Phytochemistry, 1974, Vol. 13, pp. 1071 to 1073. Pergamon Press. Printed in England.

ACTION SPECTRA FOR PHENYLALANINE AMMONIA LYASE IN *HORDEUM VULGARE**

JERRY W. MCCLURE

Department of Botany, Miami University, Oxford, OH 45056, U.S.A.

(*Received August* 1973)

Key Word Index—*Hordeum vulgare*; Gramineae; barley; flavonoid accumulation; phytochrome; action spectra.

Abstract—5-Day-old etiolated barley shoots were illuminated with monochromatic light (10 nm bandpass) and assayed for PAL 5 hr later. Action spectra for enhanced PAL levels were determined at 1·1 and 6·6 kerg.cm^{-2} between 380 and 740 nm. Peaks were found near 420, 620 and 660 nm. The action spectra further confirm phytochrome control of barley PAL. At wavelengths longer than 540 nm the action spectra for PAL are in good agreement with action spectra for barley flavonoids. Wavelengths around 420 and 440 nm are more effective for PAL than for flavonoids in barley. Photocontrol of PAL and of flavonoids in barley differ in the amount of energy required to saturate the response, the relative effectiveness of the blue part of the spectrum, and the involvement of additional photoreceptors for 3′-substitution of flavonoids.

INTRODUCTION

IN A PREVIOUS paper[1] we showed that phenylalanine ammonia lyase (PAL) levels in barley shoots were increased about 50% by illuminating dark grown seedlings with red (R) light 5 hr before harvest. Phytochrome is involved since the R light effects are partly reversed by far-red (FR) light. We also showed that 30 sec of R light (1·1 kerg.cm^{-2}.sec^{-1}) was sufficient to saturate the PAL response.

The only previously published action spectrum for PAL appears to be that of Nitsch and Nitsch[2] who illuminated disk of *Helianthus tuberosus* L. for 24 hr with monochromatic light at an intensity of 5 kerg.cm^{-2}.sec^{-1} and found a major peak at 440 nm and a very slight peak at around 680 nm. This 43 J.cm^{-2} action spectrum for *H. tuberosus* has many of the characteristics expected for a High Intensity Response, a response usually saturated by 1 J.cm^{-2} of blue (B), or occasionally FR, light.[3,4] Thus the only published action spectrum for PAL would appear to have little relationship to a low energy phytochrome control of PAL in barley.[1]

As I was interested in determining lower limits of photocontrol for barley PAL, and in comparing the action spectrum of PAL with that of the barley flavonoids,[5] action spectra were done at both 1·1 and 6·6 kerg.cm^{-2} for PAL in 5-day-old barley shoots.

* Part IV in the series "Phenolic Biosynthesis in Barley Seedlings". For Part III see Ref. 1.

[1] MCCLURE, J. W. (1974) *Phytochemistry* **13**, 1065.
[2] NITSCH, C. and NITSCH, J. P. (1966) *Compt. Rend.* **262**, 1102.
[3] BORTHWICK, H. A., HENDRICKS, S. B., SCHNEIDER, M. J., TAYLORSON, R. B. and TOOLE, V. K. (1969) *Proc. Nat. Acad. Sci. U.S.* **64**, 479.
[4] MOHR, H. (1972) *Lectures on Photomorphogenesis*, Springer, New York.
[5] CARLIN, R. M. and MCCLURE, J. W. (1973) *Phytochemistry* **12**, 1009.

RESULTS

The action spectra, done in triplicate and on two different occasions, are shown in Fig. 1. Values are corrected for daily variation and for fresh weight of the four-shoot samples.[1]

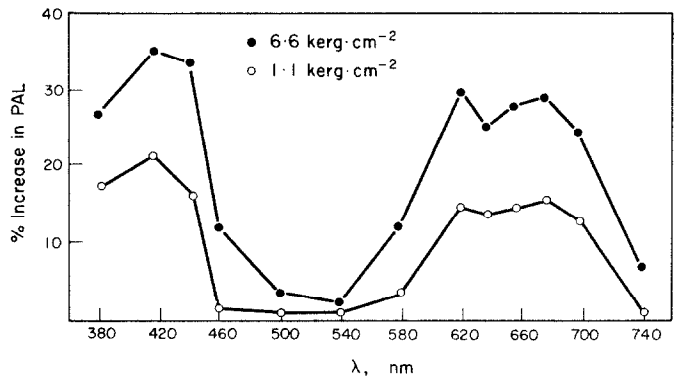

FIG. 1. 6·6 AND 1·1 kerg.cm^{-2} ACTION SPECTRA FOR THE INCREASE IN PAL LEVELS IN 5-DAY-OLD *Hordeum vulgare* SHOOTS.
Previously etiolated plants were given light from a monochrometer, returned to the dark for 5 hr, and harvested. Each point is the average of 6–12 determinations.

DISCUSSION

Linearity of response in the action spectra is evident from the similarity of the two curves where the response to 1·1 kerg.cm^{-2} is *ca* 60% that of 6·6 kerg.cm^{-2} at all wavelengths. Furthermore, 33 kerg.cm^{-2} of R[1] or of blue (McClure, unpublished) light saturate PAL increase at about 45% above that of the dark controls. Apparently photoincreased PAL in 5-day-old barley shoots is saturated at some point between 6·6 and 33 kerg.cm^{-2} of total light energy in the B and R regions of the spectrum.

When the PAL action spectra are compared to the flavonoid action spectra[5] there are general similarities between them. There is a good fit between the spectra for saponarin and PAL at wavelengths longer than 540 nm, both showing peaks at around 620 and 660 nm.

As the 620 and 660 nm peaks for saponarin are equally reversible by FR light, and since both saponarin[6] and PAL[1] are controlled by low energy R FR reversible light treatments, it is evident that phytochrome control of PAL plays a major role in controlling accumulation of the flavonoid saponarin in barley shoots.

Blue wavelengths, in the region between 420 and 440 nm, are more effective in controlling PAL (Fig. 1) than saponarin.[5] In contrast, wavelengths between 440 and 540 nm are much more effective for saponarin than for PAL. For example, 420 and 520 nm light are approximately equally effective for saponarin yet PAL is increased about 35% by 6·6 kerg.cm^{-2} of 420 nm light and not influenced by 520 nm light. It is possible that some photoacceptor other than phytochrome, absorbing in the 420–440 nm region, is involved with PAL but not with saponarin. Or in other words, there appear to be photocontrols for saponarin in addition to control of PAL.

When one compares these studies on PAL with earlier studies on flavonoids[5,6] several features emerge: (1) The amount of light required to saturate increases of PAL is far less

[6] McClure, J. W. and Wilson, K. G. (1970) *Phytochemistry* **9**, 763.

than the amount needed for maximal flavonoid production; (2) PAL is more responsive to blue wavelengths than are the flavonoids, suggesting the involvement of additional photoreceptors for one or both of these aspects of phenolic metabolism; (3) and the dissimilarity of the spectra for PAL and the flavonoids lutonarin and its 3′-methyl ether suggest a specific photocontrol for 3′-substitution.

Thus, while phytochrome controls both PAL and flavonoids in barley shoots, there is no direct relationship between higher levels of PAL and increased accumulation of flavonoids.

EXPERIMENTAL

Atlas 46 barley (*Hordeum vulgare* L.) was grown on water-saturated vermiculite in 23° incubators in a darkroom as previously described.[6] Monochromatic light (bandpass of 10 nm at 500 nm) was obtained from a Bausch & Lomb high intensity grating monochrometer used in previous action spectra determinations.[5] The PAL assay was a spectrophotometric assay at 290 nm.[1] Dark control values of PAL varied in a linear fashion from 65 to 42 in four plumule samples weighing 0·220 and 0·340 g respectively. PAL levels in this paper are corrected for daily variation and weight of the tissues, and are reported as per cent change from dark control plants grown and processed along with the experimental plants.

Acknowledgements—This work was supported by the National Science Foundation (Grant GB 16636) and by funds from the Miami University Research Committee. The expert and dedicated technical assistance of Mrs. Mary M. Saunders and Mrs. Marjorie Smith is gratefully acknowledged.