KINETICS OF LIGHT-INDUCED ABSORBANCE CHANGES IN RHODOSPIRILLUM RUBRUM EXTRACTS*

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This paper is a report of the kinetics of absorbance changes induced in extracts of R. rubrum during and following infrared illumination (Smith and Baltscheffsky, 1959; Geller and Lipmann, 1960). R. rubrum extracts, purified by a novel procedure, show absorbance changes which are modified strikingly in the course of activation of photophosphorylation. The effects suggest that common components may be involved in both the absorbance changes and photophosphorylation.

Methods - R. rubrum strain S-1 cells were grown in the light, harvested, and crude extracts made with the French pressure cell as previously described (Geller, 1962).

Crude extracts were purified at 0-5°, using the biphasic dextran-methyl cellulose partition system of Albertsson (1960). In this system the chlorophyllous R. rubrum particles are concentrated in the bottom phase. Aqueous 1% (w/w) methyl cellulose 4000 (Fisher Chemical Co.) and 10% (w/w) Dextran 2000 (Pharmacia) were added to crude extract to give final concentrations of 0.68% and 0.2% (w/w) respectively. Duplicate "blank" tubes, containing 0.2 M glycylglycine pH 8 instead of crude extracts, were prepared at the same time. The tubes were mixed thoroughly and centrifuged 20 min at 37,000 xg. The resulting bottom fluid phases

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were removed by capillary pipette; bottom phase from each crude extract tube was mixed with top phase from a "blank" tube, and the centrifugation was repeated. The bottom phase finally obtained was diluted with glycylglycine to a volume equal to that of the crude extract used and centrifuged 1 hr at 100,000 xg. The precipitate was resuspended in the same volume of buffer and the centrifugation repeated. The washed precipitate was stored in glycylglycine in the dark at 0°.

Absorbance changes were measured at 25° with a cuvette which had a 10 mm optical path for the analyzing beam; the cuvette was irradiated at 90° to the analyzing beam with infrared light (light path 3.5 mm). The infrared light, 0.7-1.0 μ, 1.0×10^6 ergs cm^{-2} sec^{-1} (measured with a YSI Kettering Model 65 Radiometer, Yellow Springs Instrument Co.), was provided by a tungsten lamp filtered by flowing water (2 cm) and Corning Filter No. 2600. This produced maximal changes in absorbance. Difference spectra, relative to unilluminated reference cuvettes, were determined in a Cary Model 14 Recording Spectrophotometer. The kinetics of the light induced absorbance changes were determined with a single beam recording photometer (band width, 2 mμ) employing an oscilloscope triggered by a shutter controlling the infrared light. This equipment will be described in detail in a subsequent paper.

Each cuvette contained R. rubrum particles (0.5 mg protein (Lowry, et al., 1951) and 20 mμmoles bacteriochlorophyll (Cohen-Bazire, et al., 1957)) in 1 ml of 50 mM glycylglycine, 5 mM ADP, 10 mM $MgCl_2$, and 5 mM potassium phosphate, final pH 7.8. The absorbance at 880 mμ of this suspension was 3.0.

Results - Infrared light induced absorbance changes in these R. rubrum extracts, as noted in previous work (Geller and Lipmann,

1960). However, unlike previous results, the photo-induced absorbance changes were only partially reversed when the light was turned off, provided the illumination period exceeded ten seconds. The deficit in absorbance reached a maximum after illumination for 3 min. Upon addition of succinate (1 mM) either prior to (Fig. 1) or following irradiation, the light-induced absorbance changes were almost completely reversible.

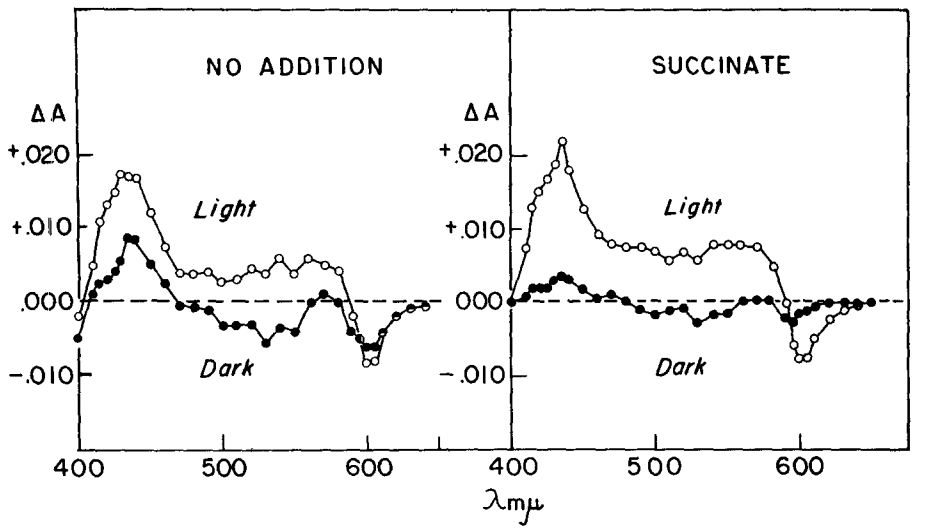

Figure 1. Effect of succinate (1 mM) upon the reversibility of light minus dark difference spectra. The base line (A = 0.000) was obtained, followed by the difference of absorption during infrared illumination ("light"). Following 3-1/2 min illumination, the light was turned off and the difference in absorption again determined ("dark") within 2 min. The "dark" curve was stable for at least 9 min.

The effects were analyzed by measurements of the kinetics of the "light-on" and "light-off" reactions at selected wavelengths: 420 and 435 mμ (Bartsch, 1963), 428 mμ (Smith and Baltscheffsky, 1959) and 605 mμ (Geller and Lipmann, 1960). The "light-on" reactions at these wavelengths had first order reaction constants which ranged from 500 to 700 sec^{-1} at the light intensity used. The "light-on" constants were not changed by succinate or prolonged prior irradiation.

On the other hand, the magnitude of the "light-off" changes was decreased by continuous irradiation and increased by succinate (or DPNH), as reflected in Fig. 1. The rate of the "light-off" reaction, which was at least two orders of magnitude slower than the "light-on", was markedly influenced by prolonged irradiation, and by succinate (or DPNH). The results are illustrated by tracings of an oscilloscope recording of the "light-off" reaction at 435 mμ (Fig. 2). In the absence of succinate,

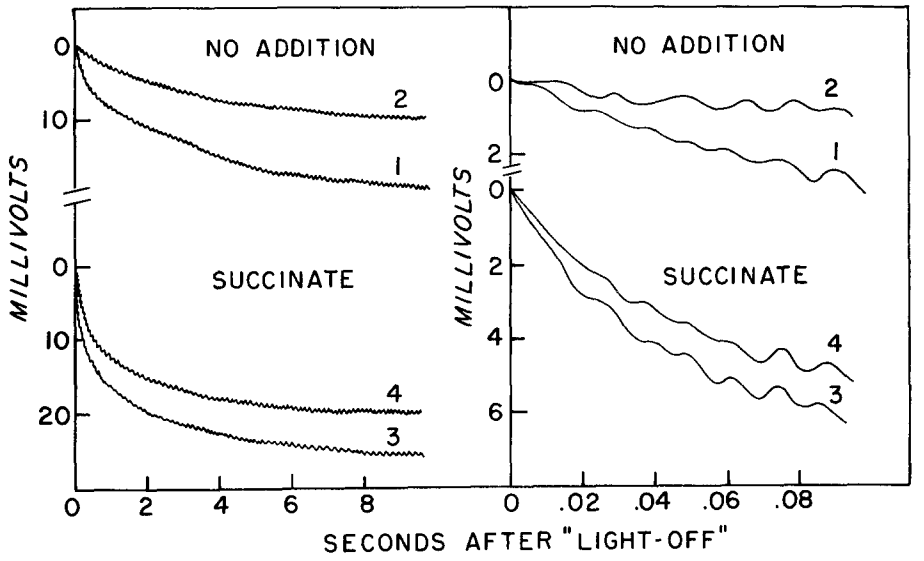

Figure 2. Effect of prolonged infrared irradiation on the "light-off" reaction at 435 mμ: variation in signal (in millivolts) with time following "light-off" after a 10 sec (curves 1 and 3) or 3 min period (curves 2 and 4) of illumination in the presence (curves 3 and 4) or absence (curves 1 and 2) of 1 mM succinate.

prolonged irradiation induced a profound depression in rate. A parallel experiment in the presence of succinate showed that the rate was only slightly depressed by prolonged irradiation.

An example of the evaluation of the kinetics of the initial 100 msec and the overall (0-10 sec) "light-off" reaction is shown in Fig. 3. After irradiation in the absence of succinate,

the reaction followed first order kinetics. When this preparation was irradiated again in the presence of succinate, the subsequent "light-off" reaction was polyphasic. The initial 100

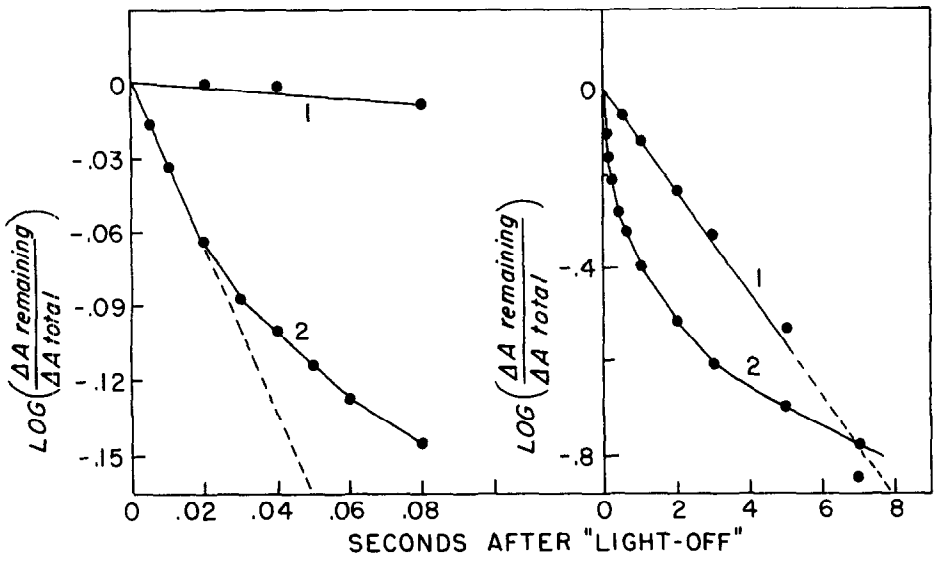

Figure 3. The effect of succinate upon the kinetics at 435 mμ, following "light-off", after 3 min illumination. In the absence of succinate (curve 1) the total change in absorbance (Δ A total) was -0.0038. Succinate (1 mM) was added and the cuvette was illuminated again for 3 min (curve 2); Δ A total was -0.0100. The values of k (for the first 20 msec) were 0.5 and 7.5 sec^{-1}, respectively (0.24 sec^{-1} for 0 to 10 sec in the absence of succinate). Δ A total was determined as the magnitude of the "light-on" reaction after a 30 sec dark "recovery" period following the radiation pretreatment.

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msec (20% of the total reaction) consisted of a rapid reaction initially following a first order course, with a distinct break in rate at 20-40 msec. Following the first 100 msec, the reaction rate rapidly declined.

It should be noted that the "light-off" kinetics at various selected wavelengths (vide infra) did not differ significantly: with succinate, 6-8 sec^{-1} (for the first 20 msec) and without succinate, 0.4-0.5 sec^{-1} (for the first 20 msec) or 0.24 to 0.27 sec^{-1} (0-10 sec).

The significance of the effect of succinate on the "light-off" reaction has been examined by determining the concentration of succinate (and DPNH) required at 435 mμ. The rate constants (for the initial phase) and the magnitude of the changes in absorbance increased in parallel fashion, requiring a minimum of 10 μM succinate or DPNH for maximum effect. For photophosphorylation (measured in air at 30° for 30 min, using the medium given in Methods and previous techniques (Geller and Lipmann, 1960), the same preparation had K_m values of 20 and 80 μM for succinate and DPNH, respectively, with a maximum velocity of 11 μmoles ATP formed/mg protein/hr (by Lineweaver-Burk plot).

It was of interest to note that TPNH (at 10 μM) did not support photophosphorylation or stimulate the "light-off" reaction. This level of TPNH did not affect the stimulatory effects of DPNH.

Discussion - The difference spectra (Fig. 1) show that prolonged irradiation of unsupplemented R. rubrum extracts leads to the loss of a portion of the "light-off" reaction. The restoration of the complete "light-off" reaction by succinate (or DPNH) would suggest that the effect of continuous illumination represents the consumption of endogenous activators. Furthermore, the requirement of photophosphorylation and the polyphasic "light-off" reaction for similar concentrations of succinate (or DPNH) suggests that common components may be involved in both absorbance changes and photophosphorylation. The slow "light-off" reaction seen in the absence of activator might then be the contribution of inactive particles or be otherwise unrelated to photophosphorylation.

Additional evidence, to be detailed in a subsequent paper,

strongly suggests that the rapid phase of the "light-off" reaction seen with succinate (or DPNH) is a measure of electron transport directly concerned with the phosphorylation process:

(1) a study of photophosphorylation in flashing light has fully confirmed the observation of Nishimura (1962) that phosphorylation occurs within a dark period of less than 100 msec following a flash; (2) a variety of inhibitors (e.g., antimycin A, oligomycin, and the carbonylcyanide phenylhydrazones), which affect the "light-off" reaction, change only the rapid phase (Geller, 1966).

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