Physicochemical Studies on Chloroplast Thylakoid Protein from *Phaseolus vulgaris*

By D. N. LEES, B. J. MAPP,* A. M. REDMOND† and L. J. ROGERS. (Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth, U.K.)

'Structural' or 'organizer' protein forms a large proportion of the total protein of the chloroplast and is believed to form the backbone of the thylakoid membranes (Criddle, 1966). The protein can be solubilized with the aid of detergents such as sodium dodecyl sulphate, and this has enabled sedimentation-velocity studies to be carried out (Biggins & Park, 1965; Criddle, 1966; Mani & Zalik, 1970). Such studies have yielded conflicting and sometimes confusing results (see Keylock, Kirk & Rogers, 1971).

The present communication reports studies on chloroplast thylakoid protein isolated from *Phaseolus vulgaris* (French-bean) seedlings by a method similar to that used for isolation of thylakoid protein from *Euglena gracilis* (Keylock *et al.* 1971). The results we report, based on some 50 sedimentation-velocity experiments, have yielded results clarifying and extending the observations previously published.

Solubilized in the presence of high concentrations of sodium dodecyl sulphate the protein migrated as a single symmetrical boundary at all protein concentrations. At protein concentrations above 3.5 mg/ml the sedimentation coefficient was 1.8–2.0S; however, at lower protein concentrations the sedimentation coefficient progressively decreased to an extrapolated $s_{20,w}$ value of approx. 0.7S at infinite dilution. No boundary characteristic of sodium dodecyl sulphate micelles alone appeared in addition to the protein boundary, suggesting that the decreasing $s_{20,w}$ values may reflect protein-sodium dodecyl sulphate complexes of changing ratio rather than dissociation of the 2S species to smaller units.

Solubilization in lower sodium dodecyl sulphate concentrations enabled identification of species of thylakoid protein sedimenting at about 5.2S. Below about 2 mg/ml the 5.2S species dissociated to smaller subunits, the sedimentation coefficient of the single symmetrical boundary observed progressively decreasing to an $s_{20,w}$ value of approx. 3.6S at infinite dilution. Treatment with 6M-urea caused the 5.2S aggregates to dissociate to a species sedimenting at 3.6S over a wide range of protein

concentrations. There is evidence to suggest that under some conditions this dissociation is reversible.

The amino acid composition of the protein was in good agreement with that obtained for *Euglena* chloroplast structural protein, with some minor differences that may be especially significant in view of the different aggregation behaviour of the two proteins.

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Inhibition of the Hill Reaction and Photophosphorylation by 1,1,1-Trichloro-2,2-bis-(p-chlorophenyl)ethane (DDT)

By W. J. OWEN and L. J. ROGERS (Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth, U.K.) and J. D. HAYES. (Welsh Plant Breeding Station, Aberystwyth, U.K.)

It is known (Hayes, 1959) that certain barley varieties are susceptible to spraying with DDT [1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane] and develop a severe chlorosis some 4–10 days after treatment. The reaction to DDT is controlled by a single major gene on chromosome 7 and susceptibility is the dominant character (Hayes, 1959; Wiebe & Hayes, 1960).

Previous studies (Lawler & Rogers, 1967, 1968, 1969) have shown that DDT affects the photosynthesis of susceptible barley, as evidenced by a decrease in rate of O_2 evolution of DDT-treated leaves and diminished Hill activity of the chloroplasts isolated from treated plants. In chloroplasts isolated by a procedure adapted from Kleese (1966) 2,6-dichlorophenol-indophenol photoreduction and phenazine methosulphate-catalysed cyclic photophosphorylation were inhibited, whereas there appeared to be little effect on ferricyanide photoreduction or concomitant non-cyclic photophosphorylation.

Recently we have studied the effect of DDT on these biochemical functions in some 30 varieties of barley from widely differing parts of the world [e.g., Fimbul (Finland), Sinop 248 (Turkey), Lyallapur (India), Tang May (China), Mosdoksky H. V.

* Present address: North East London Polytechnic, London E.17, U.K.

† Present address: Imperial Chemical Industries Ltd. Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, U.K.

(Russia), Stade Paladi (Barbados), Atlas 46 (U.S.A.)). Chloroplasts were isolated from DDT-treated plants, before chlorosis began to develop, by using a medium giving chloroplast preparations exhibiting high rates of photophosphorylation (M. Plesnicar, personal communication). In all cases the effect of DDT on photosynthesis correlated with the susceptibility or resistance of the barley to DDT in greenhouse trials. In these studies chloroplasts from susceptible barley showed a parallel effect on all the investigated aspects of photosynthesis, including 2,6-dichlorophenol-indophenol and ferricyanide photoreductions and cyclic and non-cyclic photophosphorylations. The distinction between cyclic and non-cyclic photophosphorylations evident in earlier work appears to be a result of the different method of chloroplast isolation and treatment before assay of photosynthetic activities.

Most of the chloroplast preparations isolated from DDT-treated susceptible barleys showed inhibition of electron-transport activity as measured by ferricyanide photoreduction concomitant with non-cyclic photophosphorylation; however, two varieties showed no decrease in electron transport as measured by this method, even though both cyclic and non-cyclic photophosphorylations, and 2,6-dichlorophenol-indophenol and ferricyanide photoreductions measured without concomitant photophosphorylation, were inhibited. The reason for these two distinct types of effect is not clear, although the existence of a barley variety with reaction to DDT controlled independently by two genes is known (G. A. Wiebe, personal communication); hence if both genes are segregating independently three genotypes of susceptible barley differing in reaction to DDT might exist.

Other work has been concerned with the search for the 'target site' affected by DDT in the photosynthetic lamellar membrane. Chloroplast thylakoid proteins have been isolated from both susceptible and resistant types of a single variety of barley (Zephyr), the two types of barley being identical but for the distinction in their reaction to DDT. Details of the sedimentation properties and amino acid compositions of the thylakoid proteins will be presented.

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The Effect of Isonicotinyl Hydrazide on the Greening of Maize and Barley

By M. G. GORE,* R. B. EVANS,† H. M. HILL and L. J. ROGERS. (Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth, U.K.)

A pathway has been proposed for the formation of acetyl-CoA in chloroplasts (Rogers, Shah & Goodwin, 1968; Shah & Rogers, 1969; Rogers & Goodwin, 1970) involving the sequence $\text{CO}_2 \rightarrow \text{glycollate} \rightarrow \text{glyoxylate} \rightarrow \text{glycine} \rightarrow \text{serine} \rightarrow \text{pyruvate}$. Part of the evidence for this sequence came from experiments involving inhibitors affecting reactions in the pathway, and the present communication reports some additional results obtained by using one of the inhibitors.

Isonicotinyl hydrazide (isoniazid) inhibits conversions requiring pyridoxal phosphate as cofactor. In the above sequence it might therefore be expected to affect each of the steps in the conversion of glyoxylate into pyruvate. In *Chlorella* (Pritchard, Whittingham & Griffin, 1963) and *Pisum sativum* (Marker & Whittingham, 1966) isonicotinyl hydrazide predominately inhibits the conversion of glycine into serine.

We have investigated the effect of isonicotinyl hydrazide on development of chlorophyll and carotenoid during illumination of segments of etiolated maize and barley shoots immersed in solutions of various inhibitor and metabolite concentrations. Chlorophyll and carotenoid were assayed by the method described by Kirk (1968). Maize shoots were used within 3 days of the leaves unrolling, barley shoots when they were approximately 5 in tall.

It was found that 10 mM-isonicotinyl hydrazide inhibited pigment synthesis by some 60% in maize and some 80% in barley. In maize inhibition of greening in the presence of isonicotinyl hydrazide was not overcome by addition of glycollate or glycine, but was restored by serine and by pyruvate. Results were similar in barley with the difference that only pyruvate would restore pigment formation. Isonicotinyl hydrazide thus inhibits pigment

* Present address: Department of Biological Sciences, University of East Anglia, Norwich NOR 88C, U.K.

† Present address: Department of Brewing and Biochemistry, Heriot-Watt University, Edinburgh EH1 1HX, U.K.