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ABSTRACT

A general consideration of the efficiency requirements for the quantum conversion process in photosynthesis is made. This leads to the concept that a most likely primary quantum conversion process would be an electron transfer process following molecular excitation. Model systems of the various types of known quantum conversion processes are discussed, followed by the presentation of evidence concerning the nature of the primary quantum conversion process in the photosynthetic apparatus of bacteria. That an electron transfer process is very early involved seems unambiguously established. That exciton transfer takes place following primary quantum absorption seems also well established. The present paper gives some supporting evidence that charge migration in the sense of semiconductor behavior also occurs.

A CONTRIBUTION TO THE UNDERSTANDING OF THE PRIMARY QUANTUM CONVERSION IN PHOTOSYNTHESIS*

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INTRODUCTION

One of the central physical-chemical problems in the energy conversion process of photosynthesis is the precise system in and mechanism by which the conversion of electromagnetic energy into chemical potential energy takes place. The overall efficiency, as defined by the chemical equation,

$$CO_2 + H_2O \xrightarrow{h\nu} (CH_2O)_n + O_2$$

$$\Delta F = +110 \text{ kcal/mole/}O_2$$

with which the process can be made to occur for extended periods is relatively high. Numbers varying from as high as 80%[1] to as low as 20%[2-4] have been reported. Within this overall efficiency is included a series of chemical steps whose nature is already known to us. For example, the sequence of steps beginning with carbon dioxide, reduced pyridine nucleotide and adenosine triphosphate and leading to carbohydrate is made up of a series of known reactions. We have some knowledge of the efficiency of each of them.[5] The overall thermodynamic efficiency for this chemical

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sequence turns out to be not higher than 65%. Another fraction of the chemical chain leading from water to molecular oxygen must exist, although the nature of these steps is not yet established.[6]

If, however, we assume a similar overall efficiency for that chemical series, namely, 65%, we must deal with a chemical degradation of not less than 45% in the overall process. Thus, even if the lowest value (20%) of the overall efficiency is taken, we are faced with the necessity for a minimum of 50% efficiency in the primary quantum conversion act during which the electromagnetic energy is transformed into some form of primary chemical potential. If the overall efficiency turns out to be as high as 40%, then the efficiency of the primary quantum conversion act must be over 90%.

Such high efficiencies for the conversion of electromagnetic energy into chemical potential have, as yet, not been clearly achieved in any model system whatsoever. The required high efficiency for the conversion of such a visible quantum is, perhaps, the central problem with which we have to deal. The products have a high chemical potential energy with respect to each other and are being produced by a package of electromagnetic energy only slightly larger than that chemical potential. This fact requires that there can be only a very small barrier to their back reaction. This further seems to require that they be readily, easily and rapidly physically separated to prevent the back reaction.

A brief examination of the variety of photo processes which conceivably could be playing a role in the primary quantum conversion of photosynthesis seems worth making. We will not here be concerned with the purely physical processes which occur prior to quantum conversion. These include (1) the

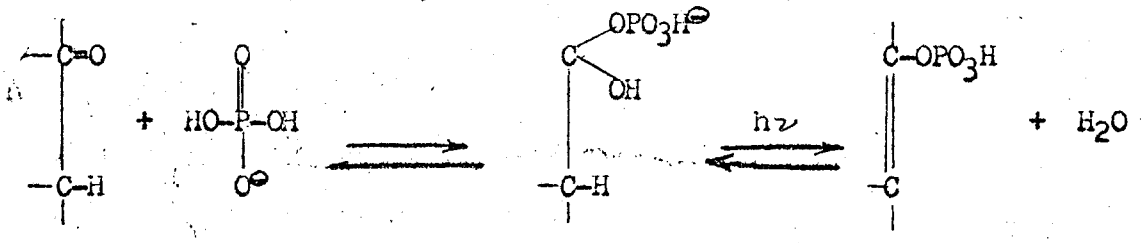
excitation process itself leading to electronic excitation in a molecule or molecular system, followed by (2) the migration of electronic excitation either as exciton or by resonance transfer to the site of conversion. We would consider both of these as purely physical transfer acts with the energy stored in the form of electronic excitation. At the conversion site, by definition, the electronic excitation is transformed into chemically definable species which upon reaction could liberate useful energy in an amount equal to, or less than, the initially absorbed quantum. The fraction by which this is less than that of the initially absorbed quantum would be considered the efficiency of the primary quantum conversion act itself.

In general, the primary products of the quantum conversion may be considered in two possible classes: The class in which the products are uncharged and the class in which the products are charged. Many examples of the first type are known, but in none of them is the fraction of the quantum stored as chemical potential very large.

Uncharged Particles

A whole series of isomerization reactions have been achieved photochemically. One of the earliest and most thoroughly studied is the *trans* to *cis* conversion of an olefin or any other simple double bond.[7,8] Here the *cis* form frequently has a few kcal more energy stored in it than the *trans*, but always the light quantum required to achieve the transformation is many times that energetic. The difference is used to overcome the high energy barrier between the two forms. Such a process of *cis-trans* photoisomerization is currently considered the triggering mechanism in vision.[9]

Another reaction type which has been much investigated photochemically involves the addition of a polar linkage across an olefin or the elimination of a polar molecule from an adjacent pair of atoms to produce an olefin. The molecule added, or eliminated, is frequently water. However, the analogy for undissociated phosphoric acid is clear. A specific case in point is the photoinduced hydration of cystosine.[10,11] Here the energy of the absorbed quantum is $\sim$90 kcal while the free energy stored in the product cannot be more than a few kcal. A hypothetical case for which no model is known would be a dehydration of the phosphoric acid addition product across a carbonyl compound, as follows:



A very common and well-known photochemical conversion process involves the dissociation of a bond. For example, the dissociation of an alkyl iodide into an alkyl radical and an iodine atom, or the dissociation of a carbonyl compound into a carbonyl radical and corresponding radical fragment. Here, also, the absorbed quantum is generally larger than the energy stored in the reaction products,[12] and even when it is not, back reaction prevents significant energy storage.

In recent years many examples of bond formation have been investigated. This bond formation may involve two separate molecules, such as the dimerization of thymine,[13,14] or a whole variety of olefins to give cyclobutane derivatives or other cyclic products. The bond formation may involve two parts of the same molecule, giving rise to still other cyclic (or acylic) products.[8]

Charged Particles

A second major class of transformations involves the formation of charged particles, and here the model chemistry is not so well developed. That the absorption of a quantum by a molecule in the gas phase can lead directly to photoionization has long been known.[15] In fact, the long wave limit for the photoproduction of conductivity in a gas has been used as a means of directly measuring the ionization potential of the molecules of that gas. The same procedures have been used to measure the direct photoionization from the surface of a solid, either a metallic surface, in which case the so-called "work function" is observed,[15] or from a molecular crystal, in which case something related to the ionization potential of the molecules of which the crystal is made, may be observed.[16] In all of these cases one of the products of the photoreaction is a free electron. It is quite clear that such a system as this has a greater possibility of leading to a highly efficient conversion process, largely because one of the products is the almost mass-less free electron which can more readily escape from its remaining cation before recombination. In fact, when the electron is photoejected from a solid surface into a vacuum, the geometry of the system makes possible the collection of the charge and its return in an external circuit to do work.

The possibility that charge separation may occur in a solid, or condensed, system is also well established, particularly for semiconducting atomic lattices. In these cases, however, the absorption

process is presumed to lead directly to the excitation of an electron from a bound state into a conduction state. This is recognized not only by the photoconduction phenomenon itself but by the very nature of the absorption spectra of such systems. The possibility that direct photoexcitation into a conduction band might occur in the condensed system of a photosynthetic apparatus has frequently been considered and rejected on the grounds that the absorption spectra of these systems too closely resembles the absorption spectra of the isolated molecules to allow the possibility for direct excitation into a conduction band to be occurring. In these cases, therefore, it seems that molecular excitation is a better description of the phenomenon of light absorption, followed by exciton migration to the site of ionization, if charge separation is to occur.

Quite clearly, if photoionization is occurring in the condensed photosynthetic apparatus, it cannot be precisely the same process as photoionization of an electron into a vacuum. Here the electron ejected from one molecule must immediately find an orbital in another molecule, so the ionization process is, in effect, an electron transfer process in which an electron is transferred from the donor system into the acceptor system. A number of models of such electron transfer in condensed systems are known. The most recent examples of them are, perhaps, the electron transfer reactions presumed, and indirectly demonstrably occurring, as a result of light absorption in the charge transfer band of a whole variety of molecule complexes.[17] Such electron transfer reactions occurring in isolated charge transfer molecule complexes in general do not lead to efficient energy storage, the reason being that the recombination in an isolated molecular complex is too

rapid and efficient. This difficulty may be overcome by incorporating the molecular complex into an ordered, or partially ordered, system in such a way that the charge migration leading to charge separation over more than one molecular diameter may occur by a "hopping" mechanism between molecular orbitals.[18-21] Once the separation of charge has proceeded to the extent of several molecular diameters we may speak of the quantum as having been converted into chemical potential, since the products thus produced now have a small energy barrier for recombination but may have a smaller one for reaction in other directions, thus leading to a long time chemical potential storage. At this stage it may very well be possible not only to demonstrate the long-lived existence of such high energy chemical products but eventually to actually isolate them, and efforts in this direction are well underway.

The model of quantum absorbing exciton migration to the site of a localized electron transfer followed by charge migration, at least over several molecules, seems thus to be one of the most likely system for achieving the high efficiency of quantum conversion into chemical potential which is required for the photosynthetic system. Evidence that such a process is indeed occurring in the photosynthetic apparatus is accumulating,[22-28], and some new contributions to it follow.

Light-induced electron paramagnetic resonance (EPR) signals have been observed in photosynthetic systems for many years. These observations have been discussed in several recent review papers.[6,29] A positive identification of the signal with a definite molecular species has proved difficult on the basis of EPR properties alone. Thus,

correlations of these properties with other physical measurements have been sought. Independent studies of absorbance changes in photosynthetic bacteria [23,26,30] have led to the tentative detection of bacteriochlorophyll positive ion, thereby leading to the working hypothesis that the EPR signal might be $BChl^{+}$.[6] This view was strengthened by recent work showing that the bacterial spin signal could be produced by chemical oxidation.[31] This paper, by means of kinetic studies of both spectroscopic systems, presents evidence which is not consistent with the above hypothesis.

EXPERIMENTAL

The kinetics studied in this paper are the EPR and absorbance transients induced by a pulse of light. The experimental arrangement used in both experiments is shown in Fig. 1. The EPR and optical spectrometers used have been described in earlier publications.[22,24] An electronically modulated neon lamp was used to induce the photo signals. Its design was kindly made available for our use by Dr. L. Piette of Varian Associates. Most of the details of its circuitry are in the literature.[32] The spectral output was primarily in the wavelength region between 580 and 720 mu. Rise and decay times were of the order of 10 7seconds. The signal resulting from a single flash was too noisy to allow the determination of the full time course of its growth and decay. To improve the signal-to-noise ratio the integration technique of Klein and Barton[33] was used. We used the com-

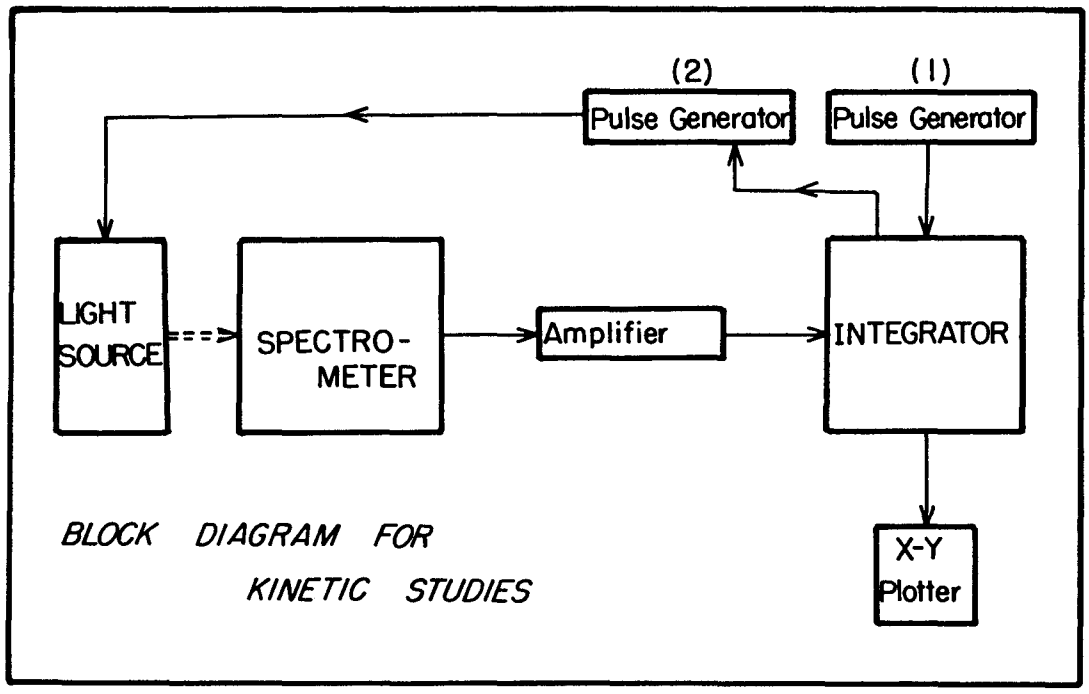


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Fig. 1. Block diagram for kinetic measurements. Operation is described in text. The appropriate spectrometer (optical or EPR) was inserted as desired.

mercial integrator, the Computer of Average Transients Model 400 (Mnemotron Corp., Pearl River, New York). The overall rise time with each spectrometer was less than 1 msec.

The sequence of events was as follows: The repetition rate was determined by a pulse generator which initiated a sweep of the integrator. The integrator triggered the lamp after a fixed delay period. The second pulse generator determined the duration of the flash. The observation time of the integrator could be set for any fraction of the repetition time. Typical flash durations were 2 seconds, repetition time 16 seconds, and integrator sweep time ranged from 1 to 16 seconds. Suitable signal-to-noise ratios were obtained after 50 to 500 events.

Chromatophores were prepared from Rhodospirillum rubrum (originally supplied by R. Y. Stanier, #1.1.1.). The samples were harvested after 5 days of growth in modified Hutner's medium,[34] using malate as substrate. The chromatophores were prepared as outlined in the paper of Loach, et al.[31] The sample was buffered at a pH of 7.5 with 0.1 N glycylglycine.

To minimize differences in the experimental conditions the same sample contained in a Varian EPR aqueous sample cell was used in both spectrometers. Typical optical densities at 880 mu were 1.5.

The following factors were found to effect rise and/or decay rates: redox potential, pH, temperature, light intensity, and the physiological state of the organisms and the preparation and storage of the chromatophores. These were held at the following values for both the EPR and absorbance experiments: +0.30 volts, 7.5 pH units, 22 ± 2°, 10^{16} photons/cm^2/sec, 5 day growth and variable storage. The light intensity was measured with a photodiode which was calibrated against a U.S. Bureau of Standards lamp.

The EPR signal, $S_{(t)}$, was measured at the point of maximum slope of the absorption curve, and is proportional to the number of observable unpaired electrons. The response of this signal to the light pulse is shown in Fig. 2. Also shown is an example of the growth of the signal when the light is turned on (when an expanded time scale is used). The growth may be described by the expression

$$S_{(t)} = S_r (1-e^{-k_r t}) \qquad (1)$$

and the decay curve by the expression

$$S_{(t)} = S_d e^{-k_d t} + S'_d e^{-k'_d t}, \text{ with } S_r = S_d + S'_d \qquad (2)$$

S_r is proportional to the steady-state of photoproduced spins, S_d and S'_d are proportional to the fraction of photoproduced spins decaying by parallel paths with unimolecular rate constants k_d and k'_d. k_r is approximately the unimolecular rate constant for spin production.

A typical absorption spectrum and a light-minus-dark difference spectrum for the chromatophores used are shown in Fig. 3. We are here concerned with the major light-minus-dark bands at 433 mu, 792 mu, 810 mu and 865 mu. The responses of these signals to light are shown in Fig. 4. The shapes of these curves can be expressed by equations of the forms of (1) and (2) above.[24] In both the EPR and the optical absorption measurements the decay rates were found to be approximately independent of the light intensity. This, together with the simple exponential behavior (Figs. 2 and ref. 24) indicates that these processes obey first-order kinetics.

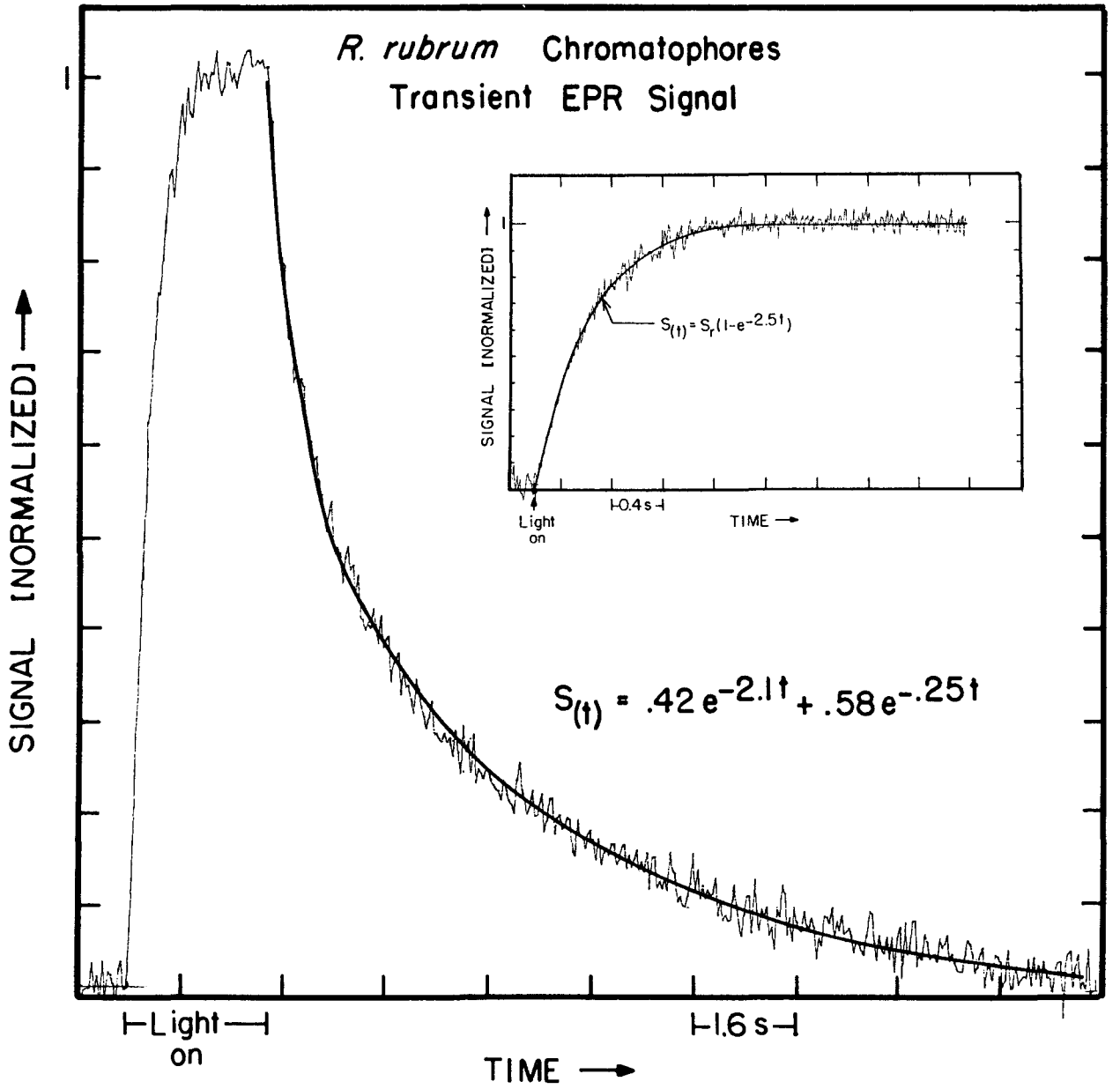

Fig. 2. Time response of the electron paramagnetic resonance (EPR) signal to light. The insert is the growth of the EPR on an expanded time scale. Also shown are exponential curves fitting the data. S_r is the normalized steady-state value of the signal.

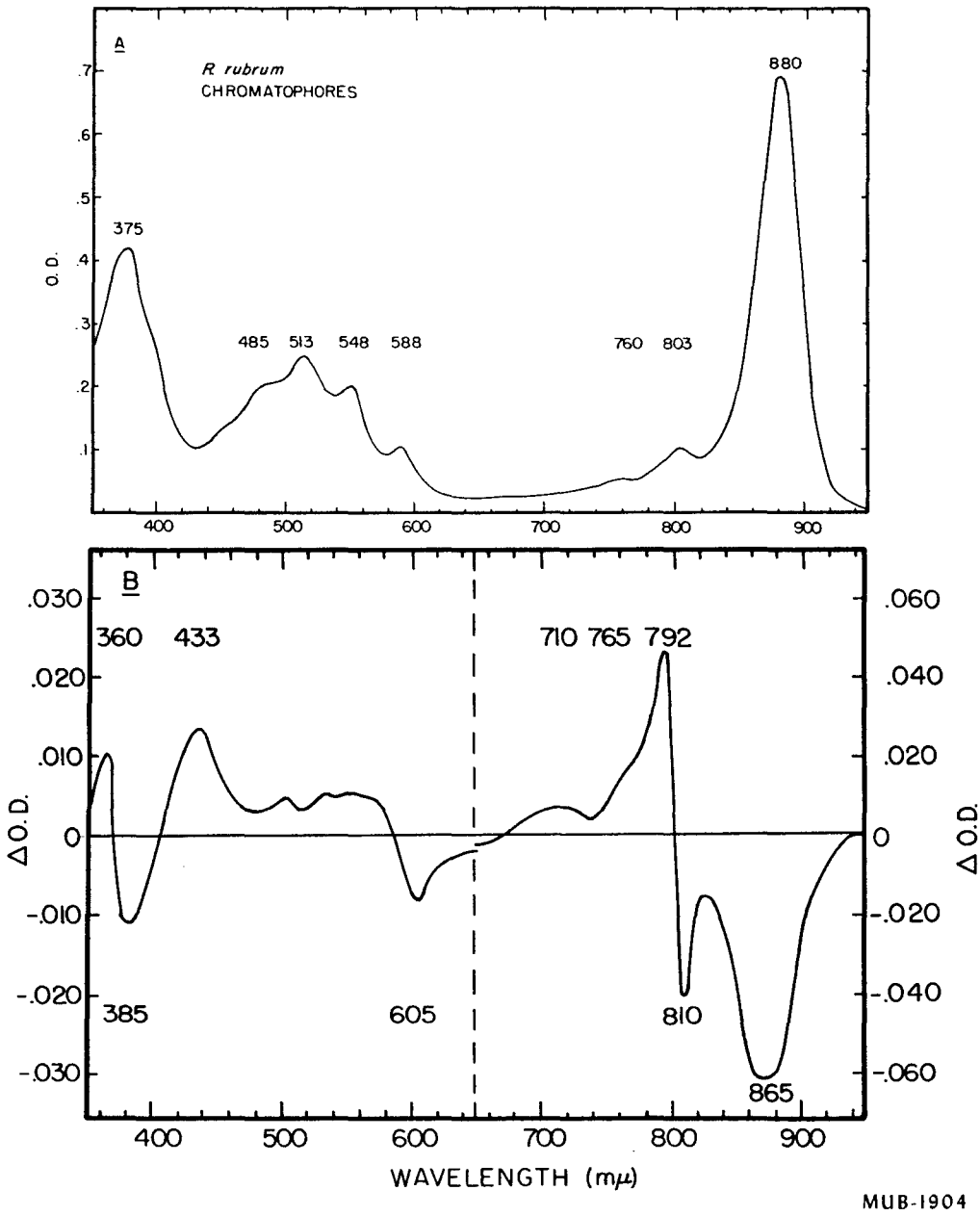

Fig. 3A. Absorption spectrum of R. rubrum chromatophores suspended in 0.01 M phosphate buffer, pH 7.13; 1 cm cuvette.

Fig. 3B. Light-induced absorption changes in R. rubrum chromatophores, whose absorbance was 2.2 at 880 mu. Excitation wavelengths were 650-900 mu for the blue absorption changes; 400-500 mu for the infrared absorption changes. Note that the absorbance scale below 650 mu is expanded twofold. For further experimental details, see Ref. 24.

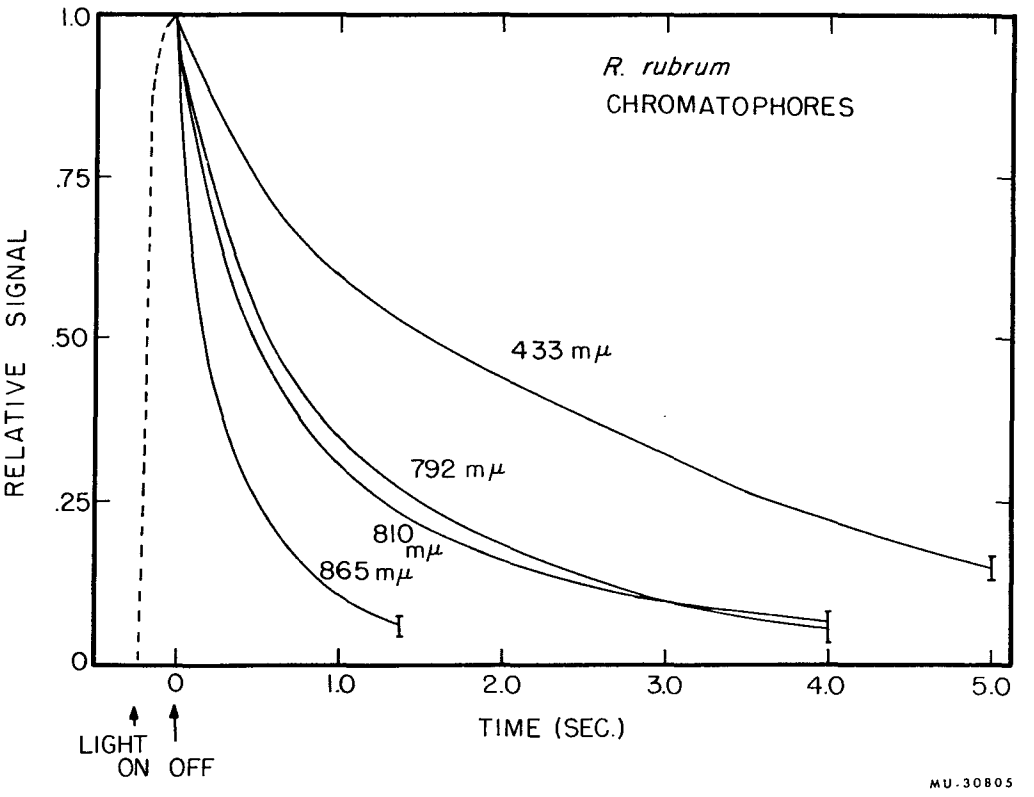

Fig. 4. Typical time response of light-induced absorbance changes in R. rubrum chromatophores. For purposes of comparison, signal heights are normalized and all signals are shown as positive. 10^{-4} M $KuFe(CN)_6$ present. E_h = + .35 volt; 0.01 M phosphate buffer, pH 7.4; O. D. at 880 mu, 0.70. Further details are given in Ref. 24.

To demonstrate the relation between the two spectroscopic observations, the time response (course) of the signals from the same sample have been plotted together (Fig. 5). The steady-state magnitude of each signal was normalized to unity.

CONCLUSIONS

The following conclusions may be drawn from this evidence:

1. The rise and decay kinetics of the spin signal are the same as the kinetics of the 433 mu absorbance changes, within experimental error. Of the major absorbance changes, only the one at 433 mu shows this close agreement. We thus assign the observed EPR signal to the molecular species which produces the 433 optical change.
2. The molecules responsible for the absorbance change at 433 mu are not the same as those molecules responsible for the absorbance change at 865 mu because of the much slower decay rate of the 433 mu band, as was earlier reported.[24]

That a relationship between optical density changes and EPR signals existed was apparent from the experiments of Clayton[26] and Loach, et al.[31] In these experiments all the light-induced optical absorbance changes and the EPR signal were removed upon oxidation and wee replaced by a "dark" signal of the same magnitude. However, no choice among the optical signals could be made, to identify the sources of the EPR signal.

The spectral position of the 865 mu band with respect to the in vivo absorption of BChl and its interaction with redox reagents has

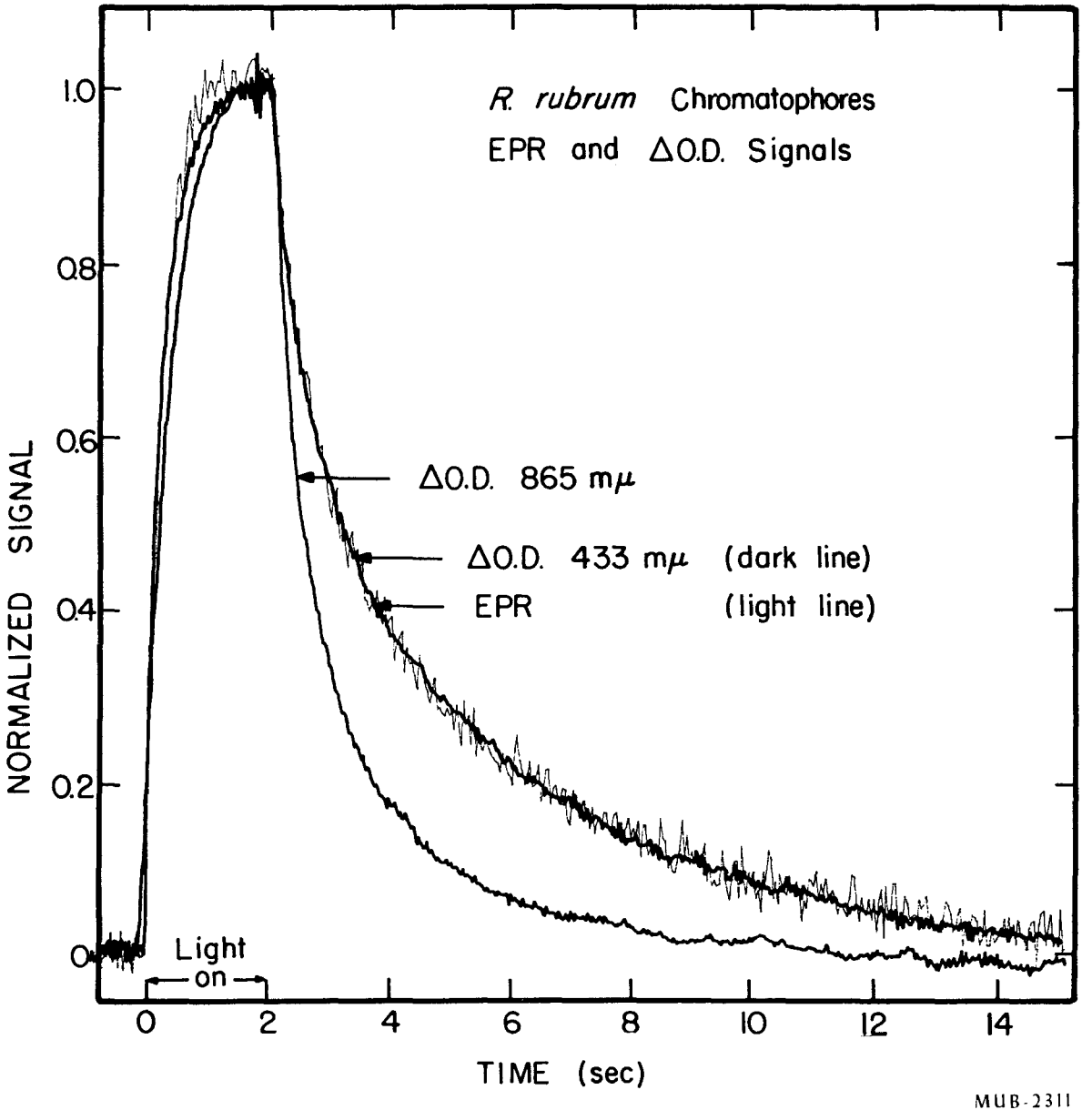

Fig. 5. Comparison of EPR and OD signals from the same sample of R. rubrum chromatophores. Experimental conditions are given in the text.

been used to identify this change with a one-electron photoinduced oxidation of BChl in the organized environment.[26,30,31] Based on this identification of the optical density change at 865 mu and the above kinetic data, oxidized bacteriochlorophyll in the organized environment of the chromatophore is not the site of the unpaired electron producing the observed electron paramagnetic resonance signal. Such a molecular species ($BChl^+$) in solution would be expected to show an EPR signal characteristic of a free radical. That we do not see one could be explained by an interaction between this electron and its environment which broadens the resonance line. Such an interaction may arise from delocalization, among several BChl molecules, of the charge associated with the oxidized BChl. A delocalization of this sort can be used as the conduction system for separation of charge in the primary quantum conversion act.

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