CATION INDUCED INCREASE IN CHLOROPHYLL FLUORESCENCE YIELD AND THE EFFECT OF ELECTRICAL CHARGE

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1. Introduction

In 1969 Homann reported that the yield of chlorophyll fluorescence from isolated chloroplasts could be increased by the addition of cations to the suspension medium [1]. Since then Murata [2–4] and many other workers [5–8] have investigated this phenomenon and it has been established that it reflects changes in the degree of energy transfer to the pigment systems serving as antenna to photosystem one (PS1) and photosystem two (PS2) [2,9,10]. The properties of this cation effect have recently been studied in some depth [11–13] and it has been argued that the fluorescence yield changes are mediated via changes in the properties of the electrical diffuse layer adjacent to the thylakoid membrane surface. In particular it seems that the positive space charge density within a few Ångströms of the surface determines the fluorescence yield and thus the degree of energy spillover between the PS2 and PS1 chlorophylls [14]. This conclusion was mainly based on the fact that it is the charge carried by the cation rather than its chemical nature which governs its effectiveness in bringing about the fluorescence yield changes [11,14]. Moreover, calculations based on the Gouy-Chapman theory for mixed electrolyte conditions could explain the characteristic dip in chlorophyll fluorescence observed when unwashed thylakoids suspended in a cation free medium are subjected to increasing concentrations of monovalent cations [11–14].

Although the theoretical approach adopted predicted that trivalent cations should be more effective at inducing the chlorophyll fluorescence changes than divalent or monovalent cations, no experimental proof has been obtained to support this. Attempts to use La^{3+} and other trivalent cations had been unsuccessful because of membrane binding effects [12]. However, in this paper we report that the trivalent Tris(ethylenediamine)cobaltic cation $(TEC)^{3+}$ can induce the characteristic fluorescence increase.

2. Materials and methods

Pea chloroplasts were isolated intact by the method of Nakatani and Barber [15] and suspended in 0.1 M sorbitol adjusted to pH 7.5 with tris(hydroxymethyl)-aminomethane (Tris).

Prior to making the fluorescence measurements the chloroplasts were osmotically shocked in 1.5 ml of distilled water in a cuvette and then 1.5 ml of 0.33 M sorbitol + 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2ethane sulphonic acid (Hepes) + 5 mM KOH, pH 7.6, at double strength was added. Chlorophyll fluorescence was excited with a beam transmitted by a filter combination consisting of Schott 4 mm BG18 and 2 mm BG38 having an intensity of 80 W/m^2. The fluorescence emission was detected by an EMI 9558B S20 photomultiplier shielded by a Balzer B-40 693-nm interference filter coupled with a Schott RG695 cut-off filter.

All experiments were carried out at room temperature in the presence of 5 μM DCMU, and salts were added by injection into the cuvette via a light-tight seal.

Chlorophyll was determined by the method of Arnon [16].

Abbreviation: DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea

3. Results and discussion

Figure 1 shows the effect of adding 2 mM $MgCl_2$ and 20 µM Tris(ethylenediamine) cobalt (III) trichloride to chloroplast thylakoids treated with DCMU and suspended in the low salt buffer containing 5 mM K^+. Like Mg^{2+}, $(TEC)^{3+}$ was found to increase the fluorescence yield and in fig.2 the concentration curves are given for the two salts and also for KCl. Earlier studies have shown that all the alkaline earth cations increase the fluorescence yield in the same concentration range. This is also true for all the alkali metal cations [12].

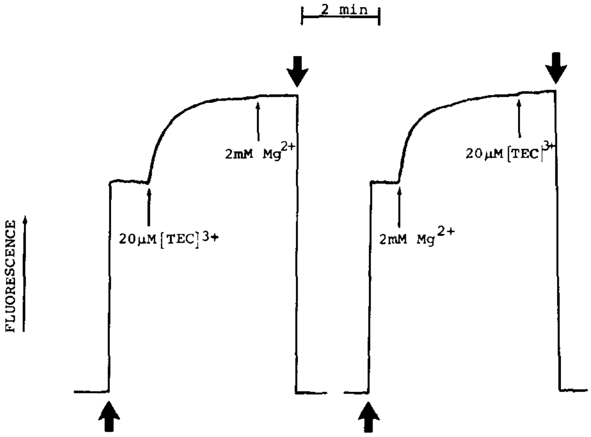

Fig.1. Increase in chlorophyll fluorescence yield of isolated thylakoids treated with 5 µM DCMU on addition of 20 µM Tris(ethylenediamine)cobaltic trichloride and 2 mM $MgCl_2$. Chlorophyll concentration was 5 µg/ml and the experiment was conducted at room temperature.

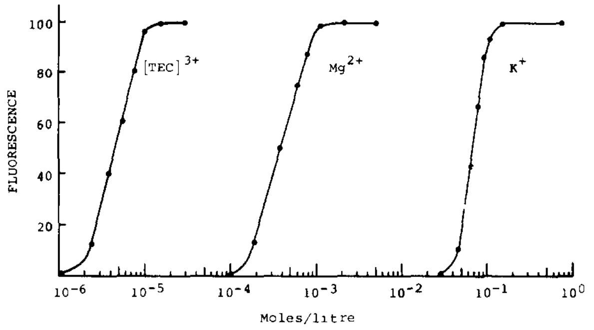

Fig.2. Concentration requirements for the cation induced increase in fluorescence yield showing the differential effect of the Tris (ethylenediamine)cobaltic cation $(TEC)^{3+}$, Mg^{2+} and K^+. Other conditions as for fig.1. 100% increase corresponds to the addition of 2 mM Mg^{2+}. All salts were added as their chlorides.

In earlier papers we have argued that when the thylakoids are suspended in a medium which contains only low levels of monovalent cations then the low fluorescence yield observed is due to the fact that the positive charge density immediately adjacent to the membrane surface is at a minimum [11,14]. Under these conditions monovalent cations are the predominant ionic species in the diffuse layer. Their displacement from this surface layer will occur when polyvalent cations are added to the bulk solution. Using the Gouy-Chapman theory and the Boltzmann expression it is possible to predict the cation exchanges which would occur at the membrane surface under the conditions employed in our experiments. From the Gouy-Chapman theory the surface potential (ψ_0) can be calculated using the following expressions for mixed electrolytes [17,18]. For a mixture of two monovalent salts having bulk concentrations $C'_{1\alpha}$ and $C'_{2\alpha}$ mol/l:

$$4\,(C'_{1\alpha}+C'_{2\alpha})\sinh^2\left(\frac{F\psi_0}{2RT}\right)-\frac{\sigma^2}{A^2}=0 \tag{1}$$

where σ is the surface charge density in $\mu C/cm^2$, F is the Faraday, ψ_0 is the surface potential, R is the gas constant, T is absolute temperature and $A=\left[\frac{RT\epsilon}{2\pi}\right]^{1/2}$ where ϵ is the permittivity of water.

For a mixture of monovalent salt (C'_α) and divalent salt (C''_α) the expression is:

$$2C''_\alpha\cosh^2\left(\frac{F\psi_0}{RT}\right)+C'_\alpha\cosh\left(\frac{F\psi_0}{RT}\right)-\left(2C''_\alpha+C'_\alpha+\frac{\sigma^2}{2A^2}\right)=0 \tag{2}$$

For a mixture of monovalent salt (C'_α) and trivalent salt (C'''_α) the expression is:

$$4C'''_\alpha\cosh^3\left(\frac{F\psi_0}{RT}\right)+(C'_\alpha-3C'''_\alpha)\cosh\left(\frac{F\psi_0}{RT}\right)-\left(C'_\alpha+C'''_\alpha+\frac{\sigma^2}{2A^2}\right)=0 \tag{3}$$

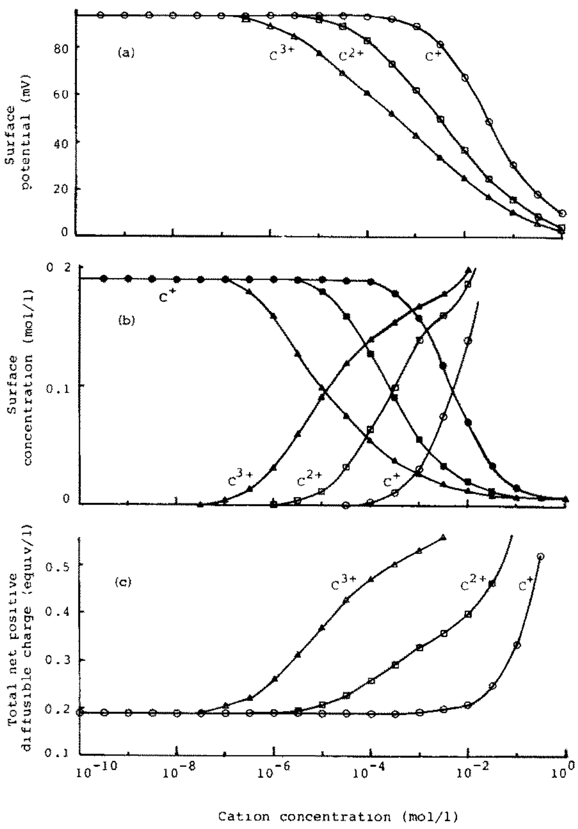

Fig.3. Theoretical curves calculated using equations 1–4 (see text). For eqn. 1, $C'_{1\alpha}$ was taken as 5 mM and for eqns 2 and 3 C'_α = 5 mM. Closed symbols in (b) are surface monovalent cation levels. Triangles, squares and circles correspond to trivalent, divalent and monovalent salt addition respectively.

Using the above expressions, taking $C'_{1\alpha}$ (in eqn. 1) and C'_α (in eqns. 2 and 3) equal to 5 mM and $\sigma = 2.5$ μC/cm^2 the curves for ψ_0 against added cation concentration shown in fig.3a are obtained. The value of 2.5 μC/cm^2 for the surface charge density has been used earlier [14] and there is experimental evidence that this value is reasonable for chloroplast thylakoids [17,19].

Now, by knowing ψ_0 and using the Boltzmann equation:

$$C_{io} = C_{i\alpha} \exp\left(\frac{-ZF\psi_0}{RT}\right)$$

it is possible to calculate the concentration of a cation species i at the surface (C_{io}) for any value of the bulk cation level ($C_{i\alpha}$) where Z is the number of charges carried by the cation.

Figure 3b shows the effectiveness of C^{3+}, C^{2+} and C^{+} species to replace the initial surface monovalent cations. Clearly both figs.3a and b demonstrate the order of effectiveness, $C^{3+} > C^{2+} > C^{+}$ as observed for the cation induced chlorophyll fluorescence changes shown in fig.2. In earlier papers we have suggested that the factor controlling the yield of chlorophyll fluorescence is the density of diffusible positive charge (positive space charge density) immediately adjacent to the membrane surface. This quantity is equal to $\sum_i ZC_{io}$, where Z is the number of charges carried by the cation and C_{io} is expressed in mol/l and its values for the various electrolyte conditions used are plotted in fig.3c.

There are many assumptions implicite in the Gouy-Chapman theory [14] and the above calculations have only been carried out for changes which occur at the very surface of the membrane. Nevertheless the analysis serves to emphasise the differential effect seen on increasing the number of charges carried by the added cation and gives further support to the hypothesis that the chlorophyll fluorescence changes associated with spillover are mediated directly via electrical effects at the thylakoid membrane surface rather than involving specific chemical binding to membrane proteins [20]. When the diffusible positive charge density is considered, the relative effect of changing the charge on the cations is more obvious, with the differences and the concentration range being comparable with the experimental results. The membrane conformational effects giving rise to the fluorescence changes could be the consequence of changes in the interaction between the fixed surface negative charges caused by variations in the local positive charge density [13].

The success obtained with $(TEC)^{3+}$ is probably due to the fact that the ligands of this cobalt complex are sufficiently strongly bonded to avoid chemical interaction of the cobalt atom with surface membrane groups (e.g. $-NH_2$, COO^-).

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