The work was done while on leave of absence in The Biodynamics Laboratory, Lawrence Radiation Laboratory, University of California, Berkeley, Calif. and was supported by the U.S. Atomic Energy Commission.

Botany Department,
University College London,
London (Great Britain)

IAN MORRIS

1 T. A. PEDERSEN, M. KIRK AND J. A. BASSHAM, *Physiol. Plantarum*, 19 (1966) 219.
2 R. G. JENSEN AND J. A. BASSHAM, *Proc. Natl. Acad. Sci. U.S.*, 56 (1966) 1095.
3 B. L. HORECKER, S. PONTREMOLY, O. ROSEN AND S. ROSEN, *Federation Proc.*, 25 (1965) 1521.
4 K. TAKETA AND B. M. POGELL, *J. Biol. Chem.*, 240 (1965) 651.
5 C. GANCEDO, M.C. SALAS, A.GINER AND A.SOLS, *Biochem. Biophys. Res. Commun.*, 20 (1965) 15.
6 A. M. UNDERWOOD AND E. A. NEWSHOLME, *Biochem. J.*, 95 (1965) 767.
7 W. J. O'SULLIVAN AND D. D. PERRIN, *Biochemistry*, 3 (1964) 18.
8 L. G. SILLEN AND A. E. MARTELL, *Chem. Soc. London Spec. Publ.*, 17 (1964).
9 J. PRIESS, M. L. WYMAN BIGGS AND E. GREENBERG, *J. Biol. Chem.*, 242 (1967) 2292.
10 D. E. ATKINSON, J. A. HATHAWAY AND E. C. SMITH, *J. Biol. Chem.*, 240 (1965) 2682.
11 M. P. GHOSH AND J. PRIESS, *J. Biol. Chem.*, 241 (1966) 4491.
12 N. GENTNER AND J. PRIESS, *Biochem. Biophys. Res. Commun.*, 27 (1967) 417.
13 B. KEECH AND G. J. BARRITT, *J. Biol. Chem.*, 242 (1967) 1983.
14 J. A. BASSHAM, M. KIRK AND R. G. JENSEN, *Biochim. Biophys. Acta*, 153 (1968) 211.

Received May 20th, 1968

Biochim. Biophys. Acta, 162 (1968) 462–464

BBA 43204

Phytochrome spectrum of Pisum leaves and stems

Reports in this journal[1] and elsewhere[2,3] indicate that the phytochrome difference spectrum obtained on etiolated Pisum (pea) leaves, or extracts of them, differs substantially from corresponding spectra obtained from other sources, including Pisum stems. These interesting observations seemed to accord with suggestions made by the writer (ref. 4, p. 318), and attempts were immediately made to confirm them in this laboratory. This communication summarizes the failure of these attempts.

At first, it seemed unnecessary to repeat the earlier procedures precisely, particularly in view of the likelihood (see later) that they might introduce errors. A difference of the magnitude reported[1] in phytochrome spectra should be easily detected by more straight-forward methods, so the following were adopted. Seedlings of *Pisum sativum* cv. Alaska were grown for 7 days in total darkness at about 26° (ref. 5). Samples of stem tissue consisted of 20–50 5-mm segments cut just below the apical hook, while leaf samples comprised 15–50 apical buds cut just above the hook and excluding as much stem tissue as possible. Immediately after cutting, the samples were packed in cylindrical aluminum cells 14 mm in diameter (*cf.* ref. 5), held on ice for about 10 min, then exposed on ice to 10–30 min of white incandescent light, about 45000 lux, to saturate protochlorophyll conversion. Spectra were then taken at ice temperature using the Biospect Model 61 (Agricultural Specialties Co., Beltsville, Md.), a single-beam recording spectrophotometer capable of measuring at high ab-

sorbances. First, the sample was saturated with red light (about 660 nm)[5] and a spectrum obtained; then it was saturated with far-red light[5] and a second spectrum obtained. These operations were repeated several times. Subtraction of the values on each red-saturated spectrum from the corresponding values on the immediately subsequent far-red saturated spectrum, at intervals of 5 nm, gave difference spectra which can be assumed to represent P_r *minus* P_{fr}.

Because of the single-beam mode, difference spectra obtained in this way are only relative. However, lack of an absolute baseline presents no problem. The two difference spectra reported for leaves and stems can be distinguished adequately in the following terms, which are derived from measurements of Fig. 1 in ref. 1 without reference to the baseline. Curve b, the "normal" spectrum of stem segments, shows a peak in ΔA at about 665 nm. If the differences $\Delta A_{650\,nm} - \Delta A_{600\,nm}$, $\Delta A_{660\,nm} - \Delta A_{600\,nm}$ and $\Delta A_{600\,nm} - \Delta A_{730\,nm}$ are calculated from this curve and converted to percentages of the maximum absorbance differences — $(\Delta A_{max} - \Delta A_{min})$ that is, total $\Delta(\Delta A)$ — they are 22, 29 and 70, respectively. Curve a, the "abnormal" leaf curve, shows a red peak at about 650 nm, and the corresponding differences, again as percentages of total $\Delta(\Delta A)$, are 15, 9 and 82, reflecting the very different shape of the curve.

The indicated parameters were calculated for the difference spectra obtained as described above on leaves and stems. Actual magnitudes of the spectra used were such that total $\Delta(\Delta A)$ ranged from 0.040 to 0.060. The results were unequivocal. The parameters derived from stem tissue agreed excellently with those expected. For example, in a typical experiment, mean values for $\Delta A_{650\,nm} - \Delta A_{600\,nm}$, $\Delta A_{660\,nm} - \Delta A_{600\,nm}$ and $\Delta A_{600\,nm} - \Delta A_{730\,nm}$, as percentages of the maximum total $\Delta(\Delta A)$, were 21, 31 and 64. Leaf tissues, however, gave essentially the same values — in a typical experiment, they were 23, 31 and 69, agreeing well with the stems and quite distinct from the 15, 9 and 82 expected, notably in peak position. In short, it proved quite impossible to distinguish between the phytochrome in etiolated leaf and stem tissue of Alaska pea seedlings by these methods: no abnormal difference spectra were found.

It thus became necessary to investigate possible sources of the discrepancy between these results. First, seedlings of the variety used in earlier work (Pisum, var. Krombek, from Nunhem Zaden N.V., Holland) when tested by the above methods, were indistinguishable from Alaska. Next, radically different procedures, resembling those used in the earlier work[1], were adopted. Samples were harvested in dim green light, given several minutes of red light and then allowed to stand for 10–30 min in darkness at about 26°, after which the first spectrum was taken. They were then immediately saturated with far-red and the second spectrum taken, all at 20–26°. Such a procedure does not, of course, give the true photochemical phytochrome difference spectrum. Rather, it gives a difference between the maximum level of P_r (the second spectrum) and a level of P_{fr} reduced by both destruction and reversion[4,6] during the 10–30 min of darkness. Nevertheless, such data were obtained; while the variability in the results with leaf tissue was extremely high, such differences in shape as appeared to exist between the spectra of leaves and stems were still not in the direction expected, since the 650-nm values in the leaf spectra were, if anything, reduced rather than increased. Otherwise, the spectra resembled those obtained by the first method. In summary, while the observations reported for stem tissue are

easy to confirm, no method so far tried in this laboratory has served to obtain the abnormal difference spectra reported[1–3] for leaf tissue.

It is difficult to provide a convincing explanation for data one cannot reproduce. All that is certain is that, if there was indeed a difference attributable to phytochrome in the spectra of leaf and stem tissues as reported, it is not a general phenomenon, and is clearly absent in the variety Alaska. However, the methods adopted here were deliberately designed to exclude complications due to the photoconversion of protochlorophyll and the subsequent nonphotochemical spectral changes[4,6,7], while the methods that yielded the abnormal spectra, at high temperatures and with no initial photosaturation, seem more susceptible to such interference. In this laboratory, as noted, the variability of the spectra derived from leaf tissue by these methods is very great, and there may also be varietal differences. All such variation seems to disappear when the samples are kept on ice and vigorously photosaturated. As for the fact that the abnormal spectra can apparently be obtained in extracts, it has in fact been acknowledged[3] that extraction media similar to those used are known to extract photoconvertible protochlorophyll. Hence, in view of the high levels of protochlorophyll present in etiolated pea leaves, and most others as well, much more rigorous evidence will be necessary before the concept of a "leaf" phytochrome, differing from that in stems and coleoptiles, can be accepted.

Work carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission and with the diligent technical assistance of ROSEMARIE DEARING and HELEN J. KELLY.

Biology Department,
Brookhaven National Laboratory,
Upton, N.Y. 11973 (U.S.A.)

WILLIAM S. HILLMAN

1 C. J. P. SPRUIT, *Biochim. Biophys. Acta*, 143 (1967) 260.
2 C. J. P. SPRUIT, *Mededel. Landbouwhogeschool Wageningen*, (1967) 67-14.
3 C. J. P. SPRUIT, *Mededel. Landbouwhogeschool Wageningen*, (1967) 67-15.
4 W. S. HILLMAN, *Ann. Rev. Plant. Physiol.*, 18 (1967) 301.
5 W. S. HILLMAN, *Physiol. Plantarium*, 18 (1965) 346.
6 H. W. SIEGELMAN AND W. L. BUTLER, *Ann. Rev. Plant Physiol.*, 16 (1965) 383.
7 G. A. AKOYONOGLOU AND H. W. SIEGELMAN, *Plant Physiol.*, 43 (1968) 66, and references.

Received June 20th, 1968

Biochim. Biophys. Acta, 162 (1968) 464–466