

The Effect of Mg^{2+} on the Reduction of NADP by an Artificial Electron Donor

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The effect of Mg^{2+} on the light-dependent reduction of NADP by an artificial electron donor has been investigated with isolated broken chloroplasts. In both short and long wavelength actinic light, addition of the salt under most conditions stimulates the yield and the saturation rate. The magnitude of the stimulation is a function of pH. The data indicate that previous interpretations of the Mg^{2+} effect in this system invoking changes in the spill-over rate between the photosystems is no longer tenable. It is suggested that addition of the salt causes activation of inactive reaction centers.

Introduction

Mono- and divalent salts profoundly affect the electron transport activities of isolated, broken chloroplasts. Mechanistic interpretations of this effect include: decreased spill-over of quanta from photosystem II to photosystem I [1, 2], increased spill-over of quanta from photosystem II to photosystem I [3], distribution of quanta between parallel light reactions and activation of photosynthetic units [4–6], increased utilization of excitation energy at the expense of radiationless transitions [5, 7, 8] and an increase in the size of the pigment array of photosystem II [9]. All interpretations rest on the observation that addition of the salts only minimally changes the absorption of light by chloroplasts. Therefore, the observed changes in the yield must have been brought about by quanta which, in the absence of salt, were either inefficiently distributed between the light reactions or wasted by de-excitation.

In this report we describe experiments concerning the effect of Mg^{2+} on the light-dependent reduction of nicotinamide adenine dinucleotide phosphate (NADP) in the presence of dichlorophenyl dimethyl urea (DCMU) and the artificial electron donor 2,6-dichlorophenol indophenol (DCPIP $_2$), a reaction which is supposedly driven by photosystem I [10]. In most circumstances, Mg^{2+} stimulated the yield as well as the saturation rate of reduction in both far red and near red light. The effect is a

function of pH. We interpret our observation in the context of results reported before [5, 6] and suggest that cations activate photosynthetic units. These utilize quanta which prior to the addition of salt were wasted.

Materials and Methods

Chloroplast were isolated from peas by a previously published method [4]. The equivalent of 20 μg chlorophyll was suspended per ml of reaction medium containing 50 mM sucrose, 10 mM NaCl, 0.25 mM NADP together with a saturating amount of spinach ferredoxin. Also contained in the medium were 10 μM DCMU, 50 μM DCPIP and 3 mM Na-ascorbate. The concentration of various buffers was 15 mM at pH values which are given in the legends.

NADP reduction was measured spectrophotometrically at 340 nm. The extinction coefficient used was $6.25 \text{ (mM cm)}^{-1}$. Actinic light from a halogen source was filtered through a broadband interference filter (transmission between 530 and 645 nm) or steep cut-off filters with 50% transmission at 695 or 715 nm.

Results

The data of Fig. 1 show the influence of Mg^{2+} on the yield of NADP reduction in low intensities of short wavelength light as a function of pH. At pH values greater than approximately 7.5, addition of the salt causes a small, but reproducible, decrease in the yield. This decrease has been reported previously for measurements at pH 7.5 and was taken as an indication for a diminished quantum input

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Abbreviations: NADP, Nicotinamide adenine dinucleotide phosphate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenol indophenol



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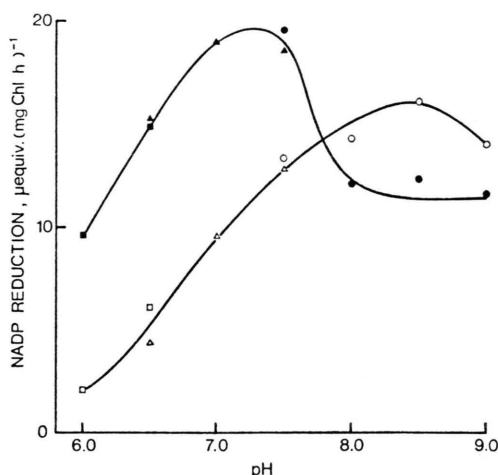


Fig. 1. Effect of Mg^{2+} on the reduction of NADP by an artificial donor as a function of pH in weak short wavelength light. (Broad-band interference filter). Open symbols: control; closed symbols: +5 mM $MgCl_2$. The following buffers were used: square: morpholino ethanesulfonic acid (MES); triangles: N-2'-hydroxy-ethylpiperazin-2-ethane sulfonic acid (HEPES); circles: N-tris (hydroxymethyl)-methyl glycine (Tricin). Other conditions are described under Methods and Materials.

into photosystem I [1]. The additional observation that the yield of DCPIP reduction or of K-ferri-cyanide reduction (both supposedly photosystem II reactions) increased led to the postulate [1, 11] that Mg^{2+} caused a decrease in the spill-over rate from photosystem II to photosystem I. Examination of Fig. 1 shows that the small decrease due to salt is restricted to pH values above 7.5. Below pH 7.5, Mg^{2+} appreciably stimulated the yield of NADP reduction. Thus, unless one postulates a change in mechanism due to a change in pH of only one unit, these data cast serious doubt on the spill-over theory.

Fig. 2 shows a similar experiment using far red actinic illumination. This light, according to present theory, is absorbed exclusively by photosystem I [10] and should therefore not be able to spill-over. The results are similar to those obtained with red light, *viz.* Mg^{2+} stimulated the yield of NADP reduction supported by an artificial electron donor and the effect is apparently a function of pH. This function is demonstrated for both actinic lights in Fig. 3.

The above data were obtained with low intensities of actinic light and reflect the relative quantum yields of the reaction. Table I list the results of experiments with saturating intensities of various

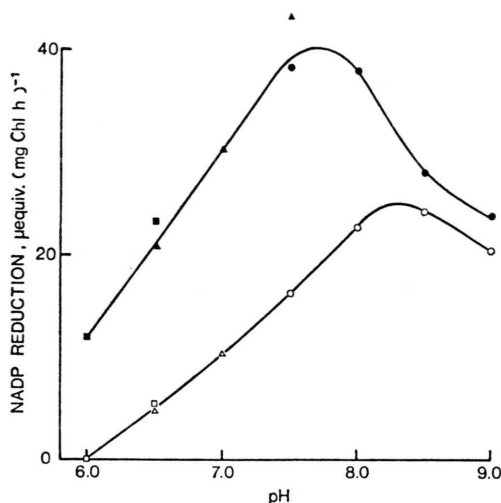


Fig. 2. Effect of Mg^{2+} on the reduction of NADP by an artificial donor as a function of pH in long wavelength light. Experimental conditions as in Fig. 1. The actinic light was filtered through a steep cut-off filter (RG 695). Symbols as in Fig. 1.

colors of light and at selected pH values. Obviously, Mg^{2+} stimulates the rate of NADP reduction in every case and to a high degree and again, the stimulation is somewhat higher at pH 6.5 than at pH 8.0.

Discussion

The data reported here indicate that Mg^{2+} stimulates the light-dependent reduction of NADP by

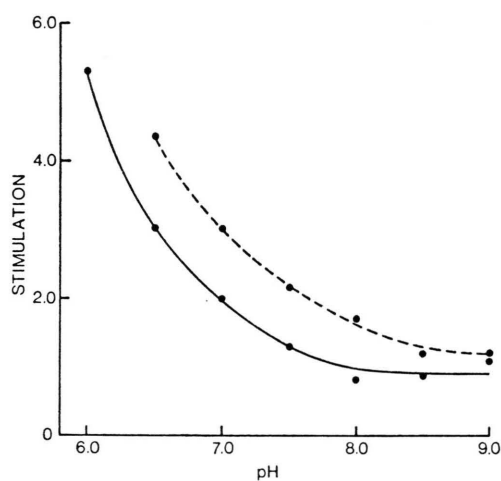


Fig. 3. Stimulation of NADP reduction by Mg^{2+} as a function of pH. The stimulation factor is the ratio of rate + Mg^{2+} /rate - Mg^{2+} . Data taken from Figs 1 and 2. Solid line: Short wavelength light dashed line: long wavelength light.

Table I. Stimulation of NADP reduction by Mg^{2+} in saturating actinic light.

Actinic light	pH 6.5			pH 8.0		
	— Mg^{2+}	+ Mg^{2+}	<i>f</i>	— Mg^{2+}	+ Mg^{2+}	<i>f</i>
IF 649	22.4	41.0	1.8	55.4	76.8	1.4
RG 695	19.2	38.4	2.0	58.2	83.2	1.4
RG 715	16.0	32.0	2.0	51.8	81.0	1.6

The numbers represent rates of NADP reduction with an artificial electron donor and are expressed as $\mu\text{equiv. [mg Chl h]}^{-1}$. *f* is the stimulation factor, the ratio of rates in the presence and absence of 5 mM $MgCl_2$, respectively. The reaction medium contained MES at pH 6.5 and tricine at pH 8.0. All other conditions as in Figs 1 and 2.

an artificial electron donor. The magnitude of the effect is dependent on the pH-value of the medium. Previously published measurements at pH 7.5 of Murata [1] showing a slight decrease have been reproduced here but our extended work suggest that the interpretation derived from this measurement, *viz.* a change in the spill-over rate between the photosystems is no longer tenable.

An alternative interpretation was postulated recently by Henkin and Sauer [9] who studied the effect of Mg^{2+} on the variable fluorescence yield and the reduction of DCPIP, both supposedly reflecting Photosystem II activity. The authors suggested that there are two distinct types of pigment arrays and that the addition of salt alters the arrangement of pigments so as to increase the size of the PS II antennae. Our data show that Mg^{2+} stimulates the reduction of NADP by the artificial

donor in short-wavelength (PS II) as well as far red (PS I) light. Therefore, the hypothesis of Henkin and Sauer [9] would have to be amended to include an increase in the size of PS I antennae as well.

In recent publications, we [5] (and others [7, 8]) have interpreted the improvement of electron transport rates with Mg^{2+} by postulating that the addition of the salt activates reaction units, *i. e.* we favour the idea that Mg^{2+} exerts its effect at the level of electron transport rather than on the pigments. The data reported here are consistent with that postulate: in both red and far-red light, we observe a similar increase in NADP reduction rates. Such an activation could conceivably be due to an increased binding of ferredoxin to the reaction center complex [12] although we point out that also the reduction of ferricyanide which does not involve ferredoxin can be stimulated by cations [5]. The alternative view that the increased NADP reduction came about by an increase in the rate constant of reaction center turnover appears unlikely, since at low light intensities, where the quantum input is the rate limiting parameter, enhancement of activity is quite pronounced.

Concerning the origin of the quanta which excite the higher concentration of traps in the presence of Mg^{2+} , we assume that they were wasted before the addition of the salt. This idea has previously been proposed by Li [8] and Malkin and Siderer [7].

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