Effects of Abscisic Acid on Photosynthesis of Protoplasts from *Petunia hybrida*

Protoplasts from plants are suitable experimental objects for investigations in which substances are taken up by cells, since chemicals can reach the cell surface directly. Therefore, it is convenient to use them for experiments concerned with effects of phytohormones on metabolism. So far, some work on the application of auxins[1-3] has been described, and recently we have made some investigations on the influence of zeatin on photosynthesis and respiration of isolated mesophyll protoplasts from *Petunia* (KULL and HOFFMANN, in preparation).

Application of abscisic acid (ABA) leads to a decrease of photosynthetic rate in treated leaves, very likely due to stomatal closure caused by the phytohormone[4-6]. Some observations[7] suggest that mesophyll photosynthesis is not affected directly. Recently, it was found that in illuminated and unilluminated dark grown *Avena* seedlings and in etio-chloroplasts of the same species, ABA reduced the levels of ribulosediphosphate carboxylase activity[8]. We studied the effect of ABA on the photosynthesis of mesophyll protoplasts from *Petunia*. The influence of ABA on the respiration of these isolated protoplasts was briefly investigated in connection with our work on zeatin.

[1] D. W. GREGORY and E. C. COCKING, J. exp. Bot. *17*, 68 (1966).
[2] J. B. POWER and E. C. COCKING, J. exp. Bot. *21*, 64 (1970).
[3] M. H. BAYER, Plant Physiol. *51*, 898 (1973).
[4] C. J. MITTELHEUSER and R. F. M. VAN STEVENINCK, Nature, Lond. *221*, 281 (1969); Planta *97*, 83 (1971).
[5] W. R. CUMMINS, H. KENDE and K. RASCHKE, Planta *99*, 347 (1971)
[6] J. POSKUTA, R. ANTOSZEWSKI and M. FALTYNOWICZ, Photosynthetica *6*, 370 (1972).
[7] R. J. JONES and T. A. MANSFIELD, Physiologia plant. *26*, 321 (1972).
[8] F. A. M. WELLBURN, A. R. WELLBURN, J. L. STODDART and K. J. TREHARNE, Planta *111*, 337 (1973).

Effect of abscisic acid on the photosynthetic rate of isolated mesophyll protoplasts from *Petunia hybrida*

Time ABA acting (h)	Concentration of ABA (racemate) 1 ppm 3.8 μM/l	5 ppm 19 μM/l	10 ppm 38 μM/l	50 ppm 190 μM/l	200 ppm 760 μM/l
1	100 ± 4.5	100 ± 4.5	100 ± 4.5	–	–
2	100 ± 4.5	100 ± 4.5	104 ± 4.5	131 ± 3.0	–
3	100 ± 4.5	103 ± 4.5	123 ± 3.0	198 ± 3.0	116 ± 3.0

Controls = 100%.

The mesophyll protoplasts from *Petunia hybrida* were produced by methods already described[9,10]. In scintillator vials 10^6 protoplasts in 1 ml medium (0.4 *M* mannitol/0.23 *M* $NaHCO_3$, ratio 95:5; with 0.22 *M* HCl brought to pH 7.2) were incubated with ABA (racemate) of different concentrations (Table) for different times (1–3 h). Then 2 μCi of $NaH^{14}CO_3$ in 100 μl 0.4 *M* mannitol were added and the probes were illuminated for exactly 10 min in an illuminated thermostate (8 × 40 W Osram-L-Warmton lamps, ca. 9000 lux, temperature 24 °C). After this, hot ethanol was added and the vials boiled for 2 min. After transferring into 50 ml graduated vessels and filling with 60% ethanol, the liquid was boiled again for 30 min, then the vessels filled up with ethanol. The activity of an equivalent of this final solution was measured in the scintillation counter in dioxane cocktail (gain 83%). Since ABA must be brought in solution with a few droplets of ammonia, the concentration of the latter rises with increasing concentration of the hormone. Therefore the respective concentrations of NH_3 were established in the controls. Always after addition of ABA or – in the controls – of NH_3 alone, the pH was adjusted again to 7.2. Due to the rising quantities of NH_3, the absolute rates of photosynthesis, measured as amount of $^{14}CO_2$ fixed, are not comparable; in the controls they decline. (In 1 set of control experiments without any NH_3 added, the rate is 2.98 μM CO_2/mg chlorophyll in 10 min; this is in good agreement with our earlier findings). Therefore the results are given as percentage of the photosynthetic rate of the respective controls. Each experiment was done 3 times together with 3 controls.

The results are shown in the Table. A rise of the photosynthetic rate caused by ABA can be seen. A marked effect needs relatively high concentrations of ABA with respect to naked protoplasts. With 1.9×10^{-4} *M*/l ABA after 3 h the increase of the photosynthesis reaches nearly 100%; with 7.6×10^{-4} *M*/l the effect is considerably lower, probably due to an inhibitory effect of very high concentrations of the phytohormone.

To a certain extent the results are surprising, considering that ABA is a senescence-promoting hormone[11,12]. The concentrations of ABA which cause a rise of the photosynthetic rate of mesophyll protoplasts are a great deal higher than those needed for closure of stomata and the subsequent inhibition of photosynthesis of leaves[6]. Therefore an unspecific effect of ABA cannot be fully excluded. The results show the effects of a phytohormone on isolated cells in this case to be different from those on the whole leaf as a target. Under normal physiological conditions ABA is effective in whole tissues, so in evolution the function of the phytohormone was determined by selection in respect to the whole system and not to single cells. We think the results are explained best, in agreement with the findings of some other authors, as an effect of ABA on membrane permeability[4,13], or the activity of ion uptake[4,14–16]. The reported[8] reduction of the activity of ribulosediphosphate carboxylase activity is due to an inhibition of plastid development and is not contrary to our findings with 'adult' cells[17].

Zusammenfassung. Inkubation mit Abscisinsäure verursacht bei Mesophyll-Protoplasten von *Petunia hybrida* einen Anstieg der Photosyntheserate.

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[9] I. Potrykus and J. Durand, Nature New Biol. *237*, 286 (1972).
[10] F. Hoffmann, Z. Pflanzenphysiol. *69*, 249 (1973).
[11] F. T. Addicott and J. L. Lyon, A. Rev. Plant Physiol. *20*, 139 (1969).
[12] B. V. Milborrow, Sci. Prog., Lond. *57*, 533 (1969).
[13] Z. Glinka and L. Reinhold, Plant Physiol. *48*, 103 (1971). – Z. Glinka, Plant Physiol. *51*, 217 (1973).
[14] T. A. Mansfield and R. J. Jones, Planta *101*, 147 (1971).
[15] R. F. M. van Steveninck, Z. Pflanzenphysiol. *67*, 282 (1972).
[16] G. N. Brown and C. Y. Sun, Physiologia plant. *28*, 412 (1973).
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