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EFFECTS OF DIVALENT METAL IONS ON CHLOROPHYLL *a* FLUORESCENCE IN ISOLATED SPINACH CHLOROPLASTS

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SUMMARY

The effects of divalent metal ions on the yields of chlorophyll *a* fluorescence were investigated in isolated spinach chloroplasts at room and liquid nitrogen temperatures. Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} and Mn^{2+} increased the yields of fluorescence emission at 684 and 695 nm from pigment system II and decreased that at 735 nm from pigment system I. Al^{3+} showed similar but less significant effects on the fluorescence yields. Zn^{2+} and Cd^{2+} showed no significant effect on the fluorescence yields at concentrations lower than 5 mM.

In accordance with the results of our previous study concerning the effects of Mg^{2+} on the excitation transfer in the chloroplasts, it was concluded that ions of alkaline earths and manganese suppress the excitation transfer from bulk chlorophyll *a* of pigment system II to that of pigment system I.

INTRODUCTION

Recent studies of chlorophyll *a* fluorescence in intact cells of some algae have shown that illumination of the organism causes changes in yields of chlorophyll *a* fluorescence which cannot be explained as a result of changes in electron transport through the two photoreactions of photosynthesis[1-5]. We discovered a light-induced change in the distribution of excitation energy between the two pigment systems in intact cells of *Porphyridium cruentum*, and proposed the concept of a control mechanism for excitation transfer, through which the organism changes the efficiency of excitation transfer between chlorophyll *a* molecules in the chloroplasts upon illumination.

MURATA[6] discovered that the addition of Mg^{2+} increased the yields of chlorophyll *a* fluorescence of pigment system II and decreased that of pigment system I, and reached the conclusion that the ions suppressed the excitation transfer from bulk chlorophyll *a* of pigment system II to that of pigment system I, *i.e.*, the step of the spillover of excitation. HOMANN[7] also observed the Mg^{2+}-induced increase in fluorescence yield at room temperature.

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We have recently investigated the effects of other divalent metal ions on the chlorophyll *a* fluorescence in spinach chloroplasts at room and liquid nitrogen temperature. It was discovered that Ca^{2+}, Sr^{2+}, Ba^{2+} and Mn^{2+}, just as Mg^{2+} in the previous study, had significant effects on the distribution of excitation energy between the two pigment systems.

METHODS

Spinach chloroplasts were prepared by the method described previously[6]. The reaction medium contained 0.4 M sucrose, 0.01 M NaCl and 0.05 M Tricine buffer adjusted to pH 7.6 by addition of NaOH. In the measurements of fluorescence emission, the chloroplast concentration was controlled so that the content of chlorophyll did not exceed 1 μg/ml in experiments with 1-cm-thick cuvettes, and 5 μg/ml with 0.2-cm-thick cuvettes.

Measurements of fluorescence at room and liquid nitrogen temperatures were performed according to the method described previously[6,8].

RESULTS

In order to eliminate the influence of photoreaction II on the fluorescence yield, 20 μM 3-(4'-chlorophenyl)-1,1-dimethylurea were always added in the present experiments. Under this condition the primary electron acceptor of photoreaction II (Q, according to DUYSENS AND SWEERS[9]) is fully reduced under illumination of the chloroplasts and there is little electron transport through photoreaction II. Therefore, there is no quenching of chlorophyll *a* fluorescence caused by photoreaction II.

At room temperature, the addition of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} or Mn^{2+} increased the yields of chlorophyll *a* fluorescence at 684 nm in spinach chloroplasts. Al^{3+} were less effective. Zn^{2+} and Cd^{2+} at concentrations lower than 5 mM were completely without effect.

Fig. 1 shows the time-course of fluorescence change on addition of $MnCl_2$ at room temperature. The fluorescence, gradually increasing, reached a steady level in 4–5 min after the addition of $MnCl_2$. Similar time-courses of fluorescence change were

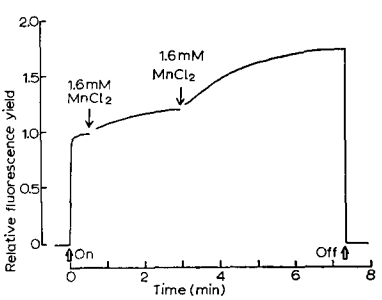

Fig. 1. Time-course of fluorescence change upon addition of $MnCl_2$ in isolated spinach chloroplasts at room temperature with 20 μM 3-(4'-chlorophenyl)-1,1-dimethylurea. Blue excitation light (3000 ergs·cm^{-2}·sec^{-1}) was obtained from an incandescent lamp through a combination of three glass filters, V-B46 (Toshiba), B-460 (Hoya Glass) and HA-50 (Hoya Glass). Fluorescence at 684 nm was isolated with an interference filter (with 16 nm half bandwidth) and a glass cutoff filter, V-R65 (Toshiba) and detected with a photomultiplier, R-236 (Hamamatsu TV).

TABLE I

EFFECTS OF SALTS ON THE FLUORESCENCE YIELD OF SPINACH CHLOROPLASTS AT ROOM TEMPERATURE

Fluorescence yields in the presence of metal ions were measured 5 min after the addition of salts. Other experimental conditions were the same as in Fig. 1.

Salts added	*Concentration (mM)*	*Relative fluorescence yield*
No addition	—	1.00
$MgCl_2$	3.2	1.82
$CaCl_2$	4.8	1.73
$SrCl_2$	4.8	1.74
$BaCl_2$	3.2	1.78
$MnCl_2$	6.4	2.07
$AlCl_3$	1.6	1.06
$ZnSO_4$	3.0	1.00
$CdSO_4$	3.0	1.00

observed on addition of alkaline earth ions. In most cases, therefore, the fluorescence yield at 5 min after addition of a salt was taken as a measure for estimating the effect of the salt, the fluorescence yield in the absence of the salt being taken as unity.

Table I shows the effects of salts on the yield of chlorophyll *a* fluorescence measured at 684 nm at room temperature. In this experiment increases in fluorescence yield as high as 70–80% were obtained on addition of salts, including $MgCl_2$, $CaCl_2$ $SrCl_2$ and $BaCl_2$ at concentrations of 3–5 mM, which were sufficient for saturating the fluorescence increase with each kind of ion (see Fig. 2). The effectiveness of the salts as measured by the ratio of the salt-induced increment to the original fluorescence yield changed from sample to sample and depended also on the period that had elapsed after the preparation of the chloroplasts. However, the relative order of effectiveness of these salts remained almost the same for any preparation of the chloroplasts used. The most significant effect in increasing the fluorescence yield was observed on addition of $MnCl_2$. The increase of fluorescence yield was 107% at a concentration of 6.4 mM of $MnCl_2$. However, a higher concentration of $MnCl_2$ was required in order to reach the maximum increase of fluorescence yield as compared with the chloride salts of alkaline earths (see Fig. 2).

The effects of $MgSO_4$ and $MnSO_4$ on the fluorescence yield were also examined. These salts were found to be similar in effectiveness to the corresponding chloride

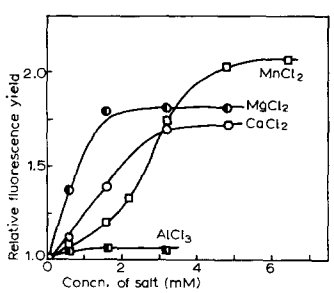

Fig. 2. Relationship between concentrations of $MgCl_2$, $CaCl_2$, $MnCl_2$ and $AlCl_3$ and fluorescence yields in spinach chloroplasts at room temperature. The values of fluorescence yields were measured 5 min after the addition of salt solutions. Other experimental conditions were the same as in Fig. 1.

salts. $AlCl_3$ had only slight effect in increasing the fluorescence yield. On the other hand, $ZnSO_4$ and $CdSO_4$ had no effect on the fluorescence yield (Table I).

The relationship between the concentration of the metal ions and the increases in fluorescence yield were investigated. Fig. 2 shows the relationship between the concentrations of salts $MgCl_2$, $CaCl_2$, $MnCl_2$ and $AlCl_3$ and the fluorescence yields at room temperature. As shown by the difference in curves for $MgCl_2$ and $CaCl_2$, Ca^{2+} was less effective in increasing the fluorescence yield than Mg^{2+}, although the fluorescence yield attained almost the same level at the saturating concentrations of these salts. The concentration for half saturation was 0.6 mM for Mg^{2+} and 1.6 mM for Ca^{2+} (averaged values of the three experiments). The effectiveness of $BaCl_2$ and $SrCl_2$ came between $MgCl_2$ and $CaCl_2$.

As exemplified by the cases of $MgCl_2$ and $CaCl_2$ at a low concentration range, the fluorescence yield increased linearly with increase in concentrations of the ions to finally attain a maximum steady level at 2–3 mM. On the other hand, $MnCl_2$ showed a rather complicated pattern of concentration dependence (Fig. 2). At low concentrations of $MnCl_2$ (0–2 mM) the fluorescence yield was not so significantly increased in comparison to alkaline earth ions. The increase, however, showed a more marked rise above 2 mM and finally attained a maximum level of fluorescence yield at 6 mM, which was much higher than the saturating concentrations in the cases of alkaline earth ions. $MnSO_4$ showed a concentration dependence similar to that of $MnCl_2$. At concentrations of 0.5–4 mM, $AlCl_3$ had a small but distinct effect in increasing the fluorescence yield.

The emission spectra of spinach chloroplasts were measured in the presence and absence of metal ions at room and liquid nitrogen temperatures. The metal ions, as chloride salts, were added 5 min before measuring the fluorescence spectra at room temperature or cooling the sample with liquid nitrogen. A comparison was made of the emission spectra with and without addition of alkaline earth or manganese ions at room temperature. The spectrum of the salt-induced increase in fluorescence yield had a peak at 685 nm and a broad shoulder at 730–740 nm. This spectrum of the fluorescence increment was similar in pattern to the fluorescence emission spectrum of the chloroplasts in the absence of the metal ions.

Fig. 3 shows the emission spectra of spinach chloroplasts at liquid nitrogen

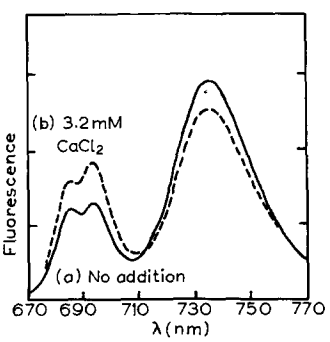

Fig. 3. Effects of $CaCl_2$ on the fluorescence emission spectrum in spinach chloroplasts at liquid nitrogen temperature. Excitation light was the same as in Fig. 1. Fluorescence was measured with 2.5 nm half bandwidth of the monochromator. (a) No $CaCl_2$ added (———); (b) 3.2 mM $CaCl_2$ added 5 min before cooling the sample with liquid nitrogen (– – – –).

temperature in the presence and absence of $CaCl_2$ (3.2 mM). The addition of salt increased the yields of the emission bands at 684 nm and 695 nm and decreased that at 735 nm. Since the emissions with peaks at 684 nm and 695 nm are emitted from pigment system II, and the emission at 735 nm from pigment system I (ref. 8), it is inferred that Ca^{2+} controls the distribution of excitation energy between the two pigment systems. These findings are the same as those discovered in our previous study on the effects of Mg^{2+} on the fluorescence spectrum in spinach chloroplasts[6].

TABLE II

EFFECTS OF METAL IONS ON THE FLUORESCENCE INTENSITIES AT 684, 695 AND 735 nm IN SPINACH CHLOROPLASTS AT LIQUID NITROGEN TEMPERATURE

The intensity at 684 nm was taken as unity for each addition of the salt. The experimental conditions were the same as shown in Fig. 3.

Metal ions added	*Concentration (mM)*	*Relative intensity of fluorescence*		
		684 nm	*695 nm*	*735 nm*
No addition	—	1.00	1.10	2.39
$MgCl_2$	3.2	1.00	1.22	1.44
$CaCl_2$	3.2	1.00	1.15	1.59
$SrCl_2$	3.2	1.00	1.18	1.46
$BaCl_2$	3.2	1.00	1.22	1.47
$MnCl_2$	3.2	1.00	1.14	1.46
$AlCl_3$	1.6	1.00	1.12	2.28

We studied the effects of other kinds of metal ions on the emission spectrum at liquid nitrogen temperature. $MgCl_2$, $SrCl_2$, $BaCl_2$, $MnCl_2$ and $AlCl_3$ had effects similar to those of $CaCl_2$; the addition of salts increased the yields of the emission bands at 684 nm and 695 nm, and decreased that at 735 nm. The results are summarized in Table II, in which the intensity of fluorescence at 684 nm in each emission spectrum was taken as unity. The fluorescence yield at 735 nm was markedly decreased as compared to that at 684 nm on addition of $MgCl_2$, $CaCl_2$, $SrCl_2$, $BaCl_2$ or $MnCl_2$. Slight increases in relative intensity of fluorescence at 695 nm were always observed on addition of these metal ions, thus indicating that the emission band at 695 nm was more markedly enhanced by the metal ions than the band at 684 nm. $AlCl_3$ showed similar, but less significant effects on the fluorescence emissions.

DISCUSSION

The experimental results of the present study show that the effects of the alkaline earth and manganese ions on the chlorophyll *a* fluorescence in spinach chloroplasts are similar in pattern to that of Mg^{2+}, which was previously studied[6], namely, the addition of these metal ions increased the yields of chlorophyll *a* fluorescence in pigment system II and decreased that in pigment system I.

The kinetic analysis of fluorescence emission in our previous study suggested that Mg^{2+} suppress the excitation transfer from bulk chlorophyll *a* of pigment system II to that of pigment system I (ref. 6).

Such changes in efficiency of excitation transfer[10] are likely to occur, since the

addition of Mg^{2+} or Ca^{2+} to the chloroplasts has been reported to induce changes in membrane structure of the chloroplast lamellae[11–13], which may result in changes in mutual orientation and distance between the absorption and emission oscillators of the chlorophyll molecules bound to the membrane structure.

The same mechanism may also be operating in the cases of other ions under investigation. The fact that the maximum extent of fluorescence increase at 684 nm obtained on addition of optimum amounts of these ions (*i.e.*, alkaline earths and manganese) was almost the same irrespective of the nature of the ions added indicates that a common mechanism is underlying the observed fluorescence change.

It has to be noticed that Zn^{2+} and Cd^{2+} did not affect the chlorophyll *a* fluorescence, a fact indicating that the capacity for the control of excitation transfer is not a property common to all the divalent ions but characteristic of alkaline earth and manganese ions. It has also to be noticed that these alkaline earth ions range in magnitude of ionic radius from 0.66 Å in Mg^{2+} to 1.35 Å in Ba^{2+}. Among these, Mg^{2+} and Ba^{2+} were found to be almost identical in their range of effective concentrations, inspite of about a 2-fold difference in ionic radii.

It was discovered in our previous study in intact cells of *Porphyridium cruentum* that the illumination of the organism changed the yields of fluorescence emission of chlorophyll *a*. When the organism had been preilluminated with light absorbed by pigment system II, the fluorescence yields of pigment system II decreased with a concomitant increase in fluorescence yield of pigment system I. Upon preillumination of pigment system I, the reverse changes in fluorescence yields of the pigment systems were observed. Similar light-induced changes in fluorescence yields were observed also in other photosynthetic organisms tested, Chlorella, Chlamydomonas, Anabaena and Porphyra (N. MURATA, unpublished data). Thus, the light-induced control of excitation transfer seems to be a general property common to most green plants.

The most plausible explanation for this phenomenon is that these ions move through the thyrakoid membrane upon illumination of the organism to induce a modification of the membrane structure, which in turn may result in a change in excitation transfer between the two pigment systems.

This hypothesis required that there is an efflux of these ions from the chloroplasts upon illumination of pigment system II and an influx upon illumination of pigment system I. The movements of ions through the thylakoid membrane upon illumination have been observed in isolated chloroplasts[14,15]. In his recent study investigating the effects of illumination on the ionic contents of chloroplasts *in vivo*, NOBEL observed the light-induced efflux of Mg^{2+} and Ca^{2+}, as well as some other kinds of ions[16]. However, it is unfortunate that information on the preferential effects of illumination of pigment systems I and II on ion exchange are still lacking at present.

It has been reported that the addition of Mn^{2+} enhanced the fluorescence yield at room temperature in chloroplasts having no, or poor, activity for water oxidation; namely heat-treated[17] or Tris-treated[17,18] chloroplasts. In these cases, Mn^{2+} seems to act as an electron donor of photoreaction II, and consequently the primary electron acceptor of photoreaction II, Q, must be in the reduced state on illumination of the chloroplasts, resulting in an increase in fluorescence yield. The effects of Mn^{2+} as seen in the present study, however, are different. Since, in the present experiments, a sufficient amount of 3-(4'-chlorophenyl)-1,1-dimethylurea was added to fresh

chloroplasts having a high activity of oxygen evolution, all the primary electron acceptor, Q, should be in the reduced state upon the illumination, and little electron flow occurs through photoreaction II. Under this condition, Mn^{2+} cannot act as electron donor of photoreaction II. These considerations led us to infer that the observed changes in fluorescence yields are caused by the action of Mn^{2+} in regulating the excitation transfer between chlorophyll *a* molecules of pigment systems I and II.

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