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THE EFFECT OF MONO- AND DIVALENT CATIONS ON THE QUANTUM YIELDS FOR ELECTRON TRANSPORT IN CHLOROPLASTS

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SUMMARY

We have previously shown (Gross, E. L. and Hess, S. C. (1973) Arch. Biochem. Biophys 159, 832–836) that low concentrations of monovalent cations (3–10 mM) caused changes in chlorophyll *a* fluorescence indicative of a transfer of excitation energy from Photosystem II to Photosystem I. These effects were reversed by divalent cations or higher concentrations of monovalent cations. In this study, we have examined the effects of cations on the relative quantum yields for Photosystem II (dichlorophenolindophenol reduction) and Photosystem I (diphenylcarbazone disproportionation) with the following results.

(1) Low concentrations of monovalent cations decreased the quantum yield for dichlorophenolindophenol reduction and increased that for diphenylcarbazone disproportionation. These results confirm that cations promote excitation energy transfer to Photosystem I.

(2) Higher concentrations of monovalent cations (30–100 mM) had no effect on electron transport. Therefore, the increases in chlorophyll *a* fluorescence observed at these concentrations may be due to a decrease in the rate constant for radiationless decay.

(3) In the absence of Tricine, divalent cations also promote energy transfer from the light harvesting pigments to Photosystem I. However, a direct inhibition of Photosystem II photochemistry cannot be ruled out.

(4) Tricine biases the system in favor of Photosystem I and divalent cations can reverse the Tricine effects.

INTRODUCTION

Murata [2–4], Homann [5] and others [6–12] showed that divalent cations regulate the distribution of excitation energy between the two photosystems of green plant photosynthesis. Murata's experiments [2–4] were carried out using 50 mM

Abbreviations: DCIP, dichlorophenolindophenol; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCMU, 3,3 dichlorophenyl-1,1-dimethyl urea.

Tricine buffer, which has been shown to cause unstacking of the chloroplast grana [13, 14]. Under these conditions excitation energy is transferred from Photosystem II to Photosystem I (alternatively, spillover from Photosystem II to Photosystem I is promoted). Addition of divalent cations or high concentrations of monovalent cations [15] reversed this situation, inhibiting the energy transfer to Photosystem I. Measurements of both chlorophyll *a* fluorescence changes and quantum yields for such reactions as DCIP and NADP reduction were used to arrive at these conclusions.

We showed [1] that at very low ionic strength (100 mM sucrose + 0.3 mM Tris base (pH 8)) the chlorophyll *a* fluorescence yield was high, indicating that transfer of excitation energy from Photosystem II to Photosystem I was inhibited. Addition of low concentrations of salts of monovalent cations such as Na^+ K^+, or Tricine buffer caused (1) unstacking of the thylakoids [16], (2) a decrease in the chlorophyll *a* fluorescence yield at room temperature and (3) an increase in the ratio of Photosystem I fluorescence observed at 735 nm [17] to that of Photosystem II at 680 nm at 77 °K. There was an equal effect on both the variable and constant parts of the fluorecence transients [18]. These results are consistent with the idea that low concentrations of monovalent cations increase the transfer of excitation energy to Photosystem I. These effects were reversed by divalent cations [1] or higher concentrations of monovalent cations which also caused restacking of the grana [16].

To test our conclusions further, we decided to study the effects of cations, under our conditions, on the relative quantum yields for Photosystem II and Photosystem I reactions separately. The results are discussed below.

MATERIALS AND METHODS

Class II chloroplasts [19] were isolated from spinach according to the method of Gross [20] and washed and resuspended in 100 mM unbuffered sucrose.

Chlorophyll concentrations were determined according to the method of Arnon [21].

DCIP reduction was monitored at 600 nm using an Aminco-Chance split-beam, dual wavelength spectrophotometer in its split-beam mode of operation. Diphenylcarbazone disproportionation was monitored at 485 nm according to the methods of Vernon and Shaw [22] and Shneyour and Avron [23]. $K_3Fe(CN)_6$ reduction was monitored at 420 nm and the reduction of dimethylbenzoquinone was determined according to the method of Saha et al. [24]. Baird Atomic interference filters were used to isolate 650, 680 and 710 nm light. A Kettering-Yellow Springs Instruments Radiometer was used to measure light intensities.

Diphenylcarbazone was obtained from Aldrich and was recrystallized from methanol, CCCP was obtained from Calbiochem, DCMU was a gift of P. Heytler. All other chemicals were of reagent grade.

RESULTS AND DISCUSSION

The effect of monovalent cations on excitation energy distribution between the two photosystems

We determined the effect of both mono- and divalent cations on the relative quantum yields for either Photosystem II or Photosystem I reactions. For Photosystem

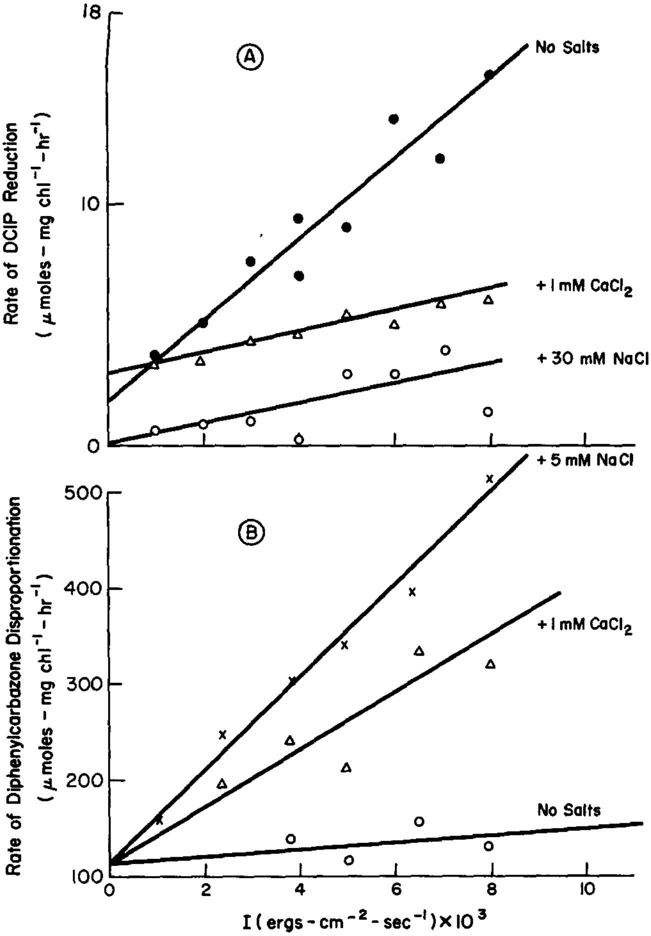

Fig. 1. The effect of cations on the relative quantum yields for DCIP reduction and diphenylcarbazone disproportionation. (A) The rate of DCIP reduction was determined as a function of light intensity using a 650 nm interference filter for chloroplasts (at 6.7 μg/ml chlorophyll) suspended in a medium containing 100 mM sucrose + 0.3 mM Tris base (final pH 8) +40 μM DCIP+other additions indicated. 10 μM CCCP was added to prevent interference by cation effects on the coupling system [30]. However, identical results were obtained in its absence. (B) 0.15 mM diphenylcarbazone (DPCN) + 100 μM DCMU was substituted for the DCIP.

II, we used either DCIP, $K_3Fe(CN)_6$ or dimethylbenzoquinone [24] as electron acceptors. Similar effects were obtained for all three electron acceptors. For Photosystem I, we used diphenylcarbazone disproportionation [22, 23]. The rates of electron transport were determined as a function of light intensity (Fig. 1). In each case, the slope of the line represents the relative quantum yield for the reaction in question. A 650 nm interference filter was used for the irradiation of chlorophyll *b*. Therefore, we were monitoring the transfer of excitation energy from the chlorophyll *b* of the light-harvesting pigment protein [25] to either Photosystem II or Photosystem I reaction centers. The concentrations of NaCl used were those which produced a maximal decrease in chlorophyll *a* fluorescence (i.e spillover would be maximal).

TABLE I

COMPARISON OF THE EFFECTS OF VARIOUS SALTS ON DCIP REDUCTION AND DIPHENYLCARBAZONE DISPROPORTIONATION

Rates of DCIP reduction and diphenylcarbazone disproportionation were determined as described for Fig. 2. The control rates were 8.6 and 82 μmol · mg chlorophyll^{-1} · h^{-1} for DCIP and diphenylcarbazone respectively. Each value represents the average of two determinations.

Salt added	Rate (% of control)	
	DCIP reduction	Diphenylcarbazone disproportionation
None	100	100
10 mM NaCl	46	167
10 mM KCl	44	157
10 mM Na^+ Tricine (pH 8)	62	230
1 mM $CaCl_2$	7	266
1 mM $MgCl_2$	29	170
1 mM $CaCl_2$+10 mM NaCl	29	188
1 mM $MgCl_2$+10 mM NaCl	–	164
1 mM Ca^{2+}+10 mM Na^+ Tricine (pH 8)	86	118

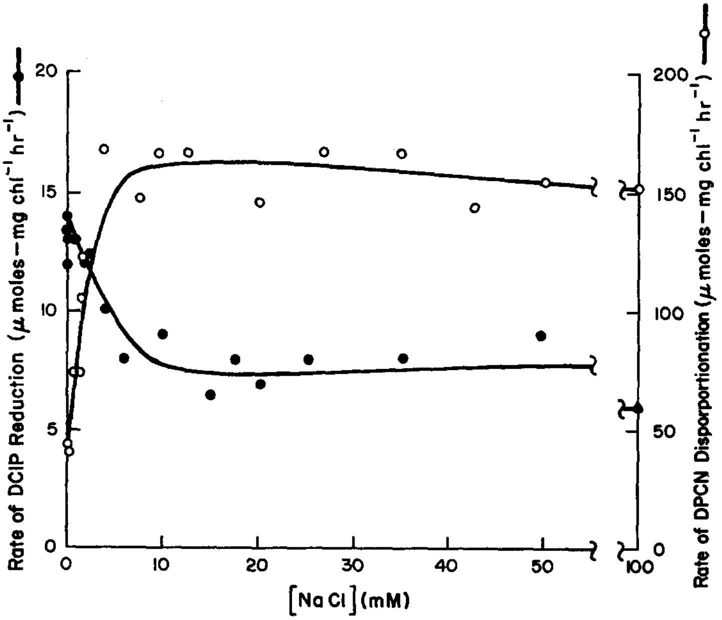

Fig. 2. The effect of varying NaCl concentration on DCIP reduction and diphenylcarbazone (DPCN) disproportionation under limiting light conditions. The conditions were the same as for Fig. 1 except that the light intensity was maintained constant at 8 · 10^4 ergs · cm^{-2} · s^{-1}.

Addition of NaCl decreased the relative quantum yield for DCIP reduction (Fig. 1) and increased that for diphenylcarbazone disproportionation. Similar results were obtained for KCl and Tricine buffer (Table 1). Therefore, the direction of the changes in the relative quantum yields for both photosystems agree with the fluorescence results.

However, if the changes in quantum yields and fluorescence are related, they must have the same salt concentration dependence. This question is complicated by the observation that the chlorphyll *a* fluorescence changes are biphasic in that chlorophyll *a* fluorescence decreases at low salt concentrations and increases again at still higher concentrations. We will discuss the two concentration regions separately.

In the low concentration region, half-maximal effects were observed between 2–3 mM NaCl concentrations for both Photosystem I and Photosystem II reactions (Fig. 2). These results agree very well with those previously obtained for chlorophyll *a* fluorescence. Therefore, we can conclude that low concentrations of monovalent cations promote the transfer of excitation energy from chlorophyll *b* to Photosystem I.

However, there is no salt effect observed at high NaCl concentrations despite the large increases in chlorphyll *a* fluorescence observed. The large increases in fluorescence may reflect a decrease in the rate constant for radiationless decay as has been suggested by Malkin [26]. Alternatively, only a very small change in the quantum yield for photochemistry might produce a large change in fluorescence yield.

The observation that monovalent cations change the relative quantum yields for electron transport indicates that one of the light steps is affected. However, we might be affecting either transfer of excitation energy from the light-harvesting pigments to the traps or the primary photochemistry itself. To answer this question, we determined the quantum yields as a function of wavelength of actinic light (Table II). Particularly in the case of diphenylcarbazone disproportionation, a much greater effect is observed when 650 nm light is used to irradiate chlorphyll *b* than when the Photosystem I traps are irradiated directly with 700 nm light. The small effect observed at 700 nm might be due to an increase in the *P*-700 turnover itself. In the case of Photosystem II, we also see a greater effect when we irradiate the light-harvesting antenna with 650 nm light than when we irradiate the reaction center directly using 680 nm light. However, the results are less clear than for Photosystem I due to the greater spectral overlap between the light-harvesting and reaction center pigments.

TABLE II

THE EFFECT OF CATIONS ON THE RATE OF DCIP REDUCTION FOR CHLOROPLASTS SUSPENDED IN TRICINE BUFFER

Chloroplasts were suspended in a medium containg 50 mM sodium-tricine buffer pH 8.2+40 μM DCIP. Other conditions were as described for Fig. 2. Each value represents the average of two separate determinations.

Salt added	Concentration (mM)	Rate of DCIP reduction (μmol · mg chlorophyll^{-1} · h^{-1})
None	0	10.1
KCl	33	17.1
$MgCl_2$	10	14.9

We can conclude from these results that monovalent cations increase the transfer of excitation energy from the light-harvesting pigments to the Photosystem I traps while simultaneously decreasing the transfer to the Photosystem II traps.

The effect of divalent cations on excitation energy distribution between the two photosystems

Divalent cations decreased the relative quantum yields for DCIP reduction and increased those for diphenylcarbazone disproportionation (Fig. 1 and Table I). These results are the opposite of those obtained by Murata [2] and Avron and Ben Hayyim [27], but their systems included Tricine buffer. When we repeated the experiments under their conditions, we obtained their results (Table II) i.e., in the presence of Tricine, both mono- and divalent cations stimulate the rate of DCIP reduction under limiting light conditions. Furthermore, divalent cations added in the presence of Tricine inhibit diphenylcarbazone disproportionation (Table I). However, it is interesting to note that divalent cations did not stimulate DCIP reduction or inhibit diphenylcarbazone disproportionation in the presence of NaCl. These results dis-

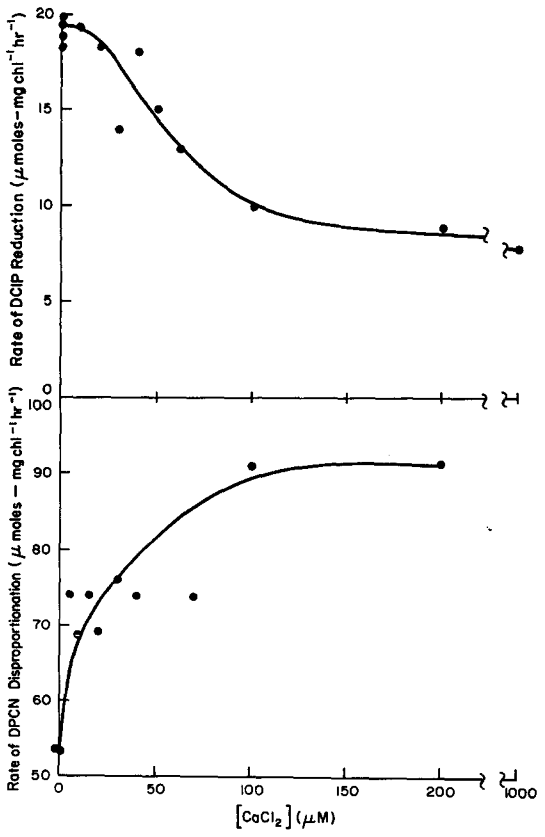

Fig. 3. The effect of varying $CaCl_2$ concentration on DCIP reduction and diphenylcarbazone (DPCN) disproportionation under light limiting conditions. The conditions were the same as for Fig. 2.

TABLE III

THE EFFECTS OF SALTS ON THE RATES OF DCIP REDUCTION AND DIPHENYLCARBAZONE DISPROPORTION AS A FUNCTION OF WAVELENGTH

The rates of DCIP reduction and DPCN disproportion were determined as described in Fig. 1 except that 650, 680 or 710 nm interference filters were used for actinic illumination. The intensities were 4.0, 1.6 and $2.4 \cdot 10^3$ erg $\cdot$ $cm^{-2} \cdot s^{-1}$ respectively. In each case, the intensity used was on the linear part of the rate vs. intensity curve. The control rates of DCIP reduction were 6.3, 5.6, 0.7 μmol $\cdot$ mg $chlorophyll^{-1} \cdot h^{-1}$ at 650, 680 and 710 nm respectively. The corresponding rates for DPCN disproportionation were 102, 113 and 91 μmol $\cdot$ mg $chlorophyll^{-1} \cdot h^{-1}$. Each case was done in duplicate. A change of 10 % was significant.

Conditions		Relative rates (% of control)		
Reaction	Salt added	Wavelength (nm):		
		650	680	710
DCIP reduction	None	100	100	100
	10 mM NaCl	73	84	85
	1 mM $CaCl_2$	20	16	77
DPCN disproportionation	None	100	100	100
	10 mM NaCl	267	198	139
	1 mM $CaCl_2$	241	176	152

agree with those obtained by Briantais et al. [7] for chloroplasts suspended in Tris buffer.

We decided to examine the effects of divalent cations in the absence of other salts in more detail. Both the decreases in DCIP reduction and the increases in diphenylcarbazone disproportionation saturated at 200 μM $CaCl_2$ (Fig. 3). Half-maximal effects occurred at 50 and 20–30 μM for DCIP reduction and diphenylcarbazone disproportionation respectively. Similar results are obtained with $MgCl_2$ (not shown). The concentration dependence for the decreases in the rate of DCIP reduction is the same as for binding of divalent cations to site II on the chloroplast membrane [30]. Therefore, divalent cation binding may be responsible for this effect.

The wavelength dependence of the divalent cation effects (Table II) shows that, in the case of diphenylcarbazone disproportionation, transfer of excitation energy from the chlorophyll *b* of the light-harvesting pigment protein to the Photosystem I traps is increased. However, in the case of Photosystem II, it is difficult to tell whether the inhibition of DCIP reduction is due solely to a decrease in excitation energy transfer or whether primary photochemistry might also be affected. It is of interest to note that divalent cations also cause inhibition of DCIP reduction in Triton TSFII [27] subchloroplast particles [28].

In the spinach chloroplast system, we can distinguish three different cases. (1) In the absence of added salts, divalent cations decrease the relative quantum yield for Photoystem II while increasing that for Photosystem I. However, there is only a slight decrease in chlorophyll *a* fluorescence. (2) In the presence of Tricine buffer, divalent cations increase the quantum yield for Photosystem II and decrease that for Photosystem I. These effects are parallelled by an increase in chlorophyll *a* fluorescence indicating an inhibition of spillover. (3) In the presence of NaCl, there are no changes in the quantum yields although fluorescence increases are observed [1]. Again, this may represent a change in the rate constant for radiationless decay.

We can explain both our results and those obtained using Tricine buffer as follows. In the absence of Tricine buffer, the excitation energy from chlorophyll *b* is transferred predominantly to Photosystem II. In this case, both mono- and divalent cations alter the system so that more of the excitation energy reaches Photosystem I. On the other hand, when Tricine is present, the excitation energy from chlorophyll *b* is predominantly transferred to Photosystem I (See Table I). In this case, the only effect that cations can have is to reverse the Tricine effects.

The question arises concerning the physiological significance of these results; particularly those obtained under low ionic strength conditions. Therefore, we calculated the concentration of free mono- and divalent cations inside the chloroplast. We used the data of Nobel [29] concerning chloroplast volumes and ion contents as well as that of Gross and Hess [30] concerning the number of binding sites for cations on the chloroplast membrane and on the stroma proteins. The values we obtained were approx. 10^{-5} M for divalent cations and 10^{-2} M for monovalent cations. These concentrations are in the range in which we observe changes in chlorophyll a fluorescence and the quantum yields for electron transport. Moreover, a small change in cation binding by either the membrane fraction or the stroma proteins could alter the internal concentration of cations which could, in turn, regulate energy transfer. Therefore, our results are of physiological significance. It is not certain what the significance of the Tricine results might be except that there might exist compounds in the chloroplast stroma which mimic its action.

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