THIRD INTERNATIONAL SEMINAR

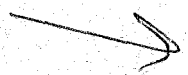
ON

ENERGY TRANSFER IN CONDENSED MATTER

Proceedings

Prague, Czechoslovakia, June 29–July 2, 1976

EDITED BY J. FIALA



UNIVERZITA KARLOVA PRAHA 1978

pp19-20

REGULATION OF EXCITATION ENERGY TRANSFER AMONG THE TWO PIGMENT SYSTEMS IN PHOTOSYNTHESIS

Govindjee and Daniel Wong
Department of Physiology and Biophysics University of Illinois, Urbana, Illinois 61801, U.S.A.

Summary

Mono- and divalent cations have been suggested to regulate excitation energy distribution among the two pigment systems in photosynthesis. We present a brief review of studies in our laboratory of this phenomenon. New data are presented on measurements of the degree of polarization of chlorophyll a fluorescence /p/ in sucrose-washed chloroplasts /SWC/, SWC plus 3-5 mM monovalent cations, and SWC plus 3-5 mM mono- and 3-5 divalent cations at different wavelengths. These results allow us to present a working hypothesis that monovalent cations increase excitation energy transfer from photosystem II to I, and decrease and increase excitation energy migration among system II and system I units, respectively. Further addition of divalent cations leads to a reversal of all these effects, i.e., excitation energy transfer from photosystem II to I decreases, and excitation energy migration among system II and system I units increases and decreases, respectively.

Introduction

Homann /1/ discovered that divalent cations increase the chlorophyll a fluorescence in isolated chloroplasts even in the presence of the electron flow inhibitor 3- /3,4-dichlorophenyl/-1, 1-dimethylurea /DCMU/. Murata and coworkers /2/ pursued this problem by measuring low temperature /77 K/ chlorophyll a emission spectra, and activity of photosystems I and II. They suggested that divalent cations cause a decrease in excitation energy transfer from photosystem II to photosystem I. This phenomenon has now been investigated in several laboratories /3-10/. Various conflicting ideas have been

proposed and the problem has not yet been resolved.

In sucrose-washed chloroplasts /SWC/, Gross and coworkers /see e.g., ref. /11/ observed that monovalent cations decreased chlorophyll a fluorescence yield at room temperature /due mostly to pigment system II/ and increased and decreased fluorescence from pigment systems I and II, respectively, at 77 K. Addition of divalent cations reversed this effect. This phenomenon was interpreted in terms of Murata's hypothesis /12/ of regulation of excitation energy distribution among the two pigment systems. Van der Meulen and Govindjee /13/ showed that in SWC from oats, lettuce, and peas, the divalent cation effects were observed even without prior addition of monovalent cations. These cation induced changes in chlorophyll a fluorescence yield could not be precisely correlated with the changes in 90° light scattering. Wydrzynski et al. /14/, on the basis of their experiments on the effects of these cations on fluorescence transients /Kautsky effect/, suggested that there was a large effect of monovalent cations on "antenna" chlorophyll a complexes in addition to their effects on reaction center chlorophyll a molecules. The divalent cations apparently reversed these effects, but in slightly different proportions. Divalent cations also caused a direct effect on system II. There was an increase in excitation energy transfer from "antenna" chlorophyll a molecules to the chlorophyll a complex associated with reaction center II. No significant effect of divalent cations on fluorescence at room temperature has been observed in isolated system I /15/ or system II particles /16/. Fixation of chloroplasts with glutaraldehyde prior to washing leads to the elimination of the divalent cation effects /16/. (Removal of coupling factor by washing chloroplasts with EDTA also eliminates the divalent cation effects.) These studies suggest that the integrity and the proximity of the pigment systems I and II are necessary /16/ for observing the regulation of excitation energy distribution among the two photosystems, and that the effects may originate by the interaction of cations, at least, partially with the "antenna" chlorophyll a complexes. Briantais, Arntzen, and coworkers /17/ have shown that the "light harvesting pigment-protein complex" is necessary for

the same phenomenon, providing credance to this conclusion.

We are now investigating the molecular mechanism of this regulation process which is quite important to the photosynthesis of plants growing /or being studied/ at low light intensities, D. Wong and Govindjee /unpublished/ have shown that the degree of polarization of chlorophyll a fluorescence, measured at 685 nm, increases when 3-5 mM monovalent cations are added to SWC, and this effect is reversed by the addition of 3-5 mM divalent cations. However, polarization of chlorophyll a fluorescence, measured at 712 nm, decreases when 3-5 mM monovalent cations are added to SWC, and this effect is reversed by the addition of 3-5 mM divalent cations. Data at 735 nm are similar but not identical to that at 685 nm. In a random suspension of chloroplasts, used in the present study, p is dependent upon the extent of energy transfer and the internal geometry of the pigment systems in the membranes /see refs. /18/ and /19/. Changes in p, caused by cation additions, may reflect only changes in the localization of energy among the different heterogenous pigment systems in the chloroplast membranes /19/. Addition of cations also cause changes in the physical organization of the thylakoid membranes. For example, Schooley and Govindjee /20/ have observed that a negative peak at 676 nm in the circular dichroism spectrum diminishes upon the addition of 3 mM monovalent cations to SWC, and is reversed by the further addition of 3 mM divalent cations. The present paper emphasizes the need of pursuing the problem of the molecular mechanism of these cation effects.

Materials and Methods

Sucrose-washed chloroplasts were prepared as described by Gross /21; also see refs. 13 and 14/ and resuspended in low ionic medium consisting of 100 mM sucrose buffered to pH 7.8 with Tris. All samples consist of 3 ml aliquots of chloroplast suspensions at a chlorophyll concentration of about 5 μg/ml placed in a quartz-glass cuvette of 1 cm pathlength. Low concentrations /3-5mM/ of NaCl and $MgCl_2$ were added to provide the cations. 5 μM DCMU was added, wherever indicated. The exciting light was ~~source~~ is from a 1000 W tungsten lamp, it was passed through a Bausch and

Lomb monochromator set at 610 nm with a bandwidth of 10 nm.

For polarization measurements, the sample was excited with light linearly polarized in the vertical direction. Fluorescence was detected with an EMI 9558 B photomultiplier placed at right angles to the direction of propagation of the exciting light. The intensities of the fluorescence polarized vertically (F_v) and horizontally (F_h) were measured and the latter corrected for systematic instrumental bias to the direction of polarization. [The correction factor was obtained from a solution of 10^{-7}M Rhodamine B in glycerol.] The ratio of the difference ($F_v - F_h$) to the sum ($F_v + F_h$) gave the degree of polarization p f chlorophyll a fluorescence.

Results

The effects of cation on the degree of polarization of chlorophyll a fluorescence in chloroplasts, reported as percent polarization, i.e., px100, are shown in tables 1-3.

Table 1 shows the effects of cations on px100 for three different batches of lettuce chloroplasts. Each number in the table is the average of 6 repetitions on the same sample and the uncertainties are the standard deviations. Fluorescence was collected through a Schott RG-665 cut-off filter with a half maximum transmission at 664 nm. We found that the addition of 3 mM NaCl to the sucrose-washed chloroplasts resulted in a 10 to 30% (mean, 12%) increase in the degree of polarization. Further addition of 3 mM $MgCl_2$ reversed this change.

Treatment	Sample 1	Sample 2	Sample 3
None	2.7 ± 0,16	2.8 ± 0.12	2.2 ± 0.11
+ 3 mM NaCl	3.0 ± 0.15	3.0 ± 0.17	2.6 ± 0.17
+ 3 mM NaCl + 3 mM $MgCl_2$	2.7 ± 0.15	2.9 ± 0.14	2.2 ± 0.19

Table 1. Effects of cations on the degree of polarization of chlorophyll a fluorescence (px100) in sucrose-washed lettuce chloroplasts (λ observation = RG 665 filter; after D. Wong and Govindjee, unpublished.)

Table 2 shows the effects of cations on px100 for pea chloroplasts. Here, again, each number in the table is the average of 6 repetitions, and the uncertainties are the standart deviations. The higher uncertainties for the NaCl treated samples are due to lowered fluorescence intensities. Fluorescence was collected either through a 680 nm interference filter with a bandwidth at half maximum transmission of 13 nm, or through a Schott RG-10 cut-off filter with a half-maximum transmission at 717 nm, or through an RG 665 cut-off filter. In this set of experiments, 5 μM DCMU was added to all the samples. We note that 5 μM DCMU did not cause any effect on p in pea chloroplasts under the experimental conditions in this paper, but could lower the value of p in algal samples, the extent of the effect being dependent on the excitation wavelength (Mar and Govindjee, /22/; also see Whitmarsh and Levine, /23/.) Results with the 680 nm filter have been confirmed on 3 separate batches of chloroplasts and with RG-665 and RG-10 filters on 2 separate batches of chloroplasts. Addition of 3 mM Na Cl to sucrose-washed chloroplasts caused an increase in polarization by 20 to 30% which was reversed by 3 mM $MgCl_2$.

Treatment	680 nm filter	RG-10 filter	RG-665 filter
None	2.5 ± 0.1	2.7 ± 0.1	2.5 ± 0.1
+ 3 mM NaCl	3.0 ± 0.4	3.4 ± 0.4	3.1 ± 0.3
+ 3 mM NaCl + 3 mM $MgCl_2$	2.2 ± 0.2	2.6 ± 0.2	2.3 ± 0.2

Table 2. Effects of cations on the degree of polarization of chlorophyll *a* fluorescence (px100) in sucrose-washed pea chloroplasts in the presence of 5 μM DCMU (after D. Wong and Govindjee, unpublished).

In summary, 3 mM NaCl caused a 10 to 30% increase in polarization of chlorophyll *a* fluorescence when added to sucrose-washed pea and lettuce chloroplasts. This effect was independent

of the presence of DCMU and was present when fluorescence was measured through 680 nm, RG-10, or RG-665 filters. 680 nm filter transmitted mainly the 685 nm emission band, RG-10--fluorescence beyond 693 nm, and RG-665--fluorescence beyond 650 nm. This result was expected because the chlorophyll a fluorescence at room temperature is from pigment system II; the RG-10 filter transmits mainly the emission from the "vibrational band" of the main electronic band at 685 nm. However, it has been shown that even at room temperature, there is some heterogeneity in chlorophyll a fluorescence (see review, ref. 4) and it should be possible to select a somewhat greater proportion of system I to system II fluorescence at wavelengths in the 695-720 nm region. Thus, we made measurements with interference filters at 685, 712, and 730 nm.

Table 3 shows our data on the cation effects on the degree of polarization of chlorophyll a fluorescence at 685, 712, and 730 nm on pea chloroplasts containing 5 μM DCMU. The wavelength of excitation in these experiments was 600 ± 16 nm. Our results revealed, in addition to confirming the results of tables 1 and 2 on the 685 nm emission band, two interesting points: (a) a higher degree of polarization of fluorescence at 712 than at 685 or 730 nm; and (b) decrease, instead of an increase, of polarization of chlorophyll a fluorescence when 5 mM NaCl was added to sucrose-washed chloroplasts, and the reversal of this effect by 5 mM $MgCl_2$. [Similar results were obtained with 3 mM NaCl and 3 mM $MgCl_2$.] In these experiments with pea chloroplasts, NaCl caused a 40 to 50% increase in polarization of chlorophyll a fluorescence at 685 and 730 nm, and a 30 to 60% decrease at 712 nm depending upon the batch of chloroplasts. [Average of results with two different batches of chloroplasts is reported in the table.] These effects were fully reversible, upon further addition of 5 mM $MgCl_2$, at 685 and 712 nm. However, the reversibility at 730 nm was not satisfactory suggesting complications in separating system I and system II fluorescence at this wavelength.

Treatment	observation, nm 685	712	730
None	2.2 ± 0.1	4.3 ± 0.3	2.9 ± 0.1
+ 5 mM NaCl	3.1 ± 0.3	2.3 ± 0.3	4.2 ± 0.2
+ 5 mM NaCl + 5 mM $MgCl_2$	2.0 ± 0.2	4.2 ± 0.3	3.8 ± 0.2

Table 3. Effect of cations on the degree of polarization of chlorophyll a fluorescence (pxl00) in pea chloroplasts treated .th 5μM DCMU (after D. Wong and Govindjee, unpublished*).

* Now published : FEBS Lett, 97, 373-377 (1979).

Discussion and Conclusions

Interpretation of the degree of polarization of chlorophyll a fluorescence in a random suspension of chloroplast membranes is complicated by the presence of anisotropic distributions of both absorbing and emitting dipoles /19/. The low degree of polarization of fluorescence has been taken as evidence for extensive excitation energy migration in photosynthetic systems (see Arnold and Meek, /25/). The non-zero polarization may be due to the fluorescence from the oriented emitting dipoles in the membrane. Any change in excitation energy transfer due to the addition of cations modifies the degree of polarization of fluorescence and may reflect the changed localization of energy in the heterogeneous pigment systems of photosynthesis.

We propose the following working hypothesis to explain our results. First, cation do not affect excitation energy transfer within individual system II or system I units, since no significant cation effects are observed in isolated system I or system II particles which do posses several chlorophyll a complexes. The only exception is a small change in excitation energy transfer from the chlorophyll a form fluorescing at 685 to that at 695 nm in system II particles at 77 K (see ref. /14/). We accept the assumption that F685 and F730 (fluorescence bands at 685 and 730 nm) at room temperature are mainly from pigment system II, and F712 has relatively more from pigment system I. At low temperature (77 K), however, F730 belongs mainly to

pigment system I (directly or by energy transfer from pigment system II) and F685 and F695 to pigment system II (see Govindjee and Yang, /26/). We also accept the idea that NaCl causes an increase in excitation energy transfer from pigment system II to pigment system I (see, e.g., Wydrzynski et al., /14/). This, we propose, leads to a decrease in excitation energy transfer among system II units and to an increase in transfer among system I units. Since F685 and F730, at room temperature, measure mainly system II fluorescence, we predict an increase in the degree of polarization of fluorescence (as observed in tables 1-3) upon addition of 3-5 mM NaCl. Since F 712, at room temperature, measures a higher contribution of system I fluorescence than F685, we predict a decrease in degree of polarization (as observed in table 3). We have accepted the idea that p at 712 nm has a higher proportion from system I than from system II because system I gives a higher degree of polarization of fluorescence than system II (see Govindjee, /24/ ; Gasanov and Govindjee, /28/). We can see from table 3 that p at 712 nm is higher than at 685 and 730 nm. Addition of 3-5 mM $MgCl_2$ reverses the NaCl effect: There is a decreased excitation energy transfer to ~~from~~ photosystem I, more energy is localized in system II units and there is greater energy exchange among system II units and a decreased energy exchange among system I units. Thus, the degree of polarization of chlorophyll a fluorescence, measured at 685 nm, decreases (as seen in tables 1-3) and that measured at 712 nm increases (as seen in table 3). The increased energy migration among system II units by the addition of $MgCl_2$ has already been reported by Briantais and coworkers*, based on their study of the shape of the fluorescence rise curve in continuous light in the presence of DCMU; this curve changes from an exponential to a sigmoid shape.

It is implicit in the above hypothesis that the energy transfer within ~~among~~ individual units (whether I or II) is by a fast mechanism. However, excitation energy exchange among units (I or II, or from II to I) is by Förster's slow mechanism. Many more experiments are needed to test the predictions of the above hypothesis. (The increased energy transfer among system I units is, however, not necessary to explain the observed results).

(Pub.) * See Moya, I., Govindjee, C. Vernotte and J.M. Briantais

References

/1/ P. Homann /1969/ Pl.Physiol., 44, 932-936

/2/ N. Murata /1969/ Biochim. Biophys. Acta, 189, 171-181

/3/ N. Murata, H. Tashiro and A. Takamiya /1970/ Biochim. Biophys. Acta, 197, 250-256

/4/ R.C. Jennings and G. Forti /1974/ Biochim. Biophys. Acta 347, 299-310

/5/ R.C. Jennings and G. Forti /1974/ Pl. Science Lett., 3, 25-33

/6/ G.H. Krause /1974/ Biochim. Biophys. Acta, 333, 301-313

/7/ H. Barber, A. Telfer and J. Nicolson /1974/. Biochim. Biophys. Acta, 357, 161-165

/8/ S. Malkin and Y. Siderer /1974/ Biochim. Biophys. Acta, 368, 422-431

/9/ J. Isaakidou and G. Papageorgiou /1975/ Arch. Biochem. Biophys., 168, 266-272

/10/ J-M. Briantais, C. Vernotte and I. Moya /1973/ Biochim. Biophys. Acta, 325, 530-538

/11/ E. Gross, T. Wydrzynski, D. Vandermeulen, and Govindjee /1974/ Proc. 3rd Internat. Congr. Photosynth. Res., Rehovot, pp. 345-361

/12/ N. Murata /1971/ Biochim. Biophys. Acta, 226, 422-432

/13/ D. Vandermeulen and Govindjee /1974/ Biochim. Biophys. Acta, 368, 61-70

/14/ T. Wydrzynski, E. Gross and Govindjee /1975/ Biochim. Biophys. Acta, 376, 151-161

/15/ N. Murata /1971/ Biochim. Biophys. Acta, 245, 365-372

/16/ P. Mohanty, B.Z. Braun and Govindjee /1972/ Biochim. Biophys. Acta,292, 459-476

/17/ J-M. Briantais and C.J. Arntzen /1976/ Personal Communication

/18/ R.S. Knox /1975/ In Bioenergetics of Photosynthesis, edited by Govindjee, Academic Press, N.Y. pp.183-221

/19/ M. Michel-Villaz /1976/ J. Theoret. Biol., 58, 113-129

/20/ R.E. Schooley and Govindjee /1976/ FEBS Letters, 65, 123-125

/21/ E. Gross /1971/ Arch. Biochem. Biophys. 147, 77-84

/22/ T. Mar and Govindjee /1971/ Proc. 2nd Internat. Congr. Photosynth. Res., Stressa, 1, 271-281

/23/ J. Whitmarsh and H.P. Levine /1974/ Biochim. Biophys. Acta, 368, 199-213

/24/ Govindjee, G. Papageorgiou and E. Rabinowitch /1973/ In Practical Fluorescence, Theory, Methods and Techniques, edited by G.G. Guilbault, Marcel Dekker Inc., N.Y. pp. 543-575

/25/ W. Arnold and E. Meek /1956/ Arch. Biochem. Biophys., 60, 82-90

/26/ Govindjee and L. Yang /1966/ J. Gen. Physiol., 49, 763-780

/27/ Govindjee /1966/ In Currents in Photosynthesis, edited by J.B. Thomas and J.H.C. Goedheer, Ad Donker Publ., Rotterdam, pp. 93-103

/28/ R. Gasanov and Govindjee /1974/ Z. Pflanzenphysiol., 72, 193-202