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Active oxygen induced protein ubiquitination in *Chlamydomonas*

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When methylviologen-treated *Chlamydomonas* cells were exposed to light, the amount of ubiquitinated proteins with molecular masses of 28- and 31-kDa were drastically changed, i.e. the former increased within 20 min illumination, while the latter decreased. Since these changes are completely dependent on illumination and suppressed by adding 3-(3,4-dichlorophenyl)-1,1-dimethylurea, it was concluded that these changes were caused by active oxygen stress. Treating cells with hydrogen peroxide did not cause such changes of ubiquitination, suggesting that the molecular species of active oxygen is a superoxide anion.

Active oxygen stress; Stress response; Methylviologen; Ubiquitin; *Chlamydomonas reinhardtii*

1. INTRODUCTION

Ubiquition, present in all eukaryotic cells, is one of the most highly conserved proteins. It has been demonstrated that conjugation of ubiquitin to short half-lived proteins is essential for their selective proteolysis by cytosolic ATP-dependent protease [1]. In this case these proteins are transiently polyubiquitinated, thus high molecular weight ubiquitin conjugants are observed. Whereas several proteins are ubiquitinated independent of their own degradation, e.g. ubiquitination of histones (H2A and H2B) has been found in several animals [2,3] and in a slime mold [4]. The amounts of these ubiquitinated histones changed depending on the various physiological conditions of the cells [4–10].

It was previously reported that a ubiquitin system exist in a unicellular green alga *Chlamydomonas reinhardtii* [9]. Immunochemical analysis revealed that two polypeptides with molecular masses of 28- and 31-kDa were predominantly ubiquitinated in *Chlamydomonas*. Since the amounts of these two ubiquitinated proteins rapidly changed when the cells were heat-treated, it was suggested that the ubiquitination of these two polypeptides had regulatory functions. The isoelectric points of these ubiquitinated proteins were strongly basic, therefore it was suggested that one or both of them might be ubiquitinated histone(s) in *Chlamydomonas*. To understand the significance of these ubiquitinations, it is useful to determine which physiological conditions cause the change of ubiquitination similar to those caused by heat stress.

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In the presented study *Chlamydomonas* cells were subjected to various stress conditions (see in the text), and it was found that active oxygen produced by methylviologen under illumination caused a significant change in the amount of ubiquitinated proteins.

2. MATERIALS AND METHODS

2.1 *Preparation of* Chlamydomonas *cells*

Chlamydomonas reinhardtii 137c (mt-) was cultured synchronically by periodical illumination as previously described [9]. Cells from the light period were used.

2.2 *Methylviologen treatment*

Cells were preincubated in darkness for 5 min with 1 mM methylviologen, then illuminated with fluorescent lamps at 5 klux. Aliquots of the cells were harvested and immediately fixed with 10% (w/v) trichloroacetic acid.

2.3 *Detection of ubiquitinated proteins in* Chlamydomonas *cells*

Ubiquitinated proteins in the methylviologen-treated *Chlamydomonas* cells were detected by radioimmunoblotting as previously described [9]. Acid-fixed cells were collected by centrifugation, washed twice with ice cooled acetone, dissolved in the SDS-PAGE sample buffer, then electrophoresed. Ubiquitinated proteins were electrotransferred to a polyvinylidene difluoride membrane, and then detected by radioimmunoblotting using an anti-ubiquitin antibody and ^{125}I-labeled *Staphylococcus aureus* protein A (<30 mCi/mg, ICN). ^{125}I labeled proteins were imaged by autoradiography on X-ray film (Fuji XR) using an intensifying screen (Cronex Lightening Plus, DuPont).

3. RESULTS AND DISCUSSION

In order to determine which stress does cause the rapid (< 10 min) change of ubiquitination similar to those observed during heat stress [9], *Chlamydomonas* cells were exposed for short-term (up to 30 min) to high light intensity (< 100 klux), darkness, UV light (256 nm and 340 nm), low-CO_2 concentration (0.03%), 100%

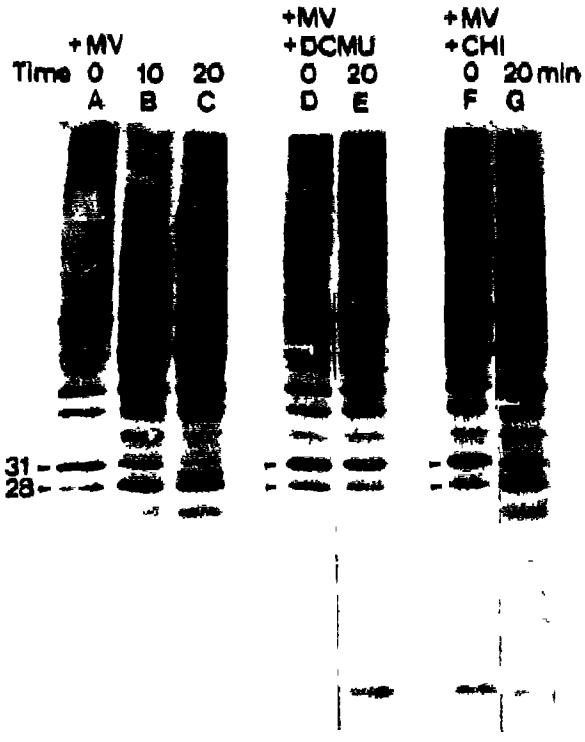

Fig. 1. Effect of methylviologen on ubiquitination of proteins in *Chlamydomonas* *Chlamydomonas* cells were preincubated in darkness for 5 min with several additives (MV: 1 mM methylviologen; DCMU, 5 μg/ml 3-(3,4-dichlorophenyl)-1,1-dimethylurea; CHI 1 μg/ml cycloheximide), then illuminated for the indicated period, and ubiquitinated proteins were detected by immunoblotting using anti-ubiquitin antibody

N_2, 100% O_2, and methylviologen. With the exception of methylviologen treatment in light, none of these treatments caused the significant change of ubiquitination.

In darkness, incubation of *Chlamydomonas* cells with 1 mg/ml methylviologen for 30 min caused no change in the ubiquitinated proteins. However, illuminating the cells after adding methylviologen resulted in very significant changes in the profile of ubiquitinated proteins. Fig. 1 shows the typical profile of ubiquitinated proteins after the above treatment in light for 10 and 20 min. The amount of 28-kDa ubiquitinated protein increased several times during the 20 min illumination, whereas the amount of 31-kDa decreased (lanes A-C). As in the heat shock experiment [9], high molecular weight polyubiquitin conjugants were observed as a spread band on the upper half of the lanes B and C.

None of these changes occurred when 5 μg/ml 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was added to inhibit photoelectron transport (lanes D and E), indicating that these changes of ubiquitination are caused by active oxygen stress.

Adding cycloheximide had no effect on these changes (lanes F and G), suggesting that de novo protein synthesis is unnecessary for initiating these change responses.

To determine either O_2^- or H_2O_2 is the active oxygen molecular species, H_2O_2 was added to the cell suspension. Since the addition of up to 1 mM of H_2O_2 had no effect, the active molecular species is believed to be O_2^-.

There are similarities between the change of ubiquitination caused by heat and active oxygen stress, i.e. both stress conditions caused an accumulation of high molecular weight ubiquitin conjugates and decrease of the 31-kDa ubiquitinated protein within 10 min. However, the initial change in the 28-kDa ubiquitinated protein was in the opposite direction, i.e. it was decreased by heat shock [9] and increased by active oxygen stress.

Although the physiological significance of these ubiquitination changes is still unclear, if these changes are important stress response features, then they may help the cells adapting to various external stresses and/or assist in the cells recovering from stress-induced damages. In order to understand the physiological significance of the changes in stress-induced protein ubiquitination, these ubiquitinated proteins must be identified.

REFERENCES

[1] Hershko, A (1988) J. Biol Chem 263, 15237-15240
[2] Goldknopf, I L. and Busch, H (1977) Proc Natl Acad Sci USA 74, 864-868
[3] West, M H P and Bonner, W M. (1980) Nucleic Acids Res. 8, 4671-4680
[4] Mueller, R D , Yasuda, H , Hatch, C L., Bonner, W.M and Bradbury, E M (1985) J Biol Chem 260, 5147-5153
[5] Nickel, B E , Roth, S.Y , Cook, R G , Allis, C D and Davie, J.R. (1987) Biochemistry 26, 4417-4421
[6] Parag, H A., Raboy, B and Kulka, R.G. (1987) EMBO J 6, 55-61
[7] Wunsch, A.M , Haas, A L. and Lough, J (1987) Dev Biol 119, 85-93
[8] Hensold, J.O , Swerdlow, P S and Housman, D E (1988) Blood 71, 1153-1156
[9] Shimogawara, K and Muto, S (1989) Plant Cell Physiol. 30, 9-16.
[10] Ferguston, D L Guikema, J A and Paulsen, G M (1990) Plant Physiol 92, 740-746