Mg^{2+}-H^+ Exchange in Chloroplast Membranes in Dark

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Protons are released from unilluminated chloroplasts after the addition of Mg^{2+}. The greatest number of protons was measured at pH 6.5 and corresponded to 0.6 mol protons per mol chlorophyll.

1. Introduction

This work was initially undertaken to investigate the effects of Mg^{2+} on light-induced proton movement and formation of proton gradient across the thylakoid membranes. In course of investigation it was observed that addition of Mg^{2+} in a chloroplast suspension kept in the dark caused extensive extrusion of protons from the membranes into the medium. This report describes the properties of $Mg^{2+} - H^+$ exchange with the conclusion that most of the protons released by Mg^{2+} were previously bound. The pH dependence of H^+ extrusion indicated that the groups involved in $Mg^{2+} - H^+$ exchange were mostly protonated at pH 6 and mostly deprotonated at pH 8.

2. Experimental

Chloroplasts were isolated from 2 – 3 week old dwarf peas (Greater Progress) grown in the greenhouse. The procedure of isolation was of Nobel [1] as modified by Rurainski and Hoch [2]. Approximately 10 gm of young leaves were ground in a chilled mortar with 10 ml of a medium containing 0.4 M sucrose, 10 mM NaCl, and 15 mM Tris-HCl (pH 7.5). The macerate was filtered through two layers of silk bolting cloth (125 mesh) and centrifuged for 50 seconds at $2000 \times g$. The pellet was taken up in a small volume of grinding medium (2 – 3 mg Chl/ml) and stored in ice. The actual experiment was started immediately after isolation and continued for not more than four hours. Chlorophyll concentration was determined by the method of Arnon [3].

Packed-cell volume was measured following the method of Shavit and Avron [4] in microcapillary tubes after centrifugation for 15 min at 13,500 rpm in an international Model MB centrifuge. Centrifugation for 20 min gave a constant packed volume. Centrifugation was performed in a cold room (4 °C).

The extent of H^+ movement in or out of the chloroplasts was calculated from the change in pH of the medium with a combination pH microelectrode (Corning 476050). The voltage signal output of the pH meter (Instrumentation Laboratory Model 245) was recorded on a Mosley 7100B recorder. For pH change measurements, chloroplasts were suspended in 10 mM NaCl which was stirred vigorously and kept at a constant temperature (12 °C) by circulation of water from a thermostat. The initial pH was adjusted with a standard 0.01 N NaOH or HCl. Quantitation of proton release was calculated from titrations with standard 0.01 N HCl in the chloroplast suspension during illumination [5].

3. Results

Upon addition of 5 mM $MgCl_2$ in a dark, unbuffered chloroplast suspension an extensive amount of H^+ was released into the medium as measured by the decrease of pH of the medium. The experiment done at initial pH 6.5 showed (Table I) that the number of protons released ranged between approximately 0.4 and 0.6 μmol per mg chlorophyll depending upon the particular chloroplast preparation. At pH 8.1 this range decreased to 0.09 to 0.13. The magnitude of proton extrusion was linearly proportional to the chlorophyll content as illustrated in experiment 1 of Table I. The dependence of proton release upon Mg^{2+} concentration is shown in Fig. 1. The half-saturation of the effect occurred around 1.0 mM $MgCl_2$.

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Table I. Proton extrusion by Mg^{2+} in dark.

Experiment	pH	Chlorophyll [µg/ml]	**Protons** extruded [mol H^+/Chl]
1	6.5	16	.65
		32	.64
		48	.60
2	6.1	25	.42
	7.1	25	.25
	8.1	25	.09
3	6.1	25	.48
	7.1	25	.28
	8.1	25	.13

Total volume of 4 ml reaction mixture contained: 0.25 mM Me viol, 10 mM NaCl, 0.4 mM TRIS. Temperatur 12 °C. $MgCl_2$ 5 mM.

Table II. Effect of Mg^{2+} on the packed volume of chloroplasts.

pH	Sucrose concen-tration [M]	Packed volume [µl per mg chlorophyll]		Ratio $\frac{+Mg}{-Mg}$
		−Mg	+Mg	
5.5	0	72	69	.96
6.1	0	75	66	.88
7.5	0	102	56	.55
	0.4	51	30	.59
7.0	0	112	71	.63
7.5	0	115	70	.61
8.0	0	117	70	.60
8.5	0	119	70	.59

All mixtures contained tricine (10 mM), NaCl (10 mM) and chloroplasts at a chlorophyll concentration of 1.0 mg per ml. $MgCl_2$ was added at a concentration of 5 mM. Packed volume was measured as described in Experimental.

The extrusion of protons does not appear to be due to osmotic shrinking of chloroplast volume because the extrusion decreased with increasing pH (Table I) while shrinking of chloroplast volume by Mg^{2+} increased with pH (Table II). At pH 5.5 or 6.1 the packed volume was essentially unchanged on addition of Mg^{2+}, but at these pH values the largest amount of protons was extruded. Although the packed volume is not an actual measure of chloroplast volume [6], it measures the relative change produced under varying experimental conditions.

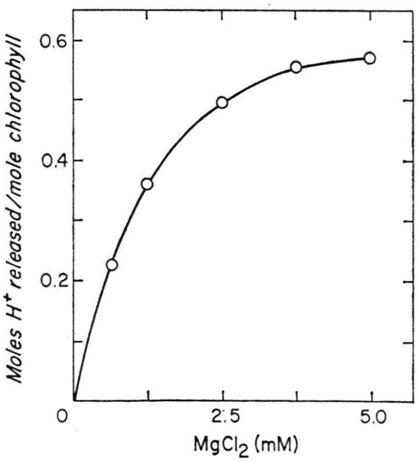

Fig. 1. Proton extrusion in dark as a function of $MgCl_2$ concentration. Experimental conditions as in Table I excepting that the initial pH was 6.5 and $MgCl_2$ was added as indicated. Each point was mean of two fresh samples.

4. Discussion

A quantitative estimation of proton release during shrinking of chloroplasts upon addition of Mg^{2+} suggests that most of the protons released by Mg^{2+} were bound. As obtained from packed volume measurement, a release of approximately 50 µl H_2O per mg chlorophyll occurred at pH 7.0 and above (Table II). If the corresponding number of protons released was 0.25 µmol Chl (Table I, pH 7.0), the pH of the extruded water would be 2.3. This value would be lower by one pH unit if the chloroplast volume was taken as 10 µl/mg Chl which was obtained from measurements of true osmotic volume [6] and Mg^{2+} is supposed to decrease the volume by 50% (Table II and ref. [4]). In other words, if the protons were free protons within the thylakoids, the internal pH was 2.3 or even less. Previous estimates of the internal chloroplast pH suggest that it is unlikely to fall below 4.0 [6].

Whether the bound protons are located on the inside or outside of the thylakoid membrane cannot be determined. The groups responsible for Mg^{2+} binding and $Mg^{2+}-H^+$ exchange do seem to influence the membrane environments that control the chloroplast structure as indicated by the Mg^{2+}-induced change in chloroplast volume. Perhaps the thickness of the membranes is also controlled by the same groups [7]. The action of Mg^{2+} may be through its ability to "screen" the fixed negative charges existing on the chloroplast membranes or, by being chelated, displace protons from chelating groups. In either of these mechanisms, protons should compete with Mg^{2+}. The decrease in chloroplast volume observed in lowering the pH (Table II) and failure of Mg^{2+} to decrease the volume at low pH support the above idea. The release of H^+ on addition of Mg^{2+} is a more direct evidence of such competition between Mg^{2+} and H^+. The number of H^+ released by Mg^{2+} is high at low pH and decreases with increasing pH of the suspending medium. This indicated that the groups responsible for $Mg^{2+} - H^+$ exchange are mostly deprotonated at high pH (~ 8), and at low pH (~ 6) most of these are protonated. This suggests that the carboxyl groups in the membranes may not be the primary source of the protons released. On the other hand, the phosphate groups in the membrane may be a main source of these protons since the pK of these groups falls in the range of pH 6 and 8, and the amount of phosphate groups available from the phospholipid alone in the membranes is reported to be approximately 0.5 μmol/mg Chl [8] which falls in the observed range of the magnitude of protons released (Table I).

It is to be noted that a similar phenomenon, *i. e.*, release of H^+ by cations, has been reported [9] with phospholipid dispersions in water. Three possibilities have been suggested to explain the observation: increased ionization of the groups caused by increased ionic strength, exchange of cation-H^+ in electrical double layer adjacent to the membrane surface, and specific binding of cations to the phospholipid. Among these the second possibility was regarded as the predominant one.

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