BIOCHEMISTRY

Insensitivity of Phytochrome Decay *in vivo* to Respiratory Uncoupling by 2,4-Dinitrophenol

PHYTOCHROME, the red, far-red photo-reversible chromoprotein that controls much of higher plant growth and development, undergoes two changes in the dark following an initial red light exposure. One change, reversion, is the transformation of the far-red absorbing form, P_{FR}, into the red absorbing form P_R. A second change, which we will call decay, is the loss of total photo-reversible phytochrome as a result of the apparent destruction of P_{FR} (ref. 1). Since there is considerable indirect evidence that P_{FR} is the active form of phytochrome, the interactions between P_{FR} decay and metabolism may be of significance in elucidating the mechanism of phytochrome action.

Butler and Lane[2] have shown that decay in etiolated *Zea* (corn) seedlings is directly proportional to oxygen concentrations between 0 per cent and 10 per cent. They further demonstrated that cyanide, carbon monoxide and azide partially inhibit both respiration and P_{FR} decay, thus suggesting that respiration and decay are closely linked. Furuya *et al.*[3], however, have shown that in coleoptiles of etiolated *Avena* (oat) seedlings ethylenediamine tetraacetic acid (EDTA) stops decay at concentrations having no effect on oxygen uptake. This suggests that respiration and phytochrome decay are not necessarily related. We have now used 2,4-dinitrophenol (DNP), an uncoupler of the respiratory chain, as a further test of the possible relationship between these processes.

Since it is well established that the uncoupling action of DNP is accompanied by a surge of oxygen uptake[4], we first determined what concentrations of DNP cause a significant increase in respiration.

The material was taken from four-day-old seedlings of *Avena sativa* cultivar 'Clintland' grown at 26° C in darkness. Leafless coleoptile segments, 5 mm in length, were cut, randomized and then placed in groups of thirty in each of the Warburg-type flasks used in the experiment. The main chamber of the flasks contained, beside the tissue, a 0·02 M sodium–potassium phosphate buffer at *p*H 6·5 with 1·5 per cent sucrose[5]. The centre well contained 10 per cent potassium hydroxide, and the side arms held either DNP or water, as the case might be. Oxygen uptake was followed at 26° C in air with a '*GME*' differential respirometer (Gilson Medical Electronics, Middleton, Wisconsin). Once the initial respiratory rate of each flask was determined over 100 min, the contents of the side arms were tipped into the main vessel. The final rate was established over 120 min after a 30-min incubation period. All data were first normalized to the initial rates for each flask. These values were then averaged for the various DNP concentrations and for the controls. With the control values taken as 100 per cent, the means obtained in this way were used to compute the net DNP effect at various concentrations[3]. Several such experiments gave values of 114, 137, 143, 154 and 135 per cent of the control for 1, 2, 3, 5 and 10×10^{-5} M DNP, respectively. These values are close to those obtained for *Zea* (corn) coleoptiles under similar conditions by Beevers[6].

We then proceeded to test the effects of uncoupling concentrations of DNP on phytochrome decay. The same type of material prepared for the respirometer experiments was used here, except that all manipulations were carried out in the dark or under a dim green safelight[7]. The segments were then incubated for 30 min in the sucrose–phosphate buffer, either with or without added DNP. After the incubation period a 5-min red-light treatment[7] was given to the appropriate segments. At 0, 1 and 2 h after the red light, twenty-five segments were quickly packed into cuvettes, 3 mm in diameter, and placed on ice. The samples were then assayed for phytochrome by methods described elsewhere[7], using a two-filter difference spectrophotometer to measure total $\Delta(\Delta OD)$ values, which are taken as estimates of total phytochrome. Results of a typical experiment are presented in Table 1 and indicate that uncoupling concentrations of DNP have no effect on decay.

Table 1. LACK OF EFFECT OF 3×10^{-5} M DNP ON PHYTOCHROME DECAY IN *Avena* COLEOPTILE SEGMENTS

Each value is the mean of three samples

Treatment	Hours after red light	Phytochrome total $10^3\ \Delta(\Delta OD)$	Phytochrome total Percentage remaining
Controls (buffer and sucrose only)	0	53·2	100
	1	32·8	62
	2	17·2	32
Plus DNP	0	51·8	100
	1	29·0	56
	2	17·0	34

Further experiments have shown that a much higher concentration of DNP, 10^{-3} M, prevents the loss of total phytochrome in both red-light-treated and dark samples. At the same time, 10^{-3} M DNP reduces the respiratory rate to about one-tenth of the control rate. These facts indicate that decay is dependent at least on an intact metabolism and also that DNP itself does not affect phytochrome absorbance.

In conclusion, our results demonstrate that respiratory chain energy conservation is not intimately related to phytochrome decay. This finding supports the view[3] that decay requires oxygen and metal activity, not as a function of the respiratory chain, but rather as a function of other, as yet unknown, reactions. The question of the physiological importance of phytochrome decay remains open.

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Activity of Amino-laevulinic Acid Synthetase in Normal and Porphyric Human Livers

ACUTE intermittent porphyria (AIP) is well known as an inborn error of porphyrin metabolism, clinically characterized by attacks of abdominal pains and frequently associated with the neurological and mental symptoms and signs. The excretion of the two kinds of porphyrin precursors, porphobilinogen (PBG) and delta-amino-laevulinic acid (ALA) in urine, suggesting the presence of a disturbance in the porphyrin metabolism, has been thought essential for the diagnosis of this disease.

Although a block in the conversion of PBG and ALA to porphyrins has been regarded as the explanation for the accumulation and urinary excretion of these compounds, Granick and Urata[1] reported a marked elevation of ALA synthetase activity in liver mitochondria in the experimental porphyria in guinea-pig induced by feeding of 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC). Recently we have had an opportunity to perform a metabolic investigation on human porphyric liver.

The patient was a man 32 years old, with a history of attacks of abdominal pain and neurological symptoms such as weakness of arms and legs lasting for a month