IMMUNOLOGICAL EVIDENCES FOR A LOCALIZATION OF SYSTEM I ON THE OUTSIDE FACE AND OF SYSTEM II ON THE INSIDE FACE OF THE CHLOROPLAST LAMELLA

J.-M. BRIANTAIS and M. PICAUD
Laboratoire de Photosynthèse du C.N.R.S., 91-Gif-sur-Yvette, France

Received 1 November 1971

1. Introduction

At least 2 extreme models can be imagined to describe the structure of the chloroplast lamella. This membrane could be made of 2 bound layers, an outside one built of units of only one of the 2 photochemical systems which shields the inside layer, made only of units of the other photosystem; or the lamella could be a single membrane built of a checker board assembly of System I and System II units. The 2 layers model was a convenient picture suggested to explain why System I-enriched subchloroplast particles are first or preferentially removed from the chloroplast lamella by a low concentration of detergents [1–3]. But a larger affinity of the detergent to System I than to System II could also explain these results. A direct verification of the 2 layers hypothesis has been tried here, using the antigen–antibody reaction. The results with corn chloroplast particles presented here are more easily interpreted in terms of the 2 layers model than the one layer picture. They indicate that antigenic substances localized on the surface of System I-enriched subchloroplast particles are present also on the outside face of the chloroplast lamella, whereas a specific antigen of the surface of System II-enriched particles is localized deeper inside the chloroplast lamella.

2. Material and methods

Three "antigens" were prepared: class II granal chloroplasts isolated from corn leaves [4], System I- and System II-enriched fractions called C 80,000 and C 10,000, respectively, obtained by treatment of chloroplast with Triton X-100 [5]. The corresponding antisera were obtained by inoculating rabbits with the freshly prepared particles according to the procedure summarized in table 1. It must be stressed that about the same amount of chloroplasts and of C 10,000 were incubated whereas, because of the low yield of the C 80,000 particles, a smaller amount of this subchloroplast fraction was injected.

The intensity of the agglutination of chloroplasts due to the antibody–antigen reactions was measured by the size of the chloroplast clumps obtained at different concentrations of the serum tested.

3. Results

3.1. *Agglutination of chloroplasts by the three sera*

From fig. 1 it is evident that the serum of rabbits vaccinated with System II-enriched particles is less effective in direct agglutination of chloroplast than the 2 other sera.

3.2. *Effect of preincubation of chloroplast antiserum with System I or System II-enriched fraction*

As it is shown in fig. 2a preincubation with System I-enriched fraction removes essentially all the antibodies of the chloroplast antiserum which are involved in the direct agglutination of chloroplasts. In contrast (fig. 2b), preincubation with the System II-enriched fraction does not change the efficiency of the serum.

North-Holland Publishing Company – Amsterdam

Table 1
Vaccination schedule.

With chloroplast	With C 80,000	With C 10,000
1) Weekly injection, armpit ganglions area in presence of complete Freund adjuvant:		
3 times, each: 1 mg Chl– 5 mg proteins–Chl *a*/Chl *b*, ratios: 2.8–3.1–2.8.	2 times, 0.133 mg Chl– 0.500 mg proteins–0.156 mg Chl, 0.580 mg proteins–Chl *a*/Chl *b*: 11.9–3.6–	3 times, each: 1 mg Chl– 3.8 mg proteins – Chl *a*/Chl *b*: 2.2–2.1–2.1.
1 week later:		
2) Weekly injection, armpit ganglions area in presence of incomplete Freund adjuvant:		
3 times, each: 1 mg Chl Chl *a*/Chl *b* = 2.8–2.7–3.3	1 time, 0.650 mg Chl– Chl *a*/Chl *b* = 4.5	3 times, each 1 mg Chl– Chl *a*/Chl *b* = 2.5–2.2–1.7.
3 weeks later:		
3) One intravenous booster, ear vein:		
1 mg Chl Chl *a*/Chl *b* = 3.0	0.326 mg Chl Chl *a*/Chl = 4.3.	1 mg Chl Chl *a*/Chl *b* = 2.1.

3.3. *Comparison of C 80,000 and C 10,000 antisera*

A qualitative comparison of these 2 sera is reported in table 2.

In part A the abilities of the sera to perform a direct agglutination of the 3 kinds of particles are compared. The table points out that:

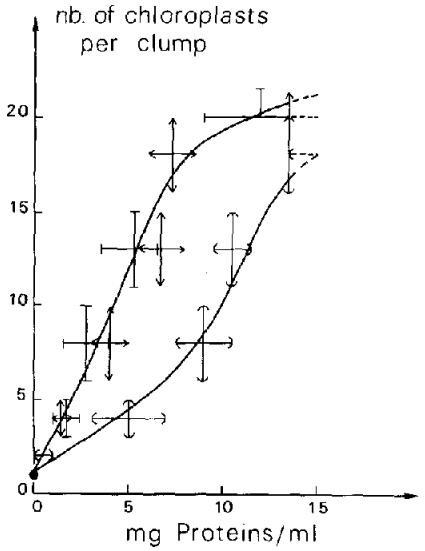

Fig. 1. Comparison of direct agglutination of chloroplasts by the 3 antisera: antichloroplast: ⊕, anti-C 80,000: ⇿, anti-C 10,000: ✠ . The concentration of corn chloroplasts corresponds to 0.5 mg Chl/ml.

(i) C 10,000 antiserum which poorly agglutinates chloroplasts, contains antibodies which induce a strong direct agglutination of System II-enriched particles (reaction 1).

(ii) C 80,000 antiserum agglutinates very well System I-enriched particles (reaction 6).

(iii) C 80,000 antiserum does not yield a direct agglutination of C 10,000 particles (reaction 5), whereas C 10,000 antiserum agglutinates C 80,000 particles (reaction 3).

In part B where preincubation assays are shown, the following main points emerge:

(i) Preincubation of C 10,000 antiserum with chloroplasts does not remove much anti-C 10,000 antibody (reaction 12).

(ii) Preincubation of C 80,000 antiserum with C 10,000 removes anti-C 80,000 antibodies (reaction 14) without any direct agglutination during the preincubation (reaction 5).

(iii) The same preincubation does not suppress the direct agglutination of chloroplasts (reaction 15).

4. Discussion

C 10,000 antiserum is less effective than the 2 other sera in performing a direct agglutination of chloroplasts. This is explained if we assume that the antigen involved in the direct agglutination of chloro-

Table 2
Antigen-antisera interactions.

(A)	Serum	Antigen	Agglutination
1	anti-C 10,000	C 10,000	++
2	Zero	C 10,000	0*
3	anti-C 10,000	C 80,000	+
4	anti-C 10,000	chloroplasts	0 or +
5	anti-C 80,000	C 10,000	0*
6	anti-C 80,000	C 80,000	++
7	anti-C 80,000	chloroplasts	++

(B)	Serum	Antigen used in preincubation	Antigen used in agglutination test	Agglutination
8	anti-C 10,000	C 10,000	C 10,000	0*
9	anti-C 10,000	C 10,000	chloroplasts	0
10	anti C 10,000	C 80,000	C 10,000	0*
11	anti-C 10,000	C 80,000	chloroplasts	0
12	anti-C 10,000	chloroplasts	C 10,000	++
13	anti C 10,000	chloroplasts	chloroplasts	0
14	anti-C 80,000	C 10,000	C 80,000	0 or +
15	anti-C 80,000	C 10,000	chloroplasts	++
16	anti-C 80,000	C 80,000	C 80,000	0
17	anti-C 80,000	C 80,000	chloroplasts	0
18	anti-C 80,000	chloroplasts	C 80,000	0
19	anti-C 80,000	chloroplasts	chloroplasts	0 or +

In part A the serum was incubated for 30 min at 35° (8.1 mg proteins/ml in the case of C 10,000 antiserum, 19.5 mg proteins/ml in the case of C 80,000 antiserum) with the different antigens (55 μg Chl/ml). In part B, the mixture was centrifuged in addition for 1 hr at 110,000 *g*. The supernatant was then incubated (5 mg protein/ml in the case of C 10,000 antiserum, 12 mg protein/ml in the case of C 80,000 antiserum) with one of the 3 antigens in a final concentration of 125 μg Chl/ml. The Chl *a*/Chl *b* ratio of the chloroplasts = 2.9, of C 80,000 = 3.8, of C 10,000 = 2.1. The measurements are only qualitative.

* C 10,000 aggregates if it is suspended in a medium of a high ionic concentration. That is the case with 0.1 M Tris-maleate, 0.4 M sucrose, pH 6.6; or 0.1 M phosphate buffer, 0.9% NaCl (= 0.15 M), pH 7.0; or with 0.01 M phosphate buffer, NaCl ≥ 0.05 M, pH 7.0. There is no aggregation if these subchloroplast particles are suspended in a 0.01 M phosphate buffer, NaCl ≤ 0.02 M, pH 7.0, in a concentrated serum blank, or if it is incubated with pancreatic lipase (Sigma, type II) 10 mg/ml in 0.1 M phosphate buffer, 0.9% NaCl, pH 7. The aggregation intensity can be estimated from the size of the aggregates obtained in a serum blank, at the same concentration as the serum tested, and must be deducted from the C 10,000 agglutination measurement. The C 80,000 fraction is very water soluble in the same 0.1 M buffers.

plasts (localized on the surface of the chloroplast lamella) is a component of the System I structure. Thus, inoculating C 10,000, which is poor in that antigen when compared to chloroplasts and System I particles, produces only small amounts of the antibody which causes direct agglutination of chloroplasts. However, it is necessary to be careful in comparing sera coming from different rabbits and therefore this comparison should be taken only as indicative.

The antigen of the C 10,000 surface, which is involved in direct agglutination of this particle by the C 10,000 antiserum (reaction 1), is absent from the surface of the chloroplast lamella (reaction 12). Vice versa, as is shown by reaction 15 and also by the lack of neutralization of the chloroplast antiserum by C 10,000, the antibody present both in the C 80,000 antiserum and the chloroplast antiserum, and which is involved in the direct agglutination of chloroplasts, corresponds to an antigen of the surface of the chloroplast lamella which is absent from the surface of C 10,000 particles.

There is, therefore, at least one antigen on the sur-

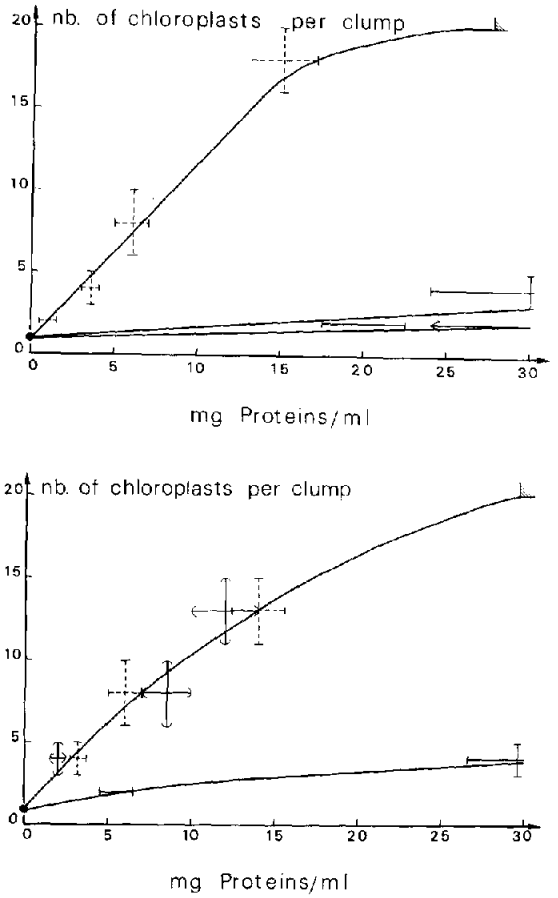

Fig. 2. Direct agglutination of chloroplasts by the chloroplast antiserum after a preincubation with C 80,000 (*a*) ⊹ or C 10,000 (*b*) ⊹ . Preincubation without any particles ⊢-⊣, and with chloroplasts ⊹ are taken as references. The samples of chloroplast antiserum at various concentrations (36, 14.4 7.2, 3.6 and 1.8 mg/ml in proteins) were incubated for 30 min at 35° in the presence of a constant concentration of antigen. The mixtures were centrifuged at 110,000 *g* for 1 hr. The supernatants were incubated for 30 min at 35° in the presence of chloroplasts, at a constant concentration in all the samples and contained *a*): 125 μg/ml; *b*): 167 μg/ml. The antigens used in preincubations were:

(*a*): C 80,000: Chl *a*/Chl *b* = 5.5, 83.3 μg Chl/ml, protein N = 47.3 μg/ml; Chloroplasts: Chl *a*/Chl *b* = 2.7, 83.3 μg Chl/ml, protein N = 66 μg/ml.
(*b*): C 10,000: Chl *a*/Chl *b* = 1.9, 167 μg Chl/ml, protein N = 97 μg/ml; chloroplasts: Chl *a*/Chl *b* = 2.5, 167 μg Chl/ml, protein N: 141 μg/ml.

face of System II-enriched particles which is inside the chloroplast lamella. Conversely, an antigen of the outside face of the thylakolid is missing in the surface of the System II-enriched fraction.

In contrast to C 10,000; C 80,000 has on its surface common antigens with the chloroplast lamella outside face. Reaction 17 and the neutralization of chloroplast antiserum by preincubation which C 80,000 indicate that the antigen responsible for direct agglutination of chloroplasts is present on the surface of System I-enriched particles. Reaction 18 shows that the chloroplast lamella has on its surface the antigen involved in the direct agglutination of System I particles by C 80,000 antiserum. Notice that the C 80,000 fraction used in this preincubation was not pure (Chl *a*/Chl *b* = 3.8) and may have contained enough C 10,000 particles (reaction 10) to neutralize the anti C 10,000 antiserum. Because of reaction 5 this explanation is more plausible than to assume that C 80,000 has the antigen for direct agglutination of C 10,000 localized on its surface. Likewise, according to reaction 9, we have to assume that C 10,000 contains still some System I structure.

So, on the 2 photochemical systems basis, there is a polarity in the structure of the chloroplast lamella. The sense of this polarity fits the necessity for the System II reductant side to be on the internal face of the membrane and for the System I terminal acceptor to be localized on the outside face of the chloroplast lamella, imposing the sense of the electric field through the membrane which determines the proton uptake. This conclusion of System I outside and System II more inside fits the results from electron microscope studies of Arntzen et al. [6], and of Wehrli et al. [7]. It agrees also with the results of Berzborn [8] and Regitz and Oettmeier [9] who localized the System I primary acceptor on the outside face of the chloroplast lamella.

Some more detailed information about the surface of the 3 structures can be deduced from these results. Since the neutralization of reaction 14 takes place without direct agglutination of C 10,000 (reaction 5), the antigen involved in direct agglutination of System I particles by C 80,000 antiserum must be also on the System II particle surface but on the bottom of a depressed area, deep enough to prohibit a direct agglutination of C 10,000. The presence of this antigen on the C 10,000 surface has been verified lately; pre-

incubation of chloroplast antiserum with C 10,000 suppresses the direct agglutination of C 80,000 by this serum. According to reactions 14 and 15, the antigen for direct agglutination of C 80,000 is different from the one involved in direct agglutination of chloroplast by the same C 80,000 antiserum. But because of reaction 18 the first one must be localized also on the surface of the chloroplast lamella. Then this antigen present on the surface of the chloroplast lamella, and which is not involved in direct agglutination of chloroplasts even in the presence of the corresponding antibody, must be on the bottom of a hole in the chloroplast lamella outside face. Thus, this antigen or hapten for direct agglutination of System I particles by C 80,000 antiserum is a common antigen present on the surface of all 3 structures. This conclusion agrees with the recent results of Radunz et al. [10] which indicate that the site of ferricyanide reduction by System II is localized on the bottom of a hole in the chloroplast lamella surface.

Moreover, reaction 17 shows that the antigen for direct agglutination of chloroplasts by C 80,000 antiserum is present on the surface of C 80,000, but because this antigen is not involved in direct agglutination of System I-enriched particles (reactions 14 and 15), it must be localized on the bottom of a depressed area of the C 80,000 particles.

So the physical separation of the 2 photochemical systems by Triton X-100 exposes in System II-enriched particles a surface which is inside the chloroplast lamella, and has reversed the relative position of 2 antigenic substances in C 80,000 vesicles [11] as compared to the chloroplast.

The comparison of the surface of the 3 kinds of particles indicates clearly a polarity of the thylakoïd membrane and leads to a preference, with some reservations, of the bilayer model for the chloroplast lamella with the external part composed of System I units, and the internal of System II units.

Acknowledgement

We are gratefull to Dr. Bazin and Dr. Houin of the CHU Créteil-94 who helped us to prepare the sera.

References

[1] J.S. Brown and J.G. Duranton, Biochim. Biophys. Acta 79 (1964) 209.
[2] J.P. Thornber, P.F. Gregory, C.A. Smith and J.L. Bailley, Biochemistry 6 (1967) 391.
[3] J.M. Briantais, Compt. Rend. 267 (1968) 2207.
[4] Y. de Kouchkovsky, Physiol. Veg. 1 (1963) 15.
[5] J.M. Briantais, Physiol. Veg. 7 (1969) 135.
[6] C.J. Arntzen, R.A. Dilley and F.L. Crane, J. Cell Biol. 43 (1969) 16.
[7] E. Wehrli, K. Muhlethaler and H. Moor, Exp. Cell Res. 59 (1970) 336.
[8] Berzborn, Progress in Photosynthesis Research I, ed. H. Metzner (1969) 106.
[9] G. Regitz and W. Oettmeier, IInd International Congress on Photosynthesis Research, 1971, in press.
[10] A. Radunz, G.H. Schmid and W. Menke, Z. Naturforsch 26b (1971) 435.
[11] G. Giraud, unpublished data.