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PIGMENT SYSTEMS AND ELECTRON TRANSPORT IN CHLOROPLASTS. II. EMERSON ENHANCEMENT IN BROKEN SPINACH CHLOROPLASTS

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SUMMARY

The Emerson enhancement effect using red illumination supplemented by far red light is a characteristic phenomenon of photosynthetic oxygen evolution by plants and algae. It has been cited as an important evidence in support of the mechanism of photosynthetic electron transport involving two light reactions operating in series. The present study confirms the occurrence of enhancement in isolated, broken spinach chloroplasts for the photoreduction of $NADP^+$ by water: $[H_2O \rightarrow NADP]$ reaction. Far red light at 700 nm is supplemented optimally with wavelengths of 650 or 670 nm. Divalent cations such as magnesium or manganese are shown to be required for enhancement to occur. The optimum concentrations of added $MgCl_2$ or $MnCl_2$ are about 7.5 mM; at concentrations below 3 mM enhancement is not obtained. The critical dependence on divalent ion concentration is felt to be the reason why the enhancement phenomenon has not been observed in some previous studies using broken chloroplasts. A role for magnesium ion is proposed in which it alters the structure of the active chloroplast membranes in a manner which controls the transfer of electronic excitation between the two photosynthetic pigment systems. These findings favor the series two-light reaction mechanism over the alternative parallel scheme.

ABBREVIATIONS:: DCIP ($DCIPH_2$), 2,6-dichlorophenolindophenol, oxidized (reduced); DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; PCy, plastocyanin; Fp, ferredoxin-$NADP^+$ reductase; CP, chloroplasts; PS I and PS II, photosystem I and photosystem II; RC I and RC II, reaction center I and reaction center II; P I and P II, pigment system I and pigment system II.

INTRODUCTION

The nature of electron transport in photosynthesis has been extensively investigated[1], but there still remain some very important differences of opinion with regard to the relationship of the light reactions and the two photosystems to electron transport in chloroplasts. In Part I of this report, we addressed the question of the number of photons required to carry out PS I, PS II or PS (I + II) reactions[2]. In this paper we report experiments designed to answer the question whether PS I and PS II are both required to transfer electrons in a series fashion in the [$H_2O \rightarrow$ NADP] reaction[1-4]. An alternative mechanism has been proposed by Knaff and Arnon in which both of the photosystems operate in independent pathways and only PS II (PS IIa + PS IIb) is involved in the [$H_2O \rightarrow$ NADP] reaction[5]. In this reaction electrons are transferred from water to $NADP^+$, leading to oxygen evolution and the reduction of $NADP^+$ to NADPH. The phosphorylation of ADP to ATP can be coupled to the [$H_2O \rightarrow$ NADP] reaction.

In Part I we reported that the [Ascorbate + $DCIPH_2 \rightarrow$ NADP] reaction, characteristic of PS I, requires only one quantum of light at 700 nm or longer wavelengths in order to transfer one electron from $DCIPH_2$ to $NADP^+$ in the presence of DCMU[2]. The [$H_2O \rightarrow$ DCIP] Hill reaction is characteristic of PS II and requires only one quantum of 630 to 660 nm light to transfer one electron from water to DCIP, provided that the reaction conditions are adjusted to give efficient spillover of excitation from P I to P II. The [$H_2O \rightarrow$ NADP] reaction requires two quanta from 620 to 678 nm to transfer one electron from H_2O to NADP under the same conditions favoring spillover[2]. Avron and Ben Hayyim reported nearly identical values for these or similar reactions[6]. However, it is impossible on the basis of action spectra and quantum requirement measurements alone to answer the question of whether

the [H_2O → NADP] reaction is driven by PS (I + II) or PS (IIa + IIb). One way of providing conclusive evidence that the [H_2O → NADP] reaction involved PS (I + II) is to demonstrate the occurrence of the Emerson enhancement effect for this reaction in chloroplasts.

Using whole cells of Chlorella and of Chroococcus, Emerson and Lewis[7,8] found a sharp decrease in the efficiency of photosynthesis with actinic light of wavelengths longer than 685 nm (red drop in efficiency). By adding a weak background of green light to the far-red light, Emerson et al. found that the combined wavelengths produced higher photosynthetic rates than the sum of the rates for the two lights used separately[9,10]. This Emerson enhancement effect is the subject of a recent review[11]. The series formulation involving two light reactions[12] gained support from these experiments. The basic idea was that far-red light, absorbed predominantly by PS I, could be supplemented or "enhanced" by adding light that was preferentially absorbed by PS II. This enhancement has been demonstrated to occur in whole cells or intact leaves in a wide variety of oxygen-evolving organisms[9-11,13-16]. Nevertheless, it was argued that the origin of enhancement in whole cells is not in the primary light-driven electron transport reactions, but is a consequence of feedback loops in the dark reaction involving the requirements of CO_2 fixation for NADPH and ATP. NADPH is produced via non-cyclic electron transport and ATP is produced, at least in part, via cyclic electron flow involving only PS I. In order to resolve the origin of enhancement, it is necessary to find out whether enhancement occurs in isolated broken chloroplasts, where CO_2 fixation is not coupled and only the immediate consequences of the light reactions would be observed.

The occurrence of significant enhancement in the [$H_2O \rightarrow$ NADP] reaction by isolated broken chloroplasts has been reported by Govindjee et al.[17,18], Gordon[19], Joliot et al.[20] and Avron and Ben-Hayyim[6]. On the other hand, Gibbs et al.[15] and McSwain and Arnon[21] studied the reduction of $NADP^+$ (and of ferricyanide) by H_2O in isolated chloroplasts and found no measurable enhancement. The lack of enhancement was interpreted as indicating no cooperation between photosystems PS I and PS II. Arnon[22] proposed that either the [Ascorbate + $DCIPH_2 \rightarrow$ NADP] or the [$H_2O \rightarrow$ NADP] reaction is driven by a single light reaction, PS I for the former and PS II for the latter. The hypothesis was that only one photon per electron transferred was required in each of these reactions. It was subsequently modified in the light of the behavior of a new photoreactive chloroplast component C550 [23,24]. The latest version of this hypothesis[5] suggests that three light reactions are involved in photosynthesis; two are in PS II and are short-wavelength light reactions, and one is in PS I and is a long-wavelength reaction. The hypothesis also states that there is no direct cooperation between PS I and PS II, and only PS II (a + b) is involved in activating the basic reaction of photosynthesis, i.e., the [$H_2O \rightarrow$ NADP] reaction.

In the present study we present further evidence that Emerson enhancement does occur in broken spinach chloroplasts for the [$H_2O \rightarrow$ NADP] reaction. We believe, however, that our findings do more than simply add one more publication to the side favoring the assignment of this reaction to the PS (I + II) scheme. We have also found what we believe to be the reason why some laboratories have been unable to observe enhancement in isolated chloroplasts despite apparently extensive and painstaking efforts.

The discovery of the cause of this variability in turn uncovers an important new phenomenon relevant to photosynthetic control mechanisms.

MATERIALS AND METHODS

Spinach and preparation of chloroplasts

Spinach (Spinacia oleracea var. early hybrid No. 7) was grown in vermiculite in a growth chamber under controlled conditions similar to those of Sauer and Park[25]: light intensity approximately 3200 f-c in 10 hr light/14 hr dark cycles, temperature ca. 18°C, leaves harvested six to eight weeks after germination. Chloroplasts isolated with sucrose isotonic solution (CP-suc) were prepared as described previously[2]. Chloroplasts isolated with NaCl isotonic solution (CP-NaCl) were prepared similarly, except that the buffer solution for the isolation was 0.35 M NaCl and 0.02 M tris buffer at pH 8.0; 0.035 M NaCl solution was used for resuspension. Chlorophyll a and b concentrations were determined as in Part I.[2]

Reagents

In addition to those chemicals described in Part I,[2] manganous chloride was obtained from J. T. Baker Chemical Co., Phillipsburg, N. J., and trizma base from Sigma Chemical Co., St. Louis, Missouri.

Apparatus and light intensity measurement

The apparatus for monitoring $NADP^+$ reduction, based on a Cary 14 spectrophotometer, was similar to that used previously[2]. Actinic lights from two identical monochromators (Bausch and Lomb, 500 mm, red-blaze grating) were brought to approximate focus on the same side of the cuvette in the sample beam. Apart from the converging lenses and an intermediate

mirror in one beam, each monochromatic beam was supplemented with appropriate short-wavelength cut-off filters[4]. The intensities of the actinic lights were measured as previously[2].

Reaction mixture and preparation of ferredoxin, NADP reductase and plastocyanin

The reaction mixture of the [Ascorbate + $DPIPH_2$ → NADP] reaction, of [H_2O → DPIP] reaction and of [H_2O → NADP] reaction were the same as described previously[2] except for those stated specifically in the text under special conditions. Saturating amounts of plastocyanin were added to each of the three reaction mixtures and saturating ferredoxin and ferredoxin-NADP reductase were added to the [H_2O → NADP] and [Ascorbate + $DPIPH_2$ → NADP] reactions, except for those cases stated specifically in the text. Ferredoxin, plastocyanin and NADP reductase were prepared from commercial spinach as described previously[2].

RESULTS

(1) [H_2O → NADP] REACTION AND ENHANCEMENT STUDIES

The rate of photoreduction of NADP as a function of incident intensity

As described previously[2], the quantum requirements for the [H_2O → NADP] reaction increased gradually as a linear function of the incident light intensity within the range studied. The quantum requirements at 650 nm and the rates of photoreduction of $NADP^+$ at 650 nm and 700 nm within this intensity range are shown in Fig. 1. Incident light intensities from zero to approximately 3.0 nanoeinsteins·cm^{-2}·sec^{-1} were used in the study of enhancement effect. Only very active chloroplasts, as in Fig. 1, were used.

Patterns of sequential presentation of two actinic lights

In order to obviate possible biases, we examined four different sequencess of presentation of the actinic lights. (A) First illumination with a red light (650 nm) to obtain the rate of the reaction, R_R, at intensity I_R; dark interval (ca. 3 min) until the rate of the back reaction became constant; illumination with 700 nm light of intensity I_{FR} to obtain a rate R_{FR}; illumination with red light of intensity I_R added to the far-red light, giving the rate R_{FR+R}. This pattern is designated $[R_R; R_{FR}, R_{FR+R}]$. A typical time course of these rates is shown in Fig. 2. (B) Using the notation adopted above, we then modified the actinic illumination to provide the sequence $[R_{FR}, R_{FR+R}; R_R]$. (C) A third pattern used was $[R_{FR}; R_R, R_{R+FR}]$. (D) A fourth pattern used was $[R_R, R_{R+FR}; R_{FR}]$. The objective of using these different patterns was to demonstrate that enhancement can be observed regardless of the order in which the actinic wavelengths are presented.

Several different measures of enhancement are used in the literature on this subject[26]. In order to facilitate comparisons with other results, we have calculated enhancement ratios based on a portion of our results in three different ways, according to the following equations:

$$E_1 = \frac{R_{FR+R} - R_R}{R_{FR}}$$

$$E_2 = \frac{R_{FR+R} - R_{FR}}{R_R}$$

$$E_3 = \frac{R_{FR+R}}{R_{FR} + R_R}$$

where R_R = the rate of the reaction with red actinic light alone.

R_{FR} = the rate of the reaction with far-red light alone.

R_{FR+R} = the rate of the reaction when both red and far-red lights were incident simultaneously.

The rates of the reaction obtained with a single sample are used to calculate the enhancement values, E_1, E_2 and E_3. The enhancement values shown in Table I were obtained using an incident light intensity, I_R, about 1.4 nanoeinsteins$\cdot cm^{-2}\cdot sec^{-1}$ at 650 nm and I_{FR} about 2.3 nanoeinsteins$\cdot cm^{-2}\cdot sec^{-1}$ at 700 nm. At these intensities, R_R is nearly four times greater than R_{FR}, and the value of E_1 is significantly larger than E_2 or E_3. At lower relative light intensities at 650 nm, when the denominators become smaller, values of E_2 and E_3 close to 2.0 are obtained. Because values of E_1, E_2 and E_3 are essentially describing the same enhancement phenomenon, we have chosen E_1 as the preferred parameter to characterize the enhancement effect for the remainder of this study.

In Table II the values of E_1 are shown for the four different sequences of illumination described above. The E_1 values obtained in the illumination sequences (A) and (B) are very similar, but E_1 of (A) is always slightly smaller than that of (B). The E_1 values obtained in the illumination sequences (C) or (D) are always larger than those obtained in (A) or (B). Therefore the illumination order (A) is the most conservative way to measure the enhancement effect among the four. We use this as the standard illumination sequence in our subsequent experiments.

Absence of enhancement with two actinic lights at 650 nm

In order to confirm the absence of unsuspected contributing effects in the enhancement study, we carried out the following control experiment: We first illuminated the sample with actinic light I at 700 nm and actinic light II at 650 nm, to obtain the E_1 value, as described in

Table I. Then we changed the wavelength of the actinic light I monochromator from 700 to 650 nm and carefully adjusted its intensity to about 0.3 nanoeinsteins$\cdot cm^{-2}\cdot sec^{-1}$, which gave the same rate of $NADP^+$ reduction as when the actinic light I at 700 nm was 2.3 nanoeinsteins cm^{-2} sec^{-1}. We then repeated the experiment and obtained E_1 in the same way as described before. The only difference is that actinic light I in the first case is at 700 nm and in the second case is at 650 nm. The results are shown in Table III. The enhancement value E_1 obtained with actinic light I at 700 nm and II at 650 nm is 2.4 to 2.6. But E_1 is less than 1.0 when both actinic lights I and II are at 650 nm. Apparently there is an enhancement effect in the former but no such effect in the latter experiment. Because the rate of the reaction is not quite a linear function of the incident light intensity, as shown in Fig. 1, the "enhancement" ratio is found to be somewhat less than 1.0 when the two actinic lights are both at 650 nm.

Repeatability of the enhancement effect in the [$H_2O \rightarrow NADP$] reaction

The rate of the reaction and the red - far red enhancement effect are closely correlated with the activity of the chloroplasts. When the rate of the reaction is within 20% of the rate shown in Fig. 1 under identical experimental conditions, the enhancement values, E_1, are very reproducible, 2.4 $\pm$0.3. The rate of the reaction decreases steadily, but slowly, when the same sample is illuminated repeatedly. For Sample 1 in Table III the rate of the reaction is $\Delta A_{340\ nm}$/min = 0.095 at the first illumination; it decreased to 0.090 at the 10th illumination. High light intensities and long illuminations tend to decrease the rate of the reaction more rapidly than do low light intensities and short illuminations.

Chloroplasts studied immediately following isolation tend to retain their activity better than do those that have been standing in the dark at 0°C for 6 hr. In our experience when the chloroplasts are fresh and their activity is high, the rate of the reaction does not decrease more than 20% of the rate at the first illumination during the course of an experiment lasting 30 min. A typical example is shown in Table IV. The rate of the reaction was $\Delta A_{340\ nm}/min = 0.117$ at the first illumination; it decreased to 0.095 at the 10th illumination. Nevertheless, E_1 at the first illumination is 2.43, and it is 2.33 at the 13th. When the rate of the reaction decreased to $\Delta A_{340\ nm}/min = 0.086$ at the 16th illumination, E_1 decreased to 1.70. We disregard the result when the rate of the reaction decreases below $\Delta A_{340\ nm}/min = 0.095$; i.e., 20% below the rate at the first illumination.

Comparison of the enhancement effect with different chloroplasts and reaction mixtures

There are two major differences between our experiments and those in which the enhancement effect fails to occur[15,21]. Chloroplasts isolated in NaCl (CP-NaCl) were used in those studies, whereas we used chloroplasts isolated in sucrose (CP-suc) in our initial studies[2]. The second difference is in the reaction mixtures. Therefore, we investigated the enhancement effect with different reaction mixtures and using both CP-suc and CP-NaCl. We call our standard reaction mixture Solution A. The second reaction mixture, Solution B, is after McSwain and Arnon[21]. The three major differences between Solutions A and B are:

Solution A: Tricine buffer, 45 mM, pH 7.5; $MgCl_2$, 7.5 mM; (ADP+Pi) not added

Solution B: Tricine buffer, 33 mM, pH 8.2; $MgCl_2$, 1.7 mM; (ADP+Pi), 3.3 mM

We examined the effect on the enhancement for each chloroplast preparation and for each reaction mixture in turn. The results are shown in Table V. We find that CP-suc and CP-NaCl were equally active in Solution A with saturating plastocyanin (PCy) and ferredoxin-NADP reductase (Fp). The enhancement values E_1 are all in the range 2.3 ± 0.2, as shown in column (1) of Table V. When Solution A is not supplemented with PCy or Fp, the CP-suc are not so active as in the former condition[2], and E_1 was ca. 1.5. However, CP-NaCl are as active in both conditions, and E_1 values are 2.4 ± 0.3. It is possible that the NaCl isolation process produces better retention of endogenous PCy and/or Fp. Because the enhancement effect could be demonstrated with both CP-suc and CP-NaCl, the discrepancy in the enhancement results cannot be due solely to the difference in the chloroplast preparations.

Next, we examined the enhancement effect using CP-suc or CP-NaCl in either Solution A or B. The results are shown in columns (1) and (5) of Table V. We find that both types of chloroplasts exhibit enhancement in Solution A with or without PCy and Fp, but no enhancement is obtained when the same CP-suc or CP-NaCl preparations are used in Solution B either with or without PCy and Fp. These results clearly show that the critical factors controlling enhancement reside in the three differences between Solutions A and B. When we change the pH from 7.5 to 8.2 or add (ADP+Pi) in Solution A [columns (2) and (3)], the values of E_1 decrease to 1.4-1.9 but enhancement is still evident. When we change the pH from 8.2 to 7.5 or do not add the (ADP+Pi) in Solution B [columns (6) and (7)], the E_1 values were below 1.0 and no enhancement could be observed.

When we changed the $MgCl_2$ concentration from 7.5 mM to 1.67 mM in Solution A [column (4)], we find that all E_1 values decrease to 1.0 or below. On the other hand, when we change the $MgCl_2$ concentration from 1.67 mM to 7.5 mM in Solution B [column (8)], the E_1 values increased from below 1.0 to about 2.0. These results clearly show that a higher concentration of $MgCl_2$, 7.5 mM, is necessary for the enhancement effect in the [$H_2O \rightarrow$ NADP] reaction using broken spinach chloroplasts.

Dependence of enhancement on the $MgCl_2$ concentration

We reported previously[2] that the rate of photoreduction of $NADP^+$ in the [$H_2O \rightarrow$ NADP] reaction is dependent upon the $MgCl_2$ concentration. Further studies now indicate a complex relationship between the optimal $MgCl_2$ concentration and the wavelength and intensity of the actinic light. The results in Fig. 3 (upper curves) show that the rate of the [$H_2O \rightarrow$ NADP] reaction reaches its maximum at 1.5 mM $MgCl_2$ when the incident actinic light at 650 nm is 1.40 nanoeinsteins cm^{-2} sec^{-1}, but the maximum rate of the reaction occurs at 7.5 mM $MgCl_2$ when the actinic light at 678 nm is 2.4 nanoeinsteins$\cdot cm^{-2} \cdot sec^{-1}$.

The enhancement effect of far-red (700 nm) light on red (650 nm) light for the [$H_2O \rightarrow$ NADP] reaction of broken chloroplasts is also a function of the $MgCl_2$ concentration (Fig. 3, lower curve). No significant enhancement is observed when $MgCl_2$ is below 3.0 mM. Enhancement reaches a maximum at 7.5 mM $MgCl_2$ ($E_1 = 2.4 \pm 0.3$), which is the same concentration of $MgCl_2$ that gives a maximum rate, R_{678}, using 678 nm light alone. At higher $MgCl_2$ concentrations (up to 15 mM) both the rate of the [$H_2O \rightarrow$ NADP] reaction at 678 nm and the enhancement value decrease. The similar dependence on $MgCl_2$ concentration of both the rate and the

enhancement effect suggest that these two features arise from a common origin affecting the state of the broken chloroplasts.

Dependence of enhancement on $MnCl_2$, NaCl and sucrose

Because of the well known ability of Mn^{2+} to replace Mg^{2+} in enzymatic reactions[27], we investigated the effect of $MnCl_2$ (as a replacement for $MgCl_2$) on the rate and the enhancement effect for the [$H_2O \rightarrow$ NADP] reaction. As shown in Fig. 4, we find that $MnCl_2$ duplicates the behavior of $MgCl_2$ (Fig. 3) in both respects.

The effect of NaCl on the rate and enhancement of the [$H_2O \rightarrow$ NADP] reaction is shown in Fig. 5. The rate increases from $\Delta A_{340\ nm}$/min = 0.14 at zero concentration to the maximum $\Delta A_{340\ nm}$/min = 0.25 at 75 mM NaCl, then decreases at higher concentrations. The enhancement ratio E_1 also increases from 0.5 ± 0.1 at zero concentration to 1.1 ± 0.1 at 75 mM NaCl and decreases at higher concentrations. Although both the rate and the enhancement are affected by NaCl concentration, no enhancement significantly greater than unity could be observed throughout the range 0 to 350 mM.

We also studied the effect of sucrose, in lieu of $MgCl_2$, on the rate and the enhancement of the [$H_2O \rightarrow$ NADP] reaction. The rate of the reaction is about $\Delta A_{340\ nm} = 0.17 \pm 0.02$, under the experimental conditions described in Fig. 5, at sucrose concentrations up to 125 mM. No enhancement effect ($E_1 \leq 1.0$) can be observed in this sucrose concentration range. At sucrose concentrations higher than 250 mM the rate as well as the enhancement ratio decrease markedly.

Dependence of enhancement on light intensity

The effect of the intensity of red light (630, 650 or 670 nm) added to a fixed intensity of far-red (700 nm) light in producing

enhancement is shown in Fig. 6. With reference to the intensity dependence of the rate of reaction at 650 nm shown in Fig. 1, the enhancement value increases as long as the rate of the reaction is in the relatively linear region of intensity dependence. The enhancement value starts to decrease as the rate of the reaction approaches light saturation at higher intensities. The enhancement effect reaches its maximum, $E_1 = 2.4 \pm 0.3$, when the red light incident at 650 nm is 1.4-1.8 nanoeinsteins cm^{-2} sec^{-1}. When the red light at 670 nm is 1.2-1.4 nanoeinsteins cm^{-2} sec^{-1} the maximum is $E_1 = 1.8 \pm 0.2$. When the red light is at 630 nm the maximum enhancement effect cannot be reached, as shown in Fig. 6. It presumably occurs at higher incident light intensities than those we studied.

Fig. 7 shows the alternative enhancement ratio, E_2, as a function of the incident light intensity, I_R, at 650 and 670 nm. At high intensities both R_{FR+R} and R_R are very large compared with R_{FR}, and the enhancement ratio E_2 is close to 1.0. As the actinic light intensity, I_R, is lowered, the denominator decreases faster than the numerator and the enhancement E_2 increases. We observe limiting values for E_2 of 2.0 and 1.6 at low light intensities when the red actinic light is at 650 nm and 670 nm, respectively.

Action spectrum of enhancement

We find positive enhancement when red light at any wavelength from 620 to 678 nm is coupled with far-red light at 700 nm, but the intensity dependence is different at each wavelength. Fig. 8 is a plot of E_1 values measured under conditions of approximately equal absorbed intensities at several wavelengths from 620 to 690 nm. We find optimal enhancement at 650 and 670 nm, where the values are $E_1 = 2.4 \pm 0.3$ and 1.8 ± 0.25, respectively. A minimum occurs near 660 nm ($E_1 = 1.4 \pm 0.2$).

Enhancement viewed as an effect on the quantum requirement for red light

In the traditional scheme for interpreting the two light requirement of electron transport in chloroplasts as a PS (I + II) reaction, enhancement can be viewed as resulting from a deficiency of photons entering PS I when only a single wavelength of actinic light in the region 620 to 678 nm is used. Throughout this wavelength range we observed zero-intensity quantum requirements for the [$H_2O \rightarrow$ NADP] reaction close to 2.0 photons absorbed per electron transferred[2]. When far-red light, which activates primarily PS I, is added at sufficient intensity that PS I is no longer strongly rate limiting, we expect to observe a corresponding decrease in the quantum requirement for the utilization of red light. The quantum requirement under enhanced conditions can be expressed by the ratio $I_R(\text{absorbed})/(R_{FR+R}-R_{FR})$.

A comparison of the quantum requirements under normal conditions (650 nm light alone) with those under enhanced conditions (650 + 700 nm light), as defined above, is shown in Fig. 9 over a range of incident intensities I_R. The intensity of far-red light used (2.3 nanoeinsteins-cm^{-2}-sec^{-1} incident) did not saturate the [$H_2O \rightarrow$ NADP] reaction by itself (Fig. 1); it was sufficient to achieve only 15% of the saturation rate for the [Ascorbate + $DCIPH_2 \rightarrow$ NADP] reaction, which does not involve the participation of PS II.

At low intensities of red light, a significant decrease in the quantum requirement of the [$H_2O \rightarrow$ NADP] reaction occurs in the enhanced versus the normal condition (Fig. 9). The quantum requirement under enhanced conditions approaches a value of 1.2 ± 0.2 einsteins absorbed-equivalent^{-1} at zero intensity of red (650 nm) light. At higher

intensities of red light, the difference between the two quantum requirements disappears. The explanation for this disappearance is probably the same as that for the behavior of E_2 shown in Fig. 7.

(2) PHOTOSYSTEM II [$H_2O \rightarrow$ DCIP] REACTION AND PHOTOSYSTEM I [ASCORBATE + $DCIPH_2 \rightarrow$ NADP] REACTION

The enhancement values, E_1 or E_2, in the [$H_2O \rightarrow$ DCIP] reaction are 1.01 ± 0.05 from 620 to 690 nm at various incident light intensities with which a background light at 700 nm at 2.3 nanoeinsteins-cm^{-2}-sec^{-1} was coupled. Thus, no appreciable enhancement effect nor any difference between the normal and differential quantum requirements is observed for the photoreduction of DCIP, in confirmation of previous studies in our laboratory[25].

For the [Ascorbate + $DCIPH_2 \rightarrow$ NADP] reaction run in the presence of DCMU the observed enhancement ratio is always less than unity. The values are a strong function of the intensity of the red light, as seen for 650 (squares) and 670 nm (circles) in Fig. 10. This behavior can be shown to be a result of the approach toward saturation of the rate of the reaction with increasing intensities of red light alone. Assuming that 650 nm photons, which are partitioned about equally between the two photosystems under these reaction conditions, are only half as effective as 700 nm photons and utilizing the linear dependence of the quantum requirement of the reaction as a function of intensity of red light[2], it is possible to calculate E_1 ratios which take into account the approach to saturation for the two wavelengths together. The agreement between the experimental points and the calculated curves (Fig. 10) is good evidence that, within experimental uncertainties, there is no two-wavelength enhancement for this reaction.

DISCUSSION

The occurrence of the Emerson red - far red enhancement effect in isolated chloroplasts has been the subject of repeated studies[11]. For the [$H_2O \rightarrow NADP$] reaction, which is the principal focus of the present study, definite enhancement has been reported by Govindjee et al.[17,18], by Joliot et al.[20] and by Avron and Ben Hayyim[6]. On the other hand, Gibbs et al.[15] and McSwain and Arnon[21] reported no measurable enhancement for the same reaction. Our studies suggest that the concentration of divalent cations (e.g., Mg^{2+}) in the reaction mixture is the principal controlling factor in determining whether enhancement can be observed.

The results summarized in Table V show that enhancement values can be affected by (1) alterations in the chloroplast preparation procedure, (2) the addition of plastocyanin and ferredoxin-$NADP^+$ reductase, (3) the pH of the reaction mixture, and (4) the addition of ADP and inorganic phosphate. Nevertheless, each of these factors can be overcome and enhancement can always be restored in the presence of 7.5 mM $MgCl_2$. By contrast, we have been unable to find any set of reaction conditions which will give enhancement when the divalent cation concentration is below about 3 mM. In retrospect, the lack of agreement in the literature reports on chloroplast enhancement can be understood largely on this basis. Govindjee et al.[17,18] and Avron and Ben Hayyim[6] were able to observe enhancement using Mg^{2+} concentrations of 7.5 and 27 mM, respectively. No enhancement was observed by Gibbs et al.[15] or by McSwain and Arnon[21] using Mg^{2+} concentrations of 2 and 1.7 mM, respectively. The results of Joliot et al.[20], who did observe enhancement in the presence of only 1 mM Mg^{2+}, are the only ones that do not correlate in this way. It may be that

there is some synergistic effect involving the high concentration of other salts (0.05 M phosphate buffer + 0.1 M KCl) that distinguishes the reaction conditions of Joliot et al.[20] from those of Gibbs et al.[15] and McSwain and Arnon[21]. This possibility remains to be investigated.

As shown in Figs. 3 and 4, added divalent cations produce an optimum not only in the enhancement effect, but also in the velocity of the [$H_2O \rightarrow$ NADP] reaction in red light alone. It might be argued that the absence of divalent cations serves only to slow down the rate-limiting step that results in light intensity saturation. In this view, the absence of enhancement at zero added divalent ion would be the fortuitous result of a compensatory decrease in E_1 because of the closer approach to light saturation in the absence of added divalent cations. Fig. 6 shows examples of the decrease of E_1 as saturating light intensities are approached. The results of McSwain and Arnon[21] argue against this interpretation of the divalent cation effect, however. At low (1.7 mM) concentrations of added $MgCl_2$ they found no enhancement to occur over a wide range of incident light intensities, such that the overall rate, $R_{650+700}$, varied by as much as 9-fold. Furthermore, added NaCl is able to increase the rate of the [$H_2O \rightarrow$ NADP] reaction (Fig. 5) even somewhat more effectively than added $MgCl_2$ or $MnCl_2$, but no enhancement values significantly greater than 1.0 are observed using NaCl. It is apparent from these results and those using added sucrose that the occurrence of enhancement depends on something more specific than the ionic or osmotic strength of the medium.

A special role for divalent cations has been proposed by Murata[28]. He observed that relatively low concentrations (2-3 mM) of Mg^{2+}, Ca^{2+} or Mn^{2+} served markedly to increase the yield of chlorophyll fluorescence from chloroplasts at room temperature. Added Mg^{2+} (3 mM) also

served to decrease the quantum yield (extrapolated to zero incident intensity) of the [Ascorbate + $DCIPH_2$ → NADP] reaction activated at 480 nm, and to increase the quantum yield of the [H_2O → DCIP] reaction --slightly at 480 nm, but markedly when activated at 695 nm. It should be noted, however, that the highest quantum yields reported by Murata are less than half those reported for the same reactions in Part I of this series[2]. Murata concluded on the basis of his findings that the role of Mg^{2+} and other divalent ions is to suppress the spillover of excitation energy from pigment system II to pigment system I.

The alternative view, namely that added Mg^{2+} enables excitation transfer between the two pigment systems, is supported by the observations of Avron and Ben-Hayyim[6] and of Rurainski et al.[29,30] that added $MgCl_2$ serves to increase significantly the quantum yield of the [H_2O → NADP] reaction extrapolated to zero light intensity. Under the assumption that excitation transfer (spillover) between the two pigment systems tends to equalize the rates of the two photoreactions, the increased quantum yields for the PS (I + II) reaction can be explained most readily if spillover occurs in the presence of $MgCl_2$ rather than in its absence. Murata's own experimental findings can be rationalized satisfactorily using this alternative view of the role of Mg^{2+}. Spillover from P I to P II in the presence of divalent cations can account for (1) increased fluorescence yield, (2) increased quantum yield for the [H_2O → DCIP] reaction, and (3) decreased quantum yield for the [Ascorbate + $DCIPH_2$ → NADP] reaction. Spillover from P I to P II will be efficient only for excitation resulting from red light, where there is no energy barrier to reaching the PS II trap. Spillover in this direction might

seem to be an unlikely process in competition with trapping within PS I. Nevertheless, such spillover must occur in order to account for the observed quantum yields of 1.0 for the [H_2O → Ferricyanide][6] and [H_2O DCIP][2] reactions using red actinic wavelengths.

Shavit and Avron have reported divalent cation-dependent shrinking and light scattering changes by illuminated broken chloroplasts[31]. Similar results have been observed by Murakami and Packer[32]. It is reasonable to suppose that these conformational changes induced by divalent cations are the basis for the effects on excitation transfer.

Models which account for the enhancement effect in the [H_2O → NADP] reaction and for the dependence on divalent cations can be constructed using either Murata's interpretation (Model A) or its converse (Model B). The models differ in the role assigned to the divalent cation and in the restrictions placed on the relative intrinsic absorptions (i.e., in the absence of spillover) of P I and P II in the red region from 620 to 680 nm. Table VI gives a listing of the postulates of the two models.

In Model A enhancement occurs in the absence of spillover because P II absorbs more than half the photons in the red region of the spectrum, and P I absorbs preferentially in the far red. This is the traditional view basic to the detailed mathematical analyses of Bannister and Vrooman[33], Malkin[34], Williams[35], and Delrieu and de Kouchkovsky[26]. In the presence of spillover red light is equilibrated between the two reaction centers, and there is no deficiency to be remedied by supplementary far red light.

In model B no enhancement occurs in the absence of spillover because the intrinsic absorptions of the two pigment systems are postulated to be identical in the red region of the spectrum. There is no imbalance to be rectified by far red light. Addition of divalent cations,

which enables spillover in this model, provides conditions favoring enhancement. The distribution of redphoton excitation between the two reaction centers, which is equal for red light alone, is altered in the presence of far red light via the spillover of some of the red excitation from P I to P II. This is in keeping with Postulate 4 of Table VI and results in the observed enhancement. That spillover is an efficient process in the enhancement studies (regardless of the model considered) is demonstrated by the results shown in Fig. 9, where red photons approach unit efficiency at low intensities and in the presence of supplementary far red light.

Neither of the two models described above is entirely satisfactory, and whichever proves to be closest to the truth will require further modifications as more is learned about the related phenomena. We have already mentioned the apparent inconsistencies between Murata's interpretation of the role of divalent cations[28] (incorporated into Model A) and the effect of $MgCl_2$ on the quantum yield of the [$H_2O \rightarrow$ NADP] reaction[6,29,30]. In addition, it is not clear why enhancement should not also be observed in the absence of divalent cations under the postulates of Model A. Spillover should permit a redistribution of red photons in the presence of far red light, according to Postulate 4 of Table VI, in which case some enhancement would be expected. None is observed. Neither the quantum yield of unity in red light for the [$H_2O \rightarrow$ DCIP] reaction in the presence of 4.5 mM $MgCl_2$ (ref. ?) nor the decrease in the quantum yield in the red in going from 0 to 27 mM $MgCl_2$ for the [Ascorbate + $DCIPH_2 \rightarrow$ NADP] reaction[6] can be reconciled with Model A. The alternative view of Model B also has its drawbacks. The assignment of equal

intrinsic absorbances in the red to P I and P II appears to be quite arbitrary and difficult to reconcile with the different absorption spectra of physically separated PS I and PS II fractions[36,37]. Both the pronounced dependence of enhancement on the wavelength of red light (Fig. 8) and the increase in quantum yield of the [$H_2O \rightarrow$ NADP] reaction upon addition of $MgCl_2$ (refs. 6, 29, 30) are difficult to reconcile with postulate (2) of Model B. Neither model can account for the observation of Avron and Ben-Hayyim[6] that the transfer of electrons from ascorbate to diquat or FMN occurs with a quantum yield of 1.0 in either red or far red light and is unaffected by added $MgCl_2$.

It seems clear that further experimental results are required before all of these difficulties can be resolved. Recent reports that PS I activity may occur in two kinetically distinct locations in broken chloroplasts[38] need to be considered in future models of enhancement and excitation transfer.

Our findings are more conclusive with respect to the parallel two photosystem hypothesis of Arnon et al.[39] and as modified by Knaff and Arnon[5]. The parallel hypothesis, by contrast with the traditional Z scheme where the two light reactions operate in series, cannot be reconciled with the observation of red - far red enhancement using isolated broken chloroplasts for the [$H_2O \rightarrow$ NADP] reaction. The failure of McSwain and Arnon to observe enhancement for this system[21], an observation which was an essential part of the justification of the parallel mechanism, is now seen to be probably the consequence of the low $MgCl_2$ concentration used in their experiments. Under their conditions, we do not observe enhancement either. Our system contained broken chloroplasts, had no

added carbon source, and did not require the components of phosphorylation in order for enhancement to be observed. Thus, it cannot be argued that enhancement occurs only in relief of an unbalance of cyclic and non-cyclic electron flow with respect to the requirements of the carbon reduction pathway for ATP and reductant. The simplest explanation of the results presented in this paper is that non-cyclic electron transport from H_2O to $NADP^+$ proceeds via two different light reactions characterized as PS I and PS II operating in series.

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TABLE I

ENHANCEMENT CALCULATED AS E_1, E_2 or E_3 FOR THE [H_2O -- NADP] REACTION BY CHLOROPLASTS

Spinach chloroplasts (CP-suc) in tricine, 45 mM, pH 7.5; $MgCl_2$, 7.5 mM; plastocyanin, ferredoxin and ferredoxin-$NADP^+$ reductase, added in saturating amounts. Experimental conditions as in Fig. 2. Illumination pattern: [R_R; R_{FR}, R_{FR+R}]. Incident intensities: 1.4 and 2.3 nanoeinsteins$\cdot cm^{-2} \cdot sec^{-1}$ at 650 and 700 nm, respectively. Definitions of E_1, E_2 and E_3 given in the text.

Sample	Rate of $NADP^+$ Reduction $\Delta A \cdot min^{-1}$ at 340 nm			E_1	E_2	E_3
	R_{650}	R_{700}	$R_{700+650}$			
1	0.117	0.0306	0.191	2.43	1.38	1.30
2	0.107	0.0305	0.182	2.46	1.42	1.32
3	0.099	0.0240	0.162	2.66	1.41	1.33

TABLE II

ENHANCEMENT OBTAINED USING DIFFERENT PATTERNS OF ILLUMINATION FOR THE [$H_2O \rightarrow$ NADP] REACTION BY CHLOROPLASTS

Reaction conditions as in Table I, but with different illumination patterns, as indicated. R, rate of $NADP^+$ reduction, $\Delta A\text{-}min^{-1}$ at 340 nm.

Sample	Illumination Pattern							
	(A) $[R_R; R_{FR}, R_{FR+R}]$				(B) $[R_{FR}, R_{FR+R}; R_R]$			
	R_{650}	R_{700}	$R_{700+650}$	E_1	R_{700}	$R_{700+650}$	R_{650}	E_1
1	0.110	0.0288	0.186	2.64	0.0288	0.186	0.105	2.84
2	0.105	0.0276	0.179	2.71	0.0284	0.189	0.109	2.82
3	0.100	0.0282	0.172	2.58	0.0284	0.182	0.105	2.74

Sample	Illumination Pattern							
	(C) $[R_{FR}; R_R, R_{R+FR}]$				(D) $[R_R, R_{R+FR}; R_{FR}]$			
	R_{700}	R_{650}	$R_{650+700}$	E_1	R_{650}	$R_{650+700}$	R_{700}	E_1
1	0.0284	0.092	0.181	3.13	0.092	0.181	0.0294	3.02
2	0.0278	0.090	0.181	3.27	0.101	0.181	0.0284	2.83
3	0.0279	0.095	0.186	3.28	0.109	0.187	0.0280	2.81

TABLE III

ENHANCEMENT E_1 WITH TWO ACTINIC LIGHTS AT DIFFERENT OR AT THE SAME WAVELENGTH FOR THE [$H_2O \rightarrow$ NADP] REACTION BY CHLOROPLASTS

Reaction conditions as in Table I. Wavelengths of illumination are indicated in the table. Three chloroplast samples were measured and twelve measurements on each were made successively in the order of presentation. R, rate of $NADP^+$ reduction, $\Delta A\text{-}min^{-1}$ at 340 nm.

Sample	R_{650}	R_{700}	$R_{700+650}$	E_1	R_{650}	R'_{650}	$R_{650+650}$	E_1
1	0.095	0.0307	0.174	2.59	0.099	0.0321	0.130	0.90
2	-	-	-	-	0.099	0.0314	0.128	0.91
3	0.095	0.0288	0.168	2.53	0.091	0.0302	0.117	0.86
1	0.092	0.0287	0.165	2.54	0.090	0.0311	0.118	0.88
2	0.093	0.0286	0.162	2.42	0.090	0.0301	0.115	0.84
3	0.089	0.0274	0.157	2.46	0.090	0.0296	0.113	0.78

TABLE IV

REPEATABILITY OF THE ENHANCEMENT EFFECT FOR THE [$H_2O \rightarrow NADP$] REACTION BY CHLOROPLASTS

Reaction conditions as in Table I. The order of the 18 measurements on a single chloroplast sample was top to bottom in each column, then left to right. R, rate of reduction of $NADP^+$, $\Delta A \cdot min^{-1}$ at 340 nm. Illumination patterns as described in the text.

Illumination Pattern		Measurement Index Number					
		1-3	4-6	7-9	10-12	13-15	16-18
	R_{650}	0.117	0.111	0.107	0.095	0.088	0.086
	R_{700}	0.0305	0.0305	0.0237	0.0253	0.0230	0.0247
	$R_{700+650}$	0.191	0.18[illegible]	0.159	0.154	0.137	0.128
(A)	E_1	2.43	2.33	2.22	2.33	2.13	1.70
(B)	E_1	-	2.63	2.45	2.73	2.60	2.20

TABLE V

ENHANCEMENT FOR THE [$H_2O \rightarrow$ NADP] REACTION BY CHLOROPLASTS IN DIFFERENT REACTION MIXTURES

Chloroplasts in Solution A [Tricine buffer, 45 mM, pH 7.5; $MgCl_2$, 7.5 mM; NADPT, 0.67 mM] or Solution B [Tricine buffer, 33 mM, pH 8.2; $MgCl_2$, 1.7 mM; ADP + Pi, 3.3 mM; $NACP^+$, 3.3 mM], and for both solutions, ferredoxin, saturating; plastocyanin (PCy), saturating or not added; ferredoxin-$NADP^+$ reductase (Fp), saturating or not added. Enhancement values determined for reactions in "normal" solutions A and B are given in columns (1) and (5), respectively. Alterations of the "normal" solutions, as indicated in columns (2), (3), (4) and (6), (7), (8) were done singly, not compounded. Enhancement values E_1 are tabulated; illumination pattern (A).

Chloroplast Preparation	PCy + Fp	Solution A				Solution B			
		Normal (1)	pH 8.2 (2)	+(ADP+Pi) (3)	$MgCl_2$ 1.67 mM (4)	Normal (5)	pH 7.5 (6)	-(ADP+Pi) (7)	$MgCl_2$ 7.5 mM (8)
CP-suc		2.32	1.62	1.80	0.92	-0.24	0.63	0.34	2.09
	+	2.14	1.39	1.44	0.84	0.25	0.26	0.27	2.23
		2.26	1.59	1.62	0.74	0.36	0.32	0.36	2.14
		1.49	-0.43	1.37	0.34	0.37	0.24	0.42	1.63
	-	1.54	-0.20	1.20	0.36	-0.21	0.63	0.53	1.37
		1.36	0.14	1.14	0.68	0.32	-0.16	0.96	1.41
CP-NaCl		2.43	1.84	1.80	0.98	0.98	0.86	1.08	2.03
	+	2.13	1.75	1.26	0.84	0.74	0.68	0.84	1.98
		2.36	1.78	1.54	1.02	0.63	0.62	0.86	1.86
		2.12	1.57	1.68	0.93	0.55	0.74	1.10	2.26
	-	2.59	1.72	1.52	1.06	0.64	0.62	0.84	1.88
		2.34	1.81	1.57	0.86	0.76	0.64	0.53	1.94

TABLE VI

ALTERNATIVE MODELS FOR THE ROLE OF DIVALENT CATIONS IN ENABLING RED - FAR RED ENHANCEMENT FOR THE [$H_2O \rightarrow$ NADP] REACTION BY BROKEN CHLOROPLASTS

Model A	Model B
1. Electron transfer between P II and P I occurs in the absence of divalent cations; not in their presence.	1. Electron transfer between P I and P II occurs in the presence of divalent cations; not in their absence.
2. Intrinsic absorption of P II is greater than that of P I in the region 620 to 680 nm.	2. Intrinsic absorption of P II is equal to that of P I in the region from 620 to 680 nm.

Both models:

3. Intrinsic absorption of P I is greater than that of P II at wavelengths longer than 690 nm.
4. Excitation transfer, when it is allowed, will occur predominantly in the direction which will enhance the activation of the reaction center that would otherwise be rate limiting.

FIGURE CAPTIONS

Fig. 1. Quantum requirements at 650 nm (o) and the rates of photoreduction of $NADP^+$ at 650 nm (□) or 700 nm (△) in the [$H_2O \rightarrow NADP$] reaction by broken chloroplasts (CP-suc) as functions of the actinic light intensity. The reaction mixture (Solution A) is given in the text. The rate of $NADP^+$ reduction was measured as the change in absorbance at 340 nm per unit time. Chlorophyll concentration 27 μg/ml.

Fig. 2. Typical time course for the photoreduction of $NADP^+$ by broken chloroplasts (CP-suc) in the [$H_2O \rightarrow NADP$] reaction using two actinic wavelengths. The reaction mixture (Solution A) is described in the text. The illumination pattern [R_R; R_{FR}, R_{FR+R}] is illustrated in this experiment; incident intensity of far-red light at 700 nm, 2.3 nanoeinsteins-cm^{-2}-sec^{-1}; incident intensity of red light at 650 nm, 1.4 nanoeinsteins-cm^{-2}-sec^{-1}. Chlorophyll concentration 27 μg/ml.

Fig. 3. Effect of the $MgCl_2$ concentration on the rate (A, upper curves) and on the enhancement E_1 (B, lower curve) of the [$H_2O \rightarrow NADP$] reaction by broken chloroplasts (CP-suc). Experimental conditions as described in Fig. 2, except $MgCl_2$ concentration varied; incident intensity of 678 nm light, 2.4 nanoeinsteins-cm^{-2}-sec^{-1}. Vertical bars show standard deviations of replicate measurements.

Fig. 4. Effect of the $MnCl_2$ concentration on the rate (A, upper curve) and on the enhancement E_1 (B, lower curve) of the [$H_2O \rightarrow NADP$] reaction by broken chloroplasts. Experimental conditions as in Fig. 3, except $MnCl_2$ in place of $MgCl_2$.

Fig. 5. Effect of the NaCl concentration on the rate (A, upper curve) and on the enhancement E_1 (B, lower curve) of the [$H_2O \rightarrow$ NADP] reaction by broken chloroplasts. Experimental conditions as in Fig. 3, except NaCl in place of $MgCl_2$.

Fig. 6. Dependence of the enhancement E_1 on actinic light intensity of red light for the [$H_2O \rightarrow$ NADP] reaction by broken chloroplasts. $E_1 = (R_{700+R}-R_R)/R_{700}$. Experimental conditions as in Fig. 2, except the incident red light intensities I_R are varied from zero to 3.2 nanoeinsteins$\cdot cm^{-2}\cdot sec^{-1}$. The three curves are for red light at 630, 650 and 670 nm, respectively, as indicated.

Fig. 7. Dependence of the enhancement E_2 on actinic light intensity of red light for the [$H_2O \rightarrow$ NADP] reaction by broken chloroplasts. $E_2 = (R_{700+R}-R_{700})/R_R$. Experimental conditions as in Fig. 6.

Fig. 8. Activation spectrum of the enhancement E_1 for the [$H_2O \rightarrow$ NADP] reaction by broken chloroplasts. $E_1 = (R_{700+R}-R_R)/R_{700}$. Measurements made at approximately equal absorbed intensities (1.15 ± 0.15) nanoeinsteins$\cdot cm^{-3}\cdot sec^{-1}$ of red light at wavelengths from 620 to 690 nm. Absorbed intensity 0.37 nanoeinsteins $cm^{-3}\cdot sec^{-1}$ at 700 nm for all measurements. Other reaction conditions as in Fig. 2.

Fig. 9. Quantum requirements under normal (o) and under enhanced (Δ) conditions for the [$H_2O \rightarrow$ NADP] reaction by broken chloroplasts. Reaction conditions as in Fig. 6. Definitions: $1/\Phi_{650}$ (normal) = I_{650}(absorbed)$/R_{650}$; and $1/\Phi_{650}$ (enhanced) = I_{650}(absorbed)$/(R_{700+650}-R_{700})$.

Fig. 10. The enhancement ratio E_1 as a function of intensity of red light absorbed for the [Ascorbate + $DCIPH_2$ → NADP] reaction. Measured values using red light at 650 nm (□) or 670 nm (o) at various intensities supplemented by far-red light at 700 nm and constant absorbed intensity (0.43 nanoeinsteins-cm^{-3}-sec^{-1}). The curves are calculated assuming no actual enhancement, but taking account of the approach to saturation of the reaction by red light alone. Chlorophyll concentration 13 μg/ml.

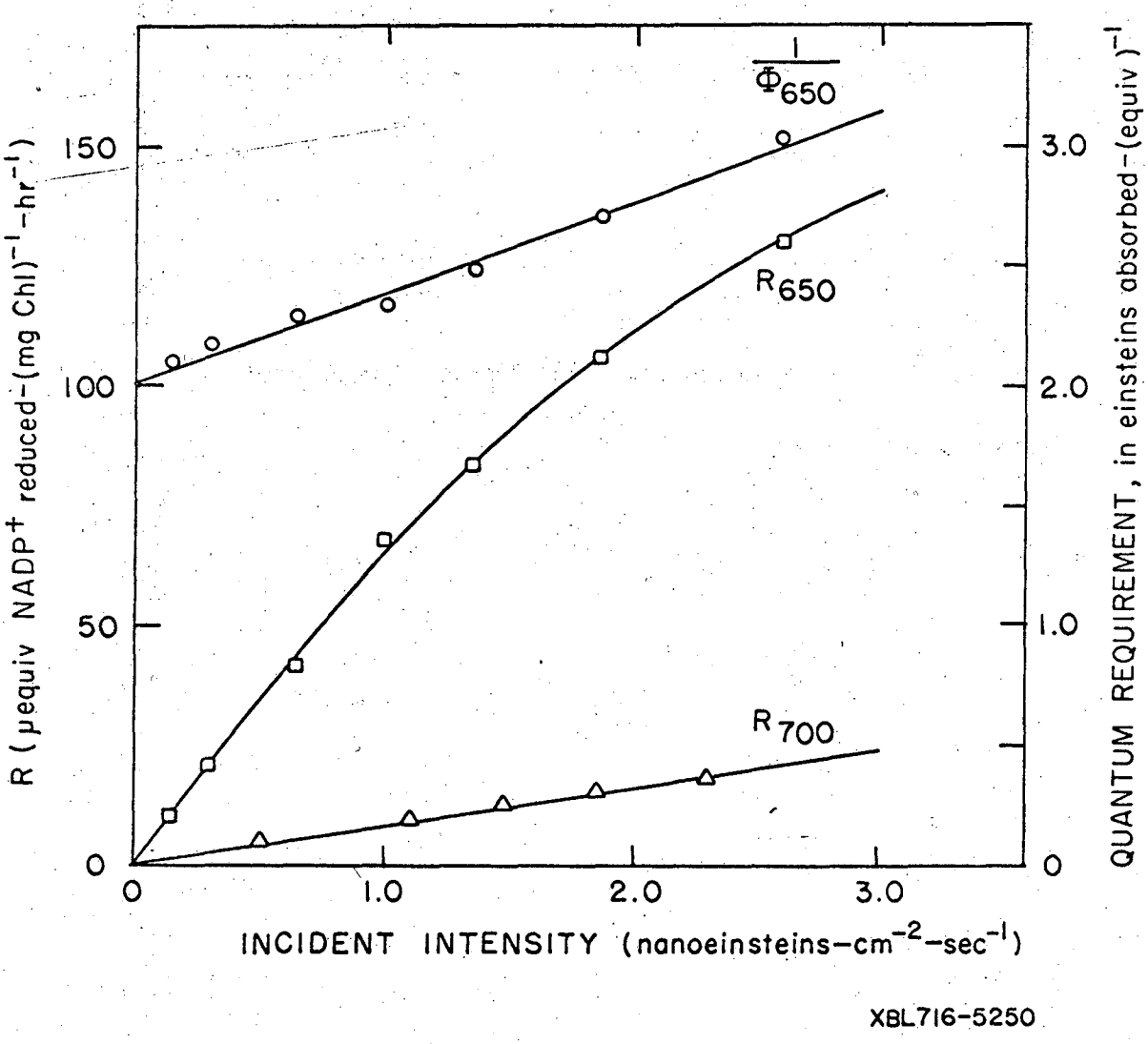
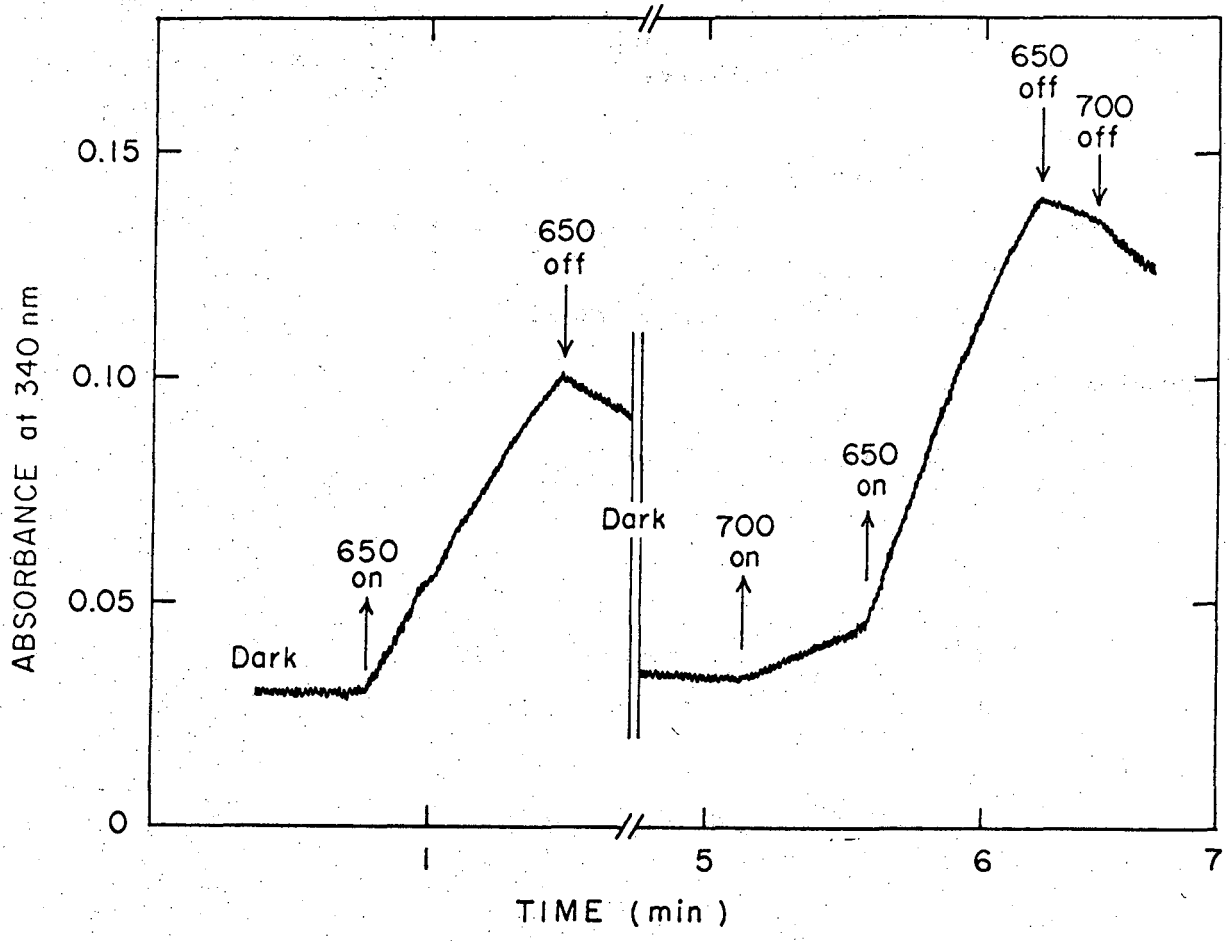

$\frac{1}{\Phi_{650}}$
R_{650}
R_{700}
150
100
50
0
3.0
2.0
1.0
0
0
1.0
2.0
3.0
R (μequiv NADP+ reduced-(mg Chl)$^{-1}$-hr^{-1})
QUANTUM REQUIREMENT, in einsteins absorbed-(equiv)$^{-1}$
INCIDENT INTENSITY (nanoeinsteins-cm^{-2}-sec^{-1})
XBL716-5250

Fig. 1



XBL716-5251

Fig. 2

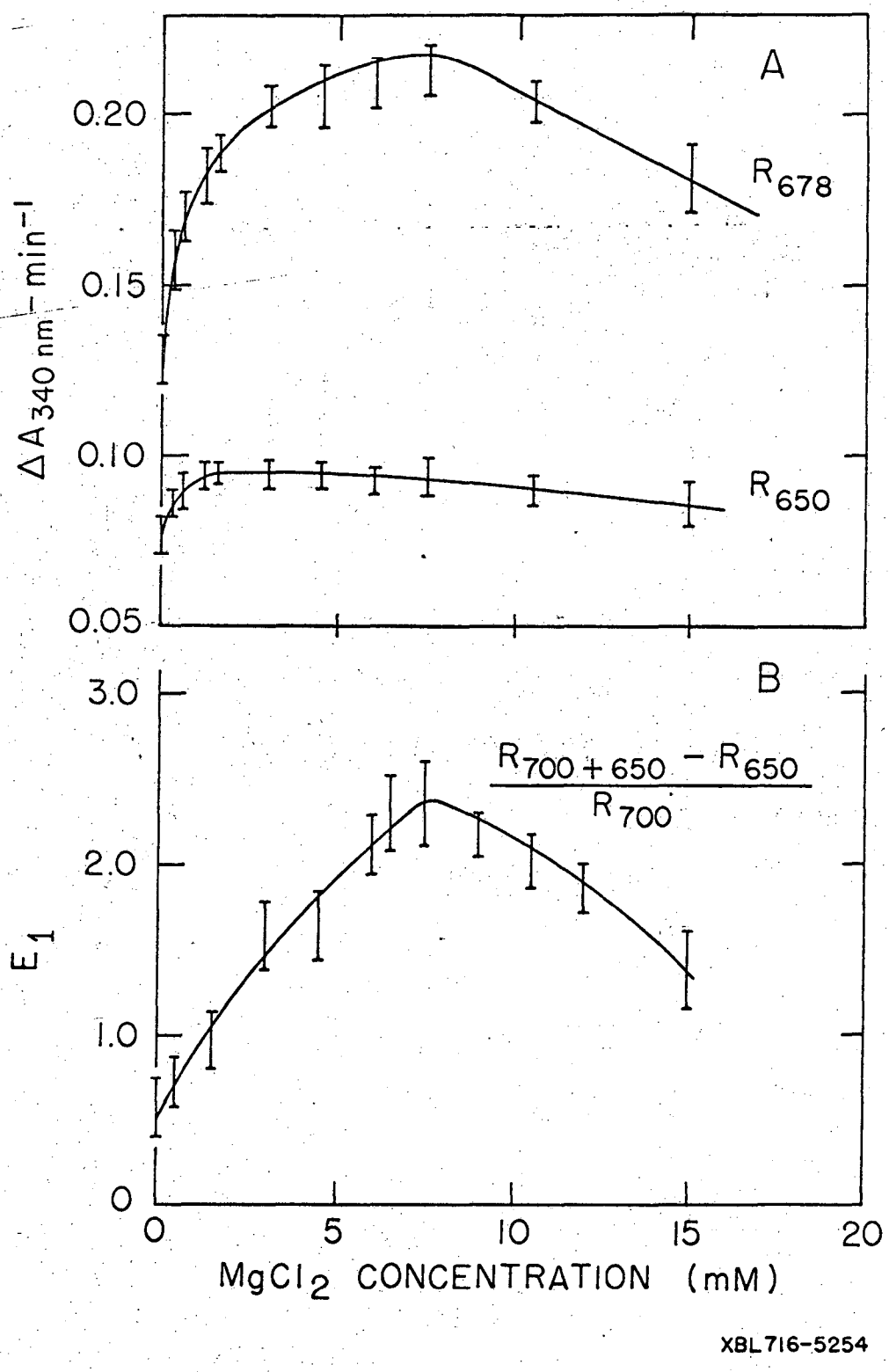

A
0.20
0.15
0.10
0.05
$\Delta A_{340\,nm}$ - min^{-1}
R_{678}
R_{650}
B
3.0
2.0
1.0
0
E_1
$\frac{R_{700+650} - R_{650}}{R_{700}}$
0
5
10
15
20
$MgCl_2$ CONCENTRATION (mM)
XBL716-5254

Fig. 3

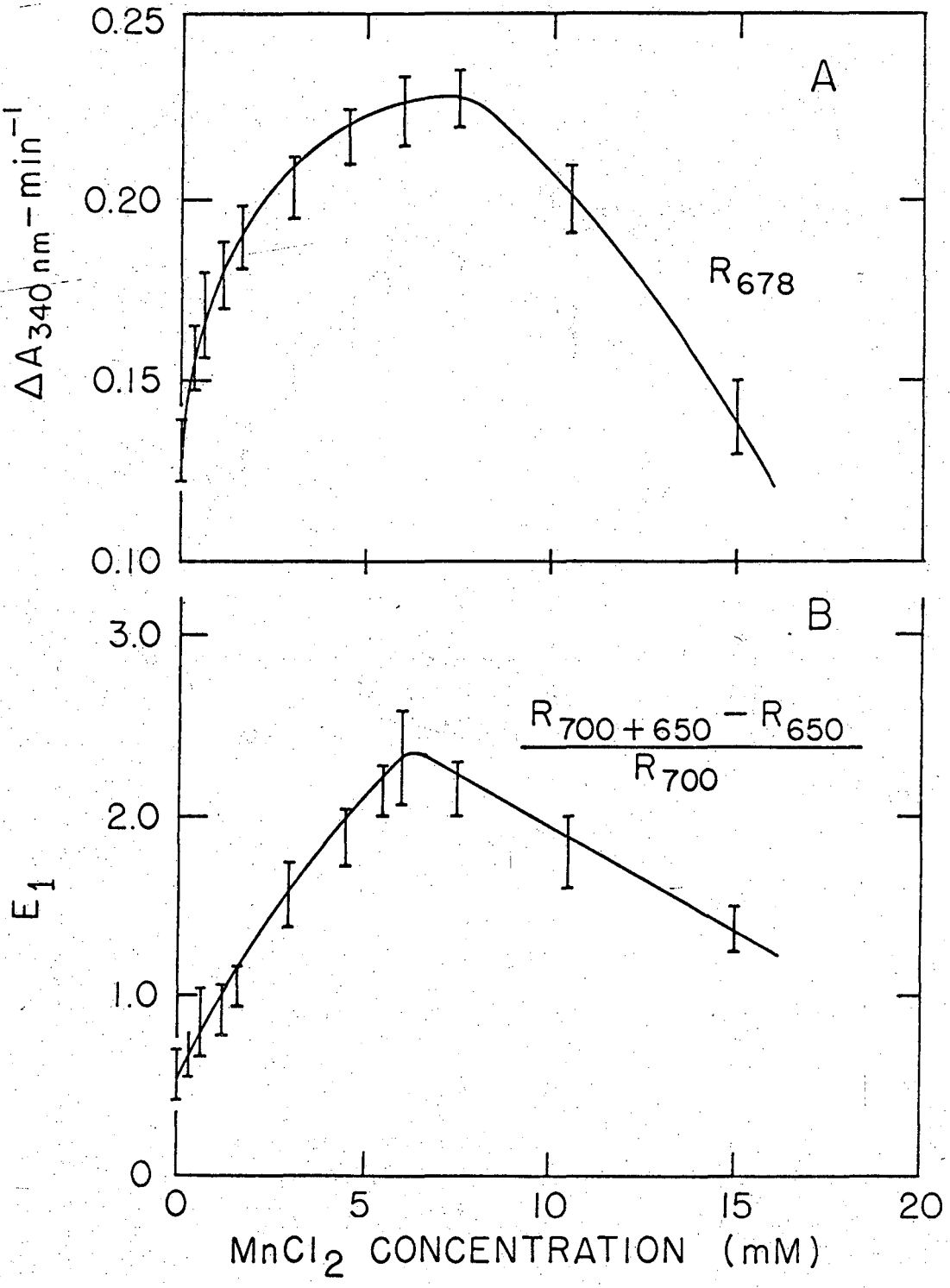

0.25
0.20
0.15
0.10
A
R_{678}
$\Delta A_{340\,nm} - min^{-1}$
3.0
2.0
1.0
0
B
$\frac{R_{700+650} - R_{650}}{R_{700}}$
E_1
0
5
10
15
20
$MnCl_2$ CONCENTRATION (mM)
XBL716-5255

Fig. 4

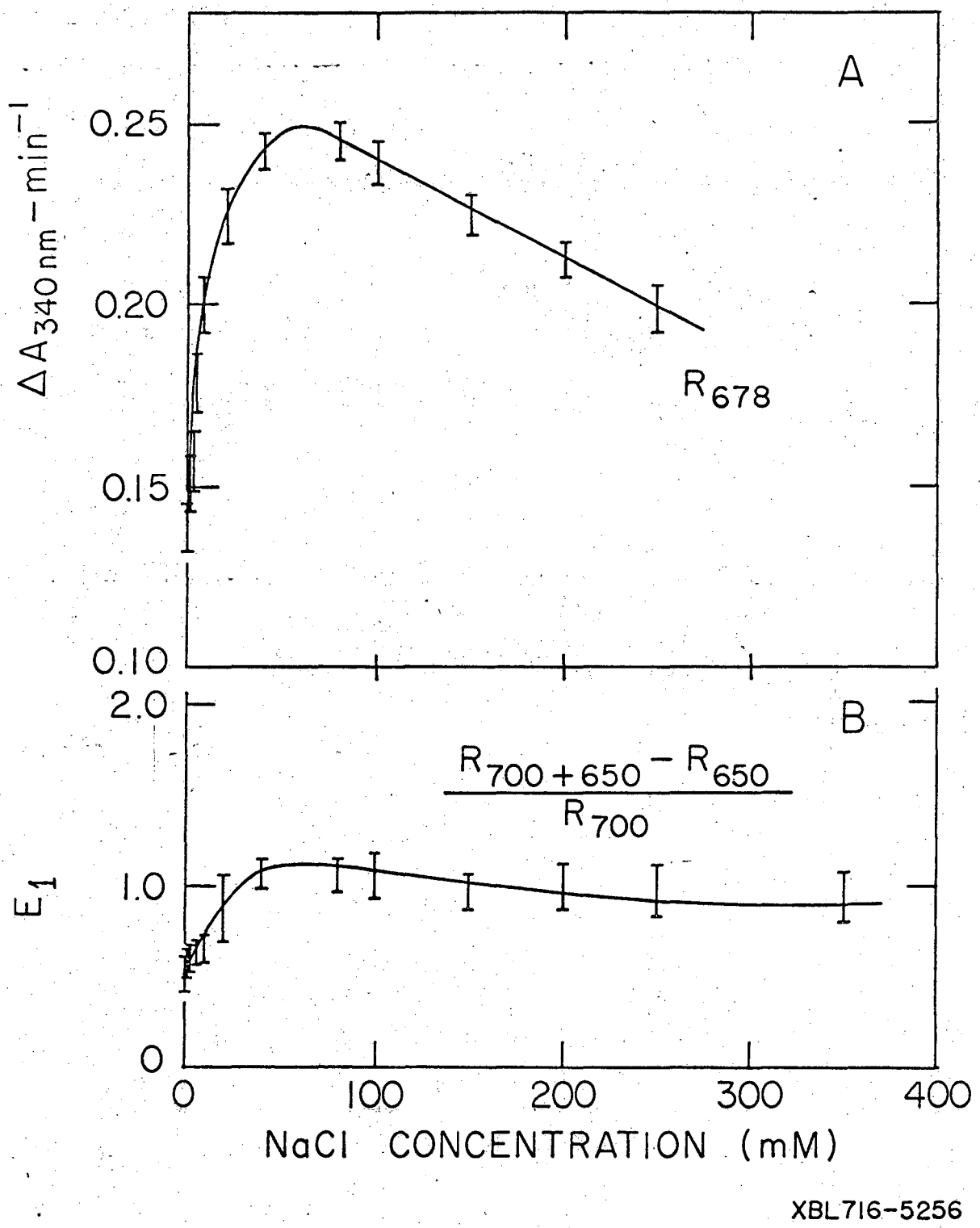
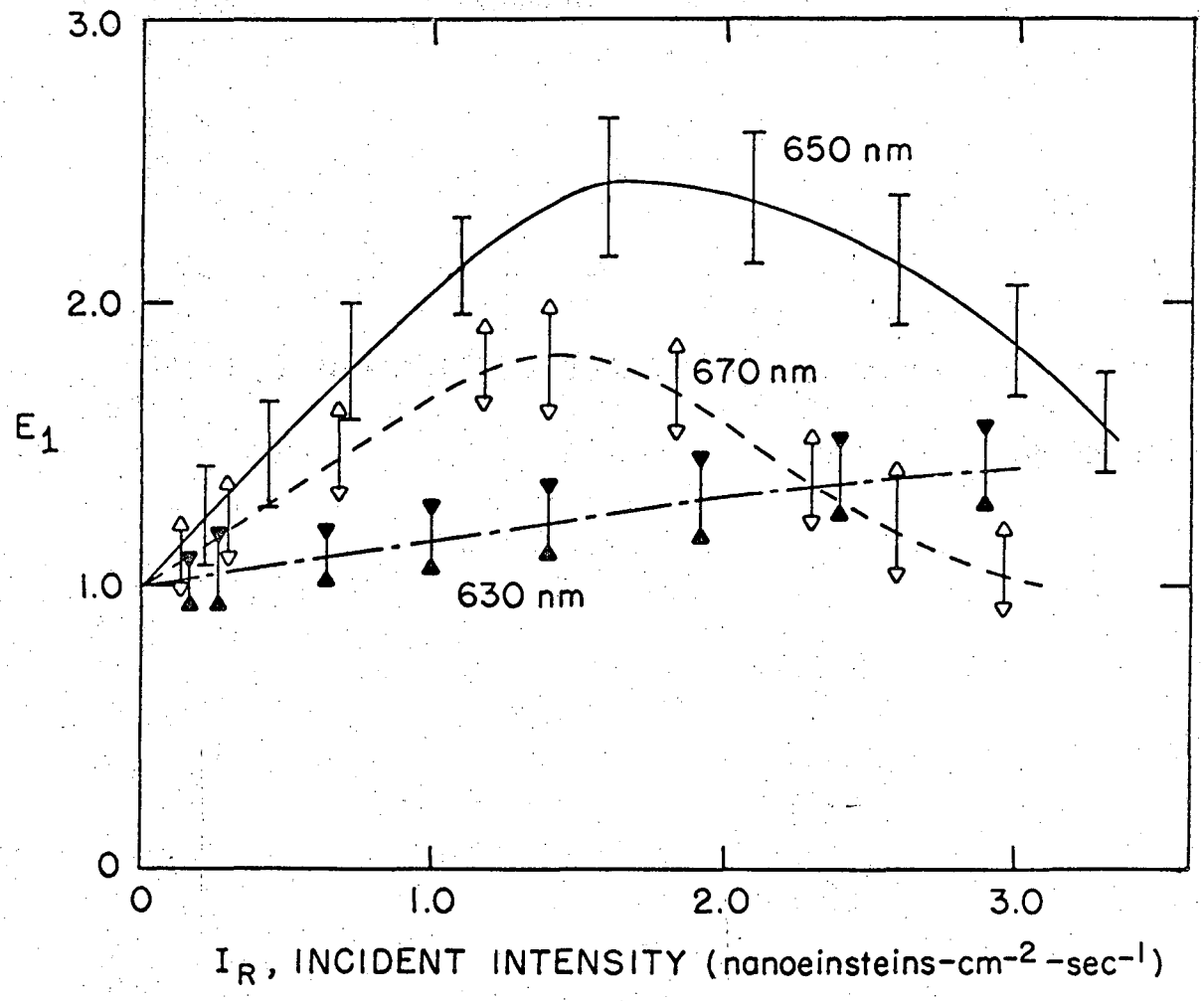
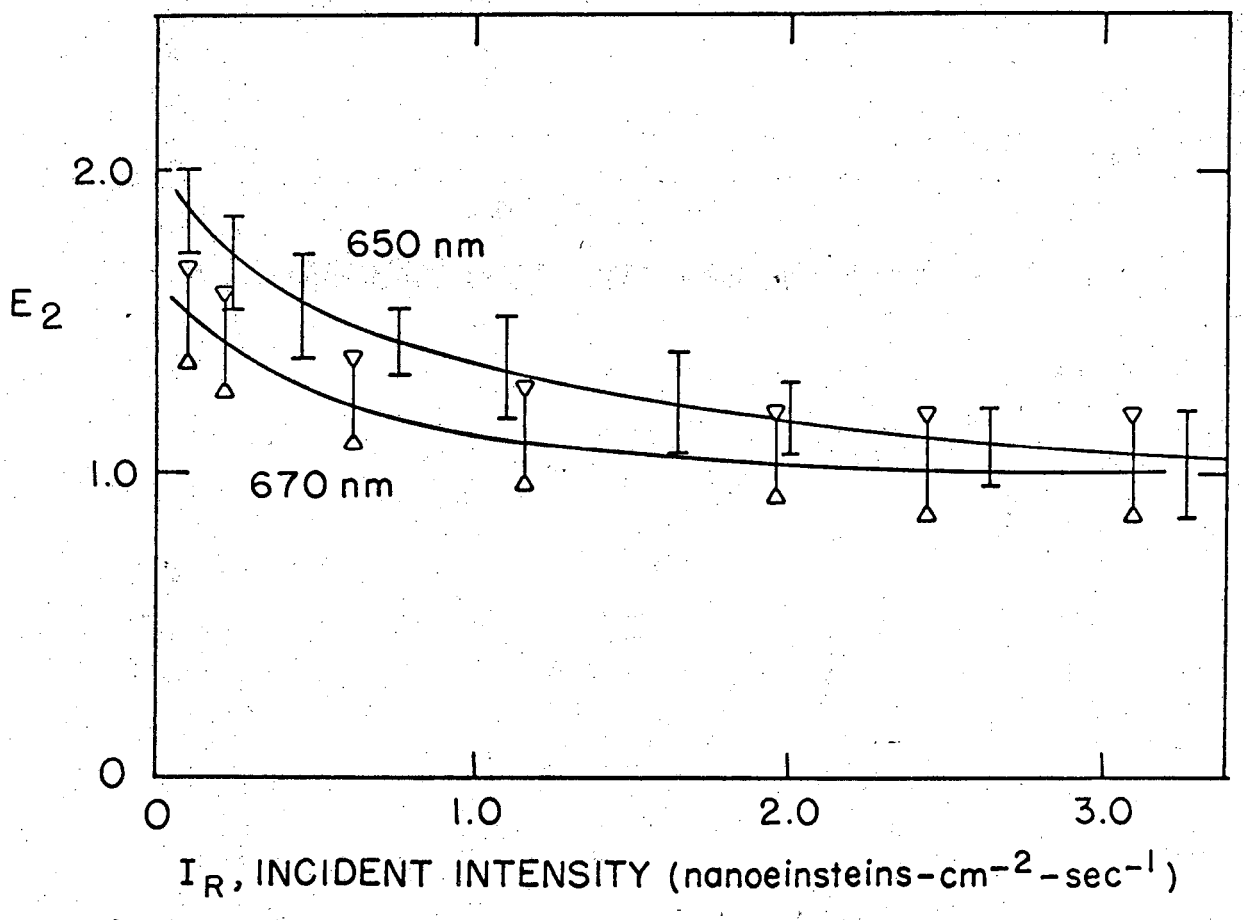

A
B
$\Delta A_{340\,nm} - min^{-1}$
0.25
0.20
0.15
0.10
R_{678}
2.0
1.0
0
E_1
$\frac{R_{700+650} - R_{650}}{R_{700}}$
0
100
200
300
400
NaCl CONCENTRATION (mM)
XBL716-5256

Fig. 5



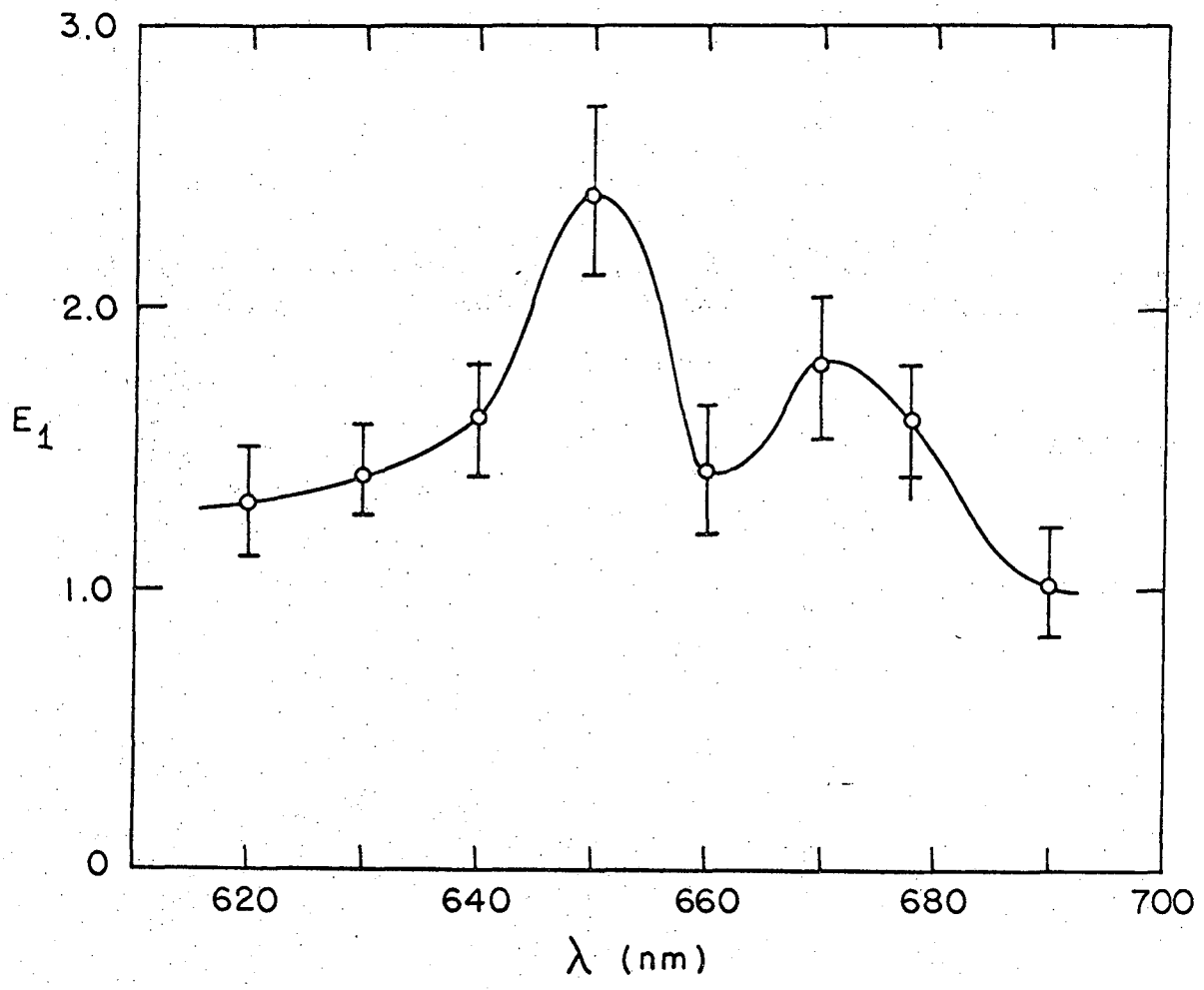
XBL 716-5247

Fig. 6



XBL716-5248

Fig. 7



XBL716-5249

Fig. 8

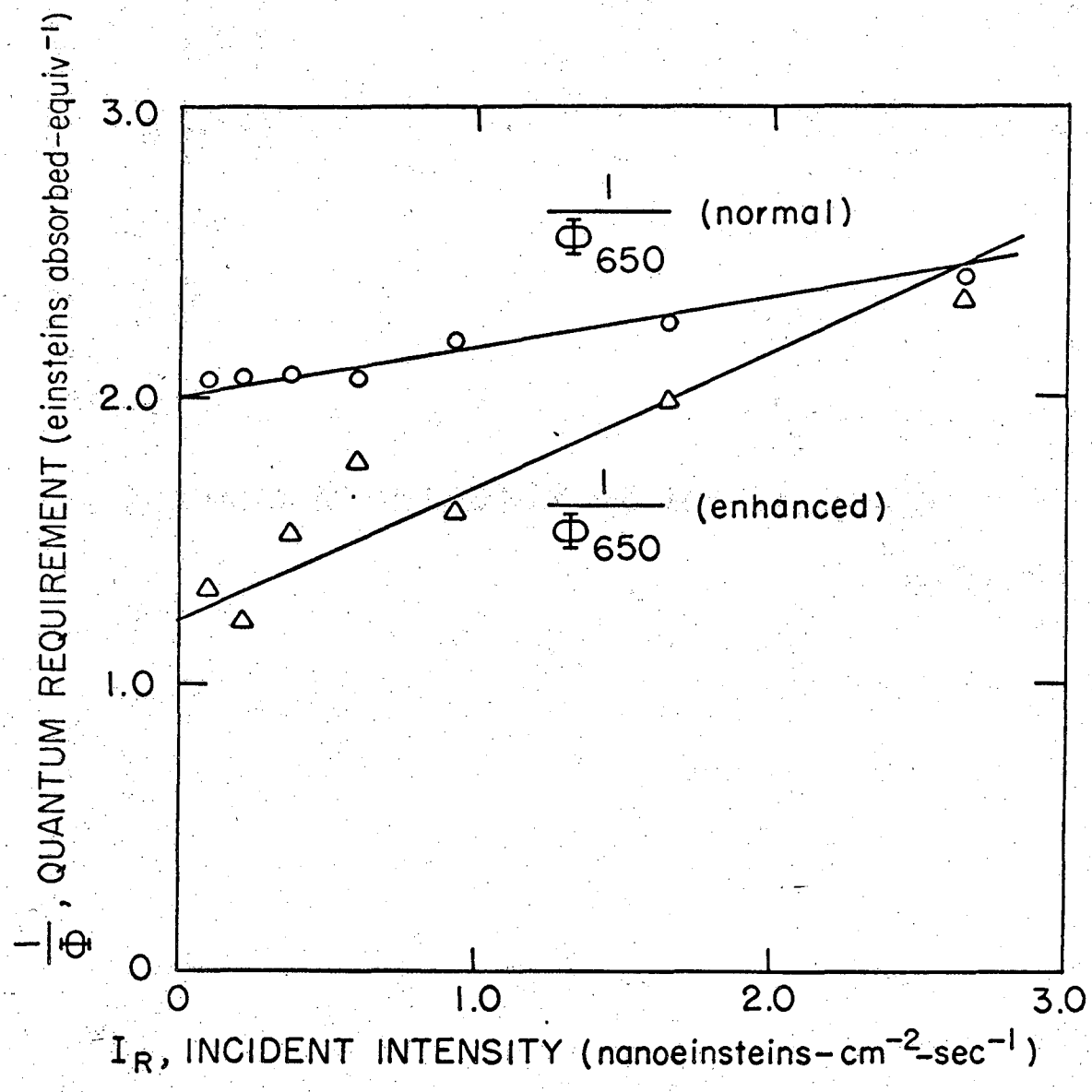

3.0
2.0
1.0
0
0
1.0
2.0
3.0
$\frac{1}{\Phi_{650}}$ (normal)
$\frac{1}{\Phi_{650}}$ (enhanced)
$\frac{1}{\Phi}$, QUANTUM REQUIREMENT (einsteins absorbed-equiv^{-1})
I_R, INCIDENT INTENSITY (nanoeinsteins-cm^{-2}-sec^{-1})
XBL716-5253

Fig. 9

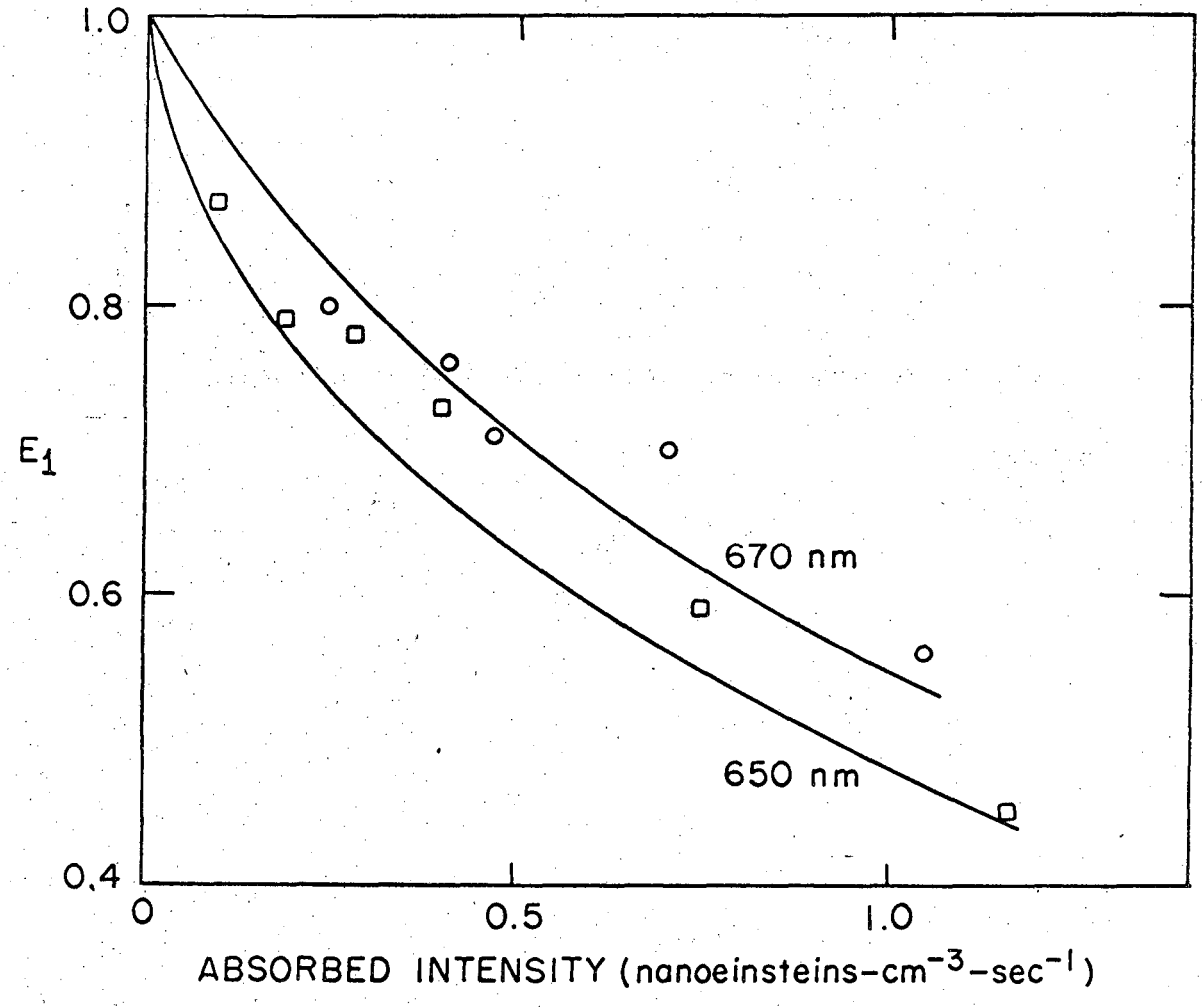

1.0
0.8
0.6
0.4
E_1
670 nm
650 nm
0
0.5
1.0
ABSORBED INTENSITY (nanoeinsteins-cm^{-3}-sec^{-1})
XBL 716-5252

Fig. 10

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