Sedimentation analysis of the Pf1 gene 5 protein

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The gene 5 protein of the filamentous bacteriophage Pf1 is a dimeric protein of $M_r \sim 30\,800$ which binds to single-stranded DNA during phage replication to form a helical nucleoprotein complex (Kneale, 1983). Earlier fluorescence studies on the protein have suggested that it is prone to aggregation (Greulich *et al.*, 1985). In this study we probe further these aggregation properties using sedimentation velocity and sedimentation equilibrium techniques. Two solvents were used: 'low salt' [a phosphate chloride buffer, pH = 6.8, with relevant proportions of Na_2HPO_4, KH_2PO_4 and NaCl made up to a combined ionic strength of 0.10 according to Green (1933)], and 'high salt' ('low salt' + 2M-KCl, I = 2.10).

Sedimentation velocity experiments were performed using an MSE Centriscan analytical ultracentrifuge equipped with scanning absorption optics and a monochromator. In the 'low salt' buffer two clear components were seen (Fig. 1*a*): the major (protein dimer) component of $s^0_{20,w}(2.6 \pm 0.10)$ S, but also an appreciable 'minor' faster component, which showed a significant concentration dependence ($s^0_{20,w} = 35.5 \pm 1.4$) S.

Low-speed sedimentation equilibrium experiments were performed using a Beckman Model E analytical ultracentrifuge incorporating Rayleigh interference optics and a 5 mW He–Ne Laser light source. A low loading concentration (~ 0.4 mg/ml) in a 30 mm path length cell was used to minimize possible effects of thermodynamic non-ideality. 'Whole cell weight average' and 'point weight average' relative molecular masses ($M^0_{r,w}$ and $M_{r,w}$, respectively) were determined as described before (Creeth & Harding, 1982). An $M^0_{r,w}$ of (37 500 ± 1000) was obtained. Throughout most of the cell, an $M_{r,w}$ of ~ 32 000 ± 3000 was evident (Fig. 1*b*), although near the cell base the presence of a small amount of low *n*-mer association products was indicated; higher order aggregates corresponding to the ~ 35.5 S component will have a sufficiently large M_r to accumulate at the cell base with the rotor speed used (14 277 rev./min) and therefore beyond optical registration.

If we assume the 2.6 S component corresponds to the dimer, with an M_r and partial specific volume, $\bar{v}$, of 30 800 and 0.734 ml/g, respectively (both calculated from protein sequence information; Maeda *et al.*, 1982), an estimate for the frictional ratio, f/f_0 (see, for example, Tanford, 1961), of ~ 1.3 is obtained. This value would appear to suggest a globular but not spherical particle. If we assume a crude value for the degree of associated solvent, or 'hydration' (~ 0.50 g of H_2O/g of protein; see Squire & Himmel, 1979), this corresponds to an axial ratio for the equivalent prolate ellipsoid of between 2:1 and 3:1. We can also estimate a lower limit for the M_r of the 35 S aggregate, assuming a spherical conformation (see, e.g. Tanford, 1961); a value of $\approx 1.5 \times 10^6$ is obtained, corresponding to ≈ 50 'dimer-units' of the protein; if it were a short helix, similar to that of the complex (Kneale *et al.*, 1982), this would correspond to at least eight helical turns.

Further sedimentation velocity experiments conducted on the protein in the presence of 2 M-KCl revealed only aggregate with an 'infinite dilution' $s_{20,w}$ of (30.0 ± 1.0) S, again with a high degree of concentration dependence; this observation is in accord with the fluorescence depolarization measurements of Greulich *et al.* (1985).

Abbreviations used: $M^0_{r,w}$, whole-cell average relative molecular mass; $M_{r,w}$, point weight average relative molecular mass.

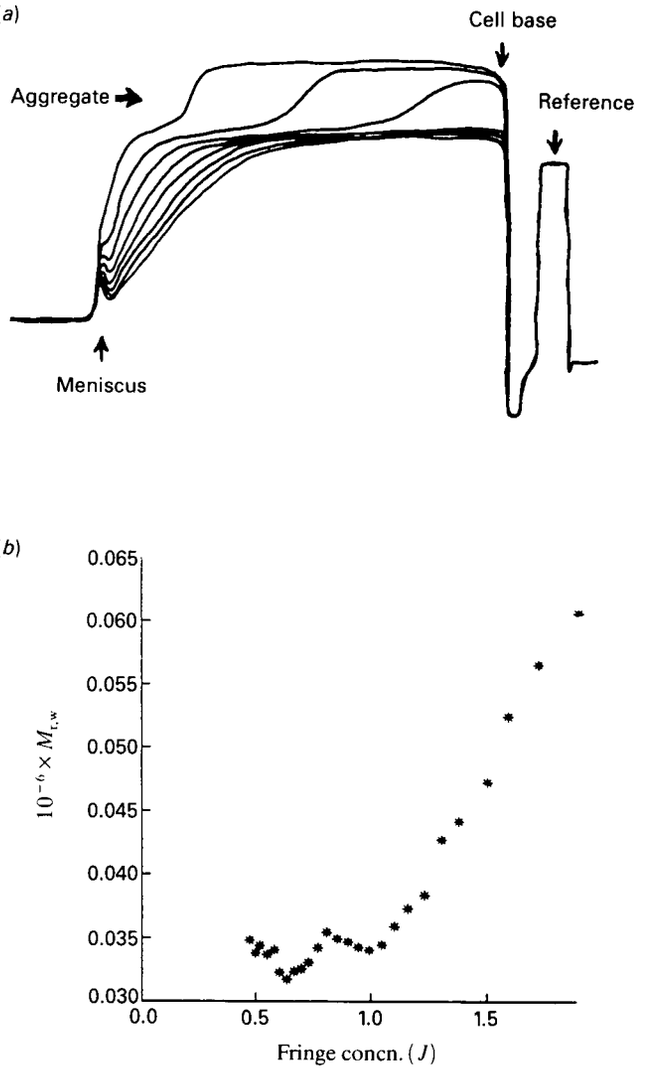

Fig. 1. *Analytical ultracentrifugation of PF1 gene 5 protein*

(*a*) Sedimentation velocity scans (at 8 min time intervals). An MSE Centriscan was used, rotor speed 40 000 rev./min, temperature 20.0°C, concentration 0.7 mg/ml. The trace records absorbance at 278 nm (vertical axis) as a function of radial displacement (horizontal axis). The direction of sedimentation is from left to right. (*b*) Plot of $M_{r,w}$ values from a low-speed sedimentation equilibrium experiment in a Beckman Model E analytical ultracentrifuge using Rayleigh Interference Optics. Rotor speed 14 277 rev./min, temperature 8.0°C. Initial loading concentration approx. 0.4 mg/ml.

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Mitochondrial calcium metabolism in *Drosophila*

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Mitochondria isolated from adult wild-type *Drosophila melanogaster* are derived mostly from flight muscle. They do not accumulate calcium by an energy-dependent process (Krall & Duke, 1979) and contain less than 5 nmol calcium mg^{-1} protein. Mitochondria from larvae do accumulate calcium at rates of about 0.2 nmol mg^{-1} protein s^{-1} and accumulate up to 60 nmol calcium mg^{-1} s^{-1}. *Drosophila* flight muscle is asynchronous, requiring that calcium released from endoplasmic reticulum (e.r.) on stimulation remains in contact with the myofibrils during as many as a thousand contractile cycles, i.e. up to 1 s. This apparently occurs because the myocytes contain only 0.5% e.r. by volume, allowing very slow reaccumulation of released calcium. The requirement for a high rate of ATP synthesis is met by the presence of mitochondria at more than 50% cell volume. If these mitochondria accumulated calcium as rapidly as mammalian liver or kidney mitochondria, only very brief bursts of contractile activity would be permitted. The loss of ability of flight muscle mitochondria to accumulate calcium occurs shortly after emergence of the adult and may be the result of either loss of inward calcium movement or an increase in its efflux rate. We have isolated a 14 kDa calcium-binding protein from rat liver mitochondria which specifically stimulates calcium uptake by protein-depleted mitochondria (Behan *et al.*, 1985). Addition of a 14 kDa protein isolated by another method to mitochondria from adult *Drosophila* caused rapid and continued cycling of calcium into and out of mitochondria. Since this protein causes only calcium uptake in protein-depleted liver mitochondria, the mechanism for rapid release of calcium must already be present in the flight muscle mitochondria, and is activated by rapid influx of calcium. This 14 kDa protein is probably the same protein as calvectin (Panfili *et al.*, 1983) which binds calcium with a K_d of 1.5×10^{-7} M. The exit mechanism is probably via a 32 kDa protein (Happel & Krall, 1979), which restores both calcium influx and efflux rates to hypotonically extracted mitochondria (Happel, 1979). The Ruthenium-Red-binding proteins (Krall *et al.*, 1985*a*) are extracted from the outer mitochondrial membrane (Krall *et al.*, 1985*b*), where they are believed to regulate calcium traffic into and out of the space(s) between outer and inner membranes. The concentration of calcium in this space determines the rate of movement of calcium through the electrogenic uniport across the inner membrane into the mitochondrial matrix space.

The 14 kDa protein is present in extracts of mitochondria prepared from larvae of *Drosophila* or from rat liver, but is not present in extracts of mitochondria from adult *Drosophila.*

We have studied the effects of hypotonic extraction on rates and amounts of calcium uptake by mitochondria from the third instar larvae from three strains of *Drosophila.* Data for controls only are given since all three strains reacted similarly to extraction and to varying calcium concentrations.

These data (Table 1) show that extraction reduces the initial rate of calcium uptake most when lower concentrations of external calcium are present. This is consistent with the idea that extraction lowers the rate of influx of calcium into the intermembrane space by removal of a calcium-specific ion channel which may or may not be voltage dependent, but is probably regulated by action of an intracellular (second) messenger.

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Table 1. *Calcium uptake of shocked and non-shocked mitochondria in late third instar stage of the wild-type (pac) strain of D. melanogaster*

	Ca^{2+} concentrations (M)			
Calcium uptake	3.3×10^{-5}	8.33×10^{-5}	1.67×10^{-4}	3.33×10^{-4}
Unshocked				
Rate (nmol mg^{-1} s^{-1})	0.104 ± 0.005	0.103 ± 0.003	0.122 ± 0.003	0.123 ± 0.004
Total (nmol mg^{-1} s^{-1})	40.6 ± 1.6	42.1 ± 1.2	48.6 ± 1.3	56.7 ± 2.0
Shocked				
Rate (nmol mg^{-1} s^{-1})	0.013 ± 0.003	0.025 ± 0.005	0.039 ± 0.005	0.092 ± 0.006
Total (nmol mg^{-1} s^{-1})	1.4 ± 0.04	3.5 ± 0.02	7.0 ± 0.02	13.6 ± 0.22