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EFFECT OF BENZYLADENINE ON PROTEIN METABOLISM IN SOYBEAN LEAF DISCS DURING SENESCENCE

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Abstract

Effect of benzyladenine (BA) on synthesis and degradation of protein in soybean leaf discs during senescence was investigated. The BA-induced retardation of decrease in protein level in soybean leaf discs parallel the retardation of decrease in chlorophyll level. Incorporation of ^{14}C-leucine into protein, as measured by total activity, specific activity and percent of total uptake, is increased by BA (0.2 mg/l) indicating a stimulation of protein synthesis. Leaf discs pre-treated with BA enhance the uptake of ^{14}C-leucine. The degradation of ^{14}C-leucine labeled protein in soybean leaf discs during chasing period is retarded by BA. It is suggested that the effect of BA in retarding protein loss in soybean leaf discs is mediated through its action in promoting protein synthesis and preventing protein degradation.

Introduction

The ability of cytokinins to retard protein degradation in senescing tissue provides the opportunity to study the action of this hormone at a biochemical level. It has been suggested that the maintenance of protein level in detached leaf segments treated with cytokinins is associated with a stimulation of protein synthesis (Beevers 1968, Osborne 1962, Wollgiehn 1967). However, other workers (Kuraishi 1968, Peterson and Huffaker 1975, Shibaoka and Thimann 1970, Tavares and Kende 1970) attributed the retardation of protein by cytokinins to its roles in preventing protein degradation. Recently, Takegami (1975) demonstrated that benzyladenine (BA) retarded protein loss by promoting synthesis and at the same time inhibiting degradation of protein. In order to examine these possibilities, the changes in protein, labeled or unlabeled, in soybean leaf discs treated with BA were studied.

Materials and Methods

Soybean (*Glycine max* L. Merr. cv. 1039) plants were grown as described

previously (Hsia and Kao 1978). Fourteen days after planting, leaf discs measuring 9 mm were punched from primary leaves and randomized. Groups of 10 leaf discs were floated on 10 ml test solution in 50 ml flasks. Incubation was carried out in darkness at 30°C for the indicated number of days. Chlorophyll, protein and α-amino nitrogen were extracted and determined as before (Kao 1980).

Protein synthesis

Twenty-five leaf discs pretreated with or without BA were floated on 10 ml ^{14}C-leucine (0.1 μCi/ml) for 3 h in darkness at 30°C. Other experiments were carried out by incubating 25 leaf discs with ^{14}C-leucine plus BA or ^{14}C-leucine alone. The ^{14}C-leucine (specific activity, 10 mCi/m mole) used was from Radiochemical Center, Amersham. Subsequently, leaf discs were rinsed three times with leucine (10^{-5} M), blotted and homogenized. The homogenate was centrifuged at 1,000×g for 30 min. To an aliquot (3 ml) of crude extract, 3 ml of 10% (w/v) trichloroacetic acid were added, and the mixture was allowed to stand for 1 hour. The precipitate collected by centrifugation at 1,000×g for 30 min was dissolved in 0.1 M NaOH. Radioactivity in soluble and protein fractions were measured by means of a liquid scintillation counter (Beckman LS-230). The counting medium consisted of dioxane, 2,5-diphenyloxazole (PPO, 5 g/l) and naphthalene (100 g/l).

Protein degradation

Twenty-five leaf discs were floated on 10 ml ^{14}C-leucine (0.1 μCi/ml) for 24 hours in darkness at 30°C. After being washed with leucine (10^{-5} M), the discs were incubated at 30°C for 5 days with or without BA (0.2 mg/l) or for 2 days in 10^{-5} M leucine with or without BA. Radioactivity in protein fraction from leaf discs before and after the 5-days or 2-days of incubation was measured as described to estimate the degree of protein degradation during the incubation period.

All experiments were repeated three times.

Results and Discussion

The retardation of decrease in protein level in soybean leaf discs by BA paralleled that of decrease in chlorophyll content and the optimum concentration was found to be 0.2-2 mg/l (Fig. 1). Thus, 0.2 mg/l of BA was used for the following experiments.

Based on total activity and specific activity, BA pretreated leaf discs increased the incorporation of ^{14}C-leucine into protein (Table 1). This agrees with earlier work using *Xanthium* (Osborne 1962), tobacco (Takegami 1975), corn (Tavares and Kende 1970) and radish (Kuraishi 1968). This effect is

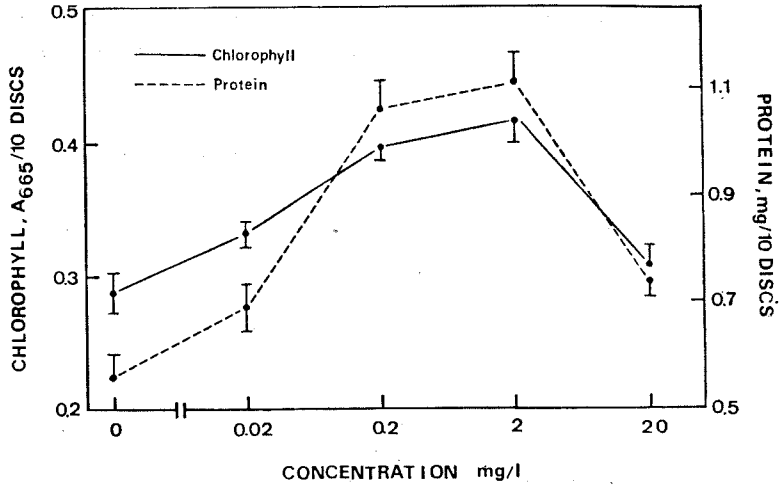

Fig. 1. Effect of BA concentrations on chlorophyll and protein contents of soybean leaf discs. All data were taken after 6 days in the dark.

also true when incorporation was measured as the percent of total uptake. Table 1 also shows that BA treated leaf discs increase the uptake of ^{14}C-leucine. Incorporation of ^{14}C-leucine is increased slightly by leaf discs treated simultaneously with BA and ^{14}C-leucine (Table 2), indicating BA promotes protein synthesis. However, this treatment decreased the uptake of ^{14}C-leucine, which is probably due to the competition of the same carrier by BA and ^{14}C-leucine for their transport into the tissue. Two other experiments of protein synthesis gave basically the same results as we reported in Tables 1 and 2.

There is no significant changes of the concentration of free amino acids, measured as soluble α-amino nitrogen, in leaf discs treated with BA or water during 3 or 6 h of incubation (Table 3). These results indicate that protein

Table 1. *Effect of BA pretreatment on the uptake of ^{14}C-leucine and incorporation of ^{14}C-leucine into protein of soybean leaf discs. Leaf discs pretreated with or without BA for 3 h. They were then labeled for 3 h with ^{14}C-leucine (0.1 μCi/ml). Total uptake means total activity (dpm) in soluble and protein fractions*

Pretreatment	Total uptake	Incorporation		
	dpm/25 discs	dpm/25 discs	dpm/mg protein	% of uptake
H_2O	20,593	6,453	6,991	31.3
BA, 0.2 mg/l	25,973	10,383	10,403	40.0

Table 2. *Effect of BA (0.2 mg/l) on the uptake of ^{14}C-leucine and incorporation of ^{14}C-leucine into protein of soybean leaf discs. Leaf discs were incubated for 3 h in darkness at 30°C*

Treatment	Total uptake	Incorporation		
	dpm/25 discs	dpm/25 discs	dpm/mg protein	% of uptake
^{14}C-leucine	91,688	16,005	9,088	17.5
BA+^{14}C-leucine	86,325	17,198	9,188	19.9

Table 3. *Effect of BA (0.2 mg/l) on α-amino nitrogen in soybean leaf discs*

Treatment	α-amino nitrogen
	A_{570}/10 discs
Freshly cut tissue	0.048±0.005
3 h H_2O	0.050±0.004
3 h BA	0.052±0.003
6 h H_2O	0.050±0.003
6 h BA	0.052±0.004

Table 4. *Effect of BA on the degradation of ^{14}C-labeled protein in senescing soybean leaf discs. Leaf discs were pre-labeled for 24 hr with ^{14}C-leucine (0.1 μCi/ml), washed thereafter with 10^{-5} M cold leucine and incubated in water for 5 days with or without BA (Experiment I) or for 2 days in 10^{-5} M cold leucine with or without BA (Experiment II)*

	Specific activity in protein
	dpm/mg protein
Experiment I	
Initial level	135,131
H_2O	91,951
BA, 0.2 mg/l	94,591
Experiment II	
Initial level	81,599
H_2O	49,500
BA, 0.2 mg/l	51,200

precursor amino acid pool is not expanded, suggesting that protein synthesis is promoted by BA.

Degradation of protein previously labeled ^{14}C-leucine is shown in Table 4. When leaf discs were incubated for 24 h in ^{14}C-leucine and subsequently rinsed and transferred to a medium containing H_2O with or without BA for 5 days, the degradation of pre-labeled protein was slower in the BA-treated discs than that in the water-treated ones (Table 4, Experiment I). This result, however, did not consider the turnover of ^{14}C-leucine itself during senescence. When discs pre-labeled with ^{14}C-leucine were incubated in leucine (10^{-5} M) with or without BA for 2 days, the degradation of pre-labeled protein in BA-treated discs was also found to be slower than that in control ones (Table 4, Experiment II). Thus, it is clear that BA retards protein degradation.

Based on the evidence presented here, it is tentatively concluded that BA retards the protein loss in soybean leaf discs during senescence by both promoting synthesis and inhibiting degradation of protein, although the mechanism of BA action on protein turnover remains to be investigated. Our conclusion is consistent with experiments of Takegami (1975), who recently demonstrated that BA not only promotes protein synthesis but also prevents protein degradation during senescence of tobacco leaf discs.

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Benzyladenine 對老化大豆葉圓片蛋白質代謝之影響

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本研究主要在探討 Benzyladenine (BA) 對老化大豆葉圓片蛋白質合成與分解之影響。BA 可延緩大豆葉圓片之葉綠素與蛋白質之分解。以總活性，比活性與佔總吸收量之百分率來表示 ^{14}C-leucine 導入蛋白質之結果顯示，BA (0.2 mg/l) 可促進蛋白質之合成。BA 同時亦可抑制 ^{14}C-leucine 標示之蛋白質分解。因此，BA 延緩大豆葉圓片蛋白質之分解，主要是由於其能促進蛋白質之合成與抑制蛋白質之分解。