A METHOD OF ESTIMATING THE MAGNITUDE OF THE LIGHT-INDUCED ELECTRICAL POTENTIAL ACROSS THE THYLAKOID MEMBRANES

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1. Introduction

Millisecond delayed light emission from spinach chloroplasts is sensitive to their high-energy state [1]. It has been argued that this sensitivity is partly due to the establishment of a light-induced electrical potential across the thylakoids [2]. The implication that the intensity of delayed light could be enhanced by developing a membrane potential had come from earlier experiments in which chloroplasts were subjected to salt gradients 10 sec after terminating the illumination [3]. More recently it has been shown that similar salt treatments can also cause a transient increase in msec delayed light [4, 5]. The object of this communication is to present a quantitative analysis of the salt induced msec delayed light with the view to obtaining a method of estimating the electrical potential created across the thylakoids of illuminated chloroplasts.

2. Materials and methods

Isolation of broken spinach chloroplasts was essentially the same as that described previously [6] except the sucrose washed preparations were finally resuspended in a small volume of medium consisting of 0.33 M sucrose and 5 mM TES* brought to pH 7.0 with 2 mM KOH. Chlorophyll concentrations were determined by the method of Arnon [7]. Prior to experimenting an appropriate quantity of stock was diluted with the above suspending medium to give a total chlorophyll concentration of 10 μg/ml.

The intensity of 1 msec delayed light was continuously measured with a rotating sector phosphoroscope similar to that used by Clayton [8]. The illumination took place for 2 msec and measuring time was 0.2 msec after a 1 msec delay. Four measurements were made per cycle. The light source was a 150 W quartz-iodine lamp used with a heat filter (Baltzer Calflex C) giving an intensity of 3×10^4 ergs $cm^{-2} sec^{-1}$ at the cuvette. The delayed light emitted was detected with an EMI 9558 photomultiplier equipped with a 2 mm Schott RG 665 filter. The current pulses from the photomultiplier were passed through a diode pump circuit with a time constant of 0.1 sec and recorded on a Honeywell or Rickadenki chart recorder.

Various additions were made during measurement by rapidly injecting 100 μl of the appropriate stock into 3 ml of suspension contained in the cuvette. Injections were made using a syringe with its needle inserted through a light-tight rubber diaphragm. This procedure gave a total mixing time of less than 200 msec and reproducible signals were obtained for particular treatments.

3. Results and discussion

In recent papers [2, 6, 9] it has been considered that the establishment of an electrical gradient across the thylakoids of the correct polarity (inside positive) can decrease the activation energy for delayed light. From this the msec emission (L) has been equated with the exponential of the potential ($\Delta\psi$)

$$\text{i.e., } L \alpha \exp(\Delta\psi) \qquad (1)$$

* *Abbreviations*

TES: N-Tris (hydroxymethyl)-methyl-2-aminoethane-sulphonic acid.

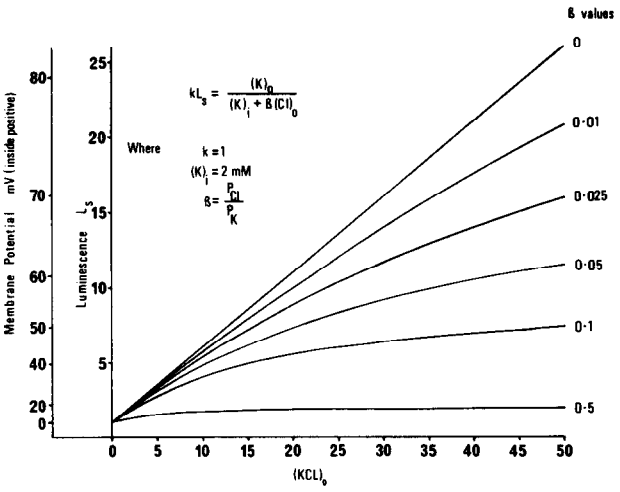

Fig. 1 Theoretical curves of initial luminescence intensity L_s induced by KCl additions for several values of β plotted according to the equation given, where k=1, $\beta = P_{Cl}/P_K$ and $(K)_i$ is assumed to be 2 mM. The magnitude of the diffusion potentials which would develop from the concentration and permeability parameters considered are also shown.

The electrical gradient ($\Delta\psi$) could either result from light-induced charge transfer processes ($\Delta\psi_1$) or from a diffusion potential created by a sudden salt addition to the chloroplast suspension ($\Delta\psi_s$);(see [3]). The size of the initial diffusion potential created after, for example, a rapid KCl addition can be estimated, assuming that K^+ and Cl^- are the main diffusing ions, from an equation derived from the Goldman theory [10].

$$\Delta\psi_s = \frac{RT}{F} \ln \frac{P_K [K]_o + P_{Cl} [Cl]_i}{P_K [K]_i + P_{Cl} [Cl]_o} \qquad (2)$$

where R, T and F have their usual meanings and P_K and P_{Cl} are the permeability coefficients.

For room temp eq. 2 becomes:

$$= 58 \log_{10} \frac{[K]_o + \beta [Cl]_i}{[K]_i + \beta [Cl]_o} \text{mV} \qquad (3)$$

where $\beta = P_{Cl}/P_K$.

In order to apply eq. 3 well washed broken chloroplasts were suspended in TES-sucrose buffer known to contain 2 mM K^+. Assuming that the initial $[Cl]_i = 0$ and $[K]_i = [K]_o = 2$ mM after a dark and cold incubation in the K^+-containing buffer for 30 min (there is support for this from flame photometric analyses) then the sudden establishment of a KCl gradient would develop a membrane potential given by eq. 4.

$$\Delta\psi_s = 58 \log \frac{[K]_o}{2 + \beta [Cl]_o} \qquad (4)$$

Substituting this equation into eq. 1

$$kL_s = \frac{[K]_o}{2 + \beta [Cl]_o} \qquad (5)$$

where k is a proportionality constant.

Clearly eq. 5 predicts that the intensity of the KCl-stimulated signals (L_s) are dependent both on the relative permeability term and the concentration of the KCl added. Theoretical curves derived from eq. 5 for various values of β and different KCl additions are shown in fig. 1. Only when β=0 is there a straight line relationship. In all other cases a curve is obtained which corresponds to a particular β value. Thus, it is possible to obtain an experimental curve to find the actual value of β for a chloroplast preparation. This approach, however, may be complicated if the msec delayed light emission already contains a component associated with a light induced membrane potential. In this case:

$$L \alpha \exp (\Delta\psi_1 + \Delta\psi_s) \qquad (6)$$

It is therefore necessary to reduce $\Delta\psi_1$ to zero and I have assumed that this can be accomplished, in the absence of light-induced ionic gradients, by adding valinomycin to the suspension. In fact, the addition of 1 μM valinomycin considerably reduces the intensity of steady-state msec delayed light as shown in trace a of fig. 2. Higher concentrations of this antibiotic caused no additional inhibition. Trace a also shows that after the valinomycin treatment an injection of KCl, to give a concentration of 50 mM, resulted in a transient increase in the emission. Assuming that the height of the KCl-induced spike represents the initial magnitude of the diffusion potential created, and that in the presence of 1 μM valinomycin $\Delta\psi_1 = 0$, then it is possible to calibrate the 50 mM KCl signal in terms of electrical units. This was

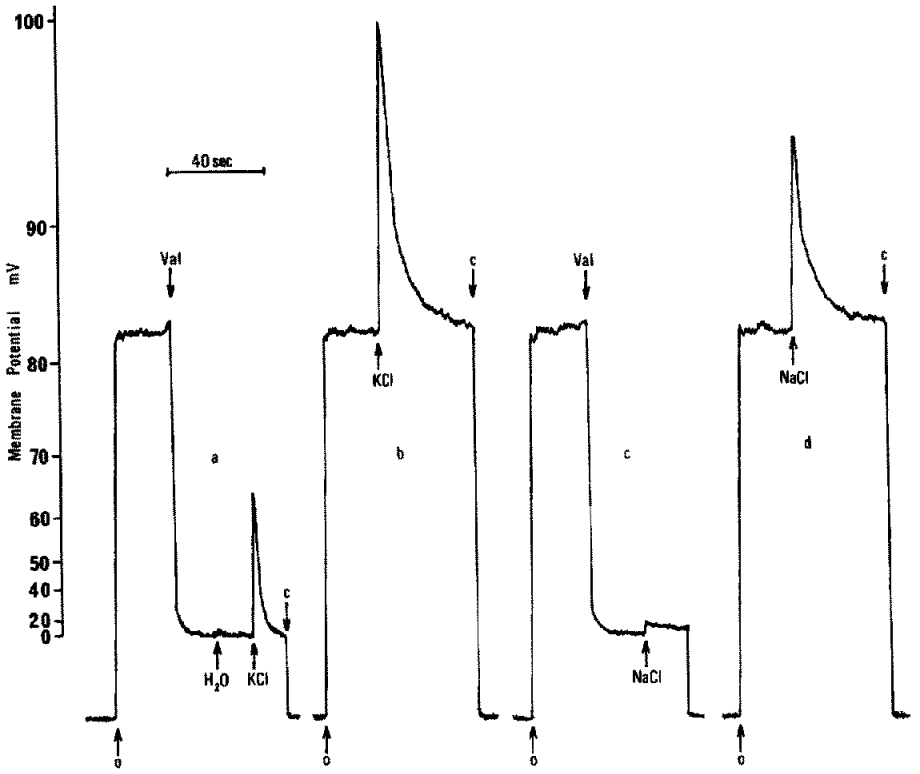

Fig. 2. The effect of valinomycin, KCl and NaCl additions on steady-state 1 msec delayed light emission from broken spinach chloroplasts. The cuvette contained 3 ml of suspension which was pre-illuminated for 2 min before opening the shutter across the photomultiplier. The various additions are indicated by arrows and were made as outlined in Methods. The valinomycin concentration was 1 μM and KCl and NaCl additions gave a final external concentration of 50 mM. Also shown is a membrane potential scale calculated using the data given in fig. 3. The opening and closing of the photomultiplier shutter is indicated by o and c, respectively

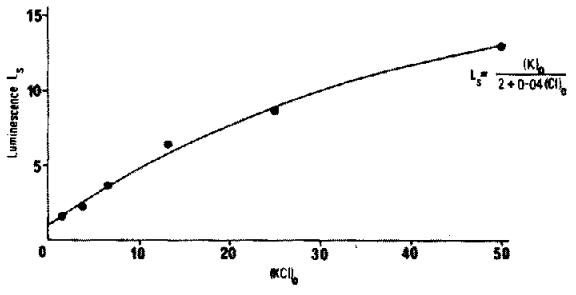

Fig. 3 Values of the initial height of the KCl-induced signals L_S obtained with chloroplasts treated with 1 μM valinomycin for various concentrations of KCl. The curve has been drawn according to the equation shown in the figure.

done by measuring the size of the signals induced with valinomycin-treated chloroplasts by a range of KCl concentrations and the results are shown in fig. 3. The curve giving the best fit for the experimental points corresponded to a β value of 0.04. Substituting this value into eq. 3 gave the maximum potential induced by a 50 mM KCl pulse as 64.5 mV. With this information it was possible to draw up the membrane potential scale shown in fig. 2. It can be seen that with this particular chloroplast suspension the valinomycin sensitive component represented an electrical potential of 83 mV (inside positive). Other chloroplast preparations gave values which fell between 75 and 105 mV under non-phosphorylating conditions and in the absence of added cofactors.

Trace b in fig. 2 shows the effect of a 50 mM KCl

pulse in the absence of valinomycin. At first sight the size of the salt-induced signal would seem larger than expected. However, because of the logarithmic relationship between L and $\Delta\psi$ a small additional change of potential above $\Delta\psi_1$ causes a considerable increase in luminescence intensity. Actually the potential developed by the 50 mM KCl addition was only 17 mV which represents a P_{Cl}/P_K of 0.3. Comparing this value with that obtained after valinomycin treatment indicates that this antibiotic increased the K^+ permeability by 7.5 times.

Under some conditions, such as low light intensity and ageing of the suspensions, the valinomycin sensitive component of msec emission can be relatively small [5] and in these cases, as would be predicted from the logarithmic relationship, the KCl luminescence signals are larger in the presence of valinomycin than in its absence.

It can be seen in trace c of fig. 3 that 50 mM NaCl additions to chloroplasts treated with valinomycin gave very small signals. The NaCl signal was more readily detected in the absence of the antibiotic. These results are expected from the above theoretical arguments and in fact, the 50 mM NaCl signal corresponded to a potential of about 12 mV. Since this gives a P_{Na}/P_{Cl} of 0.45 it seems that the chloroplasts showed a slight selectivity between K^+ and Na^+, corresponding to P_K/P_{Na} of 1.5.

Overall the results are consistent with the theoretical concepts presented and indicate that msec delayed light emission can be used as a means of estimating the size of electrical potentials across the thylakoid membranes. Similar efforts to carry out quantitative analyses of the 515 nm shift, also thought to act as an indicator of thylakoid potentials [11], has not yet been successful [12]. The main problem encountered with this approach being scattering changes induced by salt additions during the calibration procedure [13, 14]. Millisecond delayed light is not hampered by this difficulty and the analyses presented in this communication give strong support to Witt's contention [15] that a membrane potential of about 100 mV (inside positive) can be developed across the thylakoids by light-induced charge transfer processes under steady-state conditions.

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References

[1] B.C. Mayne, Photochem. Photobiol. 6 (1967) 189.
[2] C.A. Wraight and A.R. Crofts, European J. Biochem. 19 (1971) 386.
[3] J. Barber and G.P.B. Kraan, Biochim. Biophys. Acta. 197 (1970) 49.
[4] J. Barber and W.J. Varley, Inter. Congress of Photosynthesis Research, Stresa, 1971.
[5] J. Barber and W.J. Varley, Nature 1971, in press.
[6] J. Barber and W.J. Varley, J. Exp. Botany 22 (1971).
[7] D.I. Arnon, Plant Physiol. 24 (1949) 1.
[8] R.K. Clayton, Biophys. J. 9(1969) 60.
[9] A.R. Crofts, C.A. Wraight and D.E. Fleischmann, FEBS Letters 15 (1971) 89.
[10] D.E. Goldman, J. Gen. Physiol. 27 (1943) 37.
[11] W. Junge and H.T. Witt, Z. Naturforsch. 236 (1968) 244.
[12] G.R. Strichartz and B. Chance, Biochim. Biophys. Acta. (1971) in press.
[13] J. Barber, unpublished observation.
[14] W. Junge, personal communication.
[15] H.T. Witt, B. Rumberg, W. Junge, G. Doring, H.H. Stiehl, J. Weikard and C.H. Wolff, Progress in Photosynthesis Research 3 (1969) 1361.