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ON A NON-CHLOROPHYLLIC MAGNESIUM FRACTION BOUND TO PLASTIDIAL LAMELLAR PROTEINS FROM *ZEA MAYS* L.

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SUMMARY

The presence of a non-chlorophyllic magnesium fraction bound to plastidial lamellar proteins was shown by detergent solubilization of the proteins. The ratio of protein-bound magnesium to protein nitrogen is nearly constant during the greening process of the etioplasts. We demonstrated the stability of the magnesium–protein complex by dialysis against concentrations of EDTA lower than 10^{-3} M. Dissociation of this complex is a reversible phenomenon.

INTRODUCTION

Most of the magnesium in chloroplasts is bound to chlorophyll. Larkum[1] estimated that 75 % of the magnesium in the plasts is bound to the chlorophylls. Various phenomena seem to indicate that magnesium, and to a lesser degree other divalent cations play a role in the organisation of the lipoprotein complexes which constitute the chloroplastic lamellae.

Murata *et al.*[2] and Homann[3] showed that the fluorescence of isolated chloroplasts is enhanced in the presence of magnesium, but that no magnification occurs in the presence of a chelating agent such as EDTA. Murata[4,5] also showed that, at liquid nitrogen temperature, the addition of magnesium modifies the fluorescence intensity of the two photochemical systems. This change in the transfer of energy between the two photochemical systems would appear to be due to a modification in the distance and mutual orientation of the chlorophyll molecules as the membrane changes.

Brown[6], Nishida and Koshi[7] and Izawa and Good[8,9] showed that isolated chloroplasts, in the presence of salts, particularly magnesium, undergo sudden contraction distinct from an osmotic shock. Under the electron microscope these authors observed that this contraction is accompanied by modifications in the lamellar and granar structure of the chloroplasts examined.

These observations may be compared with those of Nobel[10,11], Nobel and Packer[12], Dilley and Vernon[13]. These authors showed that the illumination of isolated chloroplasts brings about a simultaneous change in the volume of chloroplasts and in the ion movement, particularly a magnesium efflux.

Further, Thompson and Weier[14] have observed that the plastids of plantlets suffering from heavy magnesium deficiency have a lamellar system practically

devoid of grana with large size starch grains and dense inclusions bound to the membranes.

Recently, Nobel and Lin[15], Packer and Murakami[16] emphasized the role of Mg^{2+} in regulation of photosynthesis and in organisation of chloroplast membrane.

The above results led us to examine the interactions between magnesium and the proteins of the chloroplast membranes.

MATERIALS AND METHODS

The maize plantlets used (INRA 260 variety) were grown on water-soaked vermiculite at a temperature of 25 °C, in day light. The etiolated plantlets were grown in the same way but in darkness. The young plantlets were lifted after 12 days. The plastid structures were separated at 4 °C by grinding the limbs in a mortar with sand in a solution of 0.35 M NaCl buffered with 0.01 M phosphate buffer at pH 7.8. The ground material was filtered through cloth and centrifuged for 1 min at 1000 × *g*. The supernatant was then centrifuged for 20 min at 3000 × *g* in order to obtain a pellet containing mainly plastids. This pellet was washed 3 times with buffer solution. The plastids thus obtained are devoid of envelope and of soluble components.

Solubilization of membrane proteins is done as described elsewhere[17]. This method allowed us to solubilize all the membrane proteins of the plastids. The solution contains less than 0.5 % of the lipids originally associated with the plastid lamellae and does not contain any detectable amount of pigments.

The protein nitrogen of the samples, wet ashed in a sand bath by the Kjeldahl method, was determined by acid titration. The pigments were extracted from the chloroplast pellet by acetone–water (80:20, v/v) until complete decoloration of the residue was observed. A 505 spectronic Bausch and Lomb spectrophotometer was used to record the absorption spectra of acetone solutions. Mac Kinney's formulae[18] were used to calculate the amounts of chlorophyll contained in these solutions. Magnesium was determined by atomic absorption in a 290 B Perkin–Elmer spectrophotometer. CD spectra were recorded on a Roussel–Jouan dichrograph II.

RESULTS

The results in Fig. 1 show that, in all the samples investigated, there is a non-chlorophyll–magnesium fraction bound to the membrane proteins of the plastids. The values of bound magnesium expressed in μg Mg per mg of protein nitrogen are on average: 4.86 $\pm$ 1.55 μg/mg for etioplasts (18 experiments); 4.36 $\pm$ 1.67 μg/mg for chloroplasts (12 experiments). These two mean values are not significantly distinct so the quantity of magnesium bound to the membrane proteins of the plastids may be considered as constant in the order of 4.5 μg Mg/mg of protein nitrogen.

Lagoutte and Duranton[17], using the Weber and Osborn method[19] of electrophoresis on polyacrylamide gel, have shown that the membrane protein solution investigated was heterogeneous and exhibited at least ten distinct bands. By filtering through a Sephadex gel, we tried to isolate the protein(s) to which the magnesium is fixed. Filtration on Sephadex G-200 (Fig. 2) reveals two peaks, the second one bearing magnesium. Further filtration of this peak on Sephadex G-75 is shown in Fig. 3. Additional filtration through Sephadex G-50 does not enable Peaks III and IV

to be fractionated. Yet each of these peaks reveals at least two distinct bands after electrophoretic analysis. The elution diagrams are quite similar for etioplasts and chloroplasts membrane proteins.

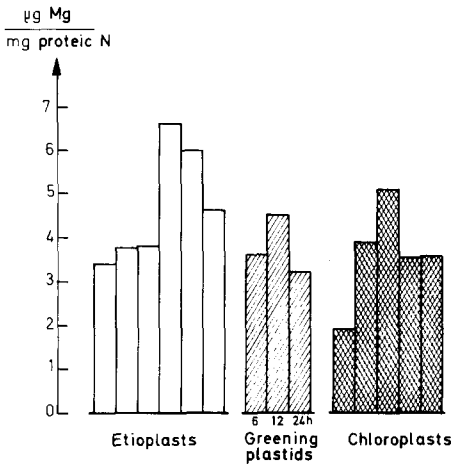

Fig. 1. Magnesium fixed to the membrane proteins of the plastids. Greening plastids were extracted from etiolated plantlets submitted to 3000 lux illumination for 6, 12 and 24 h.

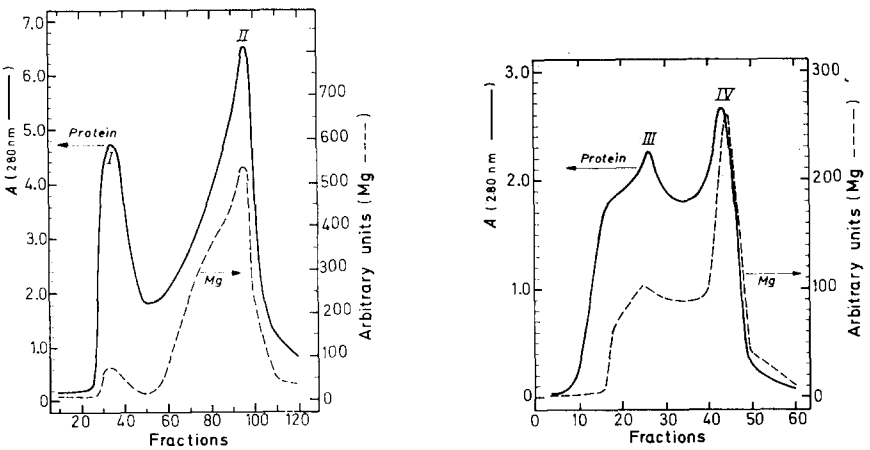

Fig. 2. Filtration through Sephadex G-200. Column: 2.4 cm × 70 cm; volume of fractions: 1.5 ml. The proteins were eluted from the column with 0.01 M phosphate buffer (pH 7.5), 0.1 % lauryl sulphate. The magnesium quantities are expressed in arbitrary units; the relationship is 0.5 µg Mg for 60 units.

Fig. 3. Filtration through Sephadex G-75 of Fraction II of the membrane proteins. Column: 1.8 cm × 45 cm. Proteins were eluted from the column with 0.01 M phosphate buffer (pH 7.5). Magnesium quantities are expressed in arbitrary units; the relationship is 0.5 µg Mg for 60 units.

Ethylene diamine tetraacetic acid, EDTA, is a powerful chelating agent. Its use may provide indications as to the nature of the interactions between magnesium and proteins. Dissociation of the magnesium–protein complex was carried out in the following way: the membrane protein solution was dialysed against a 0.1 % lauryl

sulphate solution containing EDTA, at pH 7. Further dialysis was carried out against a phosphate buffer pH 7.8, 0.1 % lauryl sulphate, to eliminate EDTA–magnesium complexes formed. The amounts of magnesium remaining fixed to the proteins were then determined.

Reassociation of magnesium with proteins is done by dialysis of the protein solution previously treated by EDTA against a $MgSO_4$ solution containing 0.1 % lauryl sulphate. Magnesium not bound to proteins is eliminated by a new dialysis against phosphate buffer. The amount of magnesium reassociating with the proteins is then determined. Dependent on the samples, EDTA concentrations were 10^{-4}, 10^{-3} or 10^{-2} M; magnesium concentrations used were 10^{-4} or 10^{-2} M. The results of these experiments are given in Table I. They are expressed in μg Mg/mg of protein nitrogen.

TABLE I

DISSOCIATION AND REASSOCIATION OF MAGNESIUM–PROTEIN COMPLEXES

The ratio of the magnesium remaining bound (dissociation) and the magnesium bound again (reassociation), to the protein nitrogen of membrane proteins is expressed in μg Mg per mg protein nitrogen

Type of samples	*Dissociation*		*Reassociation*	
	[EDTA] (M)	*Mg/N protein*	*[$MgSO_4$] (M)*	*Mg/N protein*
Etioplasts	0	3.76	10^{-2}	9.61
	10^{-4}	4.46	10^{-2}	8.53
	10^{-3}	3.25	10^{-2}	9.14
	10^{-2}	0.26	10^{-2}	6.58
	10^{-2}	0.22	10^{-4}	4.71
Chloroplasts	0	3.86	10^{-2}	12.1
	10^{-4}	4.21	10^{-2}	10.6
	10^{-3}	2.65	10^{-2}	12
	10^{-2}	0.12	10^{-4}	2.6

Gel filtration through Sephadex G-200 of the reassociated complexes shows that magnesium reassociated with proteins remains bound during filtration. It also seems that only the low molecular weight proteins, to which magnesium was originally bound are able to reassociate with it (Fig. 4).

DISCUSSION

It was possible to show that there was a non-chlorophyll magnesium fraction bound to membrane proteins by solubilizing these plastid proteins in an aqueous medium at a pH close to neutrality. The ratio between the bound magnesium and the nitrogen of the plastid membrane proteins was the same in organelles whose morphological aspects are quite different. This quantity stays appreciably constant during greening, that is the transformation of the etioplast form to the chloroplast form with intense chlorophyll synthesis. The mean value is 4.5 μg of bound magnesium per mg of protein nitrogen, or about 0.7 atom of magnesium for a protein of 25000 molecular weight (see ref. 17). However, filtration through Sephadex gel reveals high magnesium heterogeneity: Peak I (Fig. 2), which contains about 30 % of the proteins, corresponding to the five or six upper electrophoretic bands, is very poor in magnesium.

Peaks III and IV (Fig. 3) contain virtually all the magnesium bound to membrane proteins. In Peak IV the ratio between magnesium and protein reaches two atoms for one protein molecule. These values are only approximate because filtration on Sephadex gel did not completely separate the magnesium–protein complex(es).

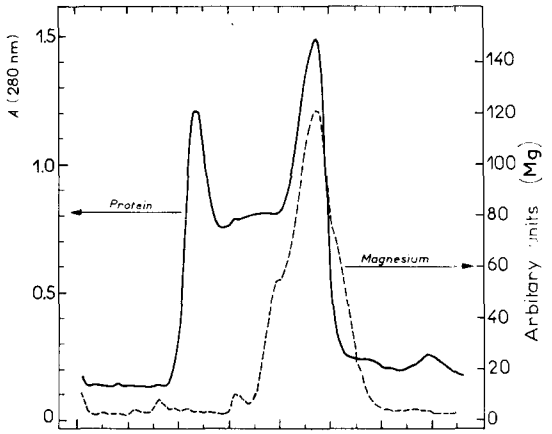

Fig. 4. Filtration of reassociated magnesium–protein complexes. Column: 1.8 cm × 45 cm; volume of the fraction: 1.5 ml. Proteins were eluted from the column with 0.01 M phosphate buffer (pH 7.5). 0.1 % lauryl sulphate. Magnesium quantities are expressed in arbitrary units; the relationship is 0.5 μg Mg for 60 units.

EDTA has a strong affinity for magnesium, but, during dialyses of membrane proteins against EDTA solutions, it was necessary to use a 10^{-2} M concentration to break the major part of the bonds between proteins and cation. This suggests two possibilities: (a) the existence of a very strong bond, probably a chelation cycle, or (b) that the sites of association between magnesium and proteins are masked. We showed that CD spectra of the membrane protein solutions, before and after dialysis against 10^{-2} M EDTA, are identical. The dialyses of membrane proteins against 10^{-4} and 10^{-3} M EDTA solutions show that increasing concentrations of EDTA enable a higher proportion of magnesium–protein complexes to be dissociated. This appears to favour the second hypothesis stated above, but it is not sufficient proof because lauryl sulphate is known to be a denaturing medium (Weber and Kuter[20]). Jirgenson and Capetillo[21] showed also that unordered polypeptide chains were ordered to a considerable degree by sodium dodecyl sulphate.

During magnesium–protein reassociation experiments at 10^{-2} M magnesium sulphate it was noticed that the amount of magnesium bound was higher than the original concentration of bound metal. The preferential binding of the reassociated magnesium to low molecular weight proteins indicates that this reassociation is not only nonspecific adsorption. It has not yet been possible to determine whether the new magnesium binding sites are identical to the original binding sites or if the magnesium is bound to sites which have been freed from other cations. EDTA is not a chelating agent specific for magnesium and preliminary experiments have shown that these membrane proteins also contain calcium, zinc and iron in the same concentration range as that of magnesium.

These results agree with those of Brown[6] and Nobel and Lin[15]. Brown gave a value of 2.7 μg of non-chlorophyll magnesium per mg of chlorophyll for isolated pea chloroplasts. Nobel and Lin showed that under illumination before isolation of plastids, the non-chlorophyll magnesium content of isolated spinach chloroplasts varies from 0.350 μM per mg chlorophyll in the dark to 0.450 μM per mg chlorophyll in the light. If we assumed a ratio of chlorophyll to protein nitrogen of 1.1–1.2 we obtained about 3 μg Mg/mg protein nitrogen, 7.8 and 9.9 μg Mg/mg protein nitrogen. In the view of our results it may be assumed that some of the physicochemical properties of plastid membranes in which magnesium is involved take place at the level of low molecular weight proteins of the membranes.

We are currently trying to obtain a magnesium–protein complex free of contaminants in order to elucidate the nature and specificity of the binding and the physiological role of such a complex.

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