UCRL-11127

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MOLECULAR ORIENTATION IN QUANTASOMES
II. ABSORPTION SPECTRA, HILL ACTIVITY AND FLUORESCENCE YIELDS

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Submitted for publication in
Biochimica et Biophysica Acta

UCRL-11127

UNIVERSITY OF CALIFORNIA
Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

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November 1963

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II. ABSORPTION SPECTRA, HILL ACTIVITY AND FLUORESCENCE YIELDS

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SUMMARY

The absorption spectrum of spinach quantasomes is compared with those of whole chloroplasts and intact leaves. Using the scattered-transmission technique, it is shown that the absorption maximum at 678.5 mμ in spinach leaves is not shifted in the preparation of isolated chloroplasts or by sonication and separation of small quantasome aggregates.

The effects of several organic compounds and surface-active agents on the absorption spectra, chlorophyll fluorescence and Hill reaction activity of quantasomes fall into two general classes. Added acetone, methanol, urea or sodium dodecylsulfate induce a loss in Hill activity at appreciably lower concentrations than are required to give a pronounced fluorescence increase or blue shift in the absorption maximum at 678 m . On the other hand, quantasomes treated with Triton X-100 retain Hill activity at higher concentrations of the non-ionic detergent than are necessary to produce pronounced absorption and fluorescence changes. The principal conclusions reached are (1) the position of the chlorophyll maximum in the red is not a sensitive measure of the ability of photosynthetic materials to carry out the Hill reaction, (2) energy diverted from normal photochemical pathways does not in general immediately appear as enhanced fluorescence,

and (3) detergent concentrations comparable to the chlorophyll concentrations of quantasome aggregates are capable of profound effects on physical properties.

INTRODUCTION

Small pigmented subunits, called quantasomes*, have been identified in spinach chloroplasts and shown to possess the ability to evolve oxygen in the presence of TCPIP or ferricyanide (Hill reaction)[1,2,3]. In the presence of the soluble, colorless stroma fraction, the quantasomes are capable of supporting CO_2 fixation as well. In addition to the photosynthetic pigments, chlorophyll a, chlorophyll b and carotenoids, quantasomes have been found to contain about 50% protein, 30% colorless lipids and a variety of possible photosynthetic electron transport agents; e.g. cytochrome b_6, plastoquinones, vitamin K, tocopherols, manganese, copper, iron, etc.[3,4]. Observations using electric dichroism have implicated oriented chlorophyll molecules in the initial photochemical act[5].

A great many studies of absorption spectra of *in vivo* photosynthetic systems have been reported[6]. Accurate spectra have been difficult to obtain owing to the large amount of light scattering generally observed in such preparations. Nevertheless, it is apparent that there exists a considerable variation among such absorption spectra in nature, including multiple forms of those absorption bands presumably associated with chlorophyll a[7,8]. Treatments with many organic reagents produce wavelength shifts of these absorption bands and lead eventually to extraction of the pigments. Similar absorption shifts resulting from the dark aging of quantasomes have been reported[9]. A rough correlation of a blue shift of the chlorophyll a red absorption maximum with loss in Hill reaction activity

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*See footnote 1, p. 27.

upon aging of quantasome material was noted previously by the authors (unpublished results).

This study is part of a continuing investigation of molecular associations related to quantum absorption and the production of chemical potential by photosynthetic systems. A detailed study of visible absorption spectra shows that the positions of the principal absorption maxima in freshly-prepared quantasome aggregates are in very close agreement with those observed for intact plant materials. The addition of various organic reagents to aqueous quantasome suspensions induces absorption shifts and leads to the loss of Hill reaction activity. Parallel studies on the relative fluorescence efficiency of quantsomes show that the energy diverted from the photosynthetic Hill reaction does not appear directly as enhanced fluorescence.

MATERIALS AND METHODS

Preparation of samples

Spinach chloroplasts and quantasomes were prepared, in general, by methods which have previously been described[1,5]. Concentrated, buffered aqueous suspensions of the photosynthetic material (A_{678} = 10) at 0°C were diluted with deionized water in order to achieve a final absorbance between 0.5 and 1 at 678 mμ for the spectrophotometric measurements of the first part of this study. The absorption spectra were taken as soon as possible, generally within a few minutes after dilution. During this interval the diluted samples were kept at room temperature.

The sample of *Chlorella pyrenoidosa* was prepared by centrifugation from its culture medium at 600 x g for 5 min., washing once with distilled water, and resuspension in a volume of water equal to 1.25 times the original.

Materials

Methanol, acetone, urea and sodium dodecylsulfate (SDS) were commercial reagent chemicals and were used without further purification. Triton X-100, obtained through the courtesy of Rohm and Haas Co., Philadelphia, is a water-soluble polyether (iso-octylphenoxy-polyethoxy-ethanol). The material is polydisperse with an average degree of polymerization of 9.7 and with approximately a Poisson distribution of components.[10] The estimated number-average molecular weight is 630.

Absorption spectra

In this study a relatively new device, commercially available as an attachment for a Cary Model 14 Spectrophotometer, was evaluated and used for measuring absorption spectra of samples exhibiting a large amount of light scattering. The apparatus, the Model 1462 Scattered-Transmission Accessory (Applied Physics Corp., Monrovia, Calif.), utilizes an end-window photomultiplier of 2 inch diameter, in close proximity to the sample and reference cuvettes. The attachment is used in place of the normal sample compartment and photomultiplier housing of the spectrophotometer, and the optical arrangement is such that transmitted and scattered light are measured over a large solid angle in the forward direction.

For the measurements to be described a red-sensitive photomultiplier (RCA 6217) was used. With this detector reliable spectra with good resolution (slit widths less than 0.2 mm corresponding to bandwidths less than 0.7 mm) were obtained from 300 to 900 mμ. The range can readily be extended into the ultraviolet to about 200 mμ if a comparable uv-sensitive photomultiplier (e.g. Dumont 7664) is used. The sample and reference cuvettes (10 x 10 mm inside square cross-section and four

transparent sides) were side by side, separated by about 1 mm, and the distance from the centers of the cuvettes to the photosensitive surface was about 12 mm. Since the effective diameter of the photosensitive surface was 42 mm, the solid angle subtended was about π steradians with respect to the centers of the cuvettes. Somewhat similar techniques have been reported in the literature[11,12].

For purposes of comparison, spectra were also determined using the standard "transmission" technique and the "opal glass" technique of Shibata, Benson and Calvin[13]. The normal cell compartment and detector chamber of the Cary Model 14 were used (RCA 1P28 Photomultiplier intercepting a beam of about 0.02 steradians from the cuvettes). When opal glass was used in the normal cell compartment, matched pieces were placed in contact with each cuvette at the side toward the photomultiplier.

Fluorescence spectra

The measurements of fluorescence were carried out using an Aminco-Bowman Spectrophotofluorometer (American Instrument Company, Silver Springs, Maryland). The instrument was provided with a red-sensitive photomultiplier (RCA 7102), which was cooled with dry ice. Activation or emission spectra were recorded using a Sanborn XY-Oscillograph. An absolute calibration of the instrument was not made ; thus, all fluorescence intensities are relative values and are not normalized at different wavelengths. Care was taken to correct for self-absorption and to avoid interference by light scattering by the chloroplast subunits. All fluorescence intensities reported in this paper represent extrapolations to infinite dilution of data taken over approximately a 100-fold concentration range, corresponding to an absorbance range of 10^{-2} to 1 for the peak at 678 mu. Furthermore, they are steady-state levels of fluorescence reached after any transients

occurring during the first few seconds of excitation have disappeared. Instrumental sensitivity was monitored using ethanolic solutions of rhodamine B base (Matheson, Coleman and Bell, B 1193)[14].

Hill reaction

Hill reaction activity was assayed manometrically using $K_3Fe(CN)_6$ or 2,4,6-trichlorophenolindophenol (TCPIP) as the oxidant. The reaction mixtures for the ferricyanide Hill reaction contained sufficient spinach quantasomes to give 0.2 to 0.4 mg/ml of chlorophyll with 20 µmoles of $K_3Fe(CN)_6$ in 3 ml of 0.03 M potassium phosphate buffer at pH 7.4. For the indophenol Hill reaction, quantasomes sufficient to give 0.009 mg of chlorophyll per ml were suspended in 3 ml of 0.03 M potassium phosphate buffer at pH 7.4, and 0.2 µmoles of TCPIP was added. The reactions were run at 15°C at a light intensity of 4000 foot candles.

RESULTS

Scattered-transmission spectra

The performance of the scattered-transmission (ST) technique of spectrophotometry is illustrated in Fig. 1, which shows a comparison of the absorption spectra obtained by three different methods applied to a single suspension of *Chlorella pyrenoidosa*. Not only is the apparent turbidity of the sample significantly lower for the ST method, compared with that obtained using direct transmission or opal glass, but the absorption maximum at 678.5 mµ is not shifted to longer wavelength or attenuated relative to the base line, as is the case in the upper curves. Although the opal glass method gives a reasonably close approximation to the "partially integrated" absorption curve of the ST method, the opal glass causes a strong reduction in the amount of light reaching the detector of the standard Cary Model 14 spectrophotometer. (This reduction was 200- to 400- fold under the conditions of these

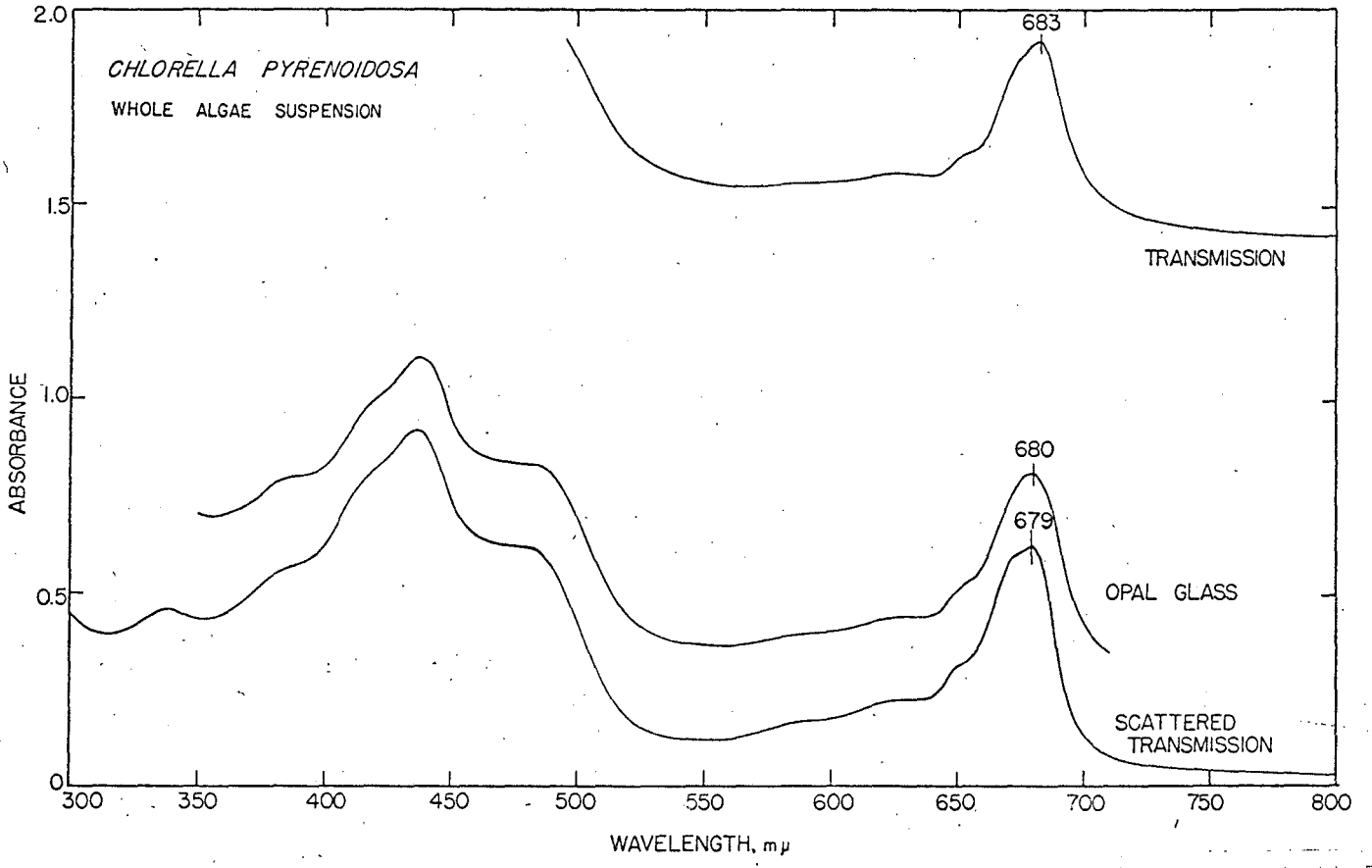

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Fig. 1. Chlorella absorption spectra - same suspension measured by three different techniques. Upper curve: transmission spectrum using standard sample and detector compartments of Cary Model Model 14 Spectrophotometer. Middle curve: opal glass adjacent to sample and reference cuvettes on exit sides; same spectrophotometer arrangement. Lower curve: scattered-transmission (ST) spectrum using Cary Model 14 Spectrophotometer with Model 1462 Scattered-Transmission Attachment.

measurements.) Under circumstances where the reference beam intensity is strongly reduced, the spectrophotometer slit widths in the red region of the spectrum become excessively large and a serious loss in spectral resolution results.

The various operations associated with the preparation of quantasomes from spinach leaves were evaluated in terms of their effect on the absorption spectra using the ST method. Fig. 2 shows A) the spectrum of a piece of intact spinach leaf in an area free from large veins and B) the spectrum of a leaf homogenate prepared in a Waring blendor (top speed for 90 sec) in 0.5 M sucrose, 0.1 M phosphate buffer (pH 7.4) and 10^{-2} M versenol at 0°C. The homogenate, strained through 8 layers of cheesecloth, was diluted 15-fold with water just before the spectral measurement. The leaf segment spectrum was run versus a cuvette containing a piece of opal glass in water; this has the effect of lowering the recorded absorbance curve approximately 0.29 unit. No correction has been made for this in the figure. The major chlorophyll a absorption band in the red appears at 678.5 ± 0.5 m in both cases, and other features in the spectra are correspondingly similar.

The spectra shown in Fig. 3 were of samples prepared in the following way: The homogenate described above was centrifuged at 200 x g for 5 min at 0°C. The resulting supernatant was centrifuged at 600 x g for 15 min at 0°C. The supernatant from this centrifugation, diluted 8-fold with water, was used for spectrum B (dashed curve) in Fig. 3. The precipitate from the second centrifugation was resuspended in 40 ml 0.5 M sucrose and 10^{-3} M phosphate buffer (pH 7.4) and centrifuged again for 15 min at 600 x g and 0°C. The precipitate of washed chloroplasts was resuspended in

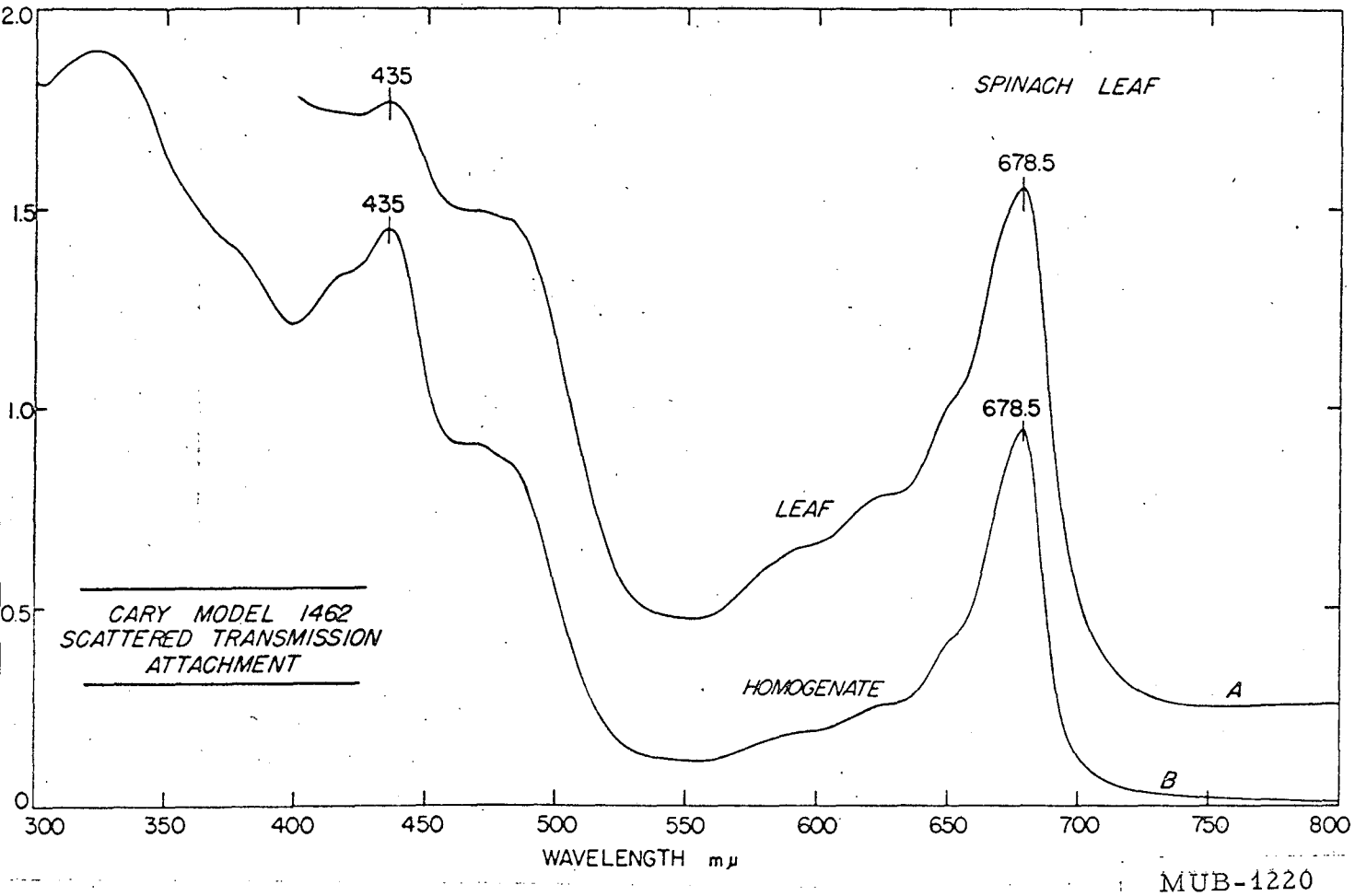

Fig. 2. Spinach leaf scattered-transmission spectra from 300 to 800 mμ. Curve A: segment (1 x 4 cm) of intact leaf immersed in water, versus opal glass in water in reference beam. Curve B: total leaf homogenate in 0.5 M sucrose, 0.1 M phosphate buffer (pH 7.4), 10^{-2} M versenol, diluted 15-fold with water just before measurement, versus water in reference cuvette.

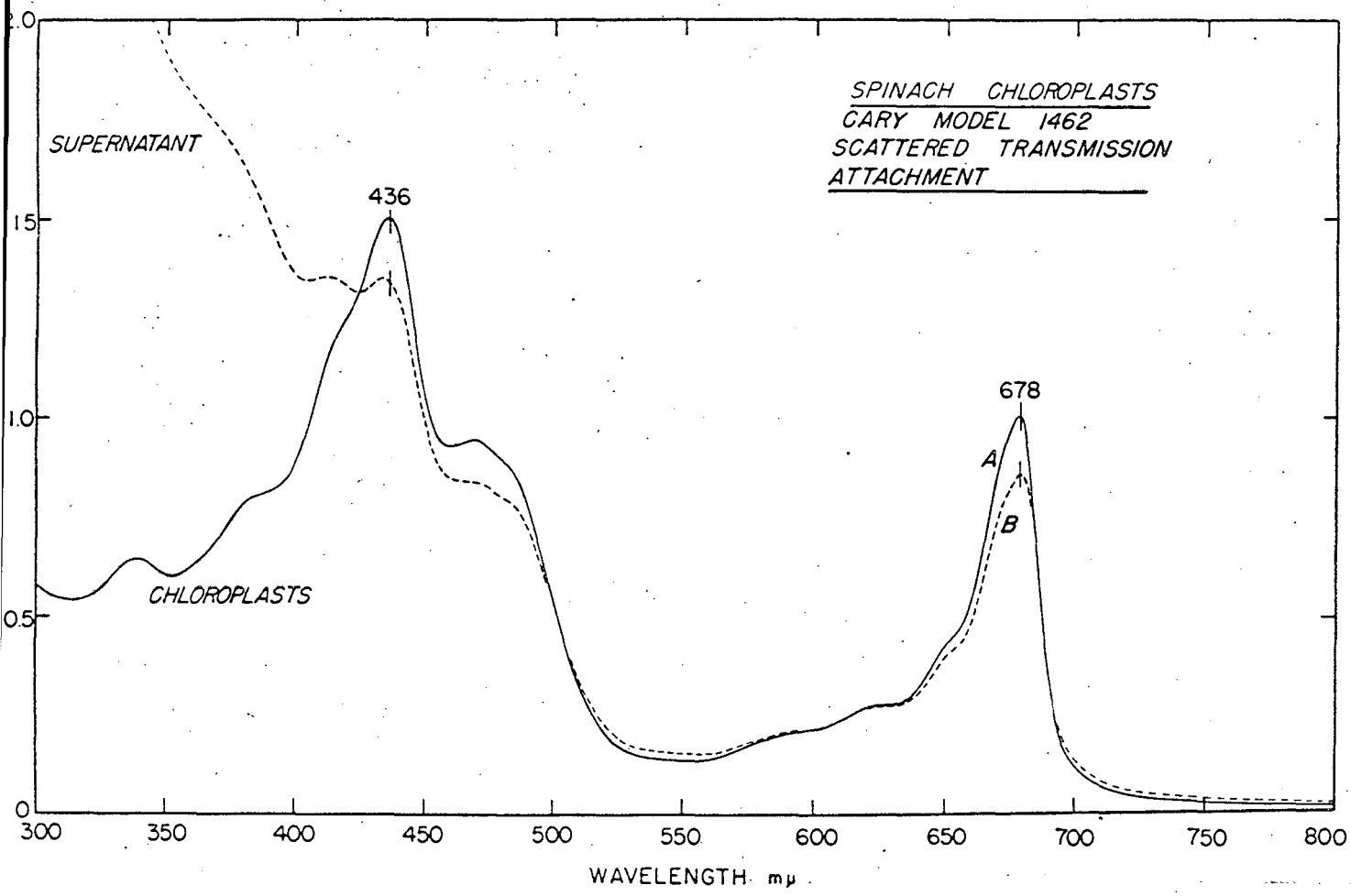

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Fig. 3. Spinach chloroplast scattered-transmission spectra, from 300 to 800 mμ. Solid curve, A: washed chloroplasts (see text) freshly suspended in distilled water, versus water in reference cuvette. Dashed curve, B: chloroplast supernatant (see text) diluted 8-fold with water, versus water.

15 ml of water. A 100-fold dilution with water of a portion of this suspension was used for spectrum A (solid curve) in Fig. 3.

For comparison purposes, a spectrum of spinach quantasome aggregates (free of soluble stroma material) is presented in Fig. 4. The preparation of this quantasome suspension has been described previously[9]. In each case in Figs. 2-4 the chlorophyll a absorption maximum appears at 678.5 mμ. Furthermore, the two curves of Fig. 3, taken together in appropriate proportion, give the principal features of the intact leaf or leaf homogenate spectra of Fig. 2. Thus, for all materials from the intact leaf to the preparation containing the smallest quantasome aggregates studied so far there seem to be no shifts of the principal absorption maxima. It is significant that activity in the form of ability to accomplish photo-induced oxygen evolution via the Hill reaction has been demonstrated for the sonicated preparations.

Absorption shifts, relative fluorescence efficiency and Hill activity with added solutes

The effects of added methanol, acetone, sodium dodecylsulfate and Triton X-100 on (a) the blue shift of the chlorophyll a red absorption maximum, (b) the relative efficiency of chlorophyll a fluorescence, and (c) the Hill reaction activity with ferricyanide (with TCPIP in the case of added acetone) are summarized in Figs. 5-8. The chloroplast material used in these studies was prepared by a procedure somewhat simplified from that used previously. Chloroplasts were isolated initially in a medium containing sodium chloride and tris buffer rather than sucrose and phosphate buffer. The general scheme is presented in Fig. 9.

Absorption shifts were measured from the peak at 678.5 mμ for an aqueous quantasome suspension to the corresponding absorption maximum of the treated suspension. This shift generally occurred with a decrease in the apparent absorption coefficient at the peak. The magnitude of the decrease never exceeded 20% in any of the systems studied. With added Triton X-100 a decrease to 90% of the initial absorption occurred at a concentration of

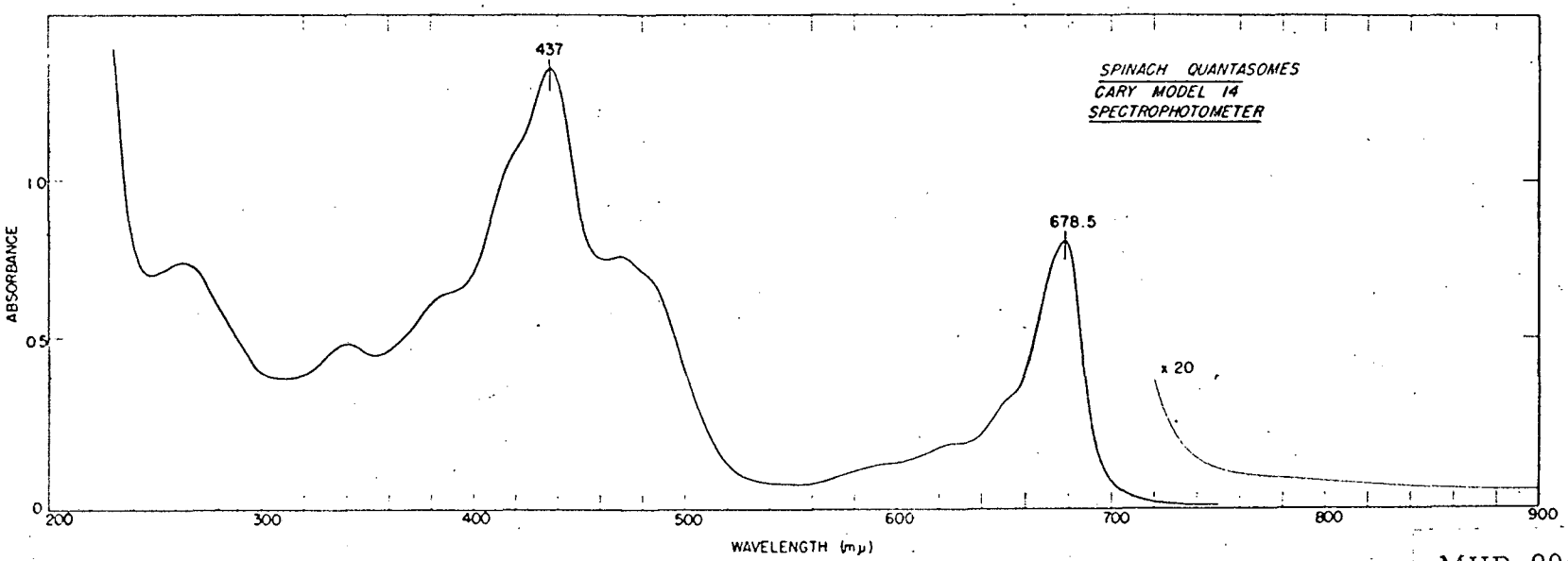

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Fig. 4. Spinach quantasome absorption spectrum from 200 to 900 mμ. Sample prepared from sonicated spinach chloroplasts; precipitate between 45,000 x g and 145,000 x g resuspended and resonicated; supernatant from subsequent 20,000 x g centrifugation. Curve at right represents 20-fold greater concentration (see ref. 9 for details).

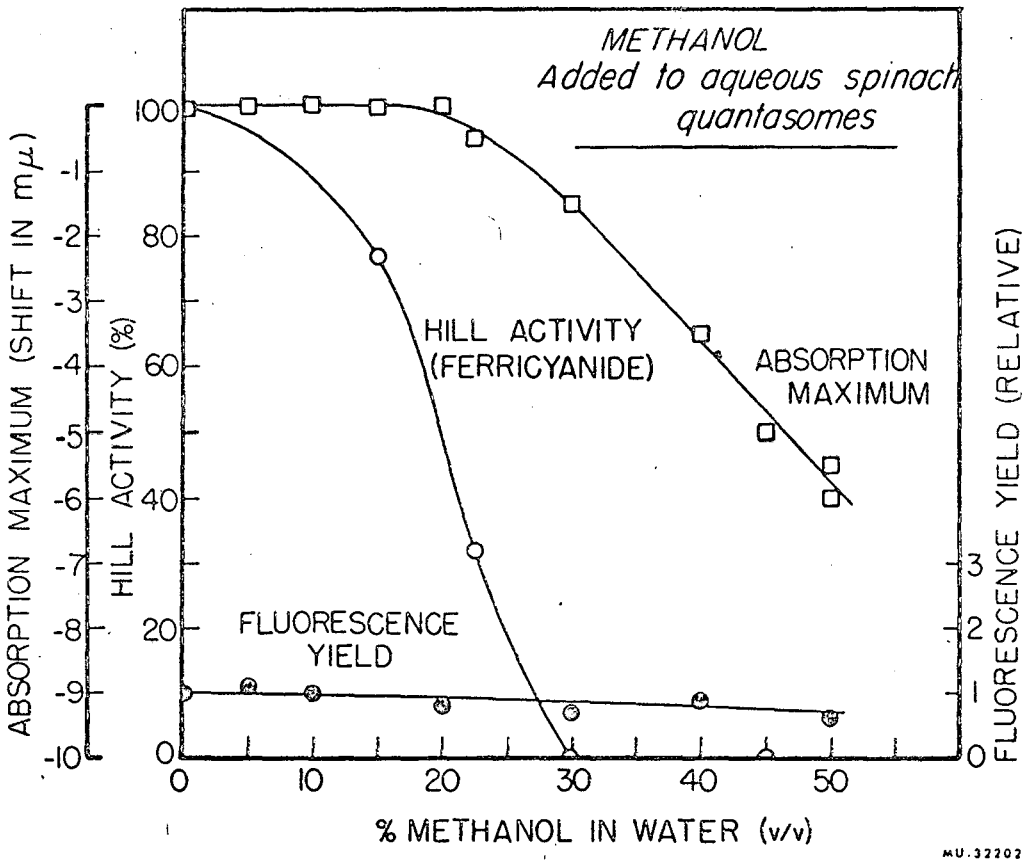

Fig. 5. Effect of methanol concentration on the ferricyanide Hill activity ○, blue shift of the absorption maximum initially at 678.5 mμ □, and fluorescence yield ⊗ of aqueous spinach quantasomes. The absolute Hill reaction activity of the aqueous suspension was 260 μl O_2 evolved/mg chlorophyll/hr.

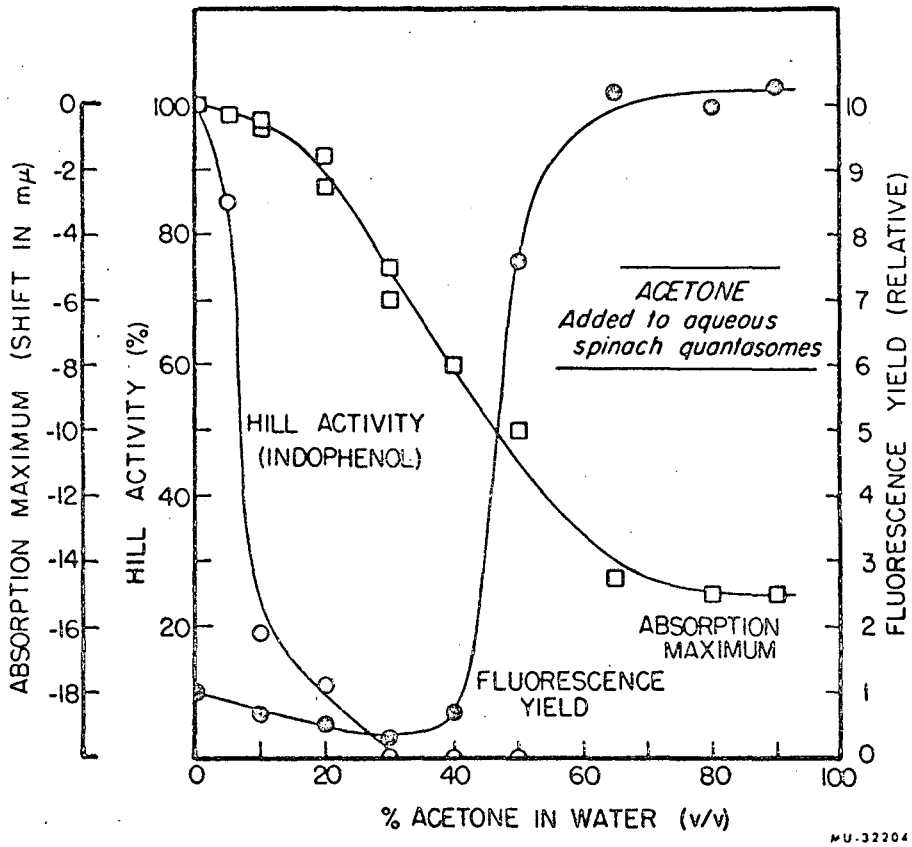

Fig. 6. Effect of acetone concentration on the indophenol Hill activity ○, the blue shift of the absorption maximum initially at 678.5 mμ □, and the fluorescence yield ⊙ of aqueous spinach quantasomes. The absolute Hill reaction activity of the aqueous suspension was 590 μmoles TCPIP reduced/mg chlorophyll/hr.

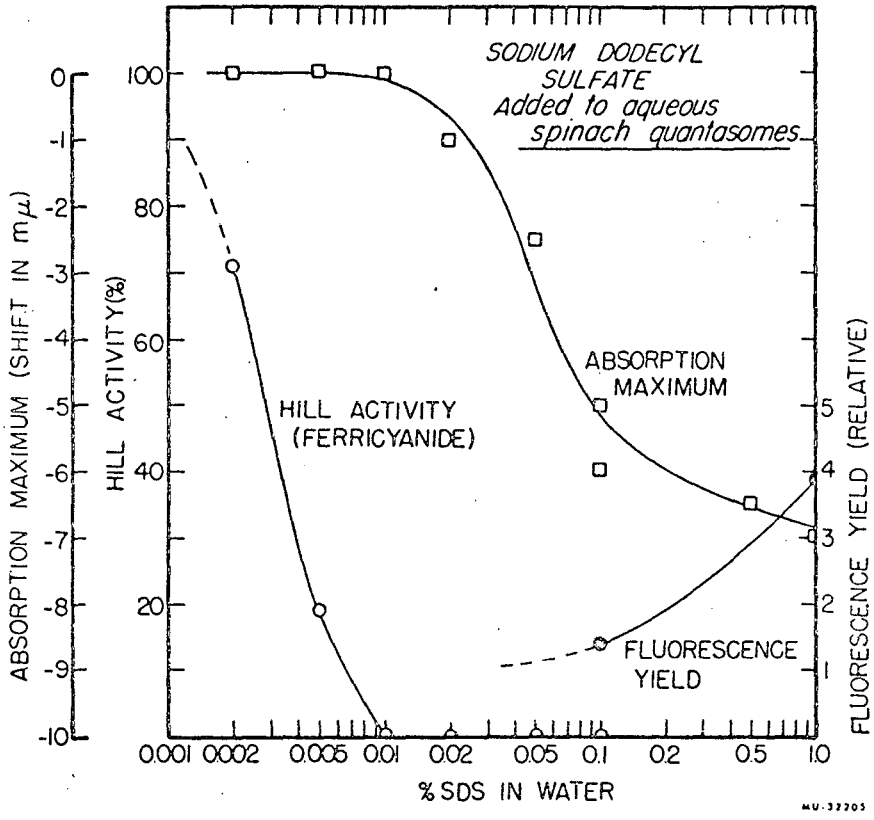

Fig. 7. Effect of sodium dodecylsulfate concentration on the ferricyanide Hill activity ○, the blue shift of the absorption maximum initially at 678.5 mμ □, and the fluorescence yield ⊗ of aqueous spinach quantasomes. The absolute Hill reaction activity of the aqueous suspension was 200 μl O_2 evolved/mg chlorophyll/hr.

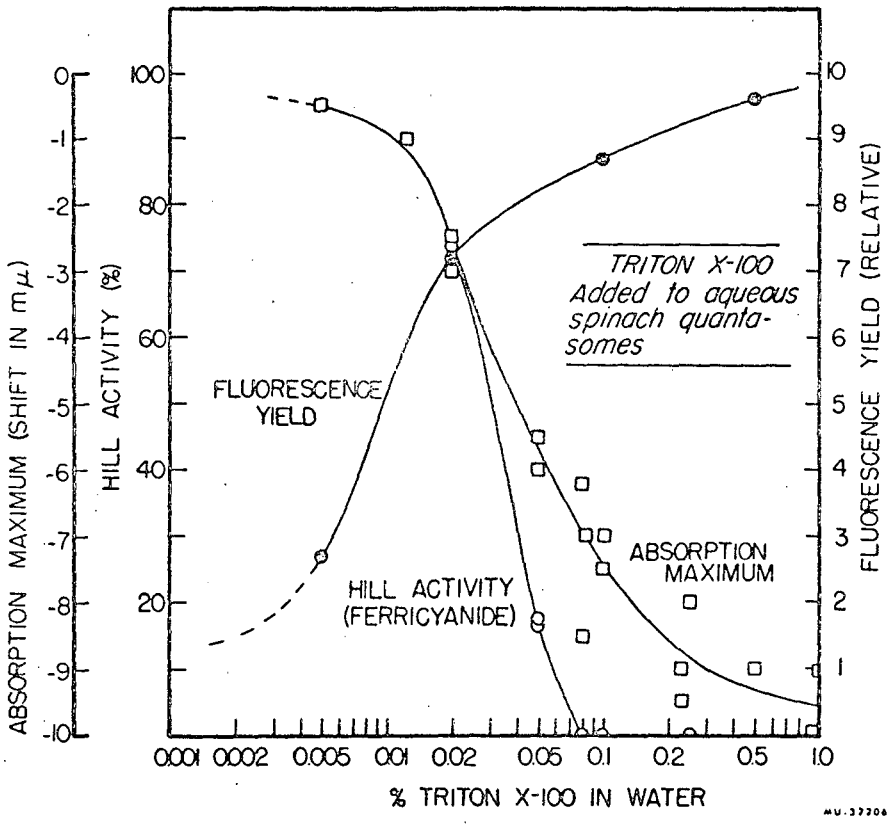

Fig. 8. Effect of Triton X-100 concentration on the ferricyanide Hill activity ○, the blue shift of the absorption maximum initially at 678.5 mμ □, and the fluorescence yield ⊙ of aqueous spinach quantasomes. The absolute Hill reaction activity of the aqueous suspension was 360 μl O_2 evolved/mg chlorophyll/hr.

Petioles were removed from 125 gm of spinach leaves. The leaves were cooled to 0°C and ground in 250 ml 0.35 M NaCl, 0.02 M tris (pH 8) for 30 seconds in a Waring blendor at full voltage. The resulting homogenate was strained through 8 layers of cheesecloth and treated as follows:

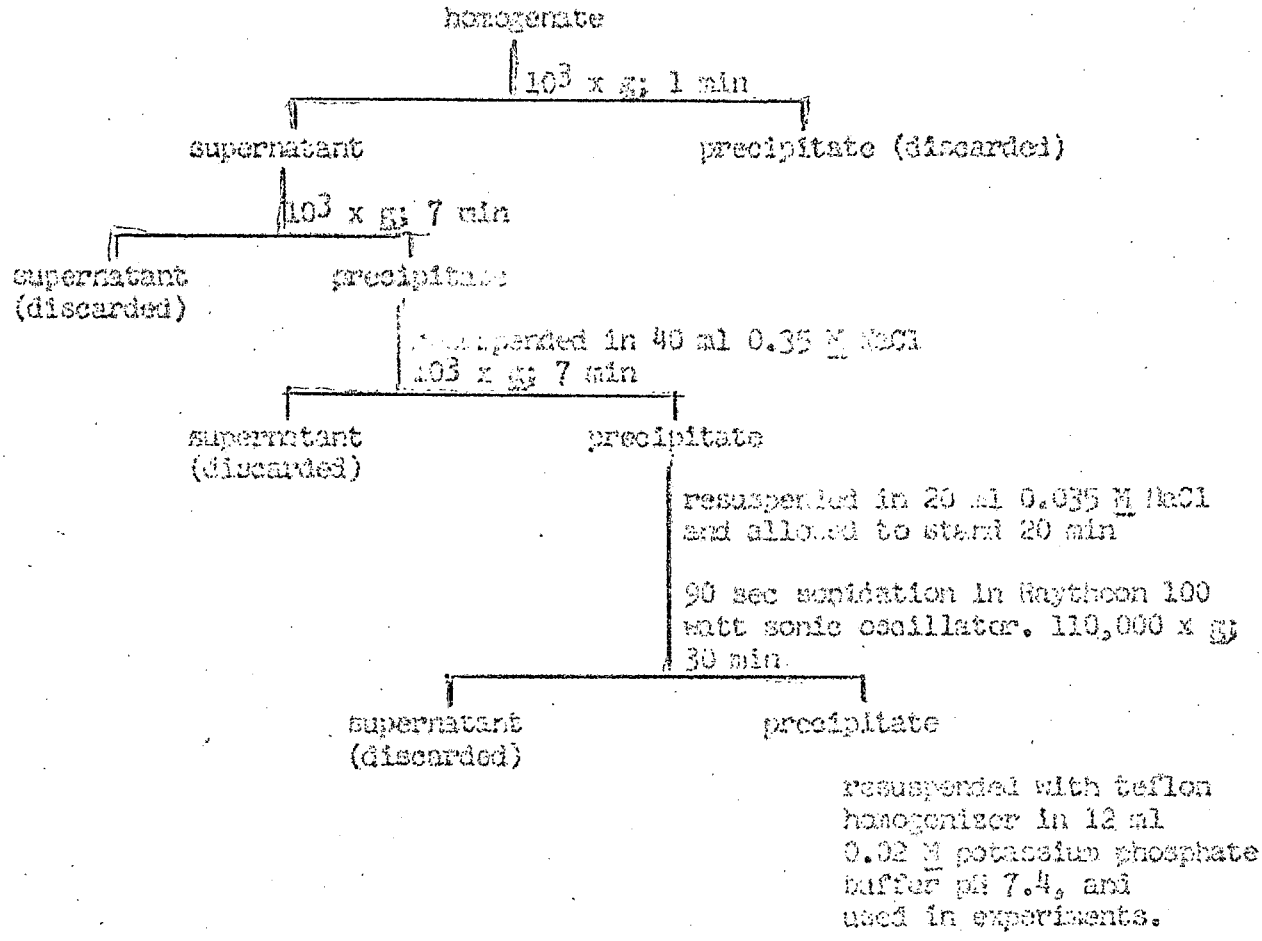

Fig. 9. Flow diagram for the preparation of chloroplast fragments.

0.05% of the detergent, followed by a subsequent increase to 96% at 1%. The minimum in absorbance occurred in the concentration region where the absorption shift was taking place most strongly (Fig. 8).

The apparent absorbance at 750 mμ for quantasome suspensions is a measure of the light scattering by the sample[9]. The level of scattering was unaffected by concentrations of acetone or methanol up to 30%, but was reduced to a very low level by 0.05% Triton X-100 and by 0.5% SDS. The highest concentrations of ethanol and acetone produced strong increases in scattering, owing to aggregation of the partially extracted lamellar protein.

Urea added as solute produced no measurable effect on the absorption spectrum at 1 M concentration and a blue shift of only 1.5 mμ at 5 M concentration.

Fluorescence measurements were made using the Aminco Spectrophotofluorometer. The wavelength of the exciting beam monochromator was set at the maximum absorption of the chlorophyll a Soret band of the treated material (430-437 mμ, depending on the solute) and the emission was observed at the wavelength of maximum fluorescence (668-681 mμ). For each concentration of added organic reagent, a series of measurements was made over a 100-fold range of concentration of quantasomes. The concentration of organic solute was kept constant throughout the dilution of the quantasomes. The ratios of fluorescence observed to absorption at the peak in the red extrapolated to a constant value at infinite dilution of quantasomes, indicating that self-absorption effects were rendered negligible. The extrapolated fluorescence/absorption ratios were normalized with respect to the corresponding ratio for an untreated aqueous quantasome suspension, and these relative values are presented in Figs. 5-8. Added urea (not shown in the Figs.) produced no effect (± 10%) on the relative fluorescence yield over the concentration range up to 5 moles/liter.

The effects of added methanol, acetone, SDS and Triton X-100 on the relative Hill reaction rates of aqueous suspensions of quantasome aggregates are presented in Figs. 5-8. Added urea produced no inhibition at a concentration of 0.1 molar and gave complete inhibition at concentrations of 0.5 molar and higher. Manometric assay using ferricyanide as the Hill oxidant was used for all the treated preparations except where acetone was added. Manometry was unsuitable as an assay for Hill reaction in the acetone-treated preparations. The spectrophotometric assay of TCPIP as a Hill oxidant was used for the acetone preparation. The spectrophotometric assay was done under conditions of light saturation and the rate was determined for the first minute of the reaction. This procedure is dictated by the spectrophotometric assay which requires a low chlorophyll concentration, and by the high absorption coefficient of the dye which necessitates a low dye concentration. The ferricyanide assay was done under non-saturating light conditions and the rate was determined over a period of 15 minutes. This procedure is necessitated by the less sensitive manometric assay. The differences in these procedures probably account for the large differences in Hill reaction rate for the ferricyanide system (200-360 µl O_2/mg chlorophyll/hr) and the indophenol system (590 µM TCPIP/mg chlorophyll/hr, i.e. ca. 6000 µl O_2/mg chlorophyll/hr).

DISCUSSION

The absorption spectra of quantasomes from spinach have very nearly the same peak wavelength values as occur in the spectra of leaves and of whole chloroplast preparations. The position of the red absorption maximum, which results primarily from absorption by chlorophyll a, is frequently used as an indication of the condition of the pigments in photosynthetic material. Our observations show

that this peak occurs at 678.5 mμ in chloroplasts and quantasomes, as well as in the intact leaf. This finding coupled with the ability of illuminated quantasomes to carry out the Hill reaction efficiently[3] and of quantasomes plus soluble colorless strona substances to support CO_2-fixation[1], is taken as evidence that the immediate environment of the pigment molecules in active quantasome preparations is not significantly different from that in the isolated chloroplasts or in the intact plant cell.

Absorption spectra of spinach leaves and of chloroplast preparations have been reported by Rabideau, French and Holt[16] and by Moss and Loomis[17]. In general, the quality of their spectra was not such that accurate wavelengths of the maxima could be determined; however, the conlcusion reached by both groups was that, within the instrumental limitations, there seemed to be no real shifts in absorption maxima in going from whole leaves to chloroplast suspensions and even to preparations of disintegrated chloroplasts. Since 1954 a number of different experimental and theoretical approaches have been studied in an effort to overcome the effects owing to light scattering in such samples. The opal glass technique of Shibata, Benson and Calvin[13] has already been mentioned. Amesz, Duysens, and Brandt have proposed a modification of the opal glass technique using a fluorescent scattering screen[18], which is particularly useful at wavelengths below 315 mμ where opal glass becomes opaque. A different approach was used by Barer, in which he altered the refractive index of the suspending medium by addition of a soluble protein until the refractive index matched that of the suspended particles[19]. The resulting improvement in the spectral discrimination of algal suspensions is limited by the fact

that the cell is not a homogeneous body with a single refractive index throughout, but contains one or more pigmented chloroplasts whose refractive index varies significantly with wavelength. Theoretical studies of these complications[20-22] have shown that the variation of refractive index with wavelength in the vicinity of the pigment absorption bands is such that light scattering is greater on the long wavelength side and less on the short wavelength side of such a band, even when the refractive index of the medium is adjusted to match that of the intracellular material exclusive of the chloroplast. The most extensive corrections which have yet been applied to such scattering-distorted spectra (by Latimer and Eubanks[22]) give curves for spinach chloroplasts which are remarkably similar to that shown in Fig. 3A, obtained by the much less laborious ST technique. In some instances spectrophotometers using Ulbricht spheres[17,23] or wide angle microscope attachments[21] have been used to obtain absorption spectra of scattering photosynthetic materials. Despite the manifold problems associated with these techniques, they have yielded some of the most reliable absorption spectra to date. An additional spectrophotometric anomally results in the flattening of absorption bands owing to the enhanced mutual shading of the pigment molecules in the suspension[24]. Amesz, Duysens and Brandt found that for Chlorella the flattening amounted to only 1% if all the scattered light within 70° of the forward direction is seen by the detector[19]. With the ST accessory used in this study the corresponding angle was 60°; therefore, the extent of flattening of the ST spectra of Chlorella or of similar pigmented structures should be little different from that observed by Amesz, Duysens and Brandt.

The advantageous features of the ST method can be summarized as follows: (1) spectral accuracy and scattering corrections comparable with

those obtained from integrating sphere measurements are achieved; (2) spectra are recorded continuously, rapidly and with the optimum wavelength resolution of the spectrophotometer, comparable to that obtained for non-scattering samples; (3) double-beam operation is utilized; (4) a wide range of wavelengths (200-1000 mμ) is available through the choice of appropriate interchangeable photomultiplier tubes (ultraviolet Dumont 7664, visible Dumont 6292, red RCA 6217, near infrared Dumont 6911).

The ability of achieving precise and reliable absorption spectra of photosynthetic materials permits a test of the hypothesis that the positions of the main chlorophyll absorption bands are a sensitive measure of the structural integrity and consequently the activity of the photochemical apparatus. Such a relationship was suggested by a rough correlation observed previously in this laboratory between the onset of a blue shift of the absorption maximum at 678 mμ with loss of Hill activity upon aging of quantasome preparations in the dark at 0°C.

The results of the present study, using added organic reagents and surfactants, demonstrate the relationship to be more complex than is suggested by the simple hypothesis above. The observations, summarized in Figs. 5-8, fall into two principal categories: (1) Acetone, methanol, urea or SDS added to aqueous quantasome suspensions inhibits the Hill reaction at significantly lower concentrations than are required to alter the position of the red absorption maximum or markedly increase the fluorescence efficiency. (2) The non-ionic detergent Triton X-100 added to aqueous quantasomes causes a striking fluorescence increase at concentrations nearly an order of magnitude less than those required to induce substantial absorption shifts or effective inhibition of the Hill reaction.

The observations of the effect of the first group of reagents on isolated chloroplast lamellar fragments (quantasomes) are in general agreement with previously published studies of the effects of these and similar reagents on Hill activity and fluorescence yield of chloroplasts; however, some important differences in observations and interpretation exist. Detailed studies of the concentration dependence of solute-induced absorption shifts have not previously been published.

Tumerman, Borisova and Rubin studied the effects of ethyl alcohol, acetone, urea and several other reagents on the indophenol Hill reaction, fluorescence yield and fluorescence lifetimes of sugar beet and tobacco chloroplasts[25]. The effect of ethanol on the Hill reaction in chloroplasts is very similar to our observations using methanol on lamellar fragments. Their observations showed, in addition, an increase in fluorescence above 20% ethanol reaching roughly ten times the initial yield at 60% ethanol. From this and other observations they reached the general conclusion that an increase in the yields (and lifetimes) of fluorescence directly accompany the decrease in photochemical activity induced by added solutes. In our study, using methanol and lamellar fragments, the fluorescence yield at 50% methanol concentration was, if anything, even less than for the aqueous suspension. The ferricyanide Hill reaction was completely inhibited at 30% methanol, however. This evidence would seem to rule out a parallel fluorescence increase and Hill activity decrease in this case.

The observations of Tumerman, et al. on the effects of acetone were not presented in detail. Their statement that complete inhibition of the Hill reaction and attainment of maximal values of fluorescence efficiency took place at concentrations of 30-50% acetone is in rough

agreement with our observations of the effect of acetone on lamellar fragments. Nevertheless, the different concentration ranges over which these two effects take place is one of the striking features shown in Fig. 6. With added urea they found no effect on the yield and duration of fluorescence at concentrations where the Hill reaction was completely inhibited. Although our results are in qualitative agreement with this statement, a closer comparison is not possible owing to the absence of concentration data in their paper. The relatively strong inhibition of the Hill reaction by urea may be explained as a specific effect on the mechanism of oxygen evolution, related to the well-known strong inhibitions of substituted ureas such as 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).

The fact that the fluorescence yields remain strikingly low even at 40% acetone and 50% methanol suggest that the pigments are not yet extracted into the solvent phase. It would appear that the absorption shifts, which begin at still lower concentrations of these reagents, are the result of alterations which precede the true extraction of the pigments. At the same time, concentrations which begin to induce absorption shifts are sufficient to have reduced Hill activity to a low level. The absorption shifts may arise from specific solute-chlorophyll interactions or they may result from a non-specific alteration of the dielectric properties of the medium in which the pigment molecules are located in the quantasomes. The positions of the chlorophyll absorption bands <u>in vitro</u> are known to be sensitive to the dipole moment and polarizability of the solvent[26].

Similar principles may be operating in the case of added SDS; however, the range of effectiveness is at much lower concentrations (0.002 to 1%) in the case of this anionic detergent. Smith and Pickels[27] found that a solubilized lipoprotein to which chlorophyll and carotenoids are bound is produced by treatment of spinach chloroplasts with 0.25% SDS. The resulting absorption shift from 677 to 670 mμ was accompanied by the conversion of chlorophyll a to pheophytin a. Ke and Clendenning confirmed this observation and showed that 0.04% SDS inhibits the Hill reaction of chloroplasts using quinone as oxidant[28]. Their data on the effect of SDS concentration on the quinone Hill activity of chloroplasts show that about 8 times as much was required as is the case for our study on the ferricyanide Hill activity of isolated lamellar fragments. Much of this extra detergent may have been interacting with the membranes and soluble colorless proteins of the chloroplasts - components which are absent in our lamellar fragment suspensions.

The addition of Triton X-100 to aqueous quantasomes causes quite a different set of consequences from those described above. Fluorescence enhancement occurs at substantially lower concentrations of the non-ionic detergent than are required to inhibit the ferricyanide Hill reaction or produce absorption shifts. It may be that Triton X-100 is interacting more strongly with a part of the chlorophyll a serving as a normally non-fluorescent accessory pigment than it is with those molecules which are at the active site associated with oxygen evolution.

The molar ratio of detergent (SDS or Triton X-100) to chlorophyll present in quantasomes can be calculated for those concentrations giving rise to pronounced inhibition of the Hill reaction. For 50% inhibition of Hill activity about 0.3 molecules of SDS or 1.6 molecules of Triton X-100

are required per chlorophyll molecule present in the quantasomes. The small magnitude of these numbers becomes even more striking considering that chlorophyll constitutes only 10.7% of the total weight of the quantasome aggregates[4]. In the case of Triton X-100, the evidence of reduction of light scattering suggests that quantasome aggregates are effectively solubilized at a detergent/chlorophyll ratio of about 2. Thus, an amount of the non-ionic detergent equivalent to about 20% of the weight of the quantasome aggregates present is sufficient for the solubilization.

Visible radiation absorbed by the photosynthetic pigments is subsequently distributed among three different pathways[29]: (1) the initiation of photochemical reactions in the synthetic chemistry of the chloroplast, perhaps through the mediation of long-lived excited states or energy traps; (2) the production of fluorescence from the lowest singlet excited state of chlorophyll a; and (3) dissipation through the processes of collisional (thermal) deactivation. A fourth possibility, the production of phosphorescent emission from a triplet or other long-lived electronic state of the pigment molecules, has never been observed with intact photosynthetic materials. Photochemical utilization under conditions of maximum photosynthetic efficiency is variously assumed to account for 30 to 70% of the absorbed energy[30]. Fluorescence yields *in vivo* account for only 1.5 to 3%. The remaining energy can, therefore, be assumed to be thermally degraded and not available for the useful energy conversion reactions.

In this study an attempt was made to determine whether the disruption of the photochemical energy conversion pathway would lead to an

enhancement of fluorescence efficiency as a direct consequence. The results show quite clearly that this does not necessarily happen. Where fluorescence is not an alternative to photosynthetic energy conversion in treated quantasomes, it must be assumed that thermal deactivation is. The quenching reactions may directly involve the electronically-excited state of the pigment molecules, perhaps explaining the decreases in fluorescence observed in some cases, or they may operate by interfering with the normal processes of the electron transport pathway.

ACKNOWLEDGEMENTS

The authors wish to thank Miss Terry Trosper for her assistance in recording some of the absorption spectra. One of us (KS) is indebted to the United States Public Health Service for a postdoctoral fellowship held for the duraction of most of this work. The project described in this paper was sponsored by the United States Atomic Energy Commission.

FOOTNOTES

1. The term "quantasome" is used loosely in this paper to refer to relatively small pigmented lamellar fragments separated from other components of sonicated chloroplasts. These fragments are actually aggregates of individual quantasomes, which have been observed as morphological features of chloroplast lamellae in the electron microscope. The term quantasome in this paper does not imply that a suspension of monomeric lamellar subunits has been prepared, or that the preparations are homogeneous in particle size.

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