Induction of Amylase
in Mustard Seedlings by Phytochrome

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Summary. In the cotyledons of mustard seedlings (Sinapis alba L.) amylase activity can be induced by phytochrome. In the dark amylase activity remains low. Gibberellin acid (GA₃) does not stimulate an increase of amylase activity in this system. Inhibitors of RNA and protein synthesis strongly inhibit the increase of amylase activity mediated by phytochrome. In gel electrophoresis amylase from mustard seedlings reveals 3 bands. The electrophoretic pattern is the same for extracts from dark-grown and from irradiated seedlings. When mustard amylases were incubated with starch the pattern of products was similar to that produced by commercially available barley β-amylase and not similar to that produced by Bacillus subtilis α-amylase.

Enzyme induction by phytochrome is well documented [8, 1, 23]. However, the mechanism of phytochrome action is still a matter of controversy [30, 25]. The present investigation was undertaken in order to test the suggestion, repeatedly emphasized by plant physiologists (e.g. recently by Galston and Davies [10]), that phytochrome exerts its function through the mediation of hormones. We have chosen the hormone gibberellin acid (GA₃) for two reasons: (1) We have found that amylase can be induced by phytochrome in the mustard seedling [7]. This phenomenon will be described in detail in the present paper. (2) It is well known that GA₃ will induce de novo synthesis of α-amylases in the aleurone layers of barley and other caryopses [9, 17]. The question is whether or not the action of Pfr in the mustard seedling (with respect to amylase induction) can be replaced by GA₃. In previous experiments we have shown [24] that in the case of hypocotyl lengthening in the mustard seedling there is no detectable interaction between Pfr (inhibitory action) and GA₃ (promotive action). However, the possibility remains that in the case of amylase induction where both effectors (Pfr and GA₃) are known to promote the response, Pfr indeed exerts its function through the mediation of a gibberellin. It will be shown that the results of the experiments provide strong evidence that at least gibberellic acid is not involved in the mediation of amylase induction by Pfr.
Materials and Methods

Standard techniques for photomorphogenic research with mustard seedlings were used [22]. The seedlings were grown at 25°C in the dark and experiments were started 36 h after sowing, which is taken as time zero in figures. The application of gibberellic acid and of the inhibitors Actinomycin D [10 μg·mL⁻¹], Puromycin dihydrochloride [100 μg·mL⁻¹] and Cycloheximide [5 μg·mL⁻¹] was performed according to the method of Lange et al. [19] during a 1-h submersion in the dark of 35-h-old seedlings. Controls were incubated in distilled water. After treatment, the seedlings were returned to standard dishes.

The standard far-red source [22], which maintains a low Pfr/Ptotal ratio in the mustard seedlings [14], was used at an irradiance of 350 μW·cm⁻². The standard red source [26] which maintains a Pfr/Ptotal ratio of about 0.8 was used at an irradiance of 75 μW·cm⁻².

Twenty seedlings or 20 pairs of cotyledons, respectively, were ground for 7 min at 2°C with 1.5 g quartz sand in a medium containing 6 ml 2 mM acetate buffer, pH 4.8, and 0.5 g insoluble polyvinylpyrrolidone [18]. The homogenate was centrifuged as described earlier [18]. The supernatant was used for the enzyme assay which was performed at 25°C and pH 4.8 essentially according to the procedure used by the Varner group [15]. However, the reaction kinetics were measured for each value of enzyme activity. The negative slope of the linear part of the reaction kinetics (between 4 and 20 min after the start of the reaction) was used as a gauge of enzyme activity. Enzyme activity is expressed as [(-μg starch)/min·pair of cotyledons]⁻¹.

The use of the biological unit (cotyledon) as a system of reference was justified previously [36]. The following results were obtained in methodological experiments: (1) Enzyme activity was proportional to the concentration of extract in the assay mixture; (2) the polyvinylpyrrolidone does not adsorb the enzyme; (3) the light treatment does not influence the enzyme activity in the extract through inhibitors or activators; (4) the enzyme activity is stable for many hours if the extract is kept close to 0°C.

Biochemicals used: The following biochemicals were obtained from Serva, Entwicklungslabor Heidelberg, Germany: Actinomycin D, α-amylase (Bacterium subtilis), and β-amylase (barley, free of α-amylase). Cycloheximide and gibberellic acid (90% activity) were obtained from Fluka A.G., Buchs, S.G., Switzerland. Puromycin dehydrochloride was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

A justification for the use of the term "amylase". The electrophoretic technique used was essentially that of Davis [6]. The gel consisted of 7.5% polyacrylamide in which 0.25% starch was included. After termination of electrophoresis the gel was incubated in a 0.2 M Na-acetate buffer leading to a pH of 4.8. For visualization of the amylase bands the gels were flooded with an I₂-KI solution. Electrophoresis revealed only 3 distinct bands, one intense and two weak ones. Re-electrophoresis of the intense band did not lead to a further resolution. If the extraction was performed at pH 7.0 (instead of 4.8) exactly the same pattern developed during electrophoresis. The electrophoretic pattern was the same for extracts from dark grown and far-red grown seedlings. It did not change with the age of the seedling. This means that during the induction of amylase by Pfr, no enzyme form is made which is not already made in the dark.

The trials to identify the mustard amylases as α- or β-amylases were not successful. The mustard amylases are inactivated by a heat treatment (15 min at 70°C) and by a prolonged treatment at pH 3.3. On the other hand the inhibitory influence of EDTA is only slight. Since the operational criteria for α-amylases include lability on a prolonged treatment at pH 3.3 as well as stability on heat treatment for 15 min