Isolation, Amino Acid Composition and Physical Properties of Chloroplast Thylakoid Protein from *Euglena gracilis*

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The physical properties of thylakoid ('structural') protein isolated from chloroplasts of higher plants have been the subject of contrasting reports (Biggins & Park, 1965; Criddle & Park, 1964; Criddle, 1966; Mani & Zalik, 1970). Biggins & Park (1965) report the behaviour of the protein in the analytical ultracentrifuge to be typical of a system exhibiting an association-dissociation equilibrium with a high tendency to aggregation. The extrapolated sedimentation coefficients of the monomer and aggregate were reported as 2.3 and 4.5S respectively. On the other hand Criddle (1966) reported an $s_{20,w}^0$ value of 2.3S and no evidence of aggregation or association over a wide range of protein concentration; and Mani & Zalik (1970), using higher concentrations of sodium dodecyl sulphate, report values of 1.2–1.3S. Lees, Mapp, Redmond & Rogers (1971) have extended these observations and shown that chloroplast thylakoid protein possesses complex association-dissociation properties under various conditions of protein concentration, detergent composition and concentration, and buffer composition and pH.

The present communication reports studies on chloroplast thylakoid protein isolated from *Euglena gracilis*. Cells were grown on a complex medium (Kirk, 1962), then ruptured by ultrasonication, and chloroplasts were isolated by discontinuous sucrose-density-gradient centrifugation. The chloroplasts were washed extensively with water to remove soluble components. Subsequent acetone precipitation and repeated washing with acetone and finally ether yielded the insoluble, virtually lipid-free, preparation termed thylakoid protein.

This protein, dissolved in buffers containing sodium dodecyl sulphate, migrated as a single symmetrical boundary in some 50 sedimentation-velocity studies on several preparations. However,

under various conditions of protein and sodium dodecyl sulphate concentration and buffer composition the single boundary differed in sedimentation coefficient in a manner characteristic of dissociation of aggregated molecules to smaller subunits. For example, in 0.1% sodium dodecyl sulphate the extrapolated $s_{20,w}^0$ value for protein concentrations greater than 3.5mg/ml was approx. 5S whereas below this protein concentration sedimentation coefficient values extrapolated to intercept the $s_{20,w}$ axis at 2.9S. Irreversible dissociation of the 5S to the 2.9S species could be achieved by treatment for a short time with 6M-urea.

In higher (1%) sodium dodecyl sulphate concentrations the thylakoid protein migrated at approx. 2S at protein concentrations greater than 3.5mg/ml. Below 3.5mg/ml sedimentation coefficient decreased with protein concentration, possibly reflecting migration of complexes of changing detergent/protein ratio, since no boundary characteristic of detergent micelles was evident in addition to the protein boundary.

Preliminary studies indicate that the 5S thylakoid protein species has a molecular weight of about 250 000–300 000; a similar value has been obtained on the basis of Sephadex G-200 gel filtration.

The amino acid composition of the thylakoid protein shows interesting features; for example, sulphur-containing amino acids are present in only very low quantities. The available results suggest that *Euglena* chloroplast thylakoid protein may well be more homogeneous than that from some other sources. Studies on this aspect and on the molecular weights of the species identified are in progress.

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