Biochimica et Biophysica Acta, 502 (1978) 183—187

BBA 47494

ENERGY TRANSFER AND SITE OF ENERGY TRAPPING IN PHOTOSYSTEM I

PHILIPPE DELEPELAIRE and PIERRE BENNOUN

Institut de Biologie Physico-Chimique, 13, rue Pierre et Marie Curie, 75005 Paris (France)

(Received October 19th, 1977)

Summary

Studies of mutants of *Chlamydomonas reinhardi* deficient in the chlorophyll · protein complex CP1 show that energy transfer occurs between at least three Photosystem I units.

Evidence for the connection of the Photosystem I units is also obtained by studying their optical cross-section in wild-type cells in which a fraction of the Photosystem I centers are inactivated.

The site of energy trapping in the Photosystem I unit is shown to be the photochemical center itself and not the surrounding long-wavelength absorbing forms of chlorophyll.

Introduction

The occurrence of energy transfer between the photosynthetic units of Photosystem II was proposed by Joliot et al [1] in order to interpret the non-exponential character of the fluorescence induction in the presence of 3-(3,4-dichlorophenyl)-1,1,dimethylurea (DCMU) or at low temperature and the non-linearity of the relation between the oxygen rate and the fraction of active Photosystem II centers.

The properties of several mutant strains of *Chlamydomonas reinhardi* possessing various amounts of Photosystem II reaction centers support this concept. It was concluded that three or more Photosystem II units are connected [2].

The problem of the interconnection of the photosynthetic units of Photosystem I has been studied by several authors.

Joliot et al. [3] failed to detect energy transfer among the Photosystem I units of isolated spinach chloroplasts whereas Borisov and Il'ina [4] claimed that energy transfer indeed occurs.

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

We have reinvestigated this question by using several mutants of *C. reinhardi* possessing various amounts of the chlorophyll · protein complex CP1. This complex contains both the reaction center chlorophyll *P*-700 and the surrounding long-wavelength absorbing forms of chlorophyll *a* Ca 690 and Ca 700 [5—7].

We have measured the optical cross-section of the Photosystem I centers in these strains to check whether the energy absorbed in a unit devoid of CP1 could be transferred to another Photosystem I unit. We also attempt to localize the site of energy trapping in the Photosystem I unit. With respect to this problem, two cases could be considered: (i) the trapping occurs at the level of the long-wavelength absorbing forms of chlorophyll surrounding *P*-700 (ii) the trapping occurs at the level of the photochemical center itself.

Materials and Methods

Chlamydomonas wild-type mutant strains were grown at 25°C under continuous illumination (250 lux) in Tris/acetate/phosphate medium [8]. The cells were suspended in a phosphate buffer 0.1 M, pH 7, containing 7% Ficoll and were used at a concentration of 25 μg chlorophyll/ml. The determination of the optical cross-section was achieved according to Joliot and Delosme [9] by measuring the 515 nm absorption change 160 μs after a short actinic flash (4 μs duration at half-height) of varying intensity. The contribution of Photosystem II was eliminated by preilluminating the algae in the presence of hydroxylamine $5 \cdot 10^{-4}$ M and DCMU $2 \cdot 10^{-5}$ M [10].

Results and Discussion

The saturation curve of the 515 nm absorption change induced by Photosystem I reaction centers is shown in Fig. 1.

The energy required in order to induce 50% of the maximum photochemical activity of Photosystem I is inversely proportional to the optical cross-section of the Photosystem I reaction centers.

One observes that in the mutant C1 which possesses only 15% of the wild-type complement of the CP1 complex [11] the optical cross-section of the remaining Photosystem I centers is enlarged by a factor of two. This increase can be interpreted by assuming that the energy absorbed in a Photosystem I unit devoid of CP1 can be transferred to another Photosystem I unit.

Such an increase has been observed in all the CP1 deficient strains that we have studied, the largest increases in the optical cross-section of the Photosystem I centers being observed in the strains containing the smallest amounts of CP1.

The relationship between the size of the Photosystem I units and the number of active Photosystem I centers is plotted in Fig. 2.

Each experimental point corresponds to the data collected on one mutant strain deficient in CP1. For each strain we have measured the 515 nm absorption change induced 160 μs after one flash of saturating intensity and one flash of low energy (4% of the saturating flash).

The number of active Photosystem I centers is given by the 515 nm change

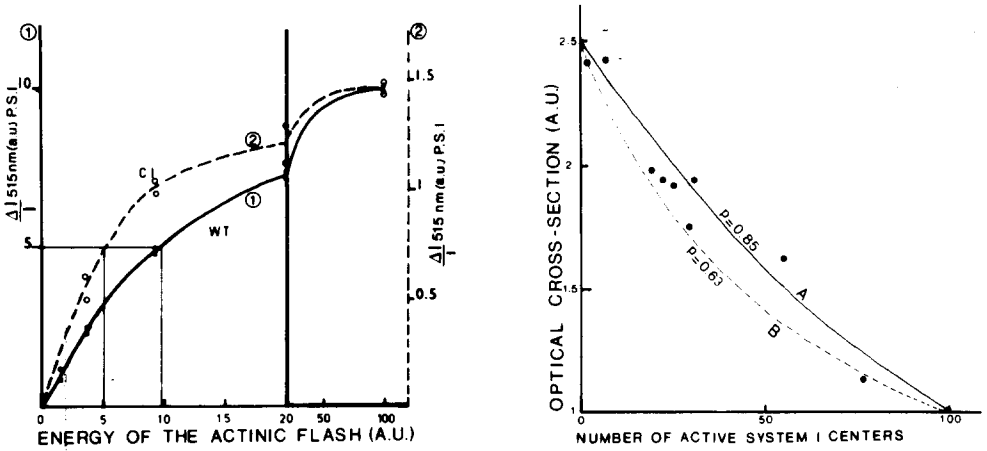

Fig. 1. Dependence of the 515 nm absorption change generated by Photosystem I on the energy of the actinic flash in *C. reinhardi* wild-type and mutant C1. The algae are preilluminated in the presence of 500 μM NH_2OH and 20 μM DCMU in order to inhibit Photosystem II. Chlorophyll concentration is 25 μg/ml. The absorption level is sampled 160 μs after the actinic flash (duration at half height = 4 μs). One observes that the energy required to develop 50% of the maximum Photosystem I photochemical activity is two times smaller in the mutant than in the wild-type. This mutant is strongly deficient in the CP1 complex and displays a maximum Photosystem I photochemical activity 15% of that of the wild-type.

Fig. 2. Variation of the optical cross-section of the photochemical centers of Photosystem I with the number of Photosystem I centers. Each experimental point corresponds to the data collected on one mutant strain deficient in CP1 in an experiment similar to that of Fig. 1. The ratio of the 515 nm change induced by a non-saturating flash (4% of the saturating flash) to the 515 nm change induced by the saturating flash is taken as a measurement of the optical cross-section of the Photosystem I reaction centers. The upper curve (A) corresponds to a model in which energy transfer occurs between three connected Photosystem I units. The lower curve (B) corresponds to a model in which energy is delocalized over all Photosystem I units.

after one saturating flash and the size of the Photosystem I unit is determined by the ratio of the 515 nm change induced by the low flash over the 515 nm change induced by the saturating flash [9,14].

We have studied in this way mutants having from 5 to 80% of the wild-type complement of CP1. Extrapolation of our data shows that a maximum increase by a factor of 2.5 of the size of the Photosystem I units is observed when the number of active Photosystem I centers approaches zero.

We have ruled out the possibility that some contribution of Photosystem II to the 515 nm change could remain in our measurements. For this purpose, we have isolated a strain carrying two mutations, one which results in the total inactivity of Photosystem II (F34, see ref. 12) and the other in a deficiency in CP1: we found essentially the same result as when the Photosystem II activity is inhibited by preilluminating with hydroxylamine and DCMU.

From these data, we conclude that in the CP1 deficient strains energy transfer occurs between at least three connected Photosystem I units.

We have plotted two theoretical curves on Fig. 2 corresponding to a model in which three Photosystem I units are connected and a model in which all the Photosystem I units are connected. For this purpose, we used the computation developed by Clayton [15].

We have assumed that the Photosystem I units devoid of CP1 are randomly distributed in the thylakoid membrane and that an excitation visiting a com-

plete Photosystem I unit is trapped in this unit with a probability of 1.

Taking into account the experimental value 2.5 as a maximum increase of the size of the Photosystem I unit, we compute a parameter p of energy transfer; p is the fraction of the energy absorbed by a Photosystem I unit devoid of active center which is transferred to the neighbouring units.

In the first model (A), we assume that energy transfer occurs within a domain of three units and that each domain is isolated: we deduce $p = 0{,}85$.

In the second model (B), we consider that all the units are connected in a two-dimensional lattice, each unit having 6 neighbours: we deduce $p = 0{,}63$.

As shown on the Fig. 2, the precision of the experimental points does not allow an exact determination of the number of Photosystem I units which are connected.

As in the mutant strains both *P*-700 and the long-wavelength absorbing forms of chlorophyll *a* are missing, these experiments do not distinguish which of the two is the site of energy trapping in Photosystem I.

To elucidate this problem, we measured the optical cross-section upon inactivating a fraction of the Photosystem I centers (see Fig. 3). In the first case, a background illumination is given to maintain 80% of the centers I in the inactive state P^+X. We observe that in this case the optical cross-section of the remaining active centers is not changed, compared to the situation where all the Photosystem I centers are active. This result is in agreement with the data of Joliot et al. on spinach chloroplasts [3].

In the second case, we have inactivated 80% of the Photosystem I centers by giving a background illumination under a nitrogen atmosphere in the presence of dithionite. Under these strong reducing conditions the Photosystem I centers are inactivated in the state PX^-. We observe that, in this case, the optical cross-section of the remaining active centers is enlarged by a factor of 2.

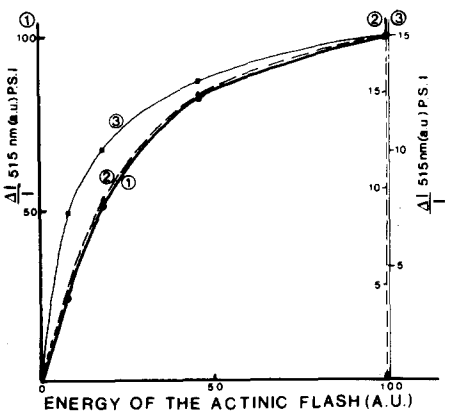

Fig. 3. Dependence of the fast 515 nm absorption change generated by Photosystem I on the energy of the actinic flash in *C. reinhardi* wild-type. 1: same conditions as in Fig. 1. 2: same conditions as in Fig. 1 except a strong background illumination is given which maintains 85% of the Photosystem I centers in inactive state P^+X. 3: same conditions as in Fig. 1 except a strong background illumination is given under a nitrogen atmosphere in the presence of 25 mM dithionite. This treatment places 82% of the Photosystem I centers in inactive state PX^-.

In these experiments, we changed the state of the photochemical center, but not the surrounding chlorophylls as was the case with the mutants. Our data show that the inactive center in state P^+X is a quencher at least as efficient as the active center PX. A quantum absorbed in a unit containing a center in state PX or P^+X is trapped in this unit. On the other hand, the inactive center in state PX^- has a lower quenching efficiency than an active center. A quantum absorbed in a Photosystem I unit containing an inactive PX^- can be transferred to another Photosystem I unit.

These data are in agreement with the report of Ikegami [13] of an increase in the fluorescence yield of Photosystem I particles induced by an illumination under reducing conditions. It is interesting to notice that on a quantitative basis the same increase in optical cross-section is observed when 80% of the CP1 is missing or when 80% of the centers are in the inactive state PX^-.

It is therefore clear that the long-wavelength absorbing forms of chlorophyll *a* are not acting as an energy trap: the actual trap of the Photosystem I unit is the photochemical center itself.

References

1 Joliot, A. and Joliot P. (1964) C.R. Acad. Sci. Paris, 258, 4622—4625
2 Joliot, P., Bennoun, P. and Joliot, A. (1973) Biochim. Biophys. Acta 305, 317—328
3 Joliot, P., Joliot, A. and Kok, B. (1968) Biochim. Biophys. Acta 153, 635—652
4 Borisov, A.Yu. and Il'ina, M.D. (1973) Biochim. Biophys. Acta 325, 240—246
5 Thornber, J.P., Alberte, R.S., Hunter, F.A., Shiozawa, J.A. and Kan, K.S. (1976) in Brookhaven Symposium Biology 28, 132—148
6 Chua, N.H., Matlin, K. and Bennoun, P. (1975) J. Cell. Biol. 67, 361—377
7 Bennoun, P. and Jupin, H. (1976) Biochim. Biophys. Acta 440, 122—130
8 Gorman, D.S. and Levine, R.P. (1965) Proc. Natl. Acad. Sci. U.S. 54, 1665—1669
9 Joliot, P. and Delosme, R. (1974) Biochim. Biophys. Acta 357, 267—284
10 Bennoun, P. (1970) Biochim. Biophys. Acta 216, 357—363
11 Bennoun, P., Girard, J. and Chua, N.H. (1977) Mol. Gen. Genet. 153, 343—348
12 Chua, N.H. and Bennoun, P. (1975) Proc. Natl. Acad. Sci. U.S. 72, 2175—2179
13 Ikegami, I. (1976) Biochim. Biophys. Acta 449, 245—258
14 Witt, H.T., Doring, G., Rumberg, B. Schmidt-Mende, P. Siegel, U. (1969) Angew. Chem. 4, 799
15 Clayton, R.K. (1967) J. Theor. Biol. 14, 173