Inhibition of Rubisco Activation by Cadmium

Kwang Soo Roh*
Department of Biology, Keimyung University
Tel: +82-53-580-5207, Fax: +82-53-580-5164, e-mail: rks@kmu.ac.kr

INTRODUCTION

Cadmium (Cd) is the important heavy metal pollutant emanating in natural and agricultural environments from various sources including industrial and municipal wastes, sewage sludge, and combustion of fossil fuels and use of agro-chemicals. Cd of high concentration is recognized as one of the most phytotoxic heavy-metal contaminants (Prasad, 1995). Cd irreversibly replaces other metal ions in essential metalloenzymes (Jackson et al., 1990).

Although not essential for plant growth, this metal is readily taken up by roots and translocated into aerial organs (Catalado et al., 1981) where it can accumulate mainly in the vacuoles to high levels (Vögeli-Lange and Wagner, 1990).

The increasing interest in environmental pollution has led to investigations of Cd uptake, and its effect on plant metabolism. Plant metabolism may be affected by Cd in different ways. Cd was found to be effective inhibitors of chlorophyll biosynthesis (Stobart et al., 1985), photosynthesis (Weigel, 1985), respiration, and the nitrogen assimilation (Petrović et al., 1990). Cd-exposed plants also showed various symptoms of injury such as chlorosis, growth inhibition, browning of root tips, and finally death (Kahle, 1993).

Ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39) (rubisco) catalyzes not only the fixation of CO_2 in photosynthetic carbon reduction, but also the fixation of O_2 in photorespiration (Miziorko and Lorimer, 1983). Ribulose-1,5-bisphosphate (RuBP) as the substrate and 3-phosphoglycerate as the product of the carboxylase reaction of rubisco are important organic substances for development and growth of plant (Josette et al., 1993).

The rubisco holoenzyme is assembled in a catalytically inactive form and is activated by the binding of activator CO_2 and Mg^{2+} to ε-amino group of Lys-201 within the active site on the large subunit (Andrews and Lorimer, 1987). This activation process of rubisco *in*

vivo is catalyzed by rubisco activase (Somerville et al., 1982) in two sequential steps of the presence of ATP (Streusand and Portis, 1987) and RuBP (Portis, 1990).

Rubisco activase promotes the dissociation of RuBP and other inhibitory sugar phosphates from decarbamylated rubisco active site in a process requiring the hydrolysis of ATP (Wang and Portis, 1992). Rubisco activase also catalyzes the removal of inhibitors such as CA1P (2-carboxyarabinitol 1-phophate) and CABP (carboxyarabinitol 1,5-bisphosphate) from the active site of rubisco (Portis, 1992). CA1P binds tightly to carbamylated rubisco (Moore and Seemann, 1994). CABP does bind to both activated sites and inactive sites of rubisco (Zhu and Jensen, 1990).

In spite of the considerable literature on this subject, however, Cd toxicity on the photosynthesis at an enzyme level are not known with any certainty.

The objectives of the present study were (i) to determine the influence of Cd on the growth *in vitro* ; (ii) to determine the effect of Cd on chlorophyll a and b; and (iii) to determine the effect of Cd on rubisco and rubisco activase by measuring activity and content.

RESULTS

This investigation was performed to study the influence of Cd on growth, and photosynthetic pigments and enzyme in tobacco (*Nicotiana tabacum* L.) and jackbean (*Canavalia ensiformis* L.). Growth inhibition by Cd was reduced by Ca, but not reduced by nitrate. Chlorophyll levels were reduced by Cd. The combination of Cd and Ca increased the chlorophyll content compared to that in plants exposed only to Cd, while the combination of Cd and nitrate reduced its compared to Cd treatment only.

Activity and content of rubisco at Cd treatment was significantly lesser than in plants receiving no treatment. These data suggest that rubisco activity was associated with an amount of rubisco protein, and that the activation and induction of rubisco is inhibited by Cd. Inhibition of both the activity and content of rubisco by Cd was reduced by Ca, but increased by nitrate. The analysis of SDS-PAGE showed that the 50 and 14.5 kD polypeptides were identified as the large and small subunits of rubisco in the preparation, respectively. The intensity of both bands at control was significantly higher than that at Cd treatment, indicating that both subunits was affected by Cd.

Under the assumption that effects of Cd on rubisco may be related to rubisco activase,

its activity and content were determined by employing ATP hydrolysis assay and ELISA. The rubisco activase activity at Cd treatment was more decreased than the control. A similar change pattern was also observed in content of rubisco activase. These data indicate that decrease of activity and content resulting by Cd was caused by activity itself and/or content of rubisco activase. Remarkable differences in the intensitiy of both the 45 kD and 41 kD band were found between at control and Cd-treatment. These results suggest that the change in the levels of rubisco activase leads to a subsequent alteration of rubisco levels.

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