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EFFECT OF CHLORIDE AND BENZOATE ANIONS ON THE DELAYED LIGHT EMISSION IN DCMU-TREATED SPINACH CHLOROPLASTS

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Abstract—Chloride anions, when added to DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea]-treated spinach chloroplasts, change the rate of decay of the delayed light emission in the seconds region but do not change the shape or the temperature dependence of the decay. Benzoate anions, on the other hand, change both the rate and the shape of the decay of the delayed light emission. These results are consistent with a model in which the membrane potential and the structure of the reaction center affect the decay kinetics of the delayed light emission in the seconds region.

INTRODUCTION

The delayed light emission of green plants in the seconds region may be caused by a back reaction between the primary stable reduced photoproduct Q^- and the primary stable oxidized photoproduct Z^+ of light reaction II. Using the decay kinetics of the primary back reaction found by Bennoun (1970), Mar and Roy (1974) have derived a theoretical equation for the kinetics of the delayed light in the seconds region that fits well with the experimental delayed-light-emission data of Jursinic and Govindjee (1972). If the model of Mar and Roy (1973) is correct, delayed light emission, denoted by L, is an exponential function of the activation energy E of the primary back reaction:

$$L = f\left[\exp\left(\frac{-E}{kT}\right)\right], \tag{1}$$

where k is Boltzmann's constant, and T is the absolute temperature. Crofts *et al.* (1971) have suggested that delayed light emission may be affected by membrane potential by furnishing a portion of the activation energy of the primary back reaction. This can be described by the equation

$$L = f\left[\exp\left(\frac{-(E-V)}{kT}\right)\right], \tag{2}$$

where V is the membrane potential. Membrane potential can be calculated from the Goldman equation (1943) as

$$V = kT \ln W, \tag{3}$$

where

$$W = \frac{\sum_A P_A[A^-]_0 + \sum_c P_C[C^+]_i}{\sum_A P_A[A^-]_i + \sum_c P_C[C^+]_0} \tag{4}$$

where P_A is the permeability of the anion A^-, P_C is the permeability of the cation C^+, i denotes the inside of the membrane, and 0 the outside. Substituting Eq. 3 into Eq. 2,

$$L = f\left[W \exp\left(\frac{-E}{kT}\right)\right]. \tag{5}$$

Hence from Eqs. 4 and 5, changing the salt concentration on either side of the membrane should change the rate of decay of the delayed light emission, but not the temperature dependence or the shape of the decay. In this report we have shown this to be experimentally true.

MATERIALS AND METHODS

Chloroplasts were isolated from spinach by the method of Miles and Jagendorf (1969). After isolation, the chloroplasts were resuspended in 0·40 *M* sucrose and 0·02 *M* Tris buffer at pH 7·8. All chloroplast samples had an absorbance of 0·5 at 680 nm. Delayed light emission was measured with the same apparatus and procedure as

that described by Jursinic and Govindjee (1972). The filters used with the exciting light were Corning CS 4-96 and 3-73. The intensity of the exciting light was 0·4 W m^{-2}, with an illumination time of 10 s. Measurements were made 15 min or longer after the addition of salt to the chloroplast solution.

RESULTS

In order to study the delayed light emission caused mainly by the primary back reaction, all chloroplast samples used were treated with 10^{-5} *M* DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] to block (Duysens and Sweers, 1963) electron transport between the primary reduced photoproduct Q^- and the next electron acceptor in the electron-transport chain. Delayed light emission measured from 5°C to 25°C in DCMU-treated chloroplasts suspended in 0·40 *M* sucrose is shown by the points in Fig. 1, with the decay curves normalized at one second after illumination. They have the same decay kinetics found by Jursinic and Govindjee (1972) for algae. The smooth curves are theoretical curves generated by the following approximate equation developed by Mar and Roy (1974)

$$L^{-1/2} = L_0^{-1/2}\left[\frac{\varphi_0}{\varphi_\infty} + \left(1-\frac{\varphi_0}{\varphi_\infty}\right)(1-p) \times \left(\frac{e^{-Ct}}{1+D(1-e^{-Ct})}\right)\right]^{-1/2} \times [e^{Ct} + D(e^{Ct}-1)] \quad (6)$$

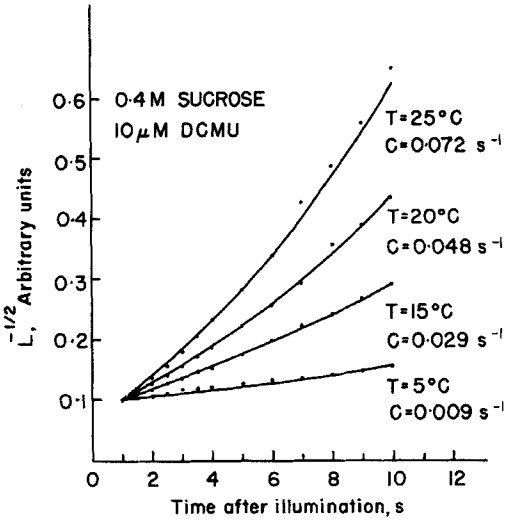

Figure 1. The reciprocal of the square root of delayed light emission ($L^{-1/2}$) as a function of time after illumination at temperatures from 5°C to 25°C. Points are experimental data and solid lines are calculated; the constant $[Q_0]D/C$ is assumed to equal 4 in all cases. Spinach chloroplasts were suspended in 0·4 *M* sucrose and 10^{-5} *M* DCMU.

where L_0 is delayed light emitted when all the Q is reduced, φ_0 is the fluorescence yield when all the Q is oxidized, φ_∞ is the fluorescence yield when all the Q is reduced, p is the probability that a quantum reaching a reaction center when Q is reduced may transfer to another center (Delosme, 1967), Q_0 is the total concentration of Q, and C and D are rate constants for the decay of Q^-;

$$\frac{d[Q^-]}{dt} = -\left\{1 + D\frac{[Q^-]}{[Q_0]}\right\} C\,[Q^-] \quad (7)$$

To calculate the theoretical curves in Fig. 1, we have assumed that $\varphi_0/\varphi_\infty = 0\,2$, and $p = 0{\cdot}5$ (Delosme, 1967). Although φ_0/φ_∞ is found to be 0·3 by Delosme (1967), we chose the value of 0·2 to account for the fluorescence that is not associated with photosystem II activity (Clayton, 1969). To fit the experimental points, D is chosen to equal 4 for all temperatures and C to equal the value indicated in the figure for each temperature measured. The theoretical curves fit the experimental points well. The delayed light emission of chloroplasts with 200 m*M* NaCl is shown by the points in Fig. 2. The theoretical curves are calculated with the same constants as for Fig. 1, with the exception of C. The value for C used at each temperature is indicated in the figure. The theoretical curves fit the experimental points well. Plotting ln C from Figs. 1 and 2 vs $1/kT$ in Fig. 3, we found that the activation energy in both cases has the same value of 0·65 eV.

The same results were found by using sodium cation instead of potassium. Different results were found, however, by using chloride instead of the

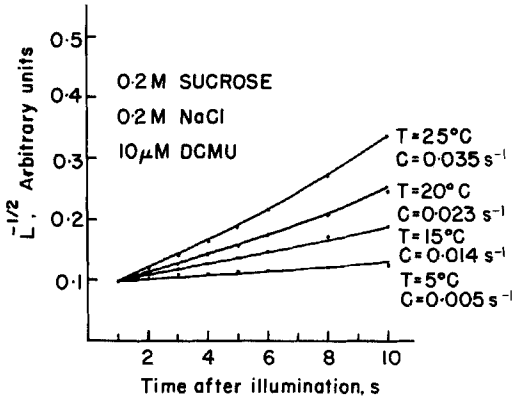

Figure 2. $L^{-1/2}$ vs time at temperatures from 5°C to 25°C in the presence of chloride. Points are experimental data and solid lines are calculated, the constant $[Q_0]D/C$ is assumed to equal 4 in all cases. Spinach chloroplasts were suspended in 0·2 *M* sucrose, 0·2 *M* NaCl and 10^{-5} *M* DCMU.

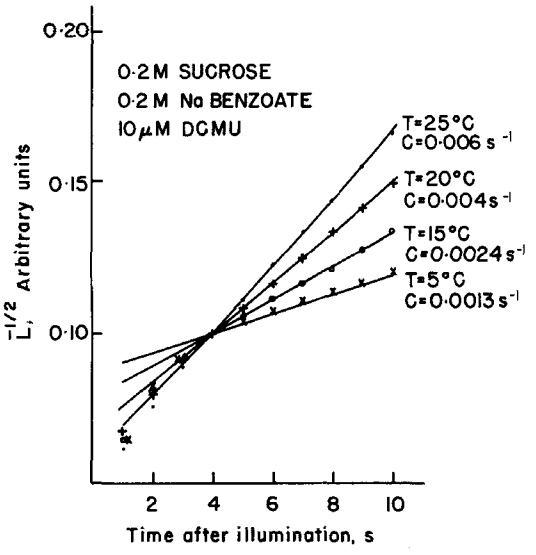

Figure 3. $L^{-1/2}$ vs time at temperatures from 5°C to 25°C in the presence of benzoate ions. Points are experimental data and solid lines are calculated; the constant $[Q_0]D/C$ is assumed to equal 19 in all cases. Spinach chloroplasts were suspended in 0·2 *M* sucrose, 0·2 *M* sodium benzoate and 10^{-5} *M* DCMU.

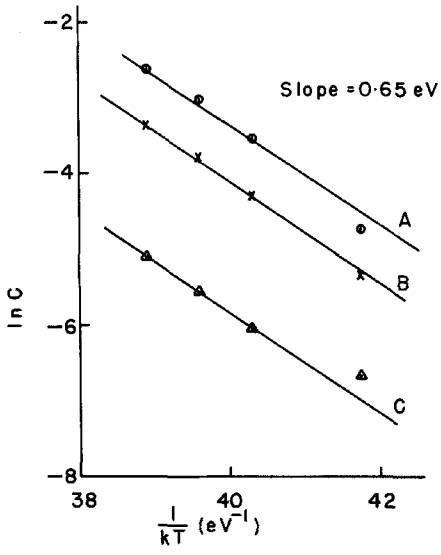

Figure 4. The rate constant of the primary back reaction *C* as a function of the reciprocal of the absolute temperature. *k* is Boltzmann's constant. Curve A: DCMU-treated chloroplasts (from Fig. 1). Curve B: DCMU-treated chloroplasts in the presence of chloride (from Fig. 2). Curve C: DCMU treated chloroplasts in the presence of benzoate ions (from Fig. 3).

benzoate anion. The experimental data for delayed light emission decay of chloroplasts suspended in 200 m*M* sodium benzoate are shown in Fig. 3. The decay curves are normalized at four seconds after illumination. The smooth curves are those generated by Eq. 6. The constants φ_0/φ_∞ and p are the same as for Figs. 1 and 2, D is set equal to 19 for all temperatures, and C at different temperatures is equal to the value indicated on the graph. The theoretical curves fit the experimental points of the decay 4s after illumination, but do not fit the decay from zero to 4s after illumination. Plotting ln C vs $1/kT$ in Fig. 4, the activation energy is again found to be 0·65 eV.

DISCUSSION

The experimental finding that the addition of sodium or potassium chloride to DCMU-treated chloroplasts changes the rate of decay of the delayed light emission, but does not change the shape of the decay curve or the temperature dependence, is consistent with the concept that addition of salt changes the membrane potential, that this in turn changes the recombination rate of the primary stable oxidant and reductant, which in turn changes the rate of delayed light emission. As shown in Eq. 5, the model also predicts that there should be no change in the shape of the decay curve or the temperature dependence, which agrees with the experimental results. The fact that the rate of decay decreases as the concentration of salt outside the chloroplast membrane increases shows that the membrane potential generated increases the activation energy needed for the primary back reaction. This agrees with Strichartz and Chance's (1971) finding that, after chloroplasts were suspended in salt solution 2 min or longer, the Δ520 nm does not go back to its initial value before the salt was added, but to a new smaller value than the initial one. This implies that the membrane potential is lowered in the new steady state, which agrees with our results.

The activation energy of 0·65 eV is in agreement with the activation energy of 0·62 eV found by Arnold and Azzi (1971) from glow curves made with cells treated with DCMU. As was pointed out by Malkin and Hardt (1973), activation energy calculated from peaks in the glow curve may be inaccurate because one has to assume an arbitrary value for the frequency factor. However, the two values are very close to each other.

Addition of sodium or potassium benzoate to chloroplasts changes both the decay rate and the shape of the decay curve. That sodium or potassium benzoate affects the delayed light emission differently than sodium or potassium chloride has also been reported by Mayne and

Hobbs (1973) and by Barber and Varley (1971). It is difficult to compare our results with theirs because our results were all done with DCMU added to the chloroplasts. This restricts Q^- to only the back reaction. Their results were all done without DCMU added. The rate of change of Q^- is more complicated. Furthermore, our results were obtained in steady-state conditions. Their results were done in non-steady-state conditions. The fact that the experimental points do not agree with the theoretical curves from zero to 4 s after illumination may be due to another component of the delayed light emission which is not caused by the primary back reaction. This is further shown by the fact that the decay after 4 s has a different temperature dependence curve than the decay from zero to 4 s. That the theoretical curves do fit the experimental points and that the decay kinetics have the same temperature dependence as chloro plasts without any salt addition, indicates that the delayed light emission component 4 s after illumination is due to the primary back reaction. In order to fit the experimental points, the constant D is changed from 4 in chloroplasts without salt to 19 for chloroplasts in benzoate. A change in D is interpreted by Mar and Roy (1974) to be a change in the entropy of the reaction center in its activated state. Hence a change in the shape of decay of the delayed light emission could be due to a change in the entropy of the reaction center, which in turn may be due to a change in the structure of the reaction center. The fact that the decay kinetics of chloroplasts in benzoate differs from chloroplasts in chloride may be due to the fact that the benzoate anions affect the structure of the reaction center as well as creating a membrane potential.

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