A MANGANESE PROTEIN COMPLEX WITHIN THE CHLOROPLAST STRUCTURES

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1. Introduction

The presence of manganese is absolutely required to obtain a normal, photosynthesizing chloroplast. Recent estimations of the content of this metal in chloroplasts of various higher plants were of one atom of manganese for 50 to 100 molecules of chlorophyll [1–5].

Two different functions are currently ascribed to manganese. The first one takes place in photosynthesis itself; within photosystem II activity, it is directly involved at the oxidant side [6–7]; here, 2/3 of the total manganese seem to be employed in oxygen evolution. The remaining 1/3 is very tightly bound and has been recognized to have a structural role, mainly in the stacking of the lamellae [8].

Among the structural proteins of the chloroplast, we have previously reported the presence of a peptide chain of mol. wt 25 000 [9,10]. This component is specifically associated with a major part of the chlorophyll and is commonly said to be characteristic of photosystem II particles [11,12].

We describe here a manganese fraction firmly bound to this protein, in the proportion of one atom of manganese for about eight molecules of protein. Considering the drastic conditions used to isolate this complex, it is likely that we are concerned with the one third fraction of manganese implied in the structuration.

2. Materials and methods

2.1. *Preparation of the structural proteins from chloroplasts*

Seeds of *Zea mays* L. (variety INRA 260), were germinated on vermiculite impregnated with distilled water in a glass house, in daylight and at a temperature of 25°C. Young plants were ready for experimentation 12 days later, at the three leaves stage.

Structural proteins were then extracted as previously described [9]. When a lipo-proteic solution was needed, we stopped the preparation procedure after the first SDS extraction (see [9]).

2.2. *Electrophoresis of the protein solutions*

Analytical electrophoresis was performed with 15% acrylamide gels in Tris buffer according to Laemmli [13] and Blattler [14], as mentioned elsewhere [15]. Proteins were stained with Coomassie Blue.

For preparative electrophoresis, we have designed a special apparatus which gave us very good results. A schematic representation of it is given in fig. 1: the composition of upper and separation gels are the same as for analytical electrophoresis; the lower gel is identical to the separation gel. Polymerization of the different gels at the required levels was obtained by layering acrylamide solutions upon an aqueous solution of saccharose at 50%. The purpose of the lower gel is not only to limit the elution chamber, but mainly to trap the proteins which are not quickly eluted. So it is possible to avoid the mixing of closely migrating proteins, and thus to obtain a good resolution.

2.3. *Manganese analysis*

The content of manganese was evaluated both by atomic absorption spectrophotometry, on a Perkin Elmer 290 B apparatus (sensitivity: 50 to 100 ng/ml), and also by radioactivity which is a more accurate

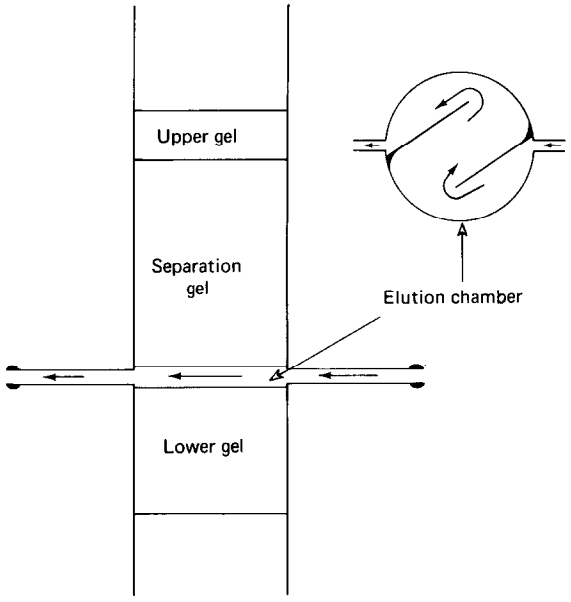

Fig. 1. A schematic drawing of the preparative electrophoresis apparatus used in this work.

technique (sensitivity 1 ng/ml). In all cases, protein solutions were first dialysed and the dialysis buffer, which contained no manganese, was used as the zero concentration solution.

2.4. *Amino-acid analysis*

These analyses were performed to estimate the exact amount of protein in solution. We used the classical automatic ion exchange chromatography, by mean of a Technicon analyser. Owing to the small quantities available, we also used a gas-chromatographic method which was about a hundred times more sensitive [16]. With both systems, internal standardization was done using Norleucine.

3. Results

We first looked for a possible repartition of manganese between the lipidic fraction and the protein components. For this purpose, we estimated the total amount of manganese in the aqueous phase, before and after extensive delipidation by acetone and by chloroform/methanol (2:1, v/v) [9]. We saw that all the manganese present in the initial lipoproteic solution was recovered in the final solution of delipidated proteins; there were no traces of manganese in lipids and pigments.

Fig. 2 shows a typical separation of the lowest protein components using the apparatus described before; we can see on the last gel that the 25 000 mol. wt. peptide chain is relatively well isolated. An attempt was made to further purify this component

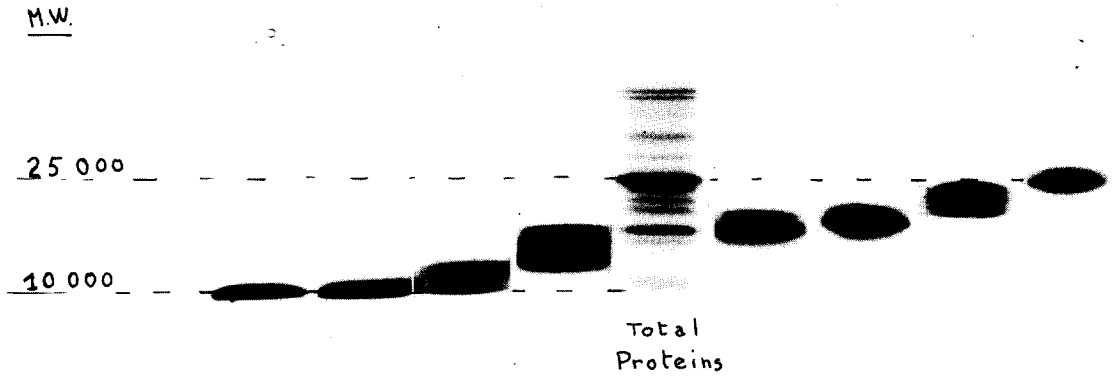

Fig. 2. Analytical electrophoresis of different fractions obtained after preparative electrophoresis in the mol. wt. range from 10 000 to 25 000.

on a column of Bio Gel P60, but it was not possible to obtain a better result this way; so, we used the first fraction without other treatments. For the quantitative determinations, we first estimated the degradation of amino acids during acid hydrolysis of the protein; it was about 10%. Then, we evaluated the contamination of the 25 000 mol. wt. component by scanning a gel previously stained for proteins; this contamination was of about 20% in weight. All these corrections gave a mean value of 240 ng of manganese for an amount of 1 mg of protein; thus, the molecular ratio was of one atom of manganese for eight 25 000 mol. wt. peptide chains.

A crude solution of all the proteins was submitted to an electrophoresis through a dialysis membrane using the same experimental conditions as for preparative electrophoresis. The concentration of manganese was then of 38 ng per mg of protein, which is about 6 times lower than the value found for the 25 000 mol. wt. component. According to this result, we can say that there is a specificity in the association between manganese and the 25 000 mol. wt. protein.

Table 1
Amino acid composition of the '25 000' protein component given as molar percent of each amino acid

Amino acid	25 000 component, % molar	mixture of all proteins from chloroplast structures [18], % molar
Asp	9.6	8.5
Thr	3.9	5.1
Ser	5.0	5.7
Glu	9.1	9.6
Pro	6.2	5.9
Gly	12.3	10.5
Ala	11.0	12.3
Val	7.1	6.9
Met	1.0	
Ile	5.1	5.7
Leu	10.5	11.1
Tyr	3.2	2.6
Phe	6.2	5.7
His	1.0	1.8
Lys	4.9	5.2
Arg	3.8	4.2

4. Discussion

One can see that drastic conditions were used in the purification of the '25 000' protein component. First, the action of a strong detergent (SDS), and then the action of an electric field in a medium which is known to remove the weakly bound fraction of manganese (Tris buffer pH. 8).

Therefore, the manganese which still remains linked to this protein is probably due to the one third fraction of this metal implied in the structuration.

Nevertheless, a very important point is that this protein, which links a part of the manganese, is also able to complex an important part of the chlorophyll [15] (and unpublished results). By comparing with the results of several authors [11,12,17], we can say that such a complex is characteristic of the photosystem II activity. So, we show here in a direct structural way that manganese is firmly associated with a component of the photosystem II. The little amounts of material available for the other peptide chains did not allow us to perform such evaluations on the other components; perhaps another fraction of the manganese is also linked to one or more other chains.

It we look at the amino acid composition of this protein (table 1), we can see that it is similar to that found for the mixture of all the different peptide chains [18]. We think that this protein, which gives the largest band after electrophoresis [9], is actually a major peptide chain of the chloroplast structures.

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