Action of Light and Streptomycin on Protein Synthesis in Cabbage Seedlings

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Abstract. The action of light on protein synthesis was examined in the cabbage seedlings, a system extensively used in the studies of anthocyanin synthesis. Continuous red and far red light have no effect on total protein content while they cause a marked decrease in the level of free amino acids in cabbage seedlings. The rate of protein synthesis, measured as incorporation of radioactively-labelled amino acids into proteins, is clearly stimulated by light. Phytochrome involvement in the light stimulation of the incorporation is also demonstrated by the red-far red reversibility of the response. The relative effectiveness of continuous red and far red light upon the incorporation of amino acids into proteins is affected by the nature of the system used to study the incorporation process. When excised cotyledons and short period of incorporation were used, continuous far red was more effective than red. However, when whole seedlings and long period of incorporation were used, red and far red were equally effective. Streptomycin causes a 10—15% decrease in the rate of incorporation of amino acids into proteins of all cellular fractions, except the plastid fraction where a much higher inhibition (30%) was observed.

It has been demonstrated that light has various effects on the nitrogen metabolism of young seedlings. It affects the mobilization and redistribution of nitrogen storage compounds and of free amino acids (HURST and SUDIA 1973). We have previously reported the effects of light and streptomycin on RNA synthesis and polyribosome formation in cabbage seedlings (YANG 1981a, b), a system that has been used in studies of photoregulation of anthocyanin synthesis (MANCINELLI et al. 1975, 1976). As a parallel study, we also examined the action of light and streptomycin on protein synthesis and free amino acid content in this seedlings. In this paper, we report: (1) Total free amino acid content and protein content in cabbage seedlings under various light treatments; (2) The action of light on the incorporation of radioactively-labelled amino acids into proteins in the cotyledons as well as in the whole seedlings of cabbage; (3) Action of streptomycin on the incorporation of amino acids into proteins of various cell fractions in cabbage seedlings.

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MATERIAL AND METHODS

Growth of Seedlings

Cabbage seeds (*Brassica oleracea*, cv. Red Acre) were germinated and grown in darkness at 20 °C in Petri dishes on two disks of Whatman No. 3 filter paper, moistened with 10 ml of distilled water or with solution as specified.

Light Sources and Illumination

Light treatments were given in growth chamber (Percival Model E-57) equipped with a far red source (fluence rate: 7.4 Wm⁻²) or a red one (fluence rate: 4 Wm⁻²). The temperature during irradiations was 20 °C. Dark controls were included in all experiments. Light treatments were started 96 h after sowing.

Extractions and Measurements of Proteins and Amino Acids

Sixty seedlings were ground with acid-washed silica for 4 min in 20 ml of Tris-HCl buffer, 0.05 M, pH 8.0, containing 140 µl of β-mercaptoethanol. The homogenates were centrifuged at 5 000 rpm for 5 min in a refrigerated centrifuge. Eight ml of the supernatants were mixed with 2 ml of 35% trichloroacetic acid, and centrifuged at 2500 rpm for 10 min. The supernatants from this centrifugation were used for the determination of amino acids, using a colorimetric assay based on the ninhydrin reaction (Moore and Stein 1948). The pellets were washed with 5% trichloroacetic acid, alcohol, 50/50 alcohol/ether mixture and ether, and then dried in air for about 10 min. The dry pellets were then dissolved in 5 ml of 2% NaOH. These final solutions were used for protein assay, using the Lowry method (Lowry et al. 1951).

Tracer Studies

Lots of 60 seeds each, sterilized with 1% Clorox and rinsed with sterile distilled water, were grown in darkness under sterile conditions. One to two hours before the beginning of the light treatments, the seedlings were transferred to sterile Petri dishes containing 5 ml of a radioactive amino acid solution (1 µCi/dish ¹⁴C-(U)-leucine or ¹⁴C-(U)-phenylalanine or 5 µCi/dish ³H-(U)-phenylalanine). At the end of the light treatments the seedlings were rinsed with distilled water for 3 min to eliminate radioactive chemicals present on the surface of the seedlings, dried on paper towels and ground for 4 min with a mortar and pestle in 20 ml of ice-cold 0.025 M Tris-HCl buffer, pH 7.5 (containing 0.005 M β-mercaptoethanol) and a small amount of acid-washed silica. The homogenates were then centrifuged at low speed; the supernatants from this centrifugation were used for the measurements of uptake and incorporation. The uptake of radioactive amino acids was measured by adding 50 µl of supernatant to vials containing 10 ml of liquid scintillation fluid (1 000 ml toluene, 500 ml Triton X-100, 8.25 g 2,5-diphenyloxazole and 0.15 g 1,4-bis-(2-(5-phenyloxazolyl))-benzene), and counting for 10 min in a Packard 3320 Liquid Scintillation Spectrophotometer. For the measurements of the incorporation of radioactive amino acids into proteins, 100 µl of the supernatant were added to 3 ml of 10% trichloroacetic acid; after 30 min the solutions were filtered through millipore filters and washed.