THE EFFECT OF LIGHT QUALITY UPON PHENYLALANINE AMMONIA-LYASE (PAL) ACTIVITY IN POTATO TUBER TISSUE SLICES

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ABSTRACT.—Phenylalanine ammonia-lyase (PAL) was extracted from both fresh and aged slices of potato tuber tissue. Using modified purification procedures, the PAL removed from potato exhibits an absolute requirement for Mg^{++} in order to obtain activity. The induction of increased levels of PAL is partially light dependent. No action spectrum was found.

Phenylalanine ammonia-lyase (PAL) catalyses the conversion of L- phenylalanine to trans-cinnamic acid. This product may then be converted by various metabolic steps into coumarin, caffeic acid, chlorogenic acid, or lignin. Since the original observation by Nutile (1945) that light could reverse the inhibition of seed germination by coumarin, numerous investigations have been concerned with the effect of light upon the activity of this enzyme. Particular attention has been focused on the involvement of phytochrome in the control and distribution of PAL activity (Attridge and Smith, 1967; Mohr et al., 1968; Bellini and Van Poucke, 1970).

Few investigations have been concerned with the level of PAL activity in storage slices. Zucker (1965) has shown that the production of chlorogenic acid is directly related to the production of the enzyme in potato and that the level of enzyme activity is light dependent. Studies using cycloheximide indicate that a *de novo* synthesis of the enzyme is involved (Zucker, 1968).

This paper shows that PAL isolated from potato tubers requires Mg^{++} for activation. Data are presented which show that the activity of the enzyme increases in physiologically aged tissue slices and that this level is enhanced by red, far-red, blue, or white light.

METHODS AND MATERIALS

Slices 8.0 x 1.0 mm were prepared from Russet white potatoes using a hand microtome. They were immediately placed in cold distilled water and were kept chilled until the initiation of the aging process. Aging was carried out at room temperature in aeration chambers fitted with Cinemoid light filters (Century Lighting, New York). The aging solution consisted of 60-0 ml of 10^{-4} M $CaSO_4$ which was renewed twice during the 24 hr aging process. Each chamber contained 120 slices, approximately 7.5 g.

TABLE 1.—*The Spectral Ranges, Light Intensities, and Length of Exposure Used to Promote PAL Synthesis.*
Except for the exposure to white light, tissue irradiation was applied at 1 hr of aging.

Light Regime	Wavelength Range (nm)	Intensity (ergs cm^{-2} sec^{-1})	Dose (ergs cm^{-2})
Red	590-700	$4.0.10^2$	(2-1/2 min) $6.0x10^5$
Far-Red	730-800	$5.4.10^4$	(1 min) $3.2x10^6$
Blue	380-550	$2.5.10^2$	(2-1/2 min) $3.8x10^5$
White (Fluorescent)	370-750	$4.4.10^3$	24 hr

The experimental tissue was allowed to age 1 hr before being illuminated. Table 1 indicates the wavelengths, intensities, and doses of irradiation employed. Two controls, one in complete darkness, and the other in continuous fluorescent white light (100 ft-c), were also used.

Phenylalanine ammonia-lyase was isolated using a modification of Rissland and Mohr (1967). The 120 slices were homogenized in the cold and dim light for 5 min in the presence of 20.0 ml of 0.1 M borate buffer, pH 8.8, 0.4 ml sulphite mixture (Verleur and van der Weyden, 1969), and $2x10^{-3}$ M $MgCl_2$. The homogenate was filtered through cheesecloth and centrifuged at 34,000 x G and -1 C for 25 min. The supernatant was then mixed for 10 min with 1.0 g of activated charcoal for every 5.0 ml of solution and recentrifuged as above. The resulting supernatant was then passed through #41 filter paper, the filtrate containing the enzyme. This enzyme solution was stable, losing only 9% of its activity after 1 day at 0 C.

The assay mixture consisted of 200 umoles of borate buffer, pH 8.8, 60 umoles of L-phenylalanine, $2x10^{-3}$ M $MgCl_2$, and 1.0 ml of enzyme extract (400-600 ug protein). The final volume was adjusted to 6.0 ml. One unit of enzyme activity was defined as 0.01 absorbance change per 100 min.

The appearance of the product was measured at 290 nm using a Hitachi 124-900 double beam grating spectrophotometer. Data were plotted by means of a Hitachi 165 recorder. Protein was measured by use of biuret reagents.

Results

The partially purified enzyme exhibits total loss of activity in the absence of $MgCl_2$. Only upon the addition of Mg^{++}does this activity appear. The combination of 3.1 umoles ml^{-1} of EDTA and $2x10^{-3}$ M $MgCl_2$ yields an enzyme activity which is only 17% of that with Mg^{++} alone.

In Table 2 are presented the results of the light treatments. Regardless of the light regime, there is at minimum an increase in specific PAL activity of 7.63 times the fresh activity. Thus, other factors inherent in the aging process are also involved in the development of increased amounts of PAL. The application of light enhances this development.

The tissue aging in continuous white light shows the greatest increase in enzyme activity, being slightly greater than twice that found in tissue aged in complete and continuous darkness. It is apparent, however, that light need

TABLE 2.—*The Effect of Light Upon the Specific Activity of Phenylalanine Ammonia-Lyase in Potato Tissue Slices.*

Light Regime	Enzyme Units*	Protein (ug/ml)	Specific Activity (Enzyme Units/ 100 ug Protein)	Increase Over	
				FRESH	DARK
(Fresh)	2.4	470	0.51	0	0
Dark	22.2	570	3.89	7.63	1.00
Red	44.0	570	7.72	15.14	1.98
Far-Red	35.7	470	7.60	14.90	1.95
Blue	40.0	510	7.84	15.37	2.01
Light	31.8	400	7.95	15.59	2.04

*An increase in absorbance at 290 nm of 0.01/100 min = 1 unit of enzyme activity.

not be continuous to obtain this enhancement. Brief doses of red, far-red, or blue light given early in the aging process are also sufficient to cause a doubling of enzyme activity. No one broad segment of the spectrum from 380-800 nm appears to be more specific than any other. Since the action spectrum is not specific, no single pigment or chromophoric group may be implicated for PAL synthesis in potato tuber tissue slices.

Discussion

Phenylalanine ammonia-lyase plays an important role in phenolic metabolism. In dormant potato tuber tissue the amount present is neglible. Upon aging of slices, however, the amount of the enzyme increases with time until a maximum is reached around 24 hr and having chlorogenic acid as a stable end product of the pathway (Zucker, 1965).

The production of phenolic compounds and their effect upon growth and development in potato and other plant tissues is not clear. It is strikingly apparent, however, that the potato tuber itself is quiescent and that upon cutting and aeration of tissue slices the metabolic activity is increased. Two important aspects of this development involve increased respiratory rates and promotion of protein synthesis (Laties, 1962; Laties, 1963; Laties, 1967).

Stimulation of cellular activity, rather than inhibition, appears to be occurring within aging potato tissue slices. Respiration is stimulated by exogenous application of low concentrations of IAA and kinetin. Endogenous gibberellins may also play an important role in the expression of these activities (Melinder, unpublished data). Classically, the phenolic compounds produced or present within plant tissue are believed to cause inhibitory effects. It is difficult, however, to conceive of a 7-15 fold increase of PAL enzyme and concomitantly having a 2-5 fold increase in respiration during aging if this enzyme influences the production of phenolic compounds which would produce deleterious effects.

The possibility may exist whereby the inhibitors produced, such as coumarin and chlorogenic acid, may be at such low concentrations during the initial stages of aging (0-24 hr) that they could act as promoting substances (Thimann and Bonner, 1949; Thimann, 1956). One possible mechanism that could be involved would be to retard the disappearance of IAA by inhibition of the IAA-oxidase system (Andreae, 1952; Henderson and Nitsch, 1962; Zenk and Müller, 1963). Conversely, trans-cinnamic acid, the initial product formed by PAL, may act as an antagonist of auxin (van Overbeek et al., 1951).

The exact nature and mechanism by which light can influence the level of PAL activity within potato slices may be as difficult to understand. This non-photosynthetic tissue responds to light by increasing protein levels (Zucker, 1963). There is, however, no concomitant effect of light or light quality upon respiration (Hanebuth, unpublished data). By 24 hr of aging in the dark the tissue slices have increased amounts of PAL. The application of continuous light for 24 hr doubles this enzymatic development (Table II). Since short exposure to light given at 1 hr of aging and followed by darkness also produces the same results, the system appears to be saturated quickly, and not reversible by the dark period.

Even though the level of PAL is increased by light, difficulty may be encountered in terming this a photomorphogenetic response. No

action spectrum is indicated and, therefore, the involvement of one particular pigment, such as phytochrome or a metallo-protein, can not be inferred.

Summary

The isolation of phenylalanine ammonia-lyase from potato tissue has been carried out. The enzyme is stable in the cold for at least one day. There appears to be an absolute requirement for Mg^{++} to obtain activity.

The enzyme appears in tissue aged in darkness but the application of light can cause this amount to double. Short doses of red, far-red, or blue light given early in the aging process are equally as effective as continuous application of white light.

Fresh tissue of the tuber contains only small amounts of the enzyme. Tissue discs physiologically aged are stimulated to produce the enzyme in amounts 7-15 fold greater than fresh tissue.

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