Degradation of the 32 kDa Photosystem II Reaction Center Protein in UV, Visible and Far Red Light Occurs Through a Common 23.5 kDa Intermediate

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Dedicated to Professor Achim Trebst on the occasion of his 60th birthday

D_1 Protein, Protein Turnover, Reaction Center, *Spirodela oligorrhiza*, UV Light

A characteristic 23.5 kDa degradation intermediate of the 32 kDa photosystem II reaction center protein is produced upon illumination in UV, visible and far red light. We suggest a similar degradation pathway is employed in these three spectral regions, even though the light can enter the system through different photoreceptors.

Introduction

The reaction center of photosystem II (PS II) consists of three proteins: The 32 kDa protein (32K, also referred to as D_1), D_2, and cytochrome b_{559} [1, 2]. Based on the crystal structure of the *R. viridis* reaction center [3], Trebst [4, 5] proposed homologous structures with five membrane spanning helices for 32K and D_2. In Trebst's model, 32K and D_2 share the special pair chlorophylls (P 680) and two pheophytins. 32K alone binds Q_b and is the site of action of PS II herbicides (*e.g.* atrazine and diuron). D_2 binds Q_a, but does not seem to interact with the herbicides.

A characteristic property of 32K is its rapid, light dependent turnover [6–8], which is inhibited by atrazine and diuron [6, 7]. Degradation of 32K is promoted by light from the UV (250–400 nm), visible (400–700 nm) and far red (700–730 nm) spectral regions; turnover is most active in UV light [9]. While the photoreceptor(s) for degradation in UV light is distinct from the one(s) used in visible and far red light [9], it is unclear if the degradation pathway(s) in UV, visible and far red light share common intermediates after light absorption.

Abbreviations: PS II; photosystem II, 32K, the 32 kDa PS II reaction center protein; 23.5K, the 23.5 kDa degradation product of 32K.

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Cleavage of 32K in visible light proceeds *via* a degradation product of 23.5 kDa (23.5K) [10]. The scission occurs in the hydrophillic, phylogenetically conserved loop between helices 4 and 5 of 32K [10, 11]. An alpha-helix destabilizing region, common to rapidly degraded proteins [12], is adjacent to the cleavage site [10]. If this region is a factor in 32K degradation, and it is important in UV, visible and far red light, then 23.5K should be produced following illumination in these three spectral regions.

Experimental

Culture conditions and radiolabeling

Spirodela oligorrhiza (Kurtz) Helgm was grown phototrophically under cool white fluorescent lamps [13]. Radiolabeling with [^{35}S]methionine in the presence of 20 μM cycloheximide was carried out as described [10]. Plants were radiolabeled for 2 h under different wavelengths of light (light sources are described below). The incident fluence rate was 6 μmol m^{-2} s^{-1}. Membrane proteins were prepared for SDS-PAGE and detected by fluorography as described by Marder *et al.* [14].

Light sources

Cool-white fluorescent tubes were used for visible light treatments. For 300 nm light, a photoreactor bulb was employed (Rayonet), which has a half-power band width of 40 nm. Emission lines of 313 and 366 nm were isolated from a 250 Watt Hg source fitted with GG19 + UG11 filters and UG1 +

WG360 filters (Schott), respectively. Light of 447, 560, 660 and 716 nm was generated with 250 Watt tungsten-halogen projectors equipped with a heat absorbing glass and the appropriate interference filter (Schott, Balzers, or Detric). The half-power band width for the interference filters was 10 to 20 nm. Photon fluence rates were measured with a quantum flux meter (LiCor) in the visible and far red, and a potassium ferrioxalate actinometer in the UV [15].

Results and Discussion

Degradation of 32K in visible light was previously shown to proceed *via* a 23.5 kDa polypeptide intermediate (23.5K) [10]. In order to determine if a similar degradation pathway(s) is used after light of different wavelengths is absorbed, we checked if 23.5K is generated in the UV, visible and far red spectral regions. *Spirodela* plants were incubated with [^{35}S]methionine in the presence of cycloheximide to specifically label chloroplast-encoded proteins [16]. Radiolabeling was carried out under several specific wavelengths of UV, visible and far red light (Fig. 1). Both 32K and 23.5K were labeled at the seven wavelengths tested from 300 to 716 nm. Moreover, the relative levels of labeling of the two proteins (ratio of 23.5K to 32K) appear to be approximately the same under all the light conditions (Fig. 1). Both 32K and 23.5K are also generated in darkness at very low levels (Fig. 1). The reason for this is unclear.

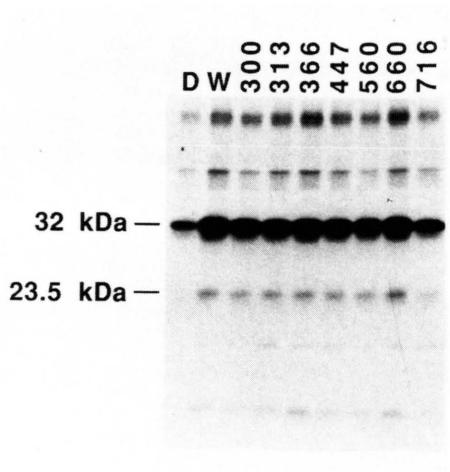

Fig. 1. Radiolabeling of 32K and 23.5K in UV, visible and far red light. *Spirodela* plants were radiolabeled with [^{35}S]methionine for 2 h in the presence of cycloheximide (20 μM). Membrane proteins were analyzed by SDS-PAGE and fluorography. Fluence rates were 6 μmol m^{-2} s^{-1}. The numbers above the gel lanes refer to the wavelength (in nm) of light used for radiolabeling. Radiolabeling was also performed in visible light (W) and darkness (D). A description of the light sources is given in experimental procedures. The positions of 32K (32 kDa) and 23.5K (23.5 kDa) are indicated.

The production of 23.5K in UV, visible and far red light is consistent with a common pathway for 32K degradation being used in the different spectral regions. In a related study, it was found that inhibitors of 32K degradation (diuron and atrazine) are equally effective in UV, visible, and far red light (Sopory *et al.*, in preparation). This also suggests that the pathway of 32K degradation is similar in the three spectral regions. If the alpha-helix destabilizing region adjacent to the cleavage site producing 23.5K is indeed a recognition site for degradation, it would appear that it is used in each spectral region. This would also imply that a similar proteolytic activity is employed in UV, visible and far red light.

A wavelength response spectrum for 32K degradation has been determined [9]. It was found that the photoreceptor(s) for 32K degradation in UV light is distinct from the one(s) used in visible and far red light. Furthermore, it was suggested that one of the UV photoreceptors is a semiquinone anion radical (probably Q_b^-), while the visible/far red photoreceptors are most likely chlorophyll and the other bulk photosynthetic pigments. The current study indicates that 32K degradation converges into a common pathway by the time 23.5K is produced, even though the activating photon is absorbed by different species. The question is, at what point does the 32K degradation process merge into a single pathway.

Because of its role as a two electron gate in PS II, Q_b exists as a semiquinone radical anion on 32K for a relatively lengthy period of time during linear photosynthetic electron flow in visible light [17]. Also, in far red light, plastosemiquinone anion is formed during cyclic electron transport around PS I [17]. In UV light, plastosemiquinone anion can absorb a photon directly [18]. Therefore, a semiquinone radical appears to be the earliest possible common intermediate in the 32K degradation process. Strikingly, the Q_b binding pocket, the alpha-helix destabilizing region and the 32K degradation site are all in close proximity in the loop between helices 4 and 5 in the model of 32K proposed by A. Trebst [4, 5].

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