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Evidence for structural distinction between PS II_α and PS II_β reaction centers

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The technique of (γ-ray) radiation inactivation has been used to estimate the functional sizes of the PS II centers (PS II_α and PS II_β) responsible for the biphasicity of the fluorescence induction curve from DCMU-inhibited spinach chloroplasts. It is found that the size of PS II_α (266 ± 5 kDa) is almost twice as large as that of PS II_β (135 ± 5 kDa). The result clearly indicates that the two types of PS II are structurally distinct and furthermore, the difference exists in the reaction center per se. Implications on the organization of PS II reaction centers are discussed.

Photosystem II; Functional size; Fluorescence induction

1. INTRODUCTION

The fluorescence induction of chlorophyll measured with DCMU-poisoned chloroplasts is thought to reflect the kinetics of the photoreduction of the PS II primary acceptor, Q [1]. The induction curve apparently is not generated by a single first-order photochemical event. An analysis of the growth of the area over the curve revealed the presence of two distinct phases: a rapid sigmoidal phase followed by a slow exponential phase [2]. The nature of these two phases has been widely studied and attributed to the two forms of PS II, termed PS II_α and PS II_β, respectively [3].

Many differences between the two types of PS II have been claimed. They differ, among other things, in their location on thylakoid membranes [4], antenna size and composition [5,6] as well as responses to herbicides and Mg^{2+} level [7,8]. The differences in their corresponding primary acceptor Q have also been reported. Unlike Q_α, Q_β has a higher midpoint potential [9] and is not associated with the two-electron gate [10]. Recently, the lack of connection between PS II_β and the plastoquinone pool has also been suggested [11].

Although most of the data point to the interpretation that PS II_α and PS II_β are structurally distinct, alternative arguments still exist. Their explanations are that the heterogeneity of the induction curve is due to incomplete DCMU blocking of some PS II centers as a result of different degrees of chloroplast integrity [12,13] or that it simply arises as a consequence of different degrees of PS II connectivity [14]. It has also been suggested that the differences between PS II_α and PS II_β could originate from different degrees of PS II-LHC interaction [15].

The technique of radiation inactivation has been successfully used to determine the molecular sizes of both soluble and membrane-bound proteins and receptors [16–18]. The method involves irradiating samples with ionizing radiation and measuring the loss of biological activity with increasing radiation dose which, by application of target theory, yields the molecular size of the structure responsible for the measured activity. The advantage of this

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Abbreviations: Chl, chlorophyll: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHC, light-harvesting complex; PS II, photosystem II

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technique is its ability to make a measurement in a crude preparation, i.e. independent of the sample purity. In addition, since the diagnostic measurement is biological activity, the technique directly reveals the functional size of the corresponding structure, rather than the relative molecular masses of individual polypeptides determined by other methods.

Here, the functional sizes of PS II_α and PS II_β were determined by use of the technique of radiation inactivation. We found that PS II_α is almost twice as large as PS II_β. The result suggests that the two types of PS II are indeed structurally distinct and furthermore, the difference is now shown to exist in the reaction center itself.

2. MATERIALS AND METHODS

Spinach was obtained from local market. Broken chloroplasts were prepared according to [19] and resuspended in 20 mM Tris-HCl (pH 7.8), 10 mM NaCl, 5 mM $MgCl_2$ and 0.2 M sucrose. Chloroplast samples (2 mg Chl/ml) were then placed in small plastic vials and frozen to −20°C. Chlorophyll concentration was estimated according to [20].

Irradiation with γ-rays was done with a ^{60}Co source (200 Ci) placed in the center of a specially designed box whose temperature was maintained at −15°C by a cryothermostat during exposure. Sample vials were put into the holes on the box making a circle around the source. The radiation dose rate (0.2 Mrad/h) of the irradiator was measured by the method of Hart and Fricke [21].

Samples were thawed immediately before fluorescence measurement. The fluorescence induction was performed at room temperature using a homemade fluorometer as described in [22]. All samples were poisoned with 10 μM DCMU. The fluorescence kinetics was analyzed according to Melis and Homann [2,3].

The theory of radiation inactivation and the method of data analysis are described in [16]. According to the theory, high-energy radiation causes primary ionizations in biological molecules; these result in damage to the molecular structure and an exponential loss of function with increasing radiation dose,

$$A = A_0 e^{-kD} \quad (1)$$

where A_0 is the population of the target of non-irradiated control; A is the survival population determined on each irradiated sample; k is a constant and D is the radiation dose.

Since the probability of a hit on the target molecule is directly proportional to its size, it can be shown that the molecular size is a linear function of the constant k. This has been developed into an empirical equation [23]:

$$\log M_r = 2.89 - \log D_{37,t} - 0.0028t \quad (2)$$

where M_r is the target size in kDa and $D_{37,t}$ ($= 1/k$) is the radiation dose (in Mrad) which, at the temperature of irradiation t, reduces the population of the target to 37% of the control value.

Each measurement (A) was converted to a percentage of the control value (A_0). The inactivation curve was calculated from the plot of data points [log (A/A_0) vs dose] by linear regression. The target size was then calculated according to eqn 2 with D_{37} obtained from the inactivation curve.

A number of standard enzymes of known molecular masses including glucose-6-phosphate dehydrogenase (104 kDa), catalase (232 kDa), glutamate dehydrogenase (320 kDa) and β-galactosidase (464 kDa) were used to test the validity of eqn 2 under our experimental conditions. Enzymes were irradiated and assayed as in [24]. The target sizes determined for these enzymes were in good agreement with their known molecular masses.

3. RESULTS AND DISCUSSION

It is generally believed that the area over the fluorescence induction curve (A_{max}) is a measure of the number of quanta utilized in the photochemical conversion of PS II [2], and hence is related to the number of PS II centers. According to Melis and Homann [3], a semilog plot of the growth of the area over the induction curve reveals the existence of two kinetically different phases which have been attributed to two forms of PS II, termed PS II_α and PS II_β, respectively. By assuming that the slow (β) component is exponential, it has been possible to estimate the relative contribution of β centers (β%) by extrapolating the slow phase to zero time. Thus, A_β ($= A_{max} \times \beta\%$) and A_α ($= A_{max} - A_\beta$) can be taken as the measure of the

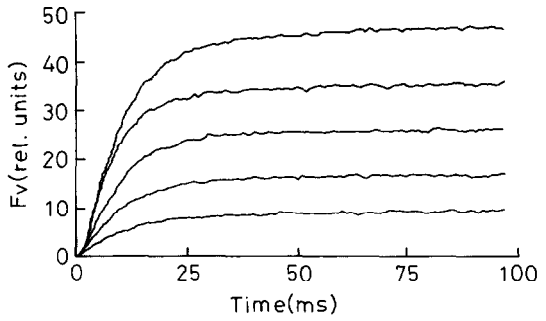

Fig.1. Fluorescence induction curves of chloroplasts irradiated by various doses of γ-rays. All samples were poisoned with 10 μM DCMU. From top to bottom: control, 0.8 Mrad, 1.6 Mrad, 3.2 Mrad and 4.8 Mrad.

numbers of PS II$_\beta$ and PS II$_\alpha$, respectively.

Because the energy deposition of ionizing radiation is so large, target theory assumes that the function of biological molecules is completely destroyed by a single hit; there are no partially damaged targets [16]. The PS II reaction center, though composed of several polypeptides, is believed to be a compact complex. We therefore assume that the center is a single target subject to 'all-or-none' type of killing by γ-rays. Under this assumption, radiation-induced decreases in A_α and A_β, which originate from the survival but completely healthy PS II centers, can be used as a gauge of the decay of PS II populations.

As shown in fig.1, γ-ray irradiation lowered the fluorescence yield of chloroplasts significantly. An analysis of the area growth over the induction curves showed that there was an increase in β% with increasing radiation dose, indicating that PS II$_\beta$ were inactivated more slowly that PS II$_\alpha$. This is depicted in fig.2a and b where decay of A_α and A_β with radiation dose is shown, respectively. Linear inactivation curves for both types of PS II suggest that they can be treated as single-sized targets, in accordance with the above-mentioned assumption. The D_{37} values obtained from the inactivation curves were used to calculate the functional sizes using eqn 2. It was found that the size of PS II$_\alpha$ (266 ± 5 kDa) is almost twice as large as that of PS II$_\beta$ (135 ± 5 kDa).

In addition, the relationship between radiation dose and three other parameters obtainable from fluorescence measurement was also investigated. The parameters are the inital fluorescence rise (F_0), and the rate constants of PS II$_\alpha$ and PS II$_\beta$ (K_α and K_β), respectively. We found that all the parameters decayed less than 20% within the dose tested (5 Mrad). The lack of significant variation in both rate constants reinforces the assumption that partial killing does not exist. The functional sizes, estimated by extrapolating the inactivation curves to high dose (not shown), were 37 kDa for F_0, 24 kDa for K_α and 26 kDa for K_β. Since F_0 originates from the light-harvesting pigment bed, whereas K_α and K_β are directly proportional to the antenna sizes of PS II$_\alpha$ and PS II$_\beta$, respectively, the functional sizes derived from these three parameters should all be related to the light-harvesting complexes (LHC), whose apoproteins have molecular sizes mainly between 25 and 30 kDa. The small molecular sizes obtained from the above-mentioned measurements suggest that all the LHC, irrespective of their location, are close to the

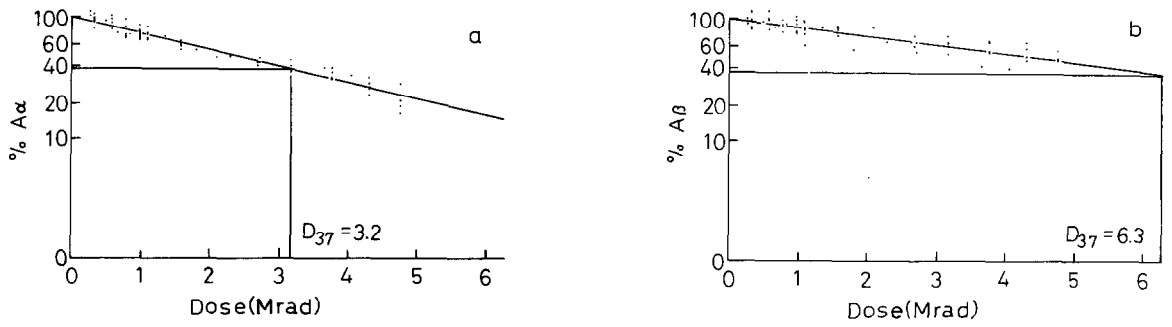

Fig.2. Semilog plots of (a) %A_α and (b) %A_β as a function of radiation dose. The inactivation lines are least-square fits of data points (70 points) from six independent measurements. The D_{37} values are also shown.

reaction centers or peripheral in the antenna system, and function as separate and 'equivalent' units, i.e. there is no hierarchy among the LHC in an antenna system.

Since the light-harvesting function of LHC decays very slowly under γ-ray irradiation, the functional sizes we found for PS II_α and PS II_β must be of reaction centers per se. Recently, the oxygen-evolving PS II core complex has been isolated [25–27]. The complex, which is mainly composed of five core proteins of 47, 43, 34, 32 and 9 kDa and in addition an extrinsic 33 kDa protein, is believed to represent the minimum machinery for oxygen evolution. Assuming that the stoichiometry of these proteins is one copy/center except for the 9 kDa protein of cytochrome b_{-559} which presents at two copies/center, the size of the PS II reaction center, which contains about 50 Chl, can be estimated to be nearly 257 kDa. This value is almost the same as that of PS II_α estimated by radiation inactivation. If this is the case, then the structure of PS II_β, which is about half the size of PS II_α, is even simpler than that of the PS II core complex we now identified. Alternatively, it has been suggested that the 32 and 34 kDa proteins constitute the reaction center, whereas the 47 and 43 kDa proteins are the non-essential antenna pigment proteins [28]. Then, it is possible that PS II_β represents the real structural minimum of the oxygen-evolving PS II, and PS II_α might present as a dimer.

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