S8-027

Regulation of photo-induced cross-linking of the D1 protein and CP43 in PSII by ATP

H Baba, S Miyake, M Ohtake, Y Yamamoto

Department of Biology, Faculty of Science, Okayama University, Okayama 700-8530, Japan

Keywords: D1 protein, photoinhibition, protein cross-linking, ATP, GTP

Introduction

When thylakoids or PSII-enriched membranes are illuminated by excessive light, the reaction center-binding protein D1 cross-links with the surrounding polypeptides. i.e., D2, the α-subunit of cytochrome b_{559} and CP43. The cross-linking has been interpreted as a process mediated by light-induced oxidation of amino acids of the D1 protein. In the acceptor-side photoinhibition mechanism, the cross-linking site of the D1 protein is probably at the stroma-exposed portion (loops) of the D1 protein. As D1, D2 and CP43 are phosphorylated proteins, and as the phosphorylation site is the threonine residue at the stroma-exposed N-terminal of each protein (Michel et al. 1988, Elich et al. 1992), it is interesting to know if phosphorylation/dephosphorylation of D1, D2 and CP43 affects the light-induced cross-linking. Here we examined the effects of ATP on light-induced cross-linking of D1 using Western blot analysis with anti-D1 and anti-phosphothreonine antibodies. ATP was shown to regulate the cross-linking of the D1 protein significantly. On the contrary to our initial expectation, the difference in the level of protein phosphorylation did not affect the cross-linking. Rather, the role of ATP as a nucleotide seems to be more important.

Materials and methods

Intact thylakoids and PSII-enriched membranes were prepared from spinach as described previously (Mullet and Chua 1983). The PSII membranes were treated with Tris where indicated (Yamamoto and Akasaka 1995). The samples were illuminated with strong visible light (3,000 μ $Em^{-2}s^{-1}$) at 25 ^{o}c for the periods indicated in the figures. SDS/urea-PAGE and Western blot analysis were carried out as previously described. The anti-phosphothreonine antibody was purchased from ZYMED (U.S.A.). The antibody against the DE-loop of the D1 protein was the gift from Dr.M.Miyao of National Institute of Agrobiological Resources, Japan. To see the effects of ATP, the nucleotide (0.4 mM) was added to the reaction mixture containing 0.4 mM sucrose, 5 mM NaCl, 10 mM $MgCl_2$, 50 mM HEPES and the samples equivalent to 0.5 mg chlorophyl (pH7.8). To hydrolyze the endogenous ATP, apyrase was added to the reaction mixture at the concentration of 6 units/ml and the whole mixture was stood at 25 ^{o}c for 30 min.

(A)

− ATP						+ ATP						(0.4 mM)
0	20	40	60	80	100	0	20	40	60	80	100	(min)

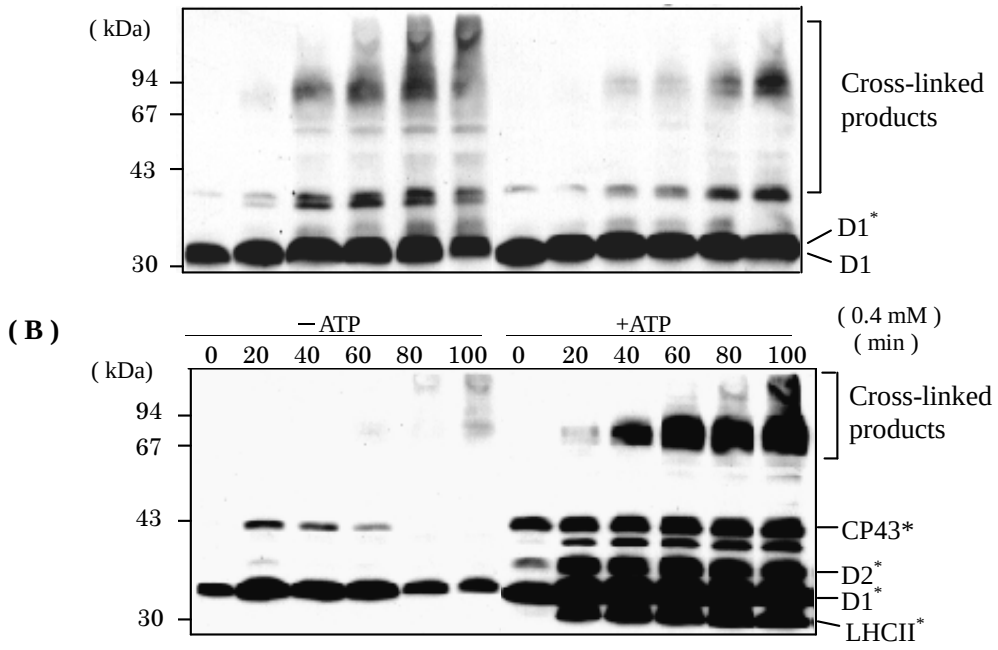
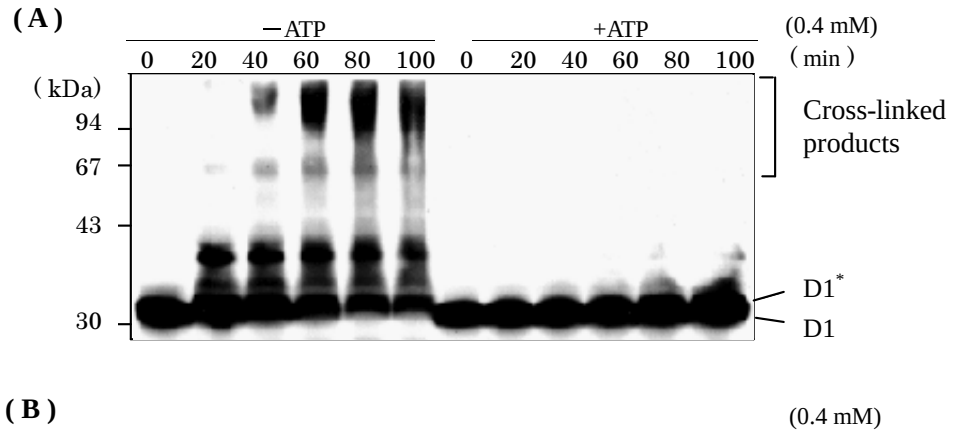


Fig.1. Effects of ATP on the light-induced cross-linking of the D1 protein in intact thylakoids. Apyrase was added to hydrolyze the endogenous ATP. The samples were illuminated with strong light (3,000 μ $Em^{-2}s^{-1}$) at 25 °c. Immunoblotting was carried out with the antibodies against D1-DE loop (A) and phosphothreonine (B).

Results

The effects of ATP on the light-induced cross-linking of the D1 protein in intact thylakoids and PSII-enriched membranes

The D1 protein of intact thylakoids and PSII-enriched membranes was already phosphorylated considerably before illumination, even through the samples were prepared in darkness from dark-adapted spinach leaves. Dark incubation or incubation under weak light of these samples did not dephosphorylate the D1 protein effectively (data not shown). When the intact thylakoids were illuminated with strong light (3,000 μ $Em^{-2}s^{-1}$), cross-linking of the D1 protein, which was estimated from Western blot analysis with the antibody against D1 DE-loop, took place both in the presence and absence of 0.4 mM ATP (Fig.1). In the presence of ATP, illumination of the intact thylakoids stimulated phosphorylation of D1, D2 and CP43. The cross-linked products of the D1 protein were also phosphorylated, but protein phosphorylation is apparently not a requirement for the protein cross-linking.



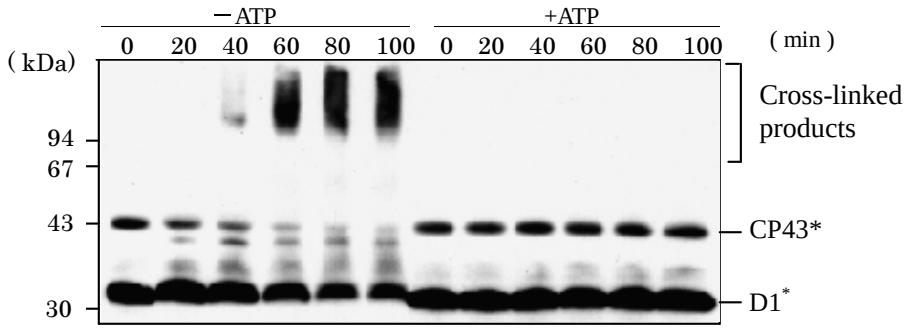


Fig.2. Effects of ATP on the light-induced cross-linking of the D1 protein in PSII membranes. The other conditions are the same as those described in the legend to Fig.1.

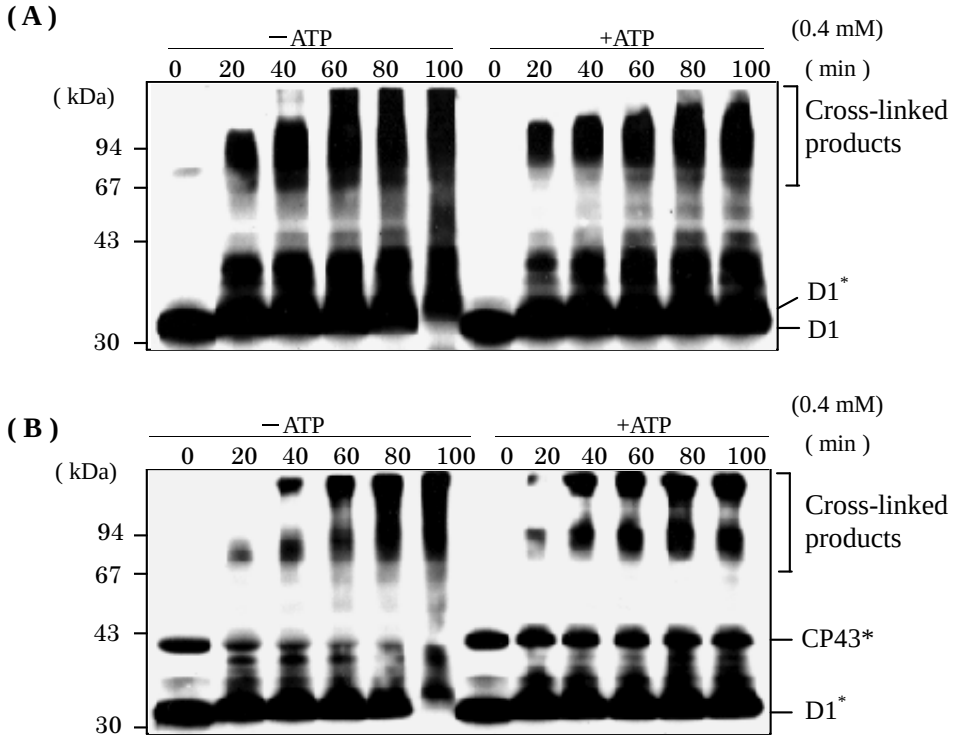


Fig.3. Effects of ATP on the light-induced cross-linking of the D1 protein in the Tris-washed PSII membranes. The other conditions are the same as those described in the legend to Fig.1.

The effects of ATP on the cross-linking of the D1 protein in PSII-enriched membranes are in contrast to that in the intact thylakoids. The addition of 0.4 mM ATP almost completely suppressed the light-induced cross-linking of the D1 protein (Fig.2). When ATP was added to the PSII membranes that had been treated with 0.8 M Tris (pH 9.0), a significant cross-linking of the D1 protein appeared again even in the presence of ATP (Fig.3). These results indicate participation of an extrinsic component(s) associated with the donor-side of PSII in the prevention of the ATP effects.

Comparison of the effect of ATP and that of GTP on the light-induced cross-linking of the D1 protein

To see if GTP has a similar effect as ATP, we examined the light-induced cross-linking of the D1 protein in the presence of 0.4 mM GTP using the intact thylakoids and PSII

membranes. The results were quite similar to those obtained with ATP. GTP suppressed the cross-linking of the D1 protein in the PSII membranes, but not in the intact thylakoids membranes (data not shown). The effects of ATP and GTP were not different at lower concentration range of the nucleotides (0－0.5mM) (data not shown).

Discussion

It has been indicated that the light-induced cross-linking of the D1 protein takes place via oxidation of amino acids in the D1 protein. In the present study, we showed that the cross-linking is suppressed efficiently by ATP or GTP. The effects of the nucleotides were clearly observed in the PSII membranes, but not in the intact thylakoids. Furthermore, the suppressive effects of the nucleotides were not apparent in the Tris-washed PSII membranes. These results suggest that these nucleotides affect the cross-linking of the D1 protein at the lumenal side. A possible candidate that mediates the nucleotide effects is OEC33, one of the extrinsic subunits of PSII. In the previous work, we suggested that the OEC33 effectively prevent the D1 protein from cross-linking. The nucleotides, such as ATP and GTP, may bind to the OEC33 and activate the protein to regulate the conformation of the D1 protein at the stromal side. The transmembrane effects of GTP on the D1 protein is also suggested by Spetea et al. (2001). Probably, ATP and/or GTP play a significant role in the regulation of the D1 turnover under light stress.

References

Elich TD, Edelman M, Mattoo AK (1992) *J. Biol. Chem.* **267**, 3523-3529.
Michel H, Hunt DF, Shabanowitz J, Bennett J (1988) *J. Biol. Chem.* **263**, 1123-1130.
Mullet JE, Chua N-H (1983) *Methods in Enzymol.* **97**, 502-509.
Spetea C, Hundal T, Andersson B (2001) *in this volume*.
Yamamoto Y and Akasaka T (1995) *Biochemistry* **43**, 9038-45.