroplasts noncyclic electron transport involves two different photoreactions. Most investigators maintain that one Photosystem I and one Photosystem II reaction cooperate in the transfer of electrons from water to NADP

(ref. 8,9,36,37) while a more recent proposal suggests that two Photosystem II light reactions cooperate in noncyclic electron transport[10-12]. This newer proposal was based on spectroscopic studies of electron carriers in spinach chloroplasts and on results derived from studies with subchloroplast fragments enriched in Photosystem II (ref. 10-12,38,23). In both of these models, plastocyanin functions as an electron carrier in the dark electron transport chain after Photosystem II and would therefore be expected to be photoreduced in red light which activates Photosystem II. Both proposals also suggest that there is a phosphorylation site in the dark chain on the Photosystem II side of plastocyanin, although experimental evidence on this point is lacking.

The findings presented in this work, that plastocyanin photoreduction proceeds in red light and is sensitive to noncyclic electron transport inhibitors, indicate that water is the source of electrons in this photoreduction. Noncyclic electron acceptors such as NADP or methyl viologen shift the steady-state level of plastocyanin to a more oxidized level in red light, providing evidence based on <u>in situ</u> measurements for the role of plastocyanin in the electron transport chain from water to NADP. A similar conclusion had previously been reached based on the requirement of plastocyanin for NADP photoreduction from water in chloroplast fragments which had been sonicated[5-7].

In the presence of phosphorylation cofactors and under conditions of noncyclic electron flow (red light, ferredoxin-NADP present), a shift in the plastocyanin steady-state was observed. In this case, plastocyanin was fully reduced as compared to 50% reduction in the absence of ADP. The addition of the uncoupler ammonium chloride produced a steady-state shift which was similar to that observed with ADP. This type of shift in the steady-state level of a carrier in chloroplasts under phosphorylating conditions or in the presence of an uncoupler was first reported by Avron and Chance[33,34] for cytochrome f and has most commonly been interpreted in terms of the "crossover" experiments discussed by Chance and Williams[39]. According to this interpretation, the addition of agents affecting phosphorylation will alter the steady-state level of electron carriers on the oxidizing and reducing side of the phosphorylation site. In the case of a linear electron transfer pathway which is noncoupled (limited by the absence of ADP), the addition of ADP would be expected to cause carriers on the oxidizing side of the phosphorylation site to become more reduced relative to the steady-state in the absence of ADP. Uncouplers would be expected to have a similar effect according to this interpretation. Applying this type of reasoning to our results with plastocyanin suggests there is a phosphorylation site on the reducing side of plastocyanin in the noncyclic electron transport chain.

The findings concerning plastocyanin photoreduction are consistent with either of the two models of noncyclic electron transport previously discussed. The models differ, however, in their predictions concerning plastocyanin photooxidation, one predicting plastocyanin photooxidation by Photosystem I (the widely accepted series hypothesis[8,9]) while the newer, parallel hypothesis predicts plastocyanin photooxidation by Photosystem II (light reaction IIa in the terminology of Knaff and Arnon) and no interaction of plastocyanin with Photosystem I.

The studies of plastocyanin photooxidation indicate that far-red light, associated with Photosystem I, can photooxidize reduced plastocyanin. Red light, which activates Photosystems I and II, is able to photooxidize plastocyanin only after the addition of inhibitors of electron flow from water (DCMU and DBMIB). In the presence of these inhibitors the amount of plastocyanin photooxidized in far-red light was the same as the amount photooxidized in red light, a finding which indicates that plastocyanin is not solely associated with Photosystem II. These results are not consistent with the parallel hypothesis in which the photooxidation of plastocyanin was assigned only to Photosystem II.

The antagonistic effect of red and far-red light on plastocyanin <u>in situ</u> is similar to the effect observed for other electron carriers in isolated spinach chloroplasts: cytochrome <u>f</u> (ref. 33,34,40,41), P700

(ref. 34,42), and plastoquinone (ref. 42,43). The oxidation-reduction behavior of these carriers after monochromatic illumination can be explained by the series hypothesis of noncyclic electron transport in which a Photosystem II light reaction interacts with a Photosystem I light reaction[8,9,36,37] but has not yet been explained by the parallel hypothesis in which two Photosystem II light reactions interact in noncyclic electron transport[10-12].

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ABBREVIATIONS: EPR, electron paramagnetic resonance; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethyl urea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone.

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TABLE I

EPR PROPERTIES OF SOLUBLE PLASTOCYANINS AND BOUND CHLOROPLAST PLASTOCYANIN

	$g_\perp$	$g_{//}$	$A_{//}$ (cm^{-1})
Soluble spinach plastocyanin	2.05	2.23	0.006
Bound chloroplast plastocyanin	2.04	2.23	0.006
Soluble Chenopodium plastocyanin (ref. 28)	2.053	2.226	0.006

LEGENDS TO FIGURES

FIG. 1. EPR spectra of soluble plastocyanin and bound chloroplast plastocyanin. High-field region of the EPR spectra of spinach plastocyanin (A) (12 nmoles/ml) and washed, broken chloroplasts (B) (1.5 mg chlorophyll/ml). In (B), 1 mM ferricyanide was added to the sample prior to freezing. The low-field region of the spectra are shown in (C) and (D). Conditions of EPR spectroscopy: Frequency, 9.30 GHz; power, 10 mW; modulation, 10 gauss, except in (D) where 18 gauss was used; temperature, 25°K; time constant, 0.1 sec; scan rate, 20 gauss/sec. Amplifier gain settings were 160 in (A), 450 in (B) 1200 in (C), and 3600 in (D).

FIG. 2. Effect of sonication on the EPR signal of bound chloroplast plastocyanin. Spectra of untreated chloroplasts (A) or sonicated chloroplasts (B) (1.5 mg chlorophyll/ml in each case) were recorded at 25°K in the presence of 1 mM ferricyanide. EPR settings as in Fig. 1 except for amplifier gain settings; these were 1200 in (A) and 2200 in (B).

FIG. 3. Photoreduction of bound plastocyanin in monochromatic light. The reaction mixture contained 50 mM Tricine, pH 8.2, 10 mM NaCl, and chloroplasts at a chlorophyll concentration of 0.5 mg/ml. Conditions for illumination and freezing of samples were as described in Methods. Conditions of EPR spectroscopy: microwave

frequency, 9.26 GHz; microwave power, 10 mW; modulation frequency, 100 KHz and amplitude, 10 gauss; time constant, 0.1 sec; scanning rate, 1000 gauss/min; temperature, 25°K. (A) Dark sample prior to illumination. (B) Sample illuminated with light of wavelength 645 nm. (C) Sample illuminated with light of wavelength 715 nm.

FIG. 4. Effect of inhibitors on the photoreduction of plastocyanin in red light. The reaction mixture contained 50 mM Tricine, pH 8.2, 10 mM NaCl, chloroplasts at a chlorophyll concentration of 0.5 mg/ml, and, where present, 10 uM DCMU or 35 uM DBMIB. Samples were illuminated with monochromatic light of wavelength 645 nm as described in Methods. Conditions of EPR spectroscopy were as in Fig. 3. (A) Dark sample prior to illumination. (B) Sample illuminated with light of wavelength 645 nm. (C) Sample illuminated with 645-nm light in the presence of DCMU. (D) Sample illuminated with 645-nm light in the presence of DBMIB.

FIG. 5. Effect of an electron acceptor on the photoreduction of plastocyanin in red light. The reaction mixture contained 50 mM Tricine, pH 7.8, 10 mM NaCl, chloroplasts at a chlorophyll concentration of 0.5 mg/ml, and, where present, 0.01 mM spinach ferredoxin and 2 mM NADP. Samples were illuminated with monochromatic light of wavelength 645 nm as described in Methods. Conditions of EPR spectroscopy were as in Fig. 3. (A) Dark sample

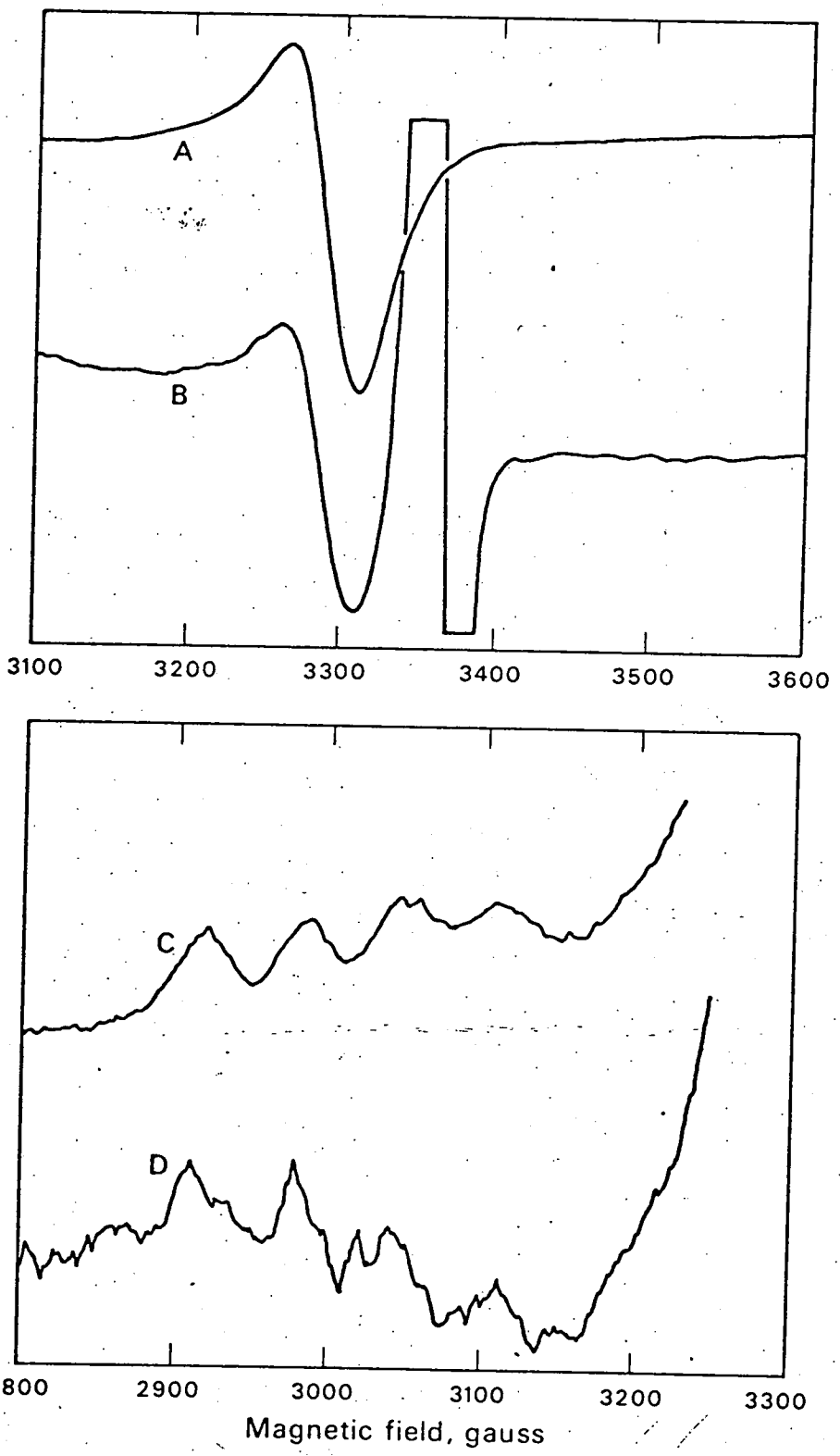
prior to illumination. (B) Sample illuminated with 645-nm light. (C) Sample illuminated with 645-nm light in the presence of ferredoxin plus NADP.

FIG. 6. Effect of phosphorylating conditions and uncoupler on photoreduction of plastocyanin in red light. The reaction mixture contained 50 mM Tricine, pH 7.8, 10 mM NaCl, chloroplasts at a chlorophyll concentration of 0.5 mg/ml, 0.01 mM spinach ferredoxin, 2 mM NADP, 5 mM $MgCl_2$, 5 mM K_2HPO_4, and, where present, 5 mM ADP or 50 mM NH_4Cl. Samples were illuminated with 645-nm light as described in Methods. Conditions of EPR spectroscopy were as in Fig. 3. (A) Sample illuminated in the presence of ferredoxin/NADP, $MgCl_2$, and K_2HPO_4. (B) Sample illuminated in the presence of ferredoxin/NADP, $MgCl_2$, K_2HPO_4, and ADP. (C) Sample illuminated in the presence of ferredoxin/NADP, $MgCl_2$, K_2HPO_4, and NH_4Cl.

FIG. 7. Photooxidation of plastocyanin in monochromatic light. The reaction mixture contained 50 mM Tricine, pH 8.2, 10 mM NaCl, 5 mM sodium ascorbate, and chloroplasts at a chlorophyll concentration of 0.5 mg/ml. Samples were illuminated as described in Methods. Conditions of EPR spectroscopy were as in Fig. 3. (A) sample prior to illumination. (B) Sample illuminated with 645-nm light. (C) Sample illuminated with 715-nm light.

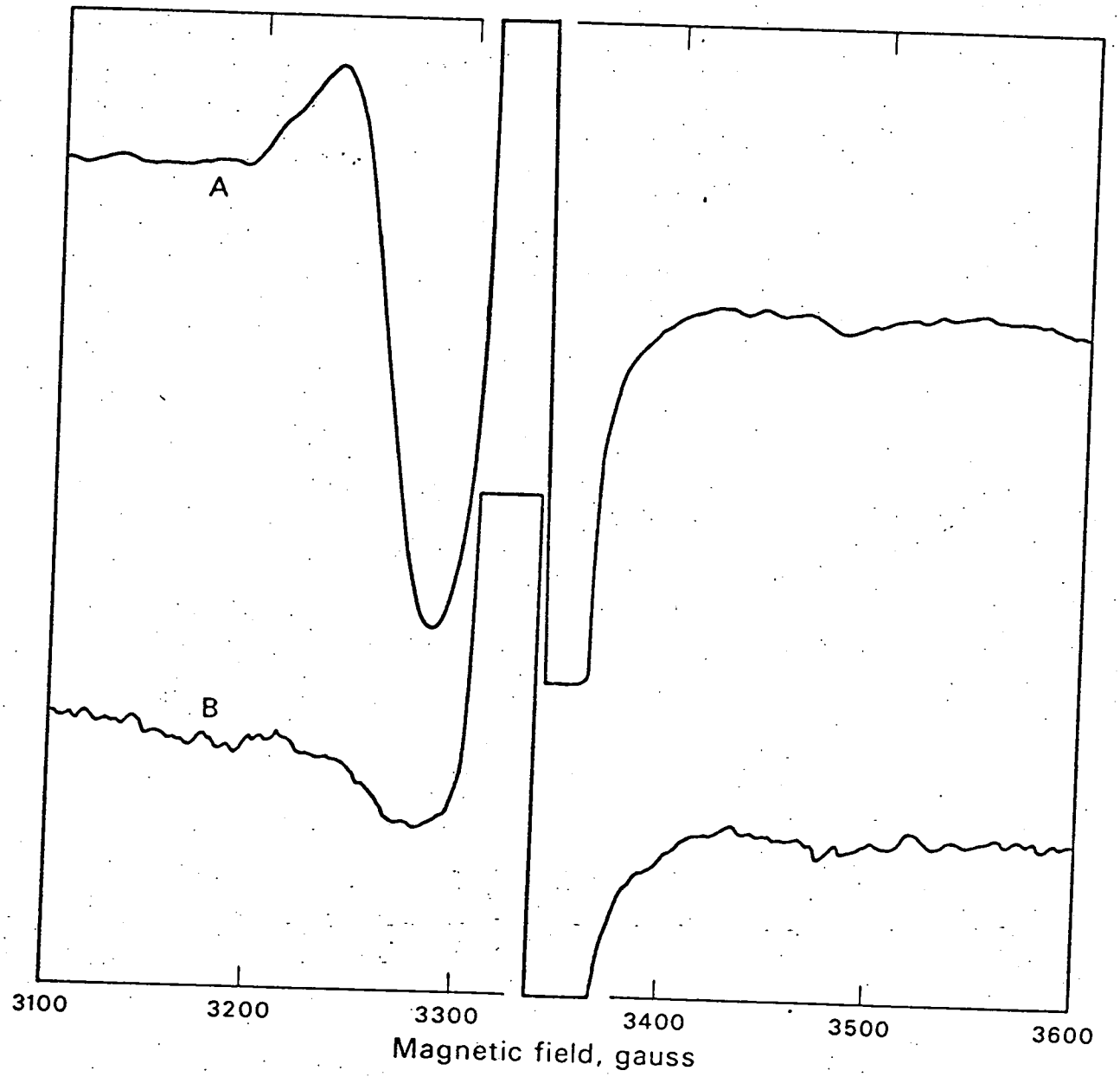
FIG. 8. Effect of DCMU on plastocyanin photooxidation in monochromatic light. The reaction mixture and illumination conditions were as in Fig. 7 except that 10 μM DCMU was present. (A) Sample prior to illumination. (B) Sample illuminated with 645-nm light in the presence of DCMU. (C) Sample illuminated with 715-nm light in the presence of DCMU.

FIG. 9. Effect of DBMIB on plastocyanin photooxidation in monochromatic light. The reaction mixture and illumination conditions were as in Fig. 7 except that 35 μM DBMIB was present. (A) Sample prior to illumination. (B) Sample illuminated with 645-nm light in the presence of DBMIB. (C) Sample illuminated with 715-nm light in the presence of DBMIB.



DBL 721-5140

Fig. 1



DBL 721-5139

Fig. 2

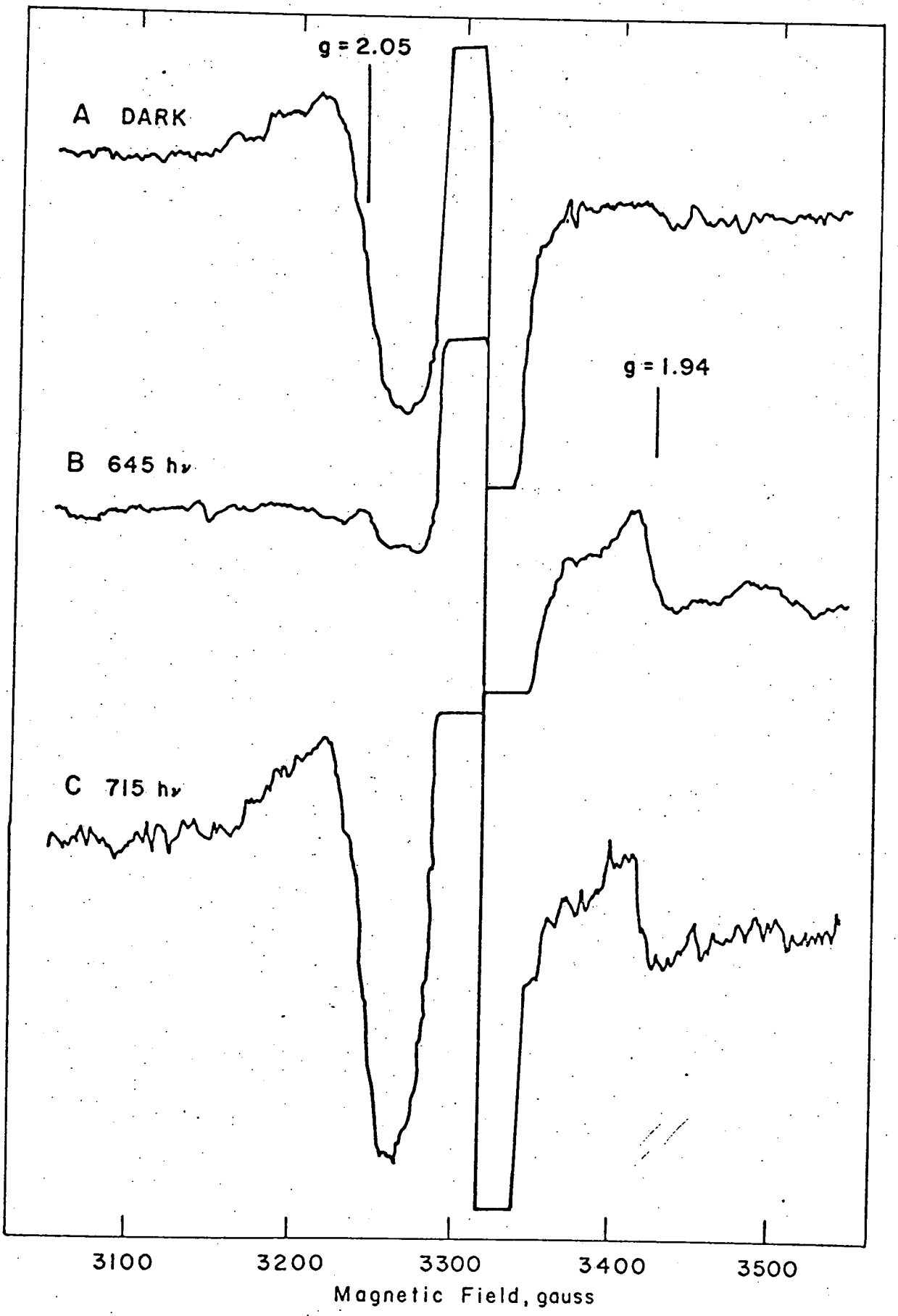

g = 2.05
A DARK
g = 1.94
B 645 hν
C 715 hν
3100
3200
3300
3400
3500
Magnetic Field, gauss

Fig. 3

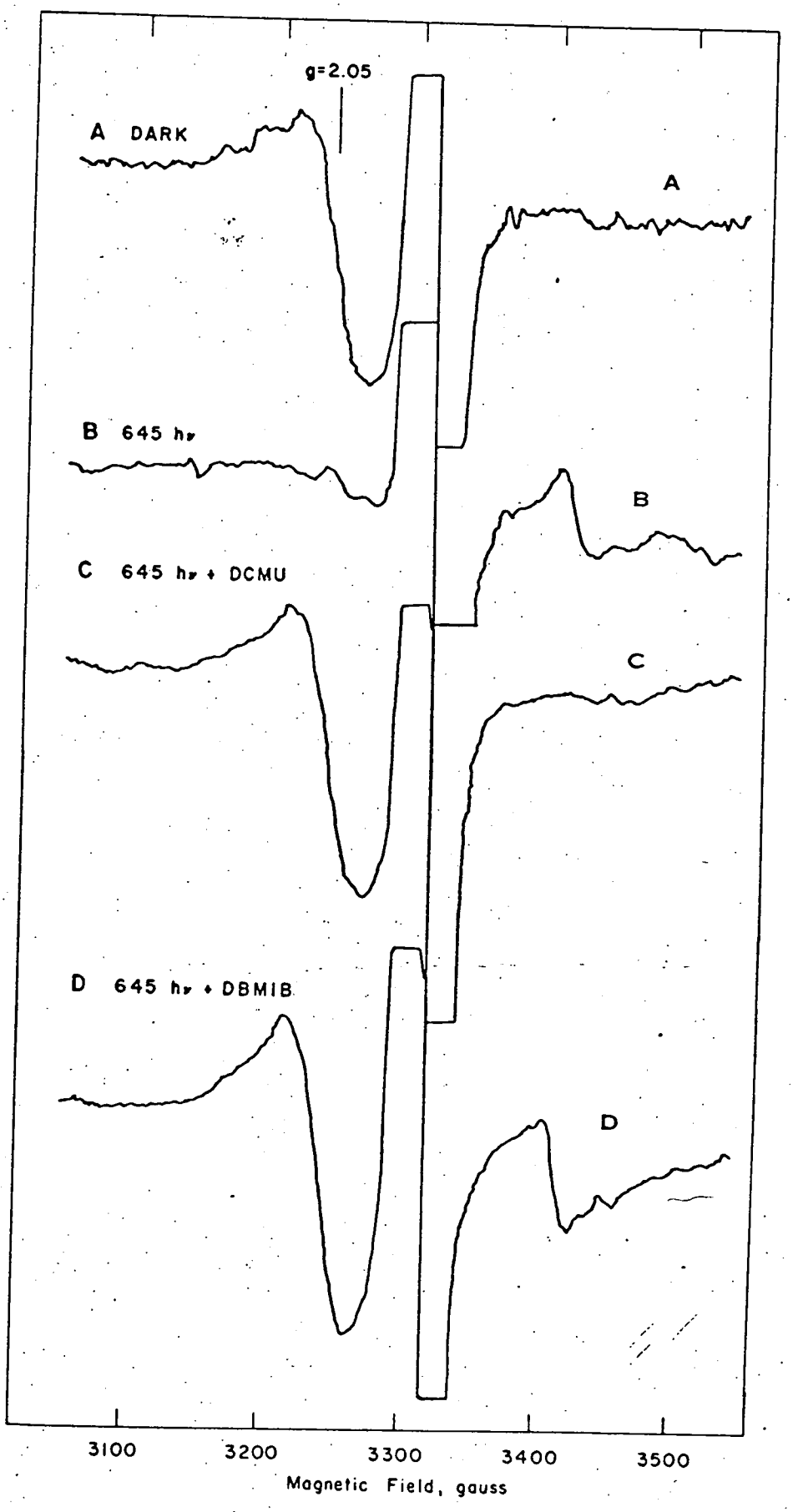

g=2.05
A DARK
A
B 645 hν
B
C 645 hν + DCMU
C
D 645 hν + DBMIB
D
3100
3200
3300
3400
3500
Magnetic Field, gauss

Fig. 4

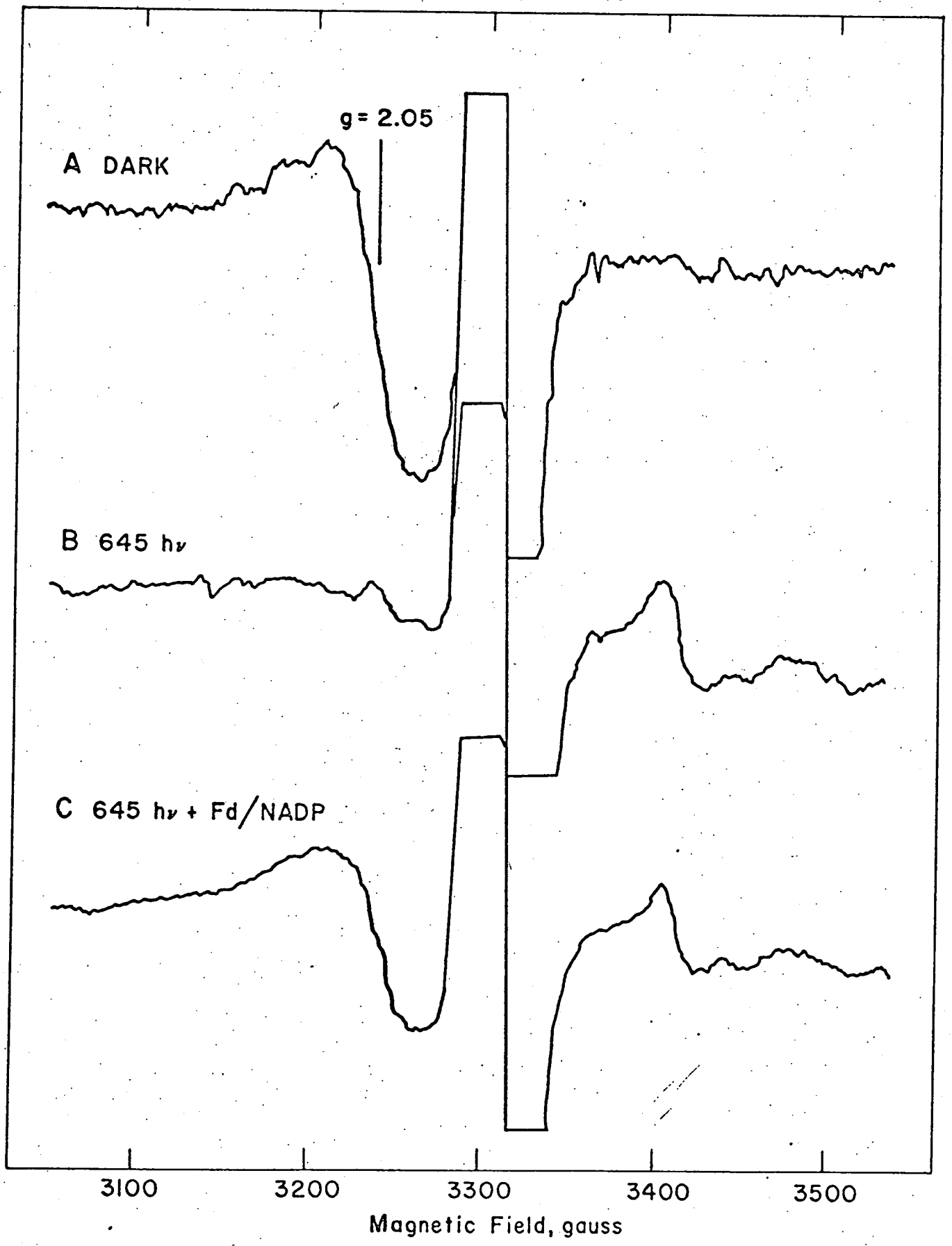

g = 2.05
A DARK
B 645 hν
C 645 hν + Fd/NADP
3100
3200
3300
3400
3500
Magnetic Field, gauss

Fig. 5

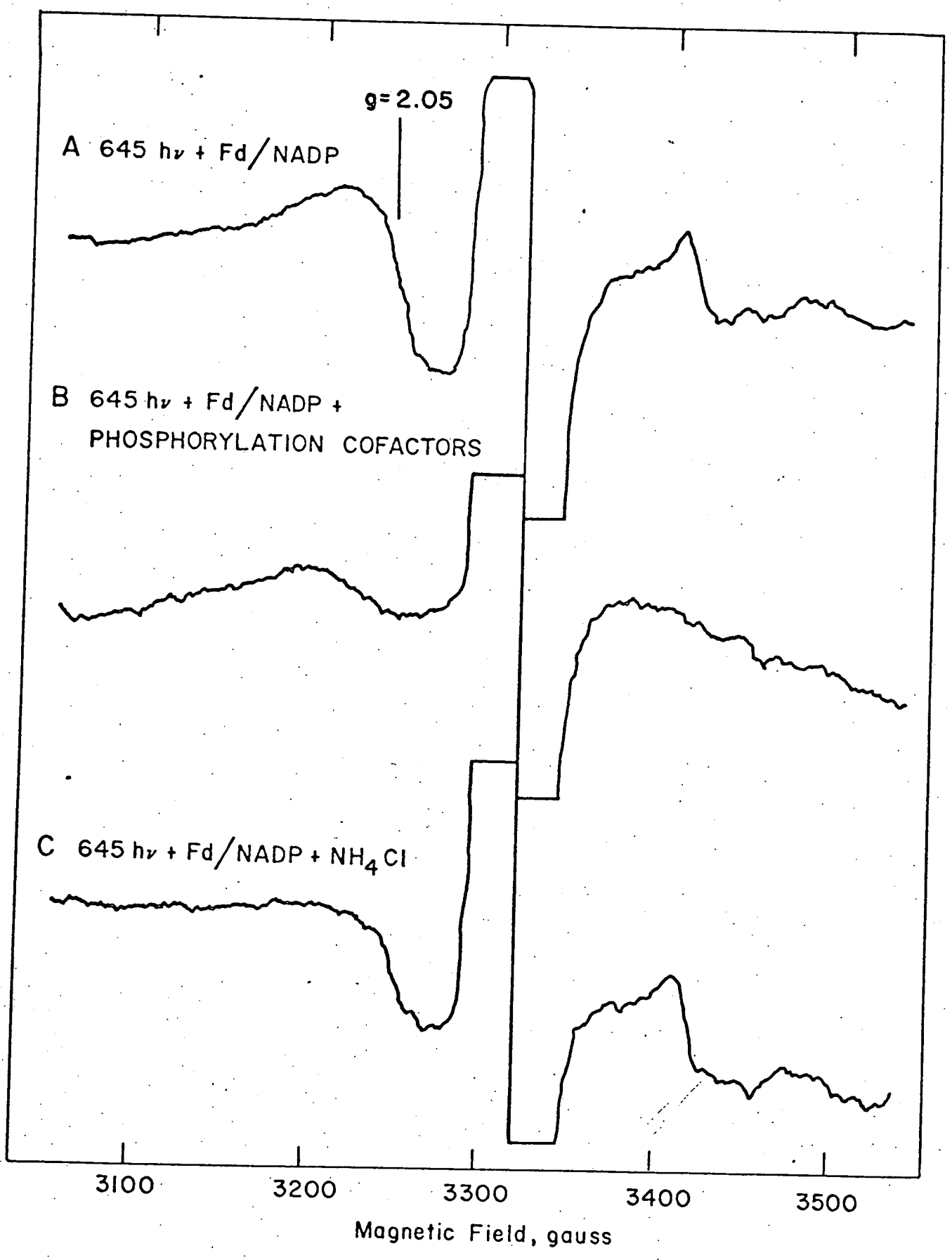

g=2.05
A 645 hν + Fd/NADP
B 645 hν + Fd/NADP +
PHOSPHORYLATION COFACTORS
C 645 hν + Fd/NADP + NH4Cl
3100
3200
3300
3400
3500
Magnetic Field, gauss

Fig. 6

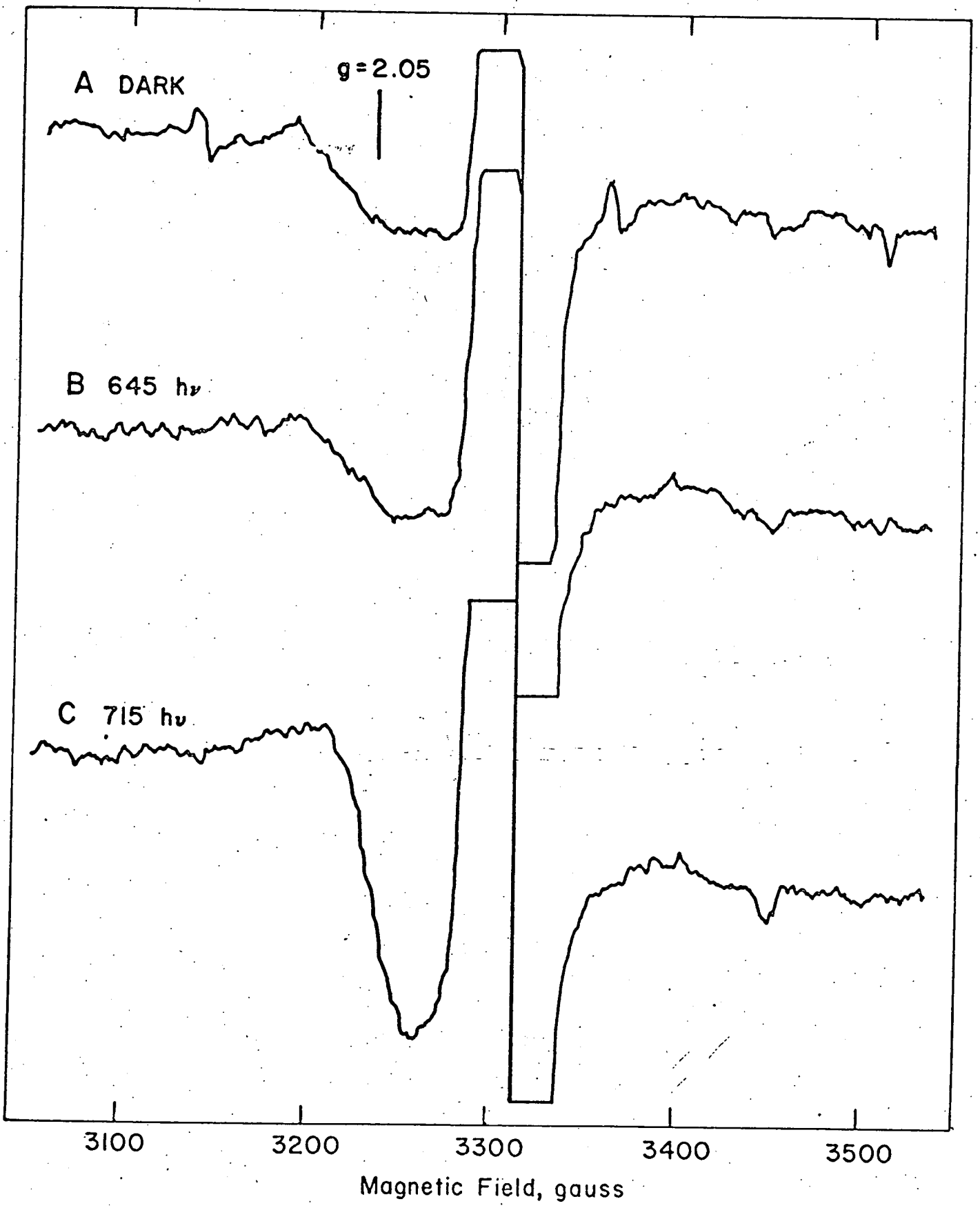

A DARK
g=2.05
B 645 hν
C 715 hν
3100
3200
3300
3400
3500
Magnetic Field, gauss

Fig. 2

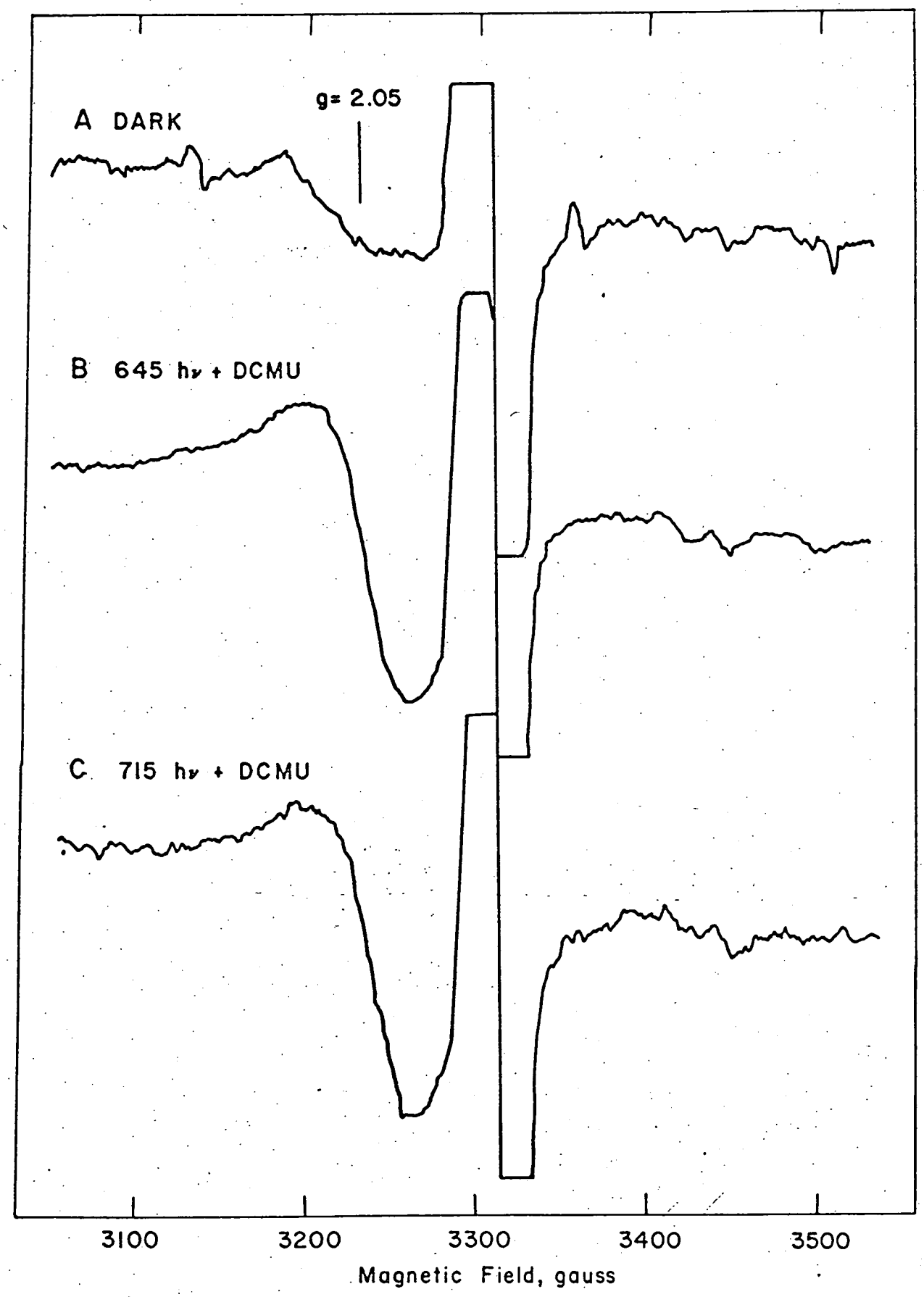

g= 2.05
A DARK
B 645 hν + DCMU
C 715 hν + DCMU
3100
3200
3300
3400
3500
Magnetic Field, gauss

Fig. 8

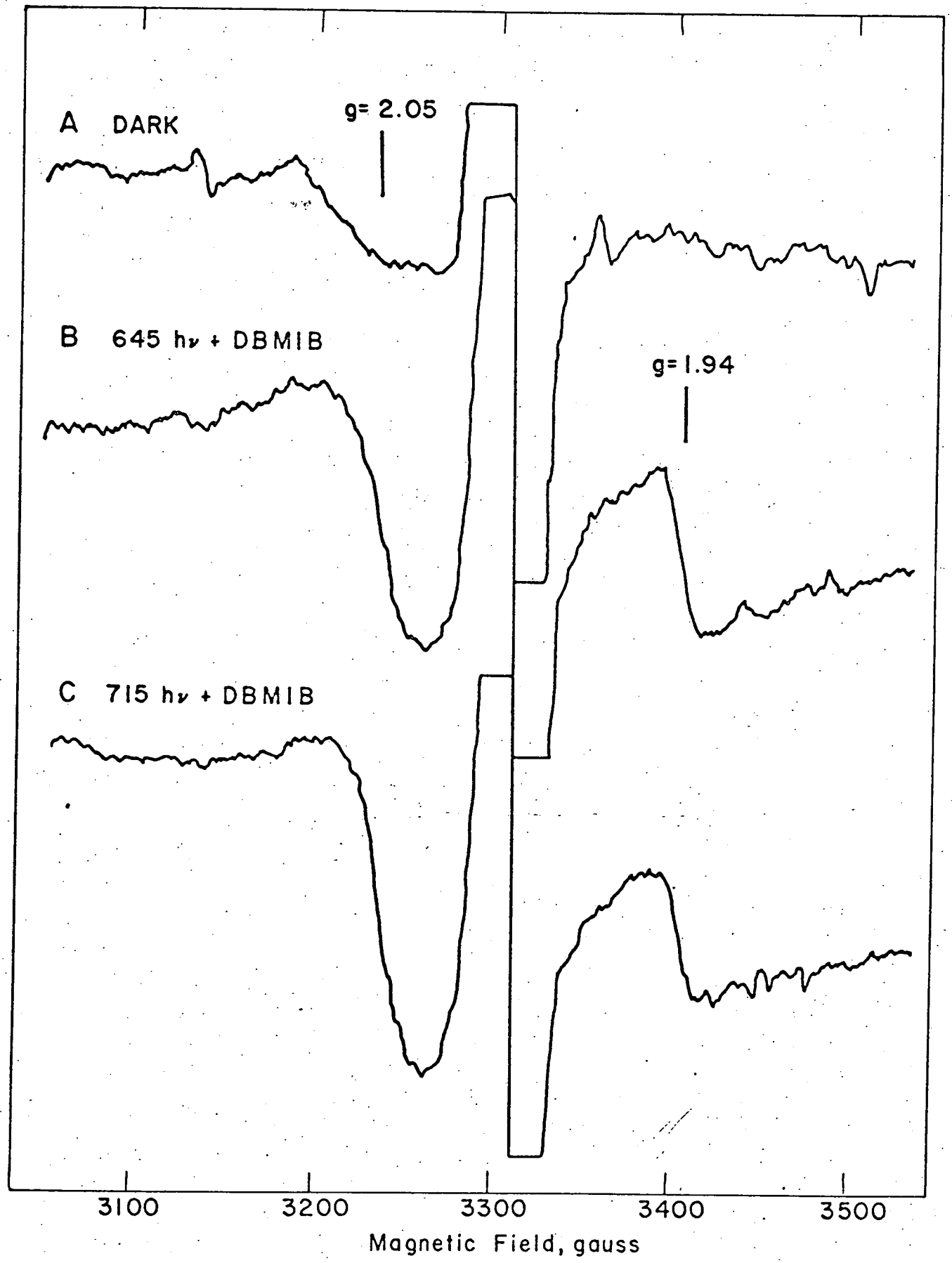

A DARK
g= 2.05
B 645 hν + DBMIB
g= 1.94
C 715 hν + DBMIB
3100
3200
3300
3400
3500
Magnetic Field, gauss

Fig. 9