CONTROL OF CHLOROPHYLL FLUORESCENCE BY THE DIFFUSE DOUBLE LAYER

J. BARBER and J. MILLS
Botany Department, Imperial College, London, S.W. 7. UK

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1. Introduction

It is now some years since it was first shown that the addition of metal cations to broken chloroplast preparations (those without outer membranes) increases the steady-state fluorescence yield [1–3]. Since in these experiments the chloroplasts had been treated with DCMU the changes were not associated with changes in the state of the photosystem two (PS2) traps and in fact have been implicated with a decrease in spillover of excitation energy from PS2 to photosystem one [2, see 4]. Recently it has been found that the cation induced fluorescence changes are more complex [5] showing antagonistic effects between low concentrations of mono- and di-valent cations. Although these cations induced effects are now well established and are important in terms of understanding the interrelationships between chlorophyll fluorescence and ionic control in the in vivo chloroplast [4,6], no satisfactory explanation has been proposed for the underlying mechanism. In this paper we present experiments and discussion aimed at getting a better understanding of the physical process involved. Cation specificity and competition studies have been interpreted in terms of simple diffuse double layer theory. Application of the Gouy-Chapman equation for solutions of mixed electrolytes indicate that the fluorescence changes are not correlated with changes in surface potential but seem to reflect the total positive charge immediately adjacent to the membrane surface.

2. Experimental

Intact chloroplasts were isolated by the method of Stokes and Walker [7] from leaves of spinach, English Cos lettuce and peas. Results were essentially identical whatever the source of material used. Before experimenting the outer membrane of the chloroplasts were disrupted by subjecting the organelles to osmotic shock with distilled water contained in the measuring cuvette. This was followed by addition of double strength suspending medium. The final suspending medium consisted of 0.1 M Sorbitol brought to pH 7.0 with Tris base, which corresponded to about 0.15 mM Tris.

Chlorophyll fluorescence was induced by a broad band blue/green beam transmitted by 2 mm Schott BG18 and 2 mm Schott BG38 broad band filters together with a Balzer Calflex C filter to give an intensity of 80 J m^{-2} sec^{-1} at the cuvette. The fluorescence emission was detected at right angles with an EMI 9558B photomultiplier, screened by a Balzer 695 nm interference filter and appropriate Schott red cut-off filter to eliminate scattered actinic light. Measurements were made with chloroplasts suspended in a 10 × 10 mm glass cuvette at a chlorophyll concentration of 5 to 15 μg ml^{-1}.

3. Results

The essential features of the cation induced fluorescence changes are shown in fig.1. The osmotically broken chloroplasts have been suspended in a medium which is essentially free of cations (see Material and methods). Initially the steady-state fluorescence level is maximum but the introduction of a low concentration of K^+ brings the fluorescence to a lower level. This lower fluorescence yield corresponds to the starting level of the experiments reported in the earlier papers [1–3]. As shown in fig.1b further addition of high concentrations of monovalent cations under

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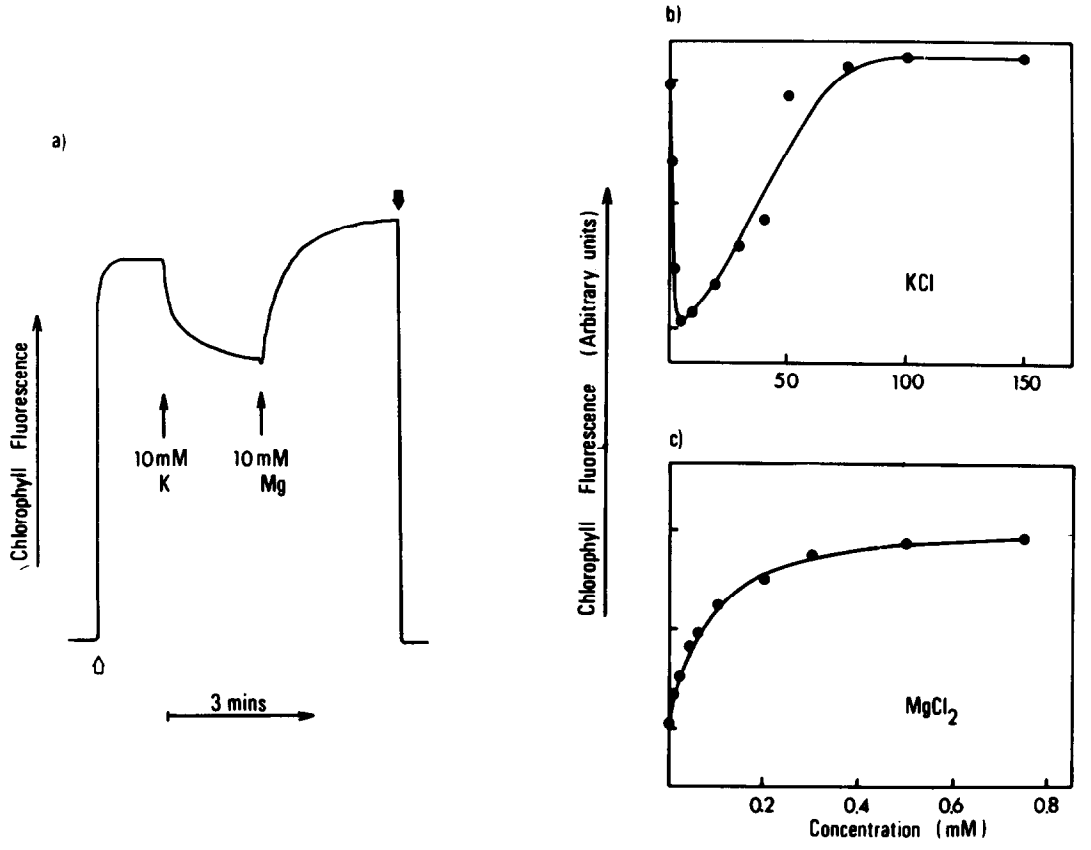

Fig.1. Effect of cations on chlorophyll fluorescence from isolated broken chloroplasts treated with 10^{-5} M DCMU. (a) Antagonistic effect of 10 mM KCl and 10 mM $MgCl_2$. (b) Concentration curve for monovalent cations, K^+. (c) Concentration curve for divalent cation, Mg^{2+}, using chloroplasts washed with 0.5 mM sodium EDTA during isolation.

these conditions increases the yield back to the higher level. A similar increase in the fluorescence yield is also seen on adding divalent cations but the levels required for maximum effect are much lower (e.g. 5 mM). The initial starting level for the experiments described in fig.1a and b depend on whether the chloroplasts have been exposed to monovalent cations during isolation. For instance, washing the preparations with 0.5 mM sodium EDTA greatly reduces the initial yield of fluorescence and under these conditions, as shown in fig.1c, very low concentrations of divalent cations restore the fluorescence to the high yield.

The fluorescence quenching induced by the addition of low levels of monovalent cations is rather unspecific. Essentially there is no difference seen between all the alkali metal cations, NH_4^+ and organic cations like choline and lysine. The subsequent rise in fluorescence observed on increasing the monovalent cation content is always seen and again there is little or no difference between the effectiveness of various inorganic and organic species. The fluorescence rise induced by divalent cations is also rather unspecific with no striking difference between the alkaline earth cations. The actual value of the C½ for the divalent cation induced rise is however dependent on the monovalent cation composition of the medium as shown in fig.2. Significantly the antagonistic or competitive effect between monovalent and divalent cations on fluorescence yield can also be seen with L-lysine (monovalent) and the related dipeptide L-lysyl-L-lysine (divalent).

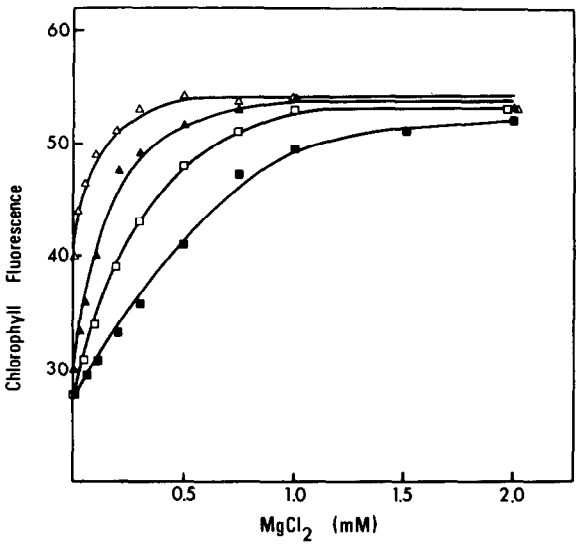

Fig.2. Competitive effect of K^+ on the Mg^{2+} stimulated increase in fluorescence yield from DCMU treated chloroplast washed with 0.5 mM sodium EDTA during isolation. Open triangles, no KCl; solid triangles, 1 mM KCl; open squares, 3 mM KCl; solid squares, 10 mM KCl.

4. Discussion

The notable feature of the above results is the significant difference seen between monovalent and divalent cations on fluorescence yet apparent lack of specificity observed between cations within these two broad groups. Although in the past these cation induced fluorescence changes have been accounted for by binding processes it seems unlikely that this is the case. On the other hand the changes are obviously not related to the ionic strength of the medium, since at low concentrations the effect of monovalent and divalent cations are antagonistic. The importance of the net charge carried by the cation suggests that chlorophyll fluorescence is controlled by electrical interactions with the chloroplast membranes. Such changes would be via the double layer which apparently exert microconformational changes in the membrane.

Below we have attempted to apply classical double layer theory to explain the observations. There are a number of indications that the thylakoid membrane is negatively charged (e.g. [8,9]). The density of the surface charges is unknown but a crude estimate can be made. It has been estimated that 1 μg of chlorophyll corresponds to about 16.7 cm^2 or 16.7×10^6 $Å^2$ [10]. According to Gross and Hess [11] the total negative site capacity is 1.2 μequiv. mg chl^{-1}. This then corresponds to 1.2 nequiv. of negative charges per 16.7 cm^2 which is equivalent to one negative charge per 230 $Å^2$ or 6.9 μCoulombs cm^{-2}. Such a negatively charged surface suspended in a medium containing electrolyte would be expected to create a diffuse double layer [12]. Thus an appreciable electrical potential can exist near the surface and decrease with increasing distance x in a direction perpendicular to the plane of the interface. The theory of double layers can be complex but one of the simplest and widely adopted approaches is that of Gouy and Chapman (see [12]). The Gouy-Chapman theory links the Boltzmann and Poisson equations and leads to an expression which relates the membrane surface potential ψ_0 (i.e. when $x = 0$) to the surface charge density q and the electrolyte concentration of the bulk solution C_0. For a Z-Z type electrolyte:

$$q = \pm \left[\frac{RTDD_0}{2\pi} \sum_i C_0^i \left(e^{\frac{-ZF\psi_0}{RT}} - 1 \right) \right]^{1/2} \quad \text{(Eq.1)}$$

where R is the gas constant, T the absolute temperature, D the relative dielectric constant, D_0 'diabattivity' of free space, Z the charge on the ion and F the Faraday. If the charged surface is suspended in a medium containing only one Z-Z electrolyte such as KCl or $MgSO_4$ then Eq. 1 reduced to

$$q = 2A\, C_0^{1/2} \sinh \left(\frac{ZF\psi_0}{2RT} \right) \quad \text{(Eq. 2)}$$

where

$$A = \left[\frac{RTD\,D_0}{2\pi} \right]^{1/2}$$

Making the appropriate numerical substitutions for 25°C Eq. 2 may be written

$$q = 11.74\, (C_0)^{1/2} \sinh \left(\frac{Z\psi_0}{51.7} \right) \quad \text{(Eq. 3)}$$

where q is in μCoulombs/cm^2, C_0 is in mol/lit and ψ_0 in mV.

Taking q = 6.93 μCoulombs/cm^2 it is found that in the presence of 10 mM monovalent cations ψ_0 = 127 mV. On increasing C_0 to 100 mM ψ_0 reduces to 71.4 mV. Thus it can be seen that as the concentration of electrolyte in the medium is increased the potential at the surface relative to the bulk becomes progressively smaller. It is pertinent to note that to obtain ψ_0 of 71.4 mV using divalent cation the external concentration needs only to be 5.6 mM. Thus the differential screening effect of monovalent and divalent cations could account for the difference in the concentration requirements of these two groups of ions on the fluorescence rise. Clearly the picture can not be that simple since this does not explain the lowering of fluorescence on adding low concentrations of monovalent cations. To understand the fluorescence lowering and antagonistic action of low concentrating of monovalent and divalent cations it is necessary to use equations appropriate for mixed solutions of electrolytes. Substitutions into Eq. 1 for a mixture of monovalent electrolyte of concentration C'_0 and divalent electrolyte of concentration C''_0 yields.

$$\frac{q^2}{A^2} = 4\,C''_0\,(\cosh^2\frac{F\psi_0}{RT} - 1) + 2\,C'_0\,(\cosh\frac{F\psi_0}{RT} - 1) \qquad \text{(Eq. 4)}$$

This can be arranged into the quadratic

$$4\,C''_0\,\cosh^2\frac{F\psi_0}{RT} + 2\,C'_0\,\cosh\frac{F\psi_0}{RT} - (4\,C''_0 + 2\,C'_0 + \frac{q^2}{A^2}) = 0 \qquad \text{(Eq. 5)}$$

Although Eq. 5 can be used to calculate ψ_0 for different values of C''_0, C'_0 and q it does not clearly explain the opposing effects of low and high concentrations of monovalent cations on fluorescence or the antagonism seen between low concentrations of monovalent and divalent cations. This is because ψ_0 is always decreased as the electrolyte composition is increased (see fig.3a). However, to attempt to explain the fluorescence changes it is necessary to use the calculated ψ_0 values to estimate the total positive diffusible charge at the membrane surface. Our

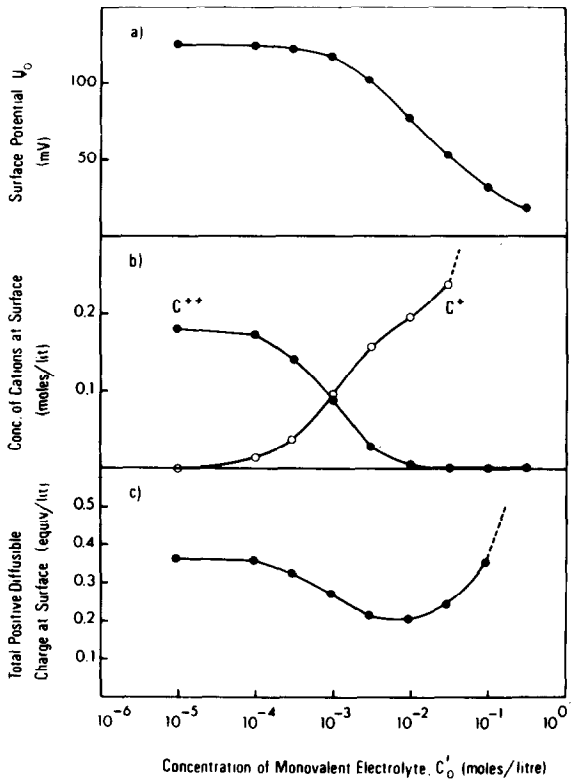

Fig.3. Calculated curves of (a) ψ_0, (b) C''_s and C'_s and (c) total positive charge at surface, using Equations 5 and 6 assuming C''_0 = 10^{-5} M and q = 2.5 μCoulombs/cm^{-2}.

experiments indicate that when the thylakoid membranes are isolated and suspended in a cation free medium the fluorescence is high because of residual divalent cations held closely to the negative charged surface during isolation (i.e. because of a large ψ_0). To use Eq. 5 we have therefore taken C''_0 to be very low using a value of 10^{-5} M. Now by changing C'_0 from 10^{-5} M to 3 X 10^{-1} M the value of ψ_0 drops (see fig.3a) and by applying the Boltzmann or Nernst expression

$$C_s = C_0 \exp(\frac{-ZF\psi_0}{RT}) \qquad \text{(Eq. 6)}$$

the surface monovalent and divalent cation concentrations (C'_s and C''_s) can be calculated. The general effect is that as the monovalent cations are added to the bulk solution the surface monovalent level

increases while for divalent cation the level drops at the surface (see fig.3b). To explain the antagonism between monovalent and divalent cations, and particularly the dip in the fluorescence level observed as the bulk monovalent cation level is increased, it is necessary to calculate the concentration of total positive charge at the surface. When this is done a dip in the curve is seen and for this to occur in the same regions as the fluorescence dip it is necessary to use a value of q of 2.5 μCoulombs cm^{-2} (a slightly lower value than that estimated above from the data of Gross and Hess).

Bearing in mind the limitation of the Gouy-Chapman theory (e.g. ignores finite size of ions and changes in activity coefficients) our analysis suggests that the high fluorescence yield is only observed when the total surface positive charge density is above a critical value. Since divalent cations even at very low concentration are 'drawn' close to the surface the reduction of total positive charge below the critical value does not occur until monovalent cations are introduced into the medium. The overall effect is that the double layer becomes more diffuse and will reduce its thickness to the original only by further addition of monovalent cations. Quantitative analysis of our data in terms of changes in the double layer thickness is difficult because the formidable task of integrating the appropriate point equation for mixed electrolytes. Finally it is worth emphasizing that the existence of surface potentials on chloroplast thylakoids can give rise to static electrical gradients across the membrane ($\Delta\psi_0$) which will be very susceptible to changes in the ionic composition of the suspending media.

Acknowledgements

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