ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 52, nr 2: 139-148
1983

Influence of growth regulators and respiration inhibitors on dark transformation of phytochrome in coleoptiles of oat seedlings

JAN KOPCEWICZ, MARIUSZ CYMERSKI, KAZIMIERZ MADELA

Laboratory of Photobiology, Department of Plant Physiology, Institute of Biology, N. Copernicus University, Gagarina 9, 87-100 Toruń, Poland

(Received: May 19, 1982)

Abstract

Irradiation with red light leads to the formation of an unstable, undergoing gradual destruction, physiologically active P_{FR} form of phytochrome in the coleoptiles of oat seedlings. Growth substances: IAA, GA_3, kinetin, ABA, ethrel as well acetylcholine do not influence the nature and rate of phytochrome dark transformation. Inhibitors of energy-producing processes such as KCN, 2,4-DNP, DCCD and antimycin A inhibit the process of dark destruction of the P_{FR} form of phytochrome.

INTRODUCTION

The influence of light, through phytochrome, on biosynthesis, release from the bound form, degradation and transport of plant hormones has been noted on frequent occasions. Phytochrome could also alter the responsiveness of cells to phytochrome (Black and Vlitos 1972). There is, however, lack of information whether reverse reactions exist, i.e. the influence of hormones on phytochrome transformations. The role of energy-producing processes in the mechanism of phytochrome transformation is also unclear.

Thus, the aim of the present study was to determine the effect of growth substances and respiration inhibitors on phytochrome transformations.

MATERIAL AND METHODS

Seeds of oat (*Avena sativa* L. Diadem variety) were soaked in distilled water for 1 hour and afterwards placed on moist sterilized sawdust of deciduous trees. Germination and cultivation of seedlings were conducted in darkness, at 26°C. According to the requirements of the experiment two variants of plant material preparation were applied.

VARIANT I — "ISOLATED COLEOPTILES"

Coleoptile tips 5-mm long were isolated from the seedlings 96 hours after sowing. The isolated coleoptiles were placed on Petri dishes containing 5 cm^3 of 0.005 M Tris-citric buffer at pH 7.0 (control) or 5 cm^3 of buffer solution of growth regulators or respiration inhibitors. The incubation was conducted in darkness, at 25°C, for 3 hours. Next, the coleoptiles were irradiated with active red light for 15 minutes. After irradiation the coleoptiles were placed in darkness, at 25°C, for 8 hours. The phytochrome content in 100 coleoptiles was measured directly before and after irradiation with red light and then at one-hour intervals.

VARIANT II — "WHOLE SEEDLINGS"

Whole seedlings were placed on glass dishes 96 hours after sowing, containing 80 cm^3 of 0.05 M Tris-citric buffer (control) or buffer solution of growth regulators. The incubation was done in darkness, at 25°C, for 24 hours. Then, the seedlings were irradiated with active red light for 15 minutes. After irradiation the seedlings were placed in darkness, at 25°C, for 8 hours. The phytochrome content in 5-mm long coleoptile tips isolated from 100 seedlings, was measured directly before and immediately after irradiation of seedlings with red light and then at one-hour intervals.

In both experiments: kinetin, indole-3-acetic acid (IAA), gibberellic acid (GA_3), abscisic acid (ABA), ethrel and acetylcholine (ACh) were applied in a concentration of 10^{-3} M.

The influence of the following respiration inhibitors was investigated only under the conditions of variant I: potassium cyanide (KCN — 1 mM), 2,4-dinitrophenol (2,4-DNP — 1 mM), dicyclohexylcarbodiimide (DCCD — 1 mM) and antimycin A (0.1 mM).

The content of phytochrome was determined spectrophotometrically on the basis of the difference in the optical density of the tissue (Δ OD) for 660 and 730 nm according to the method previously described (Butler et al. 1959, Kopcewicz and Cymerski 1982). The modified two-stream spectrophotometer SPECORD UV-VIS with a connected convertor of TEC-1 extinction, enabling numeral reading of optical density with an accuracy up to 10^{-3} was used. Red light for plant material initial irradiation was obtained from a Xenon 2500 W projector filtered through an interference filter at wavelength 660 nm. Light intensity was 170 $\mu W \times cm^{-2}$. Irradiation with active monochromatic light (660 and 730 nm) during phytochrome measurement was done with a Diaprex B-10 projector with 150 W halogen bulb.

Handling of coleoptiles and filling of the measurement cell, was conducted under dim green light on a thermostatic aluminium plate at 0°C.

RESULTS

The obtained results (Figs. 1-4) show that in both variants of experiments growth regulators do not influence, in a noticeable way, dark transformation of phytochrome. Independently of the applied growth substances, the P_{FR} form of phytochrome undergoes successive destruction (Figs. 1 and 3). The rate of destruction is similar, both for the

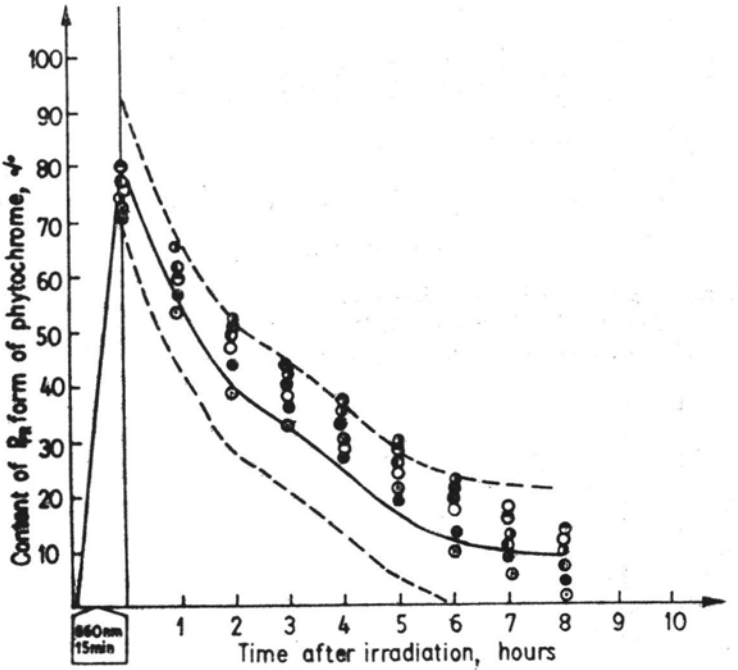

Fig. 1. Influence of growth regulators on dark transformation of P_{FR} form of phytochrome in coleoptiles of oat seedlings (Variant I). ◐ — IAA, ◓ — ABA, ● — GA_3, ○ — kinetin, ⊙ — ethrel, ◑ — ACh, — — control, = — difference statistically insignificant

coleoptiles treated with growth regulators and for control ones. Neither do growth substances exert any essential influence on the dynamics of dark transformation of the P_{FR} form of phytochrome (Figs. 2 and 4). It is, however, interesting to compare dark transformation of phytochrome between both variants of experiments (Figs. 5 and 6). The rate of destruction of the P_{FR} form of phytochrome in isolated coleoptiles (Fig. 5) is about two times lower than in those which, throughout the experiment constitute an integral part of the seedlings. Destruction of the P_R form of phytochrome (Fig. 6) takes place only under the conditions of variant II. In the case of variant I stabilization of the P_R form is observed.

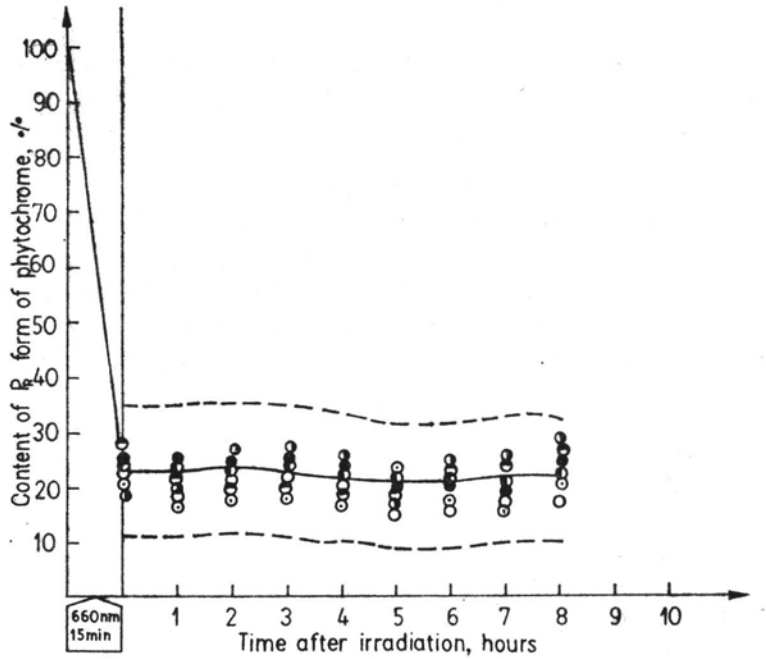

Fig. 2. Influence of growth regulators on dark transformation of P_R form of phytochrome in coleoptiles of oat seedlings (Variant I). ◐ — IAA, ◓ — ABA, ● — GA_3, ○ — kinetin, ⊙ — ethrel, ◑ — ACh, — — control, = — difference statistically insignificant

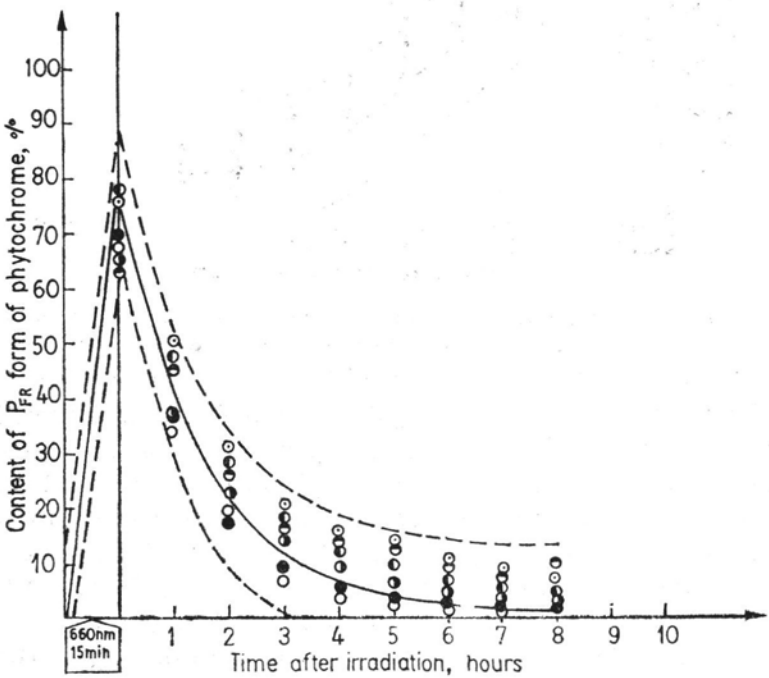

Fig. 3. Influence of growth regulators on dark transformation of P_{FR} form of phytochrome in coleoptiles of oat seedlings (Variant II). ◐ — IAA, ◓ — ABA, ● — GA_3, ○ — kinetin, ⊙ — ethrel, ◑ — ACh, — — control, = — difference statistically insignificant

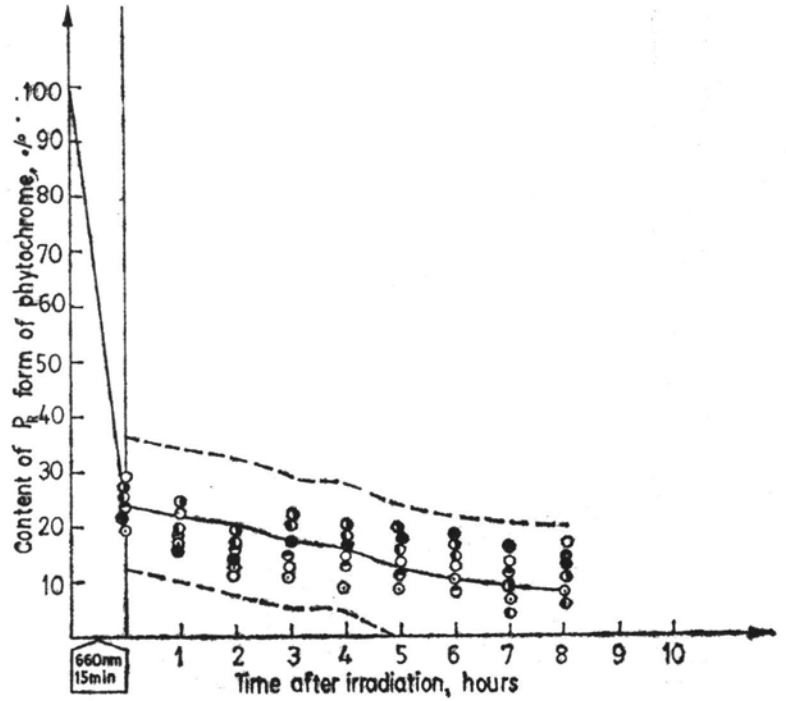

Fig. 4. Influence of growth regulators on dark transformation of P_R form of phytochrome in coleoptiles of oat seedlings (Variant II). ◐ — IAA, ◓ — ABA, ● — GA_3, ○ — kinetin, ⊙ — ethrel, ◑ — ACh, — — control, = — difference statistically insignificant

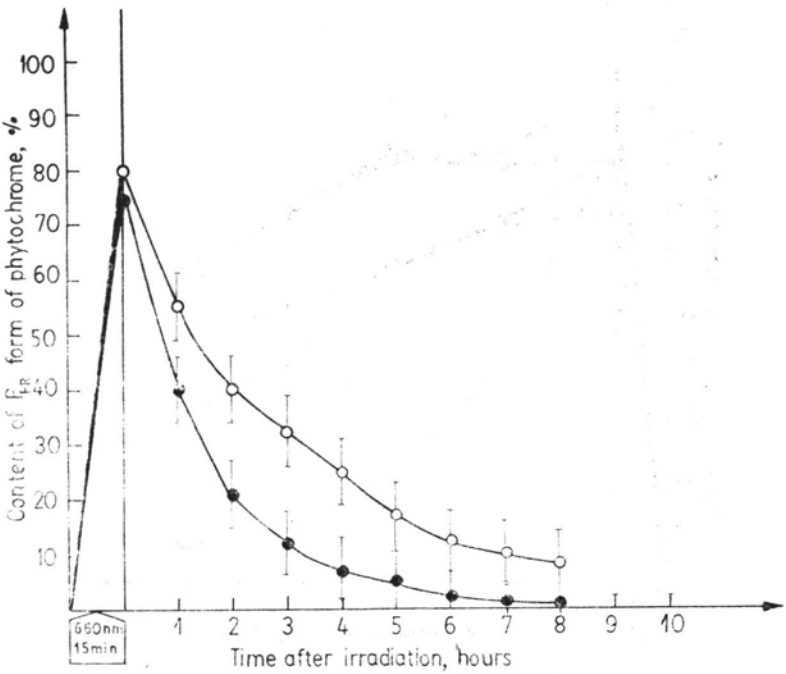

Fig. 5. Comparison of dark transformation of P_{FR} form of phytochrome for both variants of coleoptile preparation. ○ — variant I, ● — variant II

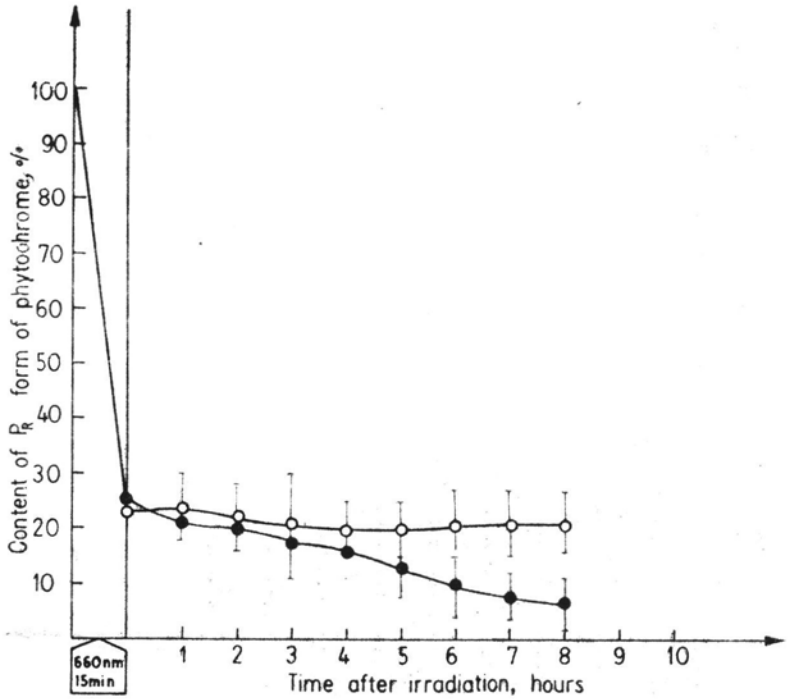

Fig. 6. Comparison of dark transformation of P_R form of phytochrome for both variants of coleoptile preparation. ○ — variant I, ● — variant II

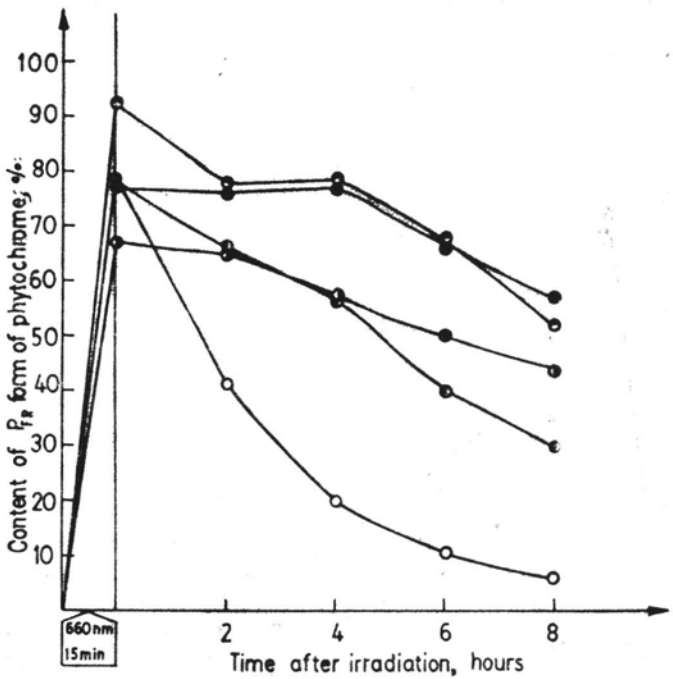

Fig. 7. Effect of respiration inhibitors on dark transformation of P_{FR} form of phytochrome in coleoptiles of oat seedlings (Variant I). ◐ — KCN, ◓ — DNP, ● — DCCD, ◑ — antimycin A, ○ — control

Respiration inhibitors influence dark transformation of phytochrome (Figs. 7-9). As seen, essential slowing down of the rate of destruction of phytochrome P_{FR} is caused by: KCN which is an inhibitor of the respiratory chain at the level of cytochrome oxidase, 2,4-DNP — a protonophore of mitochondrial membranes which reduces the membrane potential and disorganizes the phosphorylation reactions with redox

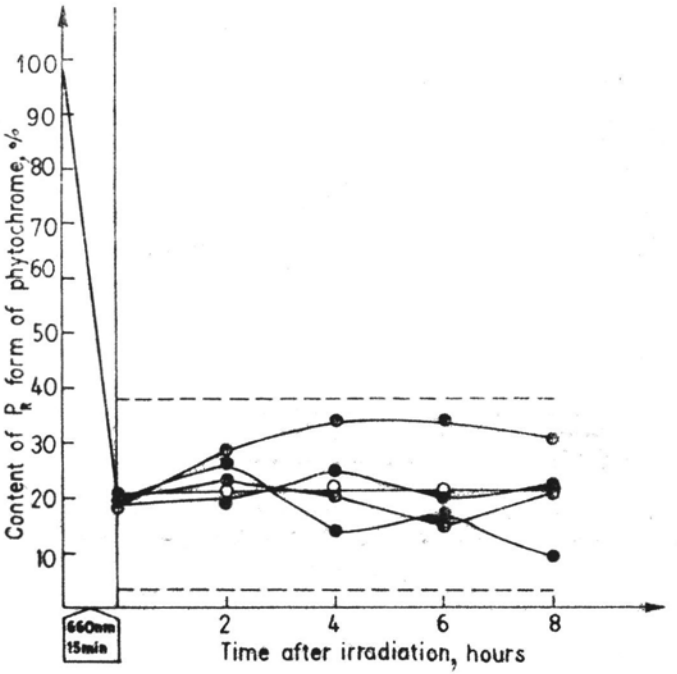

Fig. 8. Effect of respiration inhibitors on dark transformation of P_R form of phytochrome in coleoptiles of oat seedlings (Variant I). ◐ — KCN, ◓ — DNP, ● — DCCD, ◑ — antimycin A, ○ — control, = — difference statistically insignificant

ones, DCCD which is an H^+-ATPase inhibitor, and antimycin A — an inhibitor of the respiratory chain between cytochrome b and cytochrome c. Therefore, blocking of the production of energy in a plant, independently of the site of action of the inhibitor, has an essential influence on dark transformation of phytochrome. The most distinct effect retarding the destruction of phytochrome is exerted by 2,4-DNP (Figs. 7 and 8). During the first 6 hours after irradiation with red light this compound completely impeded the destruction of the total content of phytochrome (Fig. 9), causing at the same time, a slight increase in the amount of the P_R form (Fig. 8).

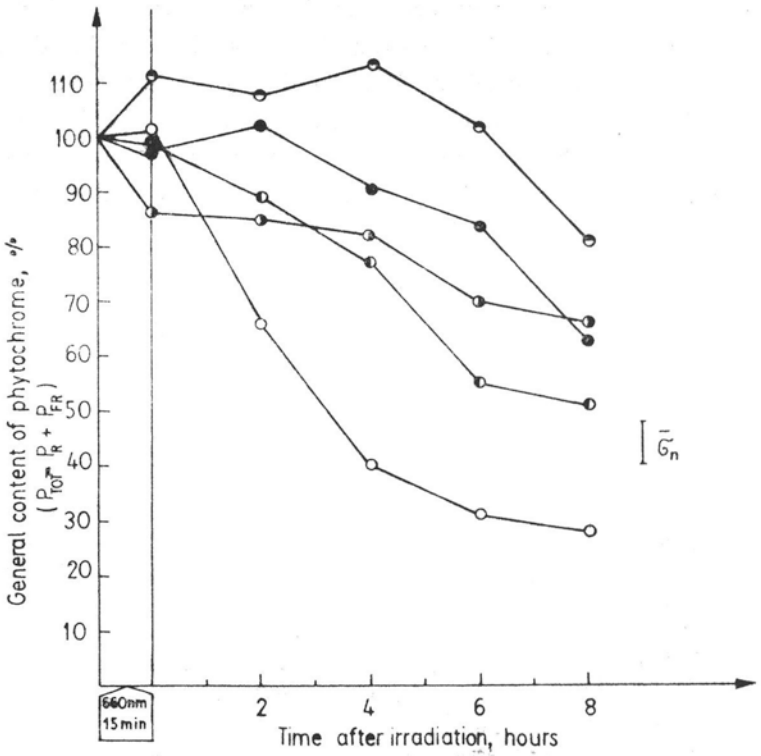

Fig. 9. Effect of respiration inhibitors on dark transformation of phytochrome ($P_{FR}+P_R$) in coleoptiles of oat seedlings (Variant I). ◐ — KCN, ◓ — DNP, ● — DCCD, ◑ — antimycin A, ○ — control

DISCUSSION

The trigger mechanism of the biological action of phytochrome appears to be the photoreversible transformation of chromoprotein between two red and far-red absorbing forms, P_R and P_{FR}. From the previous data (K o p c e w i c z and C y m e r s k i 1982) it is known that in coleoptiles of etiolated oat seedlings phytochrome occurs only as the stable P_R form. Irradiation of etiolated oat coleoptiles leads to the formation of the unstable, undergoing gradual destruction, physiologically active P_{FR} form of phytochrome. It was also found that the destruction of P_{FR} is dependent on temperature (K o p c e w i c z and C y m e r s k i 1982).

The fact that destruction of phytochrome occurs only *in vivo* (B r i g g s et al. 1968) and is dependent on temperature, allows to assume that in plant tissues there exist endogenous factors regulating this process. It was decided to check whether plant growth regulators may play this role. The examined growth substances, however, did not exhibit any essential influence on the nature and rate of dark transformation of

phytochrome (Figs. 1-4). The effect of growth regulators has not been observed, either in isolated coleoptiles or in coleoptiles of intact seedlings. In the present experiments all growth substances were applied in a concentration of 10^{-4} M. Although it is a high dose, owing to the relatively short time of incubation, however, a negative influence on the plant tissue was not observed. During preliminary experiments 10^{-4} - 10^{-6} concentrations of growth regulators were applied, but neither these had any influence on the course of phytochrome destruction.

Comparison of the kinetics of dark destruction of phytochrome shows that this process occurs most intensely in coleoptiles of intact seedlings (Fig. 5). It suggest the existence in plants of a specific stimulator of phytochrome destruction. This stimulator is not a substance regarded at present as an endogenous phytohormone, since as it has been shown above, none of the applied growth regulators influenced the rate of destruction of phytochrome. The existence of other low molecular weight substances active in this process was assumed (Shimazaki and Furuya 1975). Some enzyme or a carrier transporting the active form of phytochrome to a particular place in the cell could also be such a factor.

Pratt et al. (1974) postulate that the process of destruction may be a result of: a) compartmentation of the plant cell, b) association of phytochrome with cell membranes or cell organelles, c) destruction of a chromophoric or protein part of pigment. Furuya et al. (1965) ascertained that KCN may inhibit the process of phytochrome destruction. Our results confirm this observation. Respiration inhibitors such as KCN, 2,4-DNP, DCCD and antimycin A decrease the rate of destruction of the P_{FR} form of phytochrome. The most intensive action was exhibited by 2,4-DNP, which completely inhibited the process of destruction, causing even a slight increase in the amount of phytochrome (Fig. 9). This distinctive action of 2,4-DNP suggests a special, apart from energetic, role of mitochondria. The suppression of the potential difference of the mitochondrial membrane, by the protonophore 2,4-DNP, leads to blocking of energy production. It may be, however, that a side-effect of such a phenomenon is the release of phytochrome linked with a membrane. The possibility of formation of the complex phytochrome-mitochondrial membrane, confirms the investigations on the pelletability of phytochrome (Pratt and Marme 1976, Pratt 1978). The obtained results suggest indirectly that the process of phytochrome destruction in coleoptiles of oat seedlings may be connected with the inactivation of phytochrome by linking it with an unidentified site in the mitochondrial membrane.

Acknowledgments

Research supported by grant within project MR II/7-5.1.4.

REFERENCES

Black M., Vlitos A. J., 1972. Phytochrome and plant hormones. In: Phytochrome. K. Mitrakos and W. Shropshire Jr. (eds.), Academic Press, London-New York, pp. 517-549.

Briggs W. R., Zollinger W. D., Platz B. B., 1968. Some properties of phytochrome isolated from dark-grown oat seedlings (*Avena sativa* L.). Plant Physiol. 43: 1239-1243.

Butler W. L., Norris K. H., Siegelman H. W., Hendricks S. B., 1959. Detection, assay and preliminary purification of the pigment controlling photoresponsive development of plants. Proc. Nat. Acad. Sci. USA 45: 1703-1708.

Furuya M., Hopkins W. G., Hillman W. S., 1965. Effects of metal complexing and sulfhydryl compounds on nonphotochemical phytochrome changes *in vivo*. Arch. Biochem. Biophys. 112: 180-186.

Kopcewicz J., Cymerski M., 1982. Non-photochemical transformations of phytochrome in coleoptiles of etiolated oat seedlings. Acta Physiol. Plantarum (in press).

Pratt L. H., 1978. Molecular properties of phytochrome. Photochem. Photobiol. 27: 81-105.

Pratt L. H., Marme D., 1976. Red light enhanced phytochrome pelletability. Plant Physiol. 58: 686-692.

Pratt L. H., Kidd G. H., Coleman R. A., 1974. An immunochemical characterization of the phytochrome destruction reaction. Biochem. Biophys. Acta 365: 93-107.

Shimazaki Y., Furuya M., 1975. Isolation of a naturally occurring inhibitor for dark P_{FR} reversion from etiolated *Pisum* epicotyls. Plant Cell Physiol. 16: 623-630.

Wpływ regulatorów wzrostu i inhibitorów oddychania na ciemniowe przemiany fitochromu w koleoptylach siewek owsa

Streszczenie

Napromieniowanie czerwienią koleoptyli etiolowanych siewek owsa powoduje powstanie niestabilnej, ulegającej stopniowej destrukcji, fizjologicznie aktywnej formy fitochromu P_{FR}. Substancje wzrostowe takie jak IAA, GA_3, kinetyna, ABA, ethrel jak również i acetylocholina nie wpływają na tempo i charakter ciemniowych transformacji fitochromu. Inhibitory procesów energetycznych (KCN, 2,4-DNP, DCCD, antimycina A) hamują proces ciemniowej destrukcji fitochromu.