Biochemistry of *Chlamydomonas* flagella

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The flagella of *Chlamydomonas* can be detached from the cell bodies by calcium ions. These flagella round up and some of the axoneme protein goes into solution when they are resuspended in a buffer containing magnesium as used by Gibbons (1963) and Watson & Hynes (1966) for preserving *Tetrahymena* cilia. Flagella showing a normal transverse section and good membrane preservation may be obtained by including calcium in the magnesium buffer.

The axoneme proteins of *Chlamydomonas* flagella have different solubility properties to the previously studied *Tetrahymena* cilia (Watson *et al.* 1966; Renaud, Rowe & Gibbons 1968). A low ionic strength buffer containing *EDTA* extracts the protein of one of the central pair fibres and part of one of each of the subfibres of the outer nine. The outer nine fibres are not dissolved by dialysis against 0·6 M potassium chloride but are soluble in ionic detergents.

Approximately 50% of the total flagellar protein including all the axoneme, is dissolved by buffers containing 8 M urea, 6 M guanidine chloride. Ideally it should be possible to dissolve out of the flagella each morphological unit such as outer nine, central pair arms, etc., as has been done for *Tetrahymena* cilia (Renaud *et al.* 1968), and sea-urchin sperm tails (Stephens 1968). Recent work suggests that it may be possible to solubilize selectively the *A* and *B* subfibres of the outer nine. Also with calcium or magnesium buffers, one of the central pair can be removed leaving the outer nine intact (manuscript in preparation).

Fractionation of intact flagella into their constituent proteins can be followed by gel electrophoresis. Electrophoresis is performed under conditions where all the proteins have been split up into their respective polypeptide chains. Figure 43 (b), plate 13 shows the polyacrylamide electrophoresis of an 8 M urea extract of *Chlamydomonas* flagella (wild-type, 32°C). The electrophoretic pattern is very similar to that of *Tetrahymena* cilia (kindly prepared by Dr J. Wolfe, (figure 43, (a), plate 13) or outer fibre protein. There are two major protein zones *A* and *B* which characterize the extract. These probably come from the outer fibres and have been identified as such for *Tetrahymena*. The *A* and *B* zones of the two species have similar but not identical mobilities at pH 9·5 and 7·0. There are further protein zones migrating on the anode side of the *A* zone but only faintly visible in this urea extract. However, if the flagella are dialysed against an *EDTA*-buffer and then the soluble fraction is further dialysed against 8 M urea, enrichment of the
latter proteins is obtained. Figure 44, plate 13 demonstrates this. The gel is over-
loaded with protein to show as many minor bands as possible.

Studies are in progress to correlate the known genetic loci for flagellar synthesis
with the polypeptide chains separated by electrophoresis. So far this has been
attempted for only one locus, namely that involved in the mutation of wild
type $\rightarrow$ pf$18c$ (non-leaky, $9+0$ mutant). Comparison of the wild-type and mutant
electrophoretic patterns (figure 44) indicates that there is one additional protein in
the latter, migrating on the anode side of zone $A$. This is thought to be the mutant
protein but further data are required before it can be confirmed.

These preliminary results already provide a means of studying the kinetics of
synthesis of individual flagellar proteins and in the long term the factors controlling
their synthesis.

REFERENCES (Jacobs et al.)

Figure 43. 7.5% polyacrylamide gel electrophoresis, 8M urea. Soluble proteins from 8M urea-1% mercaptoethanol extraction: (a) Tetrahymena cilia (prepared by Dr J. Wolfe); (b) Chlamydomonas reinhardii flagella. Electrophoresis buffer: tris-glycine discontinuous system pH 9.5.

Figure 44. 7.5% Polyacrylamide gel electrophoresis, 8M urea. Soluble proteins extracted by tris-EDTA-0.01% mercaptoethanol pH 7.7 from flagella of Chlamydomonas reinhardii. (a) and (b). Strain 32c (wild-type). (c) and (d) Strain pf18c (9+0 mutant). Both samples were dialysed against tris-EDTA-8M urea 1% mercaptoethanol buffer prior to electrophoresis. Electrophoresis buffer: tris-glycine discontinuous system pH 9.5.