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THE RELATIONSHIP BETWEEN DELAYED FLUORESCENCE AND THE CAROTENOID SHIFT IN CHROMATOPHORES FROM *RHODOPSEUDOMONAS CAPSULATA*

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SUMMARY

1. The kinetics of the change in intensity of 1-ms delayed fluorescence following the onset of illumination have been studied in chromatophores of *Rhodopseudomonas capsulata* and compared with the kinetics of the light-induced carotenoid shift, and of the change in fluorescence yield. The change in intensity of delayed fluorescence, when plotted on a logarithmic scale, followed closely the kinetics of carotenoid change, but not the change in fluorescence yield.

2. The antibiotics valinomycin, nigericin and antimycin, showed qualitatively similar effects on delayed fluorescence and the carotenoid shift, but the effects on fluorescence yield were quite dissimilar.

3. The extent of the carotenoid shift, induced by KCl pulses in the presence of valinomycin was used to calibrate the change as an indicator of membrane potential. The action of nigericin on both carotenoid shift and delayed fluorescence was used to relate the two, and a linear relationship was demonstrated between the logarithm of the intensity of the delayed fluorescence and the extent of the carotenoid shift. This relationship indicated that the intensity of delayed fluorescence was proportional to the exponential of the membrane potential.

4. It is suggested that the electrical component of the transmembrane electrochemical H^+ gradient directly lowers the activation energy for emission, but that the pH component has no such effect.

INTRODUCTION

The dependence of the delayed fluorescence of chloroplasts on the high energy state was first demonstrated by Mayne [1, 2] and these observations were extended to bacterial chromatophores by Fleischmann and Clayton [3]. The latter noted parallel effects of a number of reagents on the light-induced carotenoid shift and 1-ms delayed fluorescence. Jackson and Crofts [4] showed that the carotenoid shift was proportional to the membrane potential generated across the chromatophore mem-

Abbreviations: MES, 2-(*n*-morpholino)ethane sulphonic acid.

brane by diffusion potentials, and suggested that a similar relationship might exist during illumination. In view of the similarity between the carotenoid shift and delayed fluorescence, Fleischmann and Crofts (see Fleischmann, ref. 5) proposed a mechanism of emission by which the membrane potential might act by directly lowering the activation energy for emission [6]. Observations by Fleischmann [5] support this view.

Jackson and Crofts [4] used KCl pulses in the presence of valinomycin to calibrate the steady-state membrane potential formed during illumination. This method of calibration is used in the present paper to examine the relationship between delayed fluorescence and the carotenoid shift. An exponential relationship between them would be expected if the mechanism of emission was that proposed by Crofts et al. [5, 7].

METHODS

Preparation of chromatophores

Cells of *R. capsulata* wild type (St. Louis) were grown in the medium of Sistrom [8] as previously described [9]. Chromatophores were prepared and stored essentially as described by Jackson et al. [9] except that cells were broken by extrusion through a French press at 10 tons pressure. For delayed fluorescence and fluorescence yield measurements, chromatophores were prepared in 0.1 M KCl, 20 mM 2-(*n*-morpholino)ethane sulphonic acid (MES), 5 mM $MgCl_2$, pH 6.8. For calibration of the carotenoid shift, chromatophores were prepared in 0.1 M choline chloride 20 mM MES, 5 mM $MgCl_2$, pH 6.8. Chromatophores prepared by either method showed essentially similar results.

The bacteriochlorophyll content was determined by acetone–methanol extraction as described by Clayton [10].

Measurement of delayed fluorescence

For studies of delayed fluorescence, chromatophores were suspended to about 60 $\mu g \cdot ml^{-1}$ final concentration in 2.5 ml 0.1 M KCl, 20 mM MES, pH 6.8. Delayed fluorescence was measured with a rotating sector phosphoroscope of conventional design as previously described [11], except that additional amplification and a zero offset were incorporated in the photomultiplier output circuitry.

Measurement of carotenoid shift

Carotenoid changes were observed using a dual wavelength spectrophotometer [12] with 530 and 515 nm as measuring and reference wavelengths, respectively. A Wratten 88A filter was used to cover the actinic light, and the measuring photomultiplier was screened by 1 cm of saturated $CuSO_4$ (chromatophores were suspended to 12 $\mu g \cdot ml^{-1}$ final concentration in either 2.5 ml 0.1 M KCl, 20 mM MES, pH 6.8, for light-induced carotenoid changes, or in 0.1 M choline chloride, 20 mM MES for valinomycin and KCl pulse experiments).

Measurement of fluorescence yield

Fluorescence yield was measured in a modified phosphoroscope by using a weak measuring beam chopped at about 200 cycles/s, and a D.C. actinic light source.

Fluorescence was detected at right angles to both light sources by an EMI 9659B photomultiplier connected to a lock-in amplifier (Mini-lock, MLS249A, AIM Electronics Ltd, Cambridge, Great Britain). The measuring beam was covered by Corning blue glass (9782) and neutral density filters, the actinic light by a Corning blue glass filter (9782) and the photomultiplier by a Wratten 88A filter.

MATERIALS

Simple organic and inorganic reagents were of Analar grade where obtainable, or otherwise of the highest grade commercially available. Valinomycin and antimycin were obtained from Sigma (London) Chemical Co.

Nigericin was a generous gift of Dr R. L. Harned (Commercial Solvents Corp., Ind., U.S.A.).

RESULTS

A comparison of kinetics of the carotenoid shift and delayed fluorescence

Fig. 1a shows a comparison of the rise kinetics of delayed fluorescence measured 0.8–1 ms after flashes of light in the phosphoroscope and plotted on a logarithmic scale, and the rise kinetics of the light-induced carotenoid shift on a linear scale. Fig. 1b shows a similar comparison on a longer time scale. The two processes plotted clearly follow very similar kinetics.

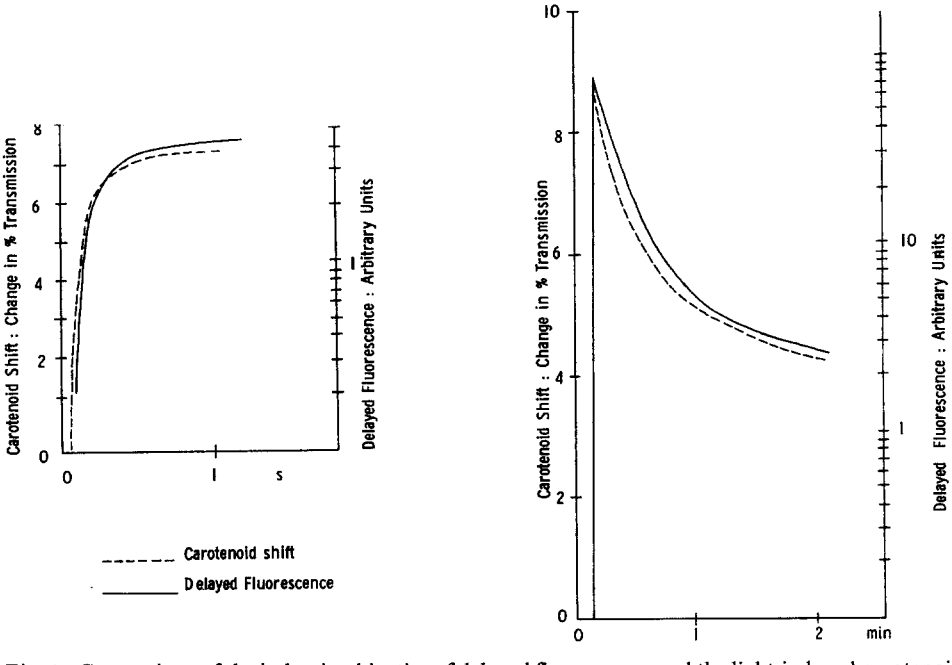

Fig. 1. Comparison of the induction kinetics of delayed fluorescence and the light-induced carotenoid shift. *R. capsulata* chromatophores suspended in 100 mM KCl, 20 mM MES, pH 6.8, to 48 μg bacteriochlorophyll/ml for delayed fluorescence, measured at 1 ms in a phosphoroscope, and 24 μg bacteriochlorophyll/ml for the carotenoid shift.

The effect of nigericin, valinomycin and antimycin on the carotenoid shift, delayed fluorescence and fluorescence

Fig. 2 shows the effect of nigericin, valinomycin and antimycin added as indicated, on the fluorescence yield, the carotenoid shift and delayed fluorescence. Nigericin stimulated the extent of both carotenoid shift and delayed fluorescence, but inhibited the fluorescence yield. Valinomycin added after nigericin inhibited the carotenoid shift and delayed fluorescence, and also the fluorescence yield. The extent of inhibition (by nigericin and valinomycin) on the fluorescence yield is equivalent to the effect of 2 μM FCCP. Valinomycin alone inhibited delayed fluorescence and the extent of inhibition was more marked than the similar inhibitory effect of valinomycin on the carotenoid shift [4]. Addition of antimycin after valinomycin, or valinomycin together with nigericin, inhibited the carotenoid shift and delayed fluorescence but stimulated the fluorescence yield.

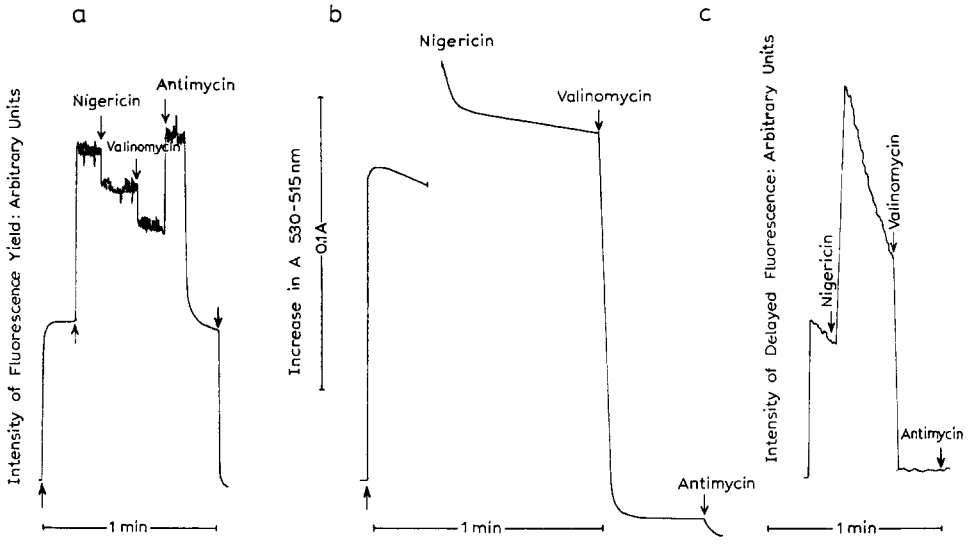

Fig. 2. Comparison of effects of ionophores and antimycin on fluorescence yield, carotenoid shift and delayed fluorescence of chromatophores of *R. capsulata*. (a) Fluorescence yield measured with a very weak chopped light, chromatophores (7.3 μg bacteriochlorophyll/ml) were suspended in 2.5 ml of 100 mM KCl, 20 mM MES, pH 6.8, 20 °C. Nigericin (0.2 μM), valinomycin (2 μM) and antimycin A (4 μM) were added where indicated; (b) Carotenoid change. Conditions as in (a) but 30 μg bacteriochlorophyll/ml; (c) Delayed fluorescence measured at 1 ms in a phosphoroscope. Conditions as in (a), but 44 μg bacteriochlorophyll/ml.

The relationship of delayed fluorescence to the carotenoid shift

It has been proposed by Jackson and Crofts [4] that the carotenoid shift is a measure of the membrane potential generated as a result of light-induced H^+ uptake. They calibrated the extent of the light-induced carotenoid shift in mV by observing the extent of the carotenoid shift induced in the dark by KCl pulses in the presence of valinomycin [4]. Briefly, addition of valinomycin to chromatophores containing K^+ and suspended in choline chloride would give rise to a diffusion potential across the membrane negative with respect to the inside, while addition of

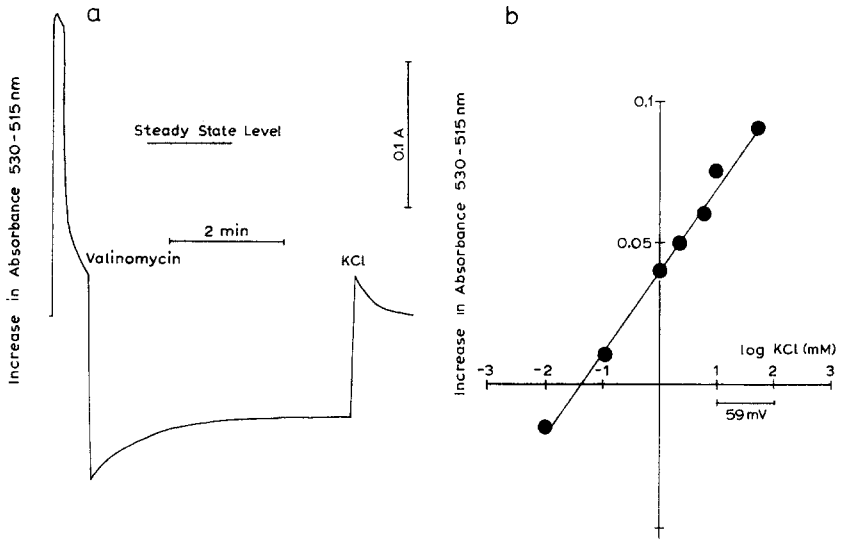

Fig. 3. Carotenoid changes induced by light and by K^+ gradients. (a) Chromatophores of *R. capsulata* (30 μg bacteriochlorophyll/ml) were suspended in 100 mM choline chloride, 20 mM MES, pH 6.8. Valinomycin (2 μM) and KCl (50 mM) were added where indicated. The steady-state level is also shown; (b) Plot of carotenoid change against K^+ concentration from experiments like those in (a).

KCl to valinomycin-treated chromatophores equilibrated in a choline chloride medium, would give rise to a potential positive with respect to the inside of the membrane. The carotenoid shifts associated with these changes are shown in Fig. 3a, together with a light-induced shift for comparison. The correlation between the carotenoid changes and the membrane potential induced by ionic gradients is shown in Fig. 3b. The extent of the carotenoid shift induced by the KCl pulse is plotted against the logarithm of the KCl concentration. From the Nernst relationship, it can be seen that the carotenoid change is proportional to the mebrane potential. The slope of the graph gives the spectral change per decade of change of K^+ concentration gradient, or (at 25 °C) per 59 mV of potential difference. From these results, a peak height of the light-induced change of 415 mV can be calculated and a steady-state value of 284 mV, as previously reported [13].

If nigericin (0.2 μM) was added either before illumination or at any point on the decay following the transient peak at the onset of illumination, a stimulation of the carotenoid change equivalent to approx. 50 mV was seen, and the new steady-state value was higher by an amount equivalent to approx. 50 mV, than in the absence of nigericin. Nigericin also stimulated the intensity of 1-ms delayed fluorescence when added either before illumination or at any point during the subsequent kinetics. If the peak height and the steady state of the delayed fluorescence before and after addition of nigericin were compared with the extent of the carotenoid shift under similar conditions, then the change in level of the delayed fluorescence could be related to the change in membrane potential indicated by the change in the carotenoid shift.

Fig. 4 shows the results of such an experiment where the logarithm of the

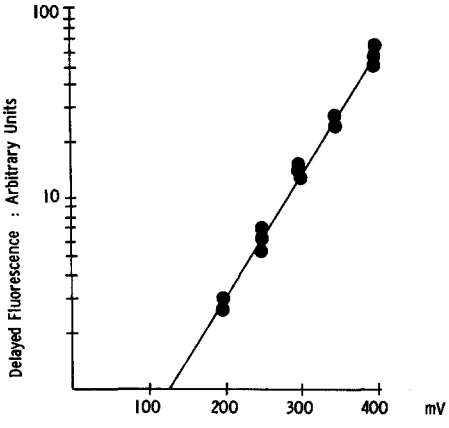

Fig. 4. The relationship between intensity of delayed fluorescence and the light-induced membrane potential. See text. Chromatophores suspended to 48 μg bacteriochlorophyll/ml in 100 mM KCl, 20 mM MES, pH 6.8. Additions of 0.2 μM nigericin.

extent of the delayed fluorescence has been plotted against the potential calibrated from the extent of the carotenoid shift. The relationship appears to be linear, and intercepts the abscissa at a point where the delayed fluorescence cannot be distinguished from the noise level.

DISCUSSION

Crofts et al. [7] suggested a model mechanism for delayed fluorescence, which was a generalisation of earlier ideas [14, 15]. This involved a dependency of the extent of delayed fluorescence on the free energy difference between the primary donor (Z/Z^+) and acceptor (Q/Q^-) of the photochemical reaction, which were envisaged as being situated on opposite sides of the membrane, so that the electrical work done in the reaction was conserved as a membrane potential. The primary donor and acceptor were assumed to be in equilibrium with secondary donor (DH/D) and acceptor (AH/A) pools which are redox couples of the H-carrier type. Treatment of this model by transition-state theory [7] led to the relationship:

$$L = \phi_{LF}\, k_a(Q)(Z) \exp\left(-(E^* - (\Delta E^{\#}_{D-A} + \Delta\Psi_{i-0} - Z\,\Delta pH_{i-0}))/kT\right) \quad (1)$$

where L is the luminescence intensity; ϕ, the fluorescence yield; Q, the primary electron acceptor; Z, the primary electron donor; D and A secondary donor and acceptor pools; E^*, the singlet energy level; ΔE^*, the redox potential difference between D and A; $\Delta\Psi$, the membrane potential; ΔpH, the pH gradient; k, the Boltzmann constant and T the absolute temperature. This simplifies to:

$$L\alpha \exp\left(\Delta E^{\#}_{D-A} + \Delta\Psi_{i-0} - Z\,\Delta pH_{i-0}\right) \quad (2)$$

We have demonstrated in this paper that

$$L\alpha \exp, \Delta\Psi_{i-0} \quad (3)$$

However, there is no indication of any involvement of a pH gradient in the energy

dependence of the delayed fluorescence, since nigericin stimulated rather than inhibited the extent. This may indicate some difference in the relation between primary and secondary donor and acceptor pools in chromatophores and chloroplasts [11, 16].

Lavorel [17] and Clayton [18] have shown that, under appropriate conditions, the intensity of the delayed fluorescence was proportional to the yield of the live, or varying prompt fluorescence in chloroplasts. This relationship clearly does not apply in chromatophores under the conditions of our experiment. However, as is shown in Eqn 1, various other components may contribute to changes in the intensity of delayed fluorescence. Our experiments suggest that, in addition to the free energy contributed by the redox changes involved in the reaction, a membrane potential of about 150 mV must exist before the delayed fluorescence intensity exceeds the "noise" level of our apparatus. In Eqn 1, the various energetic terms contributing to the reduction of the activation energy for delayed fluorescence all appear in an exponential term, so that their separate contributions cannot easily be distinguished. From the close correlation between the kinetics of the logarithm of delayed fluorescence intensity change and the carotenoid change it might be suggested that other terms in Eqn. 1 apart from the membrane potential rapidly reach a constant value, so that their changes do not contribute to the kinetics observed. If this were the case the slope of the curve of Fig. 4 would be expected to yield the proportionality factor between the logarithm of the delayed fluorescence intensity and the membrane potential changes, viz.:

$$L = \phi_{LF}\, k_a(Q)(Z) \exp\left(-(E^* - (E^{\#}_{D-A} + \Delta\Psi_{i-0} - Z\,\Delta pH_{i-0}))n/kT\right)$$
$$= K^1 \exp\left(n\Delta\Psi_{i-0}/kT\right)$$

where

$$K^1 = \phi_{LF}\, k_a(Q)(Z) \exp\left(-(E^* - (E^{\#}_{D-A} - Z\,\Delta pH_{i-0}))n/kT\right)$$
$$L_1/L_2 = \exp\left(n(\Delta\Psi_1 - \Delta\Psi_2)/kT\right)$$
$$\Delta\Psi_1 - \Delta\Psi_2 = 2.303\, kT/n \log_{10} L_1/L_2$$

Since our model anticipates the movement of a single equivalent of charge across the membrane on reversal of the photochemical reaction, we would expect a change in potential of approx. 60 mV to give a 10-fold change in intensity of delayed fluorescence. In fact from the slope of Fig. 4 it can be seen that a change in potential of approx. 140 mV was required to give a 10-fold change in intensity. We do not wish to lay too much emphasis on these values, since our apparatus did not enable us to measure the carotenoid and delayed fluorescence intensity changes under identical conditions. However, the results may indicate that the electrical field available to drive the photochemical reaction in reverse is less than that indicated by the carotenoid change. If the carotenoid change indicated a transmembrane potential, this would suggest that the photochemical reaction does not occur across the full width of the membrane.

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