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CALMODULIN-STIMULATED Ca^{2+} UPTAKE IN MICROSOMES PREPARED FROM LEAVES OF SPINACH SUBMITTED TO VARIOUS LIGHT TREATMENTS

BY

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ABSTRACT

Microsomes prepared from spinach leaves exhibit an ATP-dependent Ca^{2+} uptake. This uptake is stimulated by calmodulin in microsomes prepared during the second half of the short day light period, but calmodulin is rather ineffective during the dark period. Short irradiations with red light given on whole plants before microsome extraction enhances the subsequent stimulation of Ca^{2+} uptake by calmodulin, when far red light inhibits it.

Key words: *Spinacia* — Microsomes — Ca^{2+}-transport — phytochrome — calmodulin.

INTRODUCTION

The regulation of the level of Ca^{2+} present in cytoplasm is of considerable importance, since several biochemical and physiological processes are triggered by a raise in cytosolic Ca^{2+} concentration (Dieter and Marmé 1980; Williamson 1981). One possibility to control this level is to modulate the activity of the ATP-dependent Ca^{2+} pumps which pump Ca^{2+} out of the cytoplasm. It is known that one Ca^{2+}-ATPase is activated by calmodulin and is probably located on plasmalemma (Dieter and Marmé 1982). Microsomes prepared from leaves of light-grown spinach exhibit the capacity of accumulating $^{45}Ca^{2+}$ in the presence of ATP and Mg^{2+}. This uptake is enhanced by the addition of calmodulin (Stosic et al. 1983). The aim of the present work was to examin the possible influence of the photoperiod on the stimulation of Ca^{2+} uptake by calmodulin.

MATERIALS AND METHODS

Plants (*Spinacia oleracea*, cv Nobel) were grown under short days (8hr fluorescent white light-16 hr darkness) at 20° C and 70% RH. Fully-developed leaves of 4-week old plants were used for the experiments. Leaves were harvested at various times

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during the light-dark cycle and homogenized (1 g leaf material to 2 ml of buffer) in a glass homogenizer in 25 mM MOPS (3-[N-morpholino] propane sulfonic acid) equilibrated at pH 7.5 with tris (tris-hydroxymethylaminomethane) and containing 5 mM EGTA (ethylene glycol-bis [2-aminoethylether] N, N'-tetraacetic acid) and 10% (w/v) sucrose (buffer A). The brei was filtered through cheese cloth and centrifuged for 15 min at 1500 g and then for 15 min at 6000 g. The resulting supernatant was spun at 48000 g for 20 min and the pellet was resuspended in buffer A without EGTA (buffer B) and centrifuged again at 48000 g for 20 min. After resuspension in buffer B, the resulting pellet was used as microsomal fraction. Ca^{2+} uptake by the microsomal suspensions was measured as indicated by Stosic et al. (1983), using an incubation medium containing 5 mM $MgCl_2$, 10 μM $CaCl_2$ and 250 bq $^{45}CaCl_2$ per ml buffer B. The assay was done with and without 1 mM ATP and with and without 5 μg calmodulin at 25° C. The reaction was started by adding 50 μg of microsomal proteins. After a 30-min incubation, one ml of the assay medium was filtered through 45 μ HAWP type Millipore filters and washed with buffer B. Radioactive $^{45}Ca^{2+}$ retained on the filters was counted in a scintillation counter. Proteins were determined as described by Spector (1978). Red light was provided by a red fluorescent Philips tube TL 20W/15 (0.07 Wm^{-2} at 660 nm) and far red light by three 100 W incandescent bulbs filtered with red and blue Röhm and Haas Plexiglas and 10 cm water (0.05 W m^{-2} at 730 nm). Measurements of Ca^{2+} uptake during the photoperiod were repeated three times. Results concerning red and far red light treatments are the mean of 5 to 8 independent assays.

RESULTS AND DISCUSSION

The uptake of Ca^{2+} by microsomes from spinach leaves was assayed at various times during the photoperiod. It was measured in the absence of ATP, in the presence of ATP, and in the presence of ATP and calmodulin. Figure 1 shows that the ATP-dependent Ca^{2+} uptake fluctuates during the lightdark cycle. It is maximum at the middle of night and minimum at the middle of day. The level of "uptake" measured in the absence of ATP and calmodulin, which most likely corresponds to a binding of Ca^{2+} to membranes, also exhibits a slight fluctuation. This asumption is due to the release of accumulated Ca^{2+} from the vesicles when these vesicles were washed with the ionophore A23187 (Stosic et al., 1983) at the end of the incubation. Calmodulin activates the Ca^{2+} uptake by microsomes prepared from leaves collected during the second half of the light period, but has no effect on microsomes prepared at the middle of the night. This calmodulin activation of the uptake of Ca^{2+} in microsomes prepared from leaves is inhibited by Chlorpromazin, a potent calmodulin inhibitor (Stosic et al., 1983). Therefore, it appears that a Ca^{2+}-ATPase

exhibits a rhythm of its sensibility towards exogenously supplied calmodulin during the photoperiod. This suggests that light modifies the properties of this ATPase.

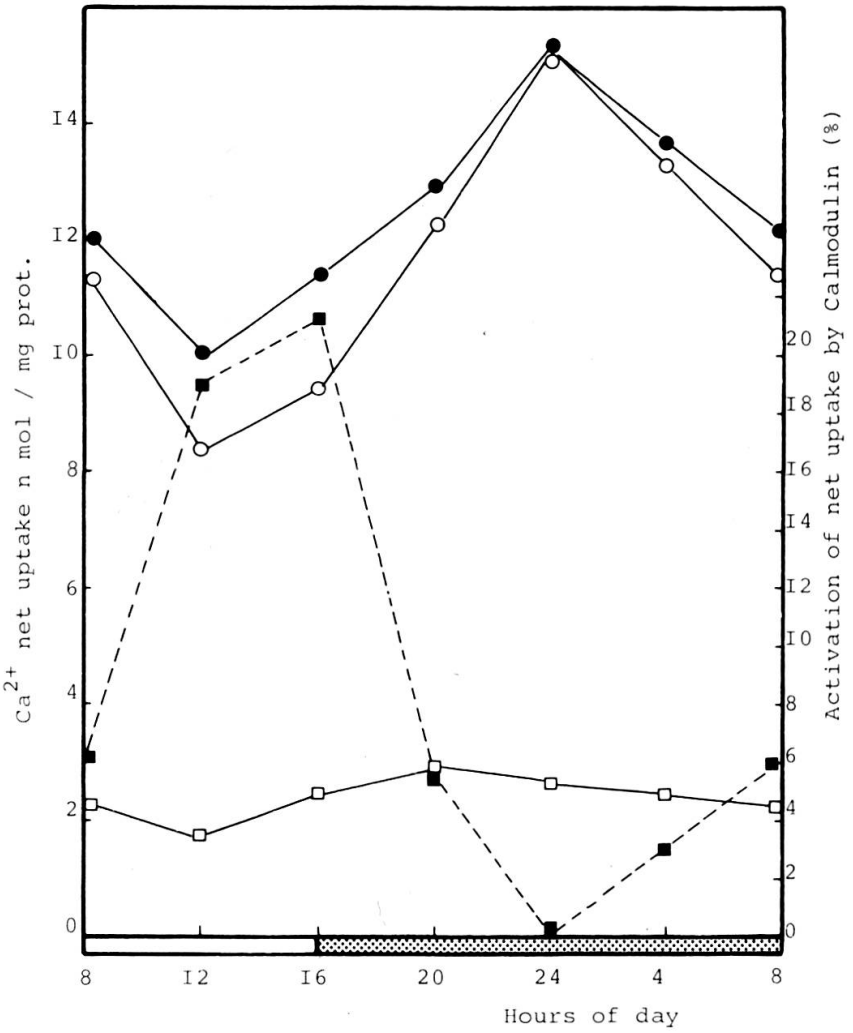

FIG. 1. — Ca^{2+} net uptake by microsomes prepared from leaves of spinach at various times during a short day cycle. Measurements were performed in absence of ATP and calmodulin (□—□), in presence of ATP (O—O), and in presence of ATP and calmodulin (●—●). The per cent of activation by calmodulin is also plotted (■- -■).

In order to test the possibility that phytochrome is implicated in the control by light of the sensitivity of the ATPase towards calmodulin, brief irradiations with red light were given to plants just before the beginning of the day or, alternatively, with far-red light at the end of day. Table 1 shows the calmodulin-dependent activation observed at the beginning or at the end of day and after the ligh ttreatments. It appears that 15 min red light (or even less) are sufficient to considerably raise the activation by calmodulin, when 5 min far red light at the end of the day lowers the activation to the level characteristic of plants assayed during the dark period.

The reversibility of the effect of red light by far red (or conversely of far red by red) was not reproducibly observed. It was previously shown that microsomes from epidermis exhibited a stronger stimulation of Ca^{2+} uptake by calmodulin than microsomes from other leaf tissues (Stosic et al., 1983). An attempt to verify that red and

TABLE 1.

Effect of 15 min red light given at the end of night or 5 min far red light given at the end of day on the stimulation of the Ca^{2+} uptake by calmodulin expressed in per cent.

	control	irradiated	
		15 min red	5 min far red
end of night	8.8 [1] ± 2.3	17.5 [1] ± 3.7	as control
end of day	17.8 [1] ± 2.6	as control	8.3 [1] ± 2.4

[1] absolute values of the uptake (in nmole Ca^{2+}/30 min mg prot) are similar to those plotted on Fig. 1.

far red light act on epidermis remains inconclusive: no significative difference between irradiated and control epidermis microsomes was observed. This may be due to the long time necessary to collect epidermis after irradiations.

These preliminary data strongly suggest that light controls a calmodulin-dependent Ca^{2+} transport in plant cells by modifying the sensitivity of a pump to calmodulin. Such a control was already reported in coleoptiles from etiolated corn (Dieter and Marmé, 1981). In that case, the Ca^{2+} uptake by microsomes from dark-grown coleoptiles was activated by calmodulin, whereas microsomes from coleoptiles deetiolated with far red light were no more activated. It was also reported that phytochrome controls the Ca^{2+} permeability of cells of *Mougeotia* (Dreyer and Weisenseel 1979) and *Avena* (Hale and Roux 1980). Apparently, calmodulin is involved in the phytochrome-mediated activation of *Onoclea* spores (Wayne and Hepler 1983). There is therefore a growing evidence that the Ca^{2+}/calmodulin system is involved in processes controlled by phytochrome.

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