Journal of Muscle Research and Cell Motility **11**, 344–350 (1990)

Self-interaction of dynein from *Tetrahymena* cilia

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Received 6 February 1990; revised 4 March 1990; accepted 4 April 1990

Summary

The molecular mass (M_r) and enzymic activity of the larger dynein species from *Tetrahymena thermophila* has been studied in the high (600 mM) to low (40 mM) ionic strength range. The apparent M_r is found to vary with both ionic strength (by sedimentation velocity and quasi elastic light scattering analysis) and with protein concentration at low ionic strength (by sedimentation equilibrium analysis). These data indicate a strong self-interaction, resulting in dimer formation under low salt conditions. There is no evidence for the formation of species of higher than dimeric mass. A molecular mass for the dynein monomer of 1.64×10^6 daltons has been determined, a value rather lower than previous published estimates.

The ATPase activity of dynein increases with increasing ionic strength. The possible relationship between this effect and the self-association phenomenon is discussed.

Introduction

The microtubule-dynein system is the universal mechanism by which eukaryotic cilia and flagella generate movement. Within the axoneme, the microtubules are arranged as 9 outer doublets surrounding 2 central single microtubules. The dynein arms have ATPase activity and emanate from the A-tubules of the doublets. Movement is effected by the dynein arms interacting transiently with the B-tubule of the adjacent doublet in an ATP-sensitive manner.

Dynein isolated from *Tetrahymena* cilia can be separated on a sucrose gradient into two fractions, which were originally termed 14S and 30S dynein from sedimentation studies (Gibbons & Rowe, 1965). These fractions have different patterns on SDS polyacrylamide gel electrophoresis, indicating that they are two distinct proteins (Porter & Johnson, 1983). Analysis of 30S dynein from *Tetrahymena*, by STEM, showed that the molecule comprised three globular heads connected by separate strands to a common basal region (Johnson & Wall, 1983). More recent estimations of the sedimentation coefficient of the 30S fraction give a value of 21S (Mitchell & Warner, 1981; Clutter *et al.*, 1983). Subsequent papers referred to this faster sedimenting species as 22S dynein, with reference to the aforementioned papers and Clutter and co-workers' unpublished results. The data presented here demonstrate that the sedimentation coefficient varies with ionic strength. Therefore it seems inappropriate to identify this protein using its s value, and so it will subsequently be referred to as simply, dynein, as recently adopted by Shimuzu *et al.*, (1989).

The mechanism by which dynein ATPase activity is utilized to generate the wide repertoire of movements achieved by eukaryotic cilia and flagella is largely unknown. The complex structure of dynein may signify that it is directly involved in the regulation of the system. Factors affecting dynein ATPase activity have been studied mainly in sea-urchin sperm. Activation occurs under various conditions, either reversibly by monovalent salts (Evans *et al.*, 1986) or EDTA, or irreversibly by the use of organic solvents, detergents or heat (Gibbons & Fronk, 1979). This paper reports on both structural and functional effects of NaCl on dynein. Using a range of hydrodynamic techniques, ionic strength-dependent self-association of dynein molecules is observed. The sedimentation coefficient of the protein is shown to increase with decreasing NaCl concentration, while the diffusion coefficient decreases under these conditions, resulting from an increase in average mass. Sedimentation equilibrium analysis supports these findings and is consistent with the protein forming dimers under low salt conditions. This structural effect is paralleled with a positive effect of salt on ATPase activity. The inter-relationship between these structural and functional changes is discussed, together with their possible physiological significance.

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0142-4319/90 $03.00 + .12

Materials and methods

Organism

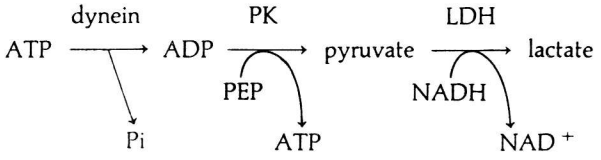
The organism used for these studies was the ciliated protozoan *Tetrahymena thermophila* SB255 which was kindly supplied by Dr J. S. Hyams. This is a mucus-deficient strain of this organism, which therefore improves cilia yield.

Isolation and purification of dynein

Tetrahymena was routinely grown in 17 l or sometimes 34 l cultures and dynein was extracted and purified from the cilia essentially as described by Porter and Johnson (1983). Following separation from 14S dynein on a 5–25% sucrose gradient, dynein was normally dialysed against 10 mM Hepes, 4 mM $MgCl_2$, 0.1 mM EGTA, pH 7.4 containing either 0.04 M or 0.6 M NaCl. A typical yield from a 17 l culture of *Tetrahymena* is 10–12 mg of dynein and 1–3 mg of 14S dynein. When necessary, dynein was concentrated using an Amicon minicon-B concentrator.

ATPase assay

Dynein ATPase activity was routinely measured using a linked assay system (adapted from Emes & Rowe, 1978). The reaction was followed by the decrease in absorbance at 340 nm, due to NADH oxidation.



(PK, pyruvate kinase; PEP phospho(enol)pyruvate; LDH lactate dehydrogenase).

The 1 ml reaction mixture typically contained 400 μM phospho(enol)pyruvate, 400 μM ATP, 250 μM NADH, 0.01 ml PK/LDH enzyme mix and 10–50 μg dynein. Assays were carried out at 20° C in 10 mM Hepes, 4 mM $MgCl_2$, 0.1 mM EGTA, pH 7.4 containing NaCl at the concentration indicated.

Sedimentation velocity measurements

Sedimentation velocity measurements were carried out using an MSE Centriscan 75 analytical ultracentrifuge at 20° C and 20 000–30 000 rev min^{-1}. The sedimentation rate of dynein (0.3–0.6 mg ml^{-1}) was analysed in 10 mM Hepes, 4mM $MgCl_2$, 0.1 mM EGTA, pH 7.4 containing NaCl as stated, in single sector cells. As the amount of protein available was limited, a relatively low loading concentration was used (0.3–0.6 mg ml^{-1}) and the measured sedimentation coefficient was taken as approximating to the infinite dilution value. Absorption optics were used, with the monochromator set at 280 nm, and the sedimentation coefficient calculated from measurements of the radial position of the boundary taken at regular intervals. All sedimentation coefficients were corrected to standard conditions (water as solvent at 20.0° C) in the usual way (Bowen & Rowe, 1970).

Diffusion coefficient measurements: quasi-elastic light scattering (QLS)

Quasi-elastic light scattering measurements were performed using Malvern 4700 light scattering equipment with a Siemens 40 mW He/Ne laser (wavelength, $\lambda = 632.8$ nm). The beam from the laser was focussed on to the centre of a 1 cm × 1 cm cuvette. The cuvette was placed at the centre of a goniometer so that the scattering angle could be varied from 5° to 90°. Diffusion coefficients showed no significant trend with changing angle so that an angle of 90° was utilized to minimise effects of dust (Godfrey *et al.*, 1982). Scattered light was collected by an EMI photomultipler via a well-collimated pinhole (aperture 100 μm) and via an amplifier–discriminator to a 64-channel Malvern autocorrelator (K7032-0S). The digital correlator output was stored on floppy disks and then sent via an Olivetti M24 computer to the University of Cambridge IBM 3081/B computer (via the JANET link) for processing. The routine used produced an accurate plot of $\log[g^{(2)}(t) - 1]$ versus time, where $g^{(2)}(t)$ is the normalized intensity correlation function. z-average (apparent translational) diffusion coefficients ($D_{T,b}$) were obtained from the limiting slope (Pusey, 1974) of this plot. The routine used produced the best least-squares fit to a linear, quadratic or cubic polynomial, and a guide to the best fit was provided by the ε function (see, for example, Teller, 1973). The routine also calculated the 'polydispersity factor' or 'PF', namely the z-averaged normalized variance of the diffusion coefficient distribution (see e.g., Pusey, 1974).

As for the sedimentation work, because of scarcity of material, for a given salt concentration, diffusion coefficients were measured only at one (low) protein concentration (0.5–1.0 mg ml^{-1}) which in each case was taken as approximating to the infinite dilution value. These values were corrected to standard conditions (water as solvent at 20.0° C) according to the usual formula (see, e.g., Tanford, 1961)

$$D_{20,w} = \frac{293.15}{T} \cdot \frac{\eta_{T,b}}{\eta_{20,w}} \cdot D_{T,b}$$

Sample times of 3 or 5 μs were used. No dependence of diffusion coefficient with sample time was observed, so a correction to zero sample time was not necessary (Godfrey *et al.*, 1982). Experimental duration times were 2–6 min, depending on the sample, and chosen so as to ensure a high number of counts to be stored in the autocorrelator channels.

In order to minimize scatter due to foreign particles, cuvettes were cleaned and samples clarified essentially as described by Godfrey *et al.*, (1982). Any such effects were shown to be negligible.

Sedimentation equilibrium analysis

Low speed equilibrium analysis was carried out using an MSE MkII analytical ultracentrifuge. Single sector 20 mm cells filled with either solvent or solution and with a solution column close to 1 mm in height were used, and photographed using phaseplate schlieren optics. A low loading concentration was used (~0.5 mg ml^{-1}) to minimize the effects of thermodynamic non-ideality. The enlarged traces were scanned using an LKB 2202 Ultroscan laser densitometer (Harding & Rowe 1988) and the increment of the solution trace similar to that obtained with interference optics is yielded by this approach (Rowe *et al.*, 1989). To ensure exact comparability of experiments in high and low salt, the experiments were optically multiplexed from within the same rotor.

Computation of point $M_{r,app}$ values was achieved by numerical differentiation of the appropriate function. Direct evaluation from the regression of $\ln((dc/dr)/r)$ yields point values for M_z.

To compute M_w values, advantage was taken of the invariance with r of M_z for dynein in high salt to derive M_w at the harmonic midpoint of the cell (and hence evaluate the otherwise unknown constant of integration of the dc/dr upon c curve) from the assumption that $M_w = M_z$ for this sample. The relationship between integrated area units and absolute mass concentration units is thus established for both samples, because of the common optics.

From an amino acid analysis of dynein, the partial specific volume $\bar{v}$ was computed to be 0.730 $cm^3.g^{-1}$.

Results

Sedimentation velocity measurements and ATPase activity of dynein

Figure 1 shows the sedimentation diagram of dynein in buffer containing 40 mM NaCl and 600 mM NaCl at a single time interval. Dynein shows a single, clearly defined sedimenting boundary at both salt concentrations, as well as at intermediate NaCl concentrations (not shown). The boundary, however, is faster moving and rather broader in low salt, which could be the result of either a change in conformation or a change in average mass. The difference is greater than would be expected from simply differences in the viscosities and densities of the two solvents.

The sedimentation coefficient and the ATPase activity of dynein was estimated over a range of NaCl concentrations and the same protein loading concentrations, and the results are plotted in Fig. 2. These data show that there is essentially a linear decrease in s value with increasing NaCl concentration over the range measured. This is paralleled by a corresponding increase in ATPase activity with increasing salt. The possible relationship between these two phenomena will be discussed later.

Diffusion coefficient estimations by QLS

Near-linear plots of the log of the normalised autocorrelation function ($Log[g^{(2)}(t) - 1]$) versus time were obtained for both salt concentrations (Fig. 3a, b).

The $D_{20,w}$ values were seen to decrease as the NaCl concentration decreased (Table 1). By combining this data with that of $s_{20,w}$ (via the Svedberg equation) this decrease in $D_{20,w}$ corresponds to an increase in the weight-average relative molecular mass, $M_{r,w}$ on lowering the salt concentration.

The 'polydispersity factor' for dynein under low salt conditions was somewhat higher (0.15 ± 0.06) than that obtained under high salt conditions (0.11 ± 0.06), which suggests that under low salt concentrations, the dynein is more heterogeneous as well as having larger molecular mass. Table 1 summarises hydrodynamic and ATPase activity data obtained from a typical dynein preparation. The monomeric molecular mass value of 1.64×10^6 daltons is a little lower than that previously obtained by the (less precise) STEM weighing technique (Johnson & Wall, 1983).

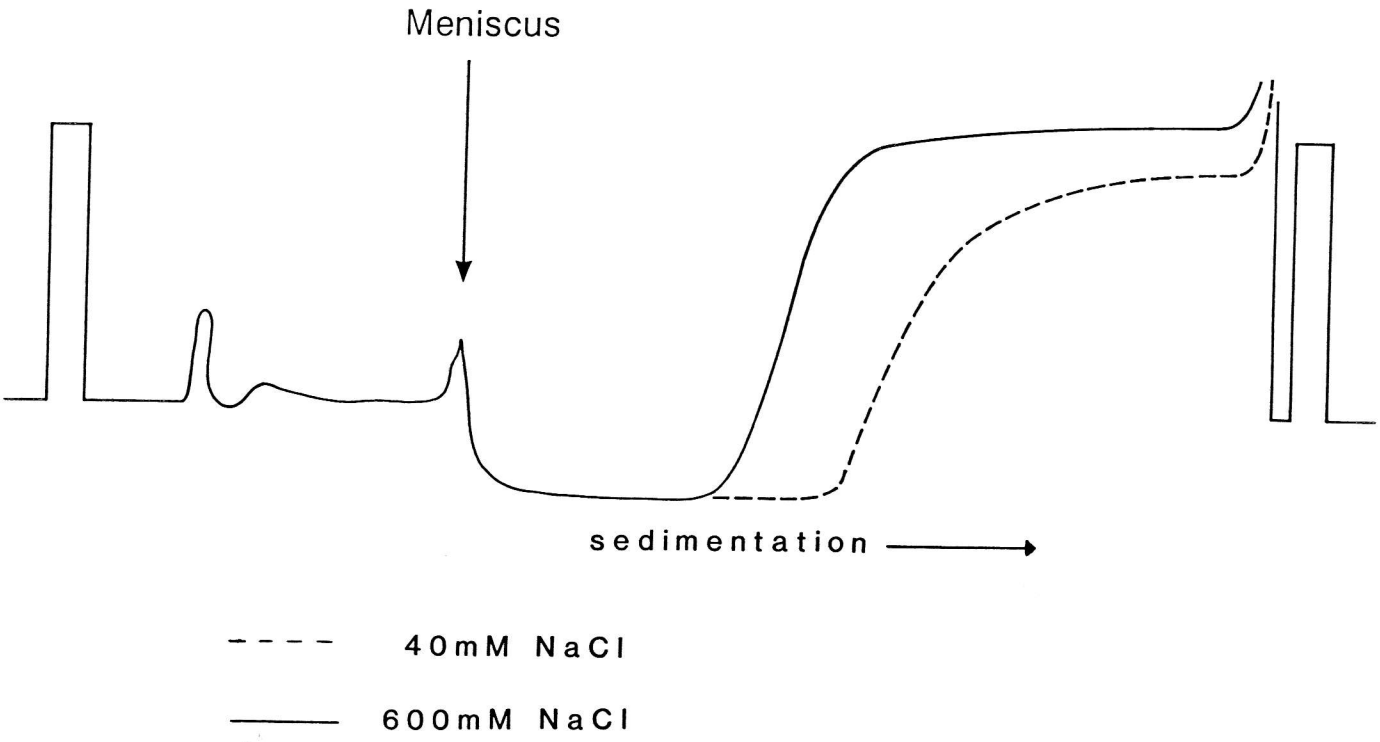


Fig. 1. Dynein (0.35 mg ml^{-1}) was sedimented at 31 000 rpm and 20° C in 10 mM Hepes, 4 mM $MgCl_2$, 0.1 mM EGTA, pH 7.4 containing 40 mM NaCl (dashed line) and 600 mM Nacl (solid line). A single boundary, recorded at the same time interval, is shown for each salt concentration.

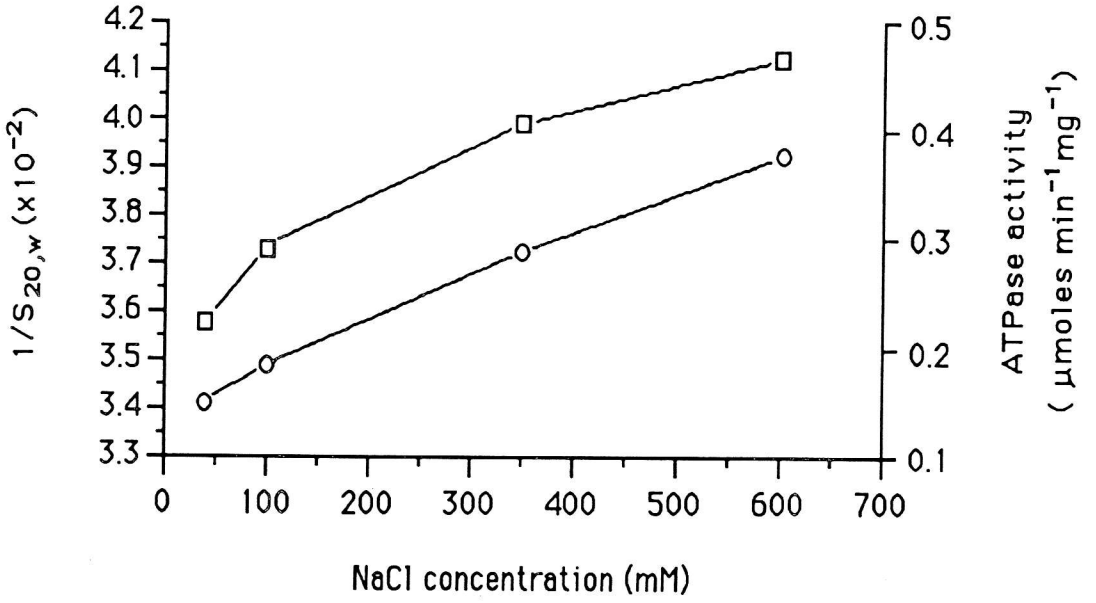


Fig. 2. The sedimentation coefficient (open squares) and ATPase activity (open circles) were measured for dynein dialysed into 10 mM Hepes, 4 mM $MgCl_2$, 0.1 mM EGTA, pH 7.4 and NaCl concentrations as shown. The protein loading concentration was the same for each sedimentation estimation (0.3 mg ml^{-1}).

Sedimentation equilibrium analysis

Sedimentation equilibrium measurements confirmed that the average M_r of dynein increases with lowering NaCl concentration. The dependence of M_w upon solute concentration differs markedly for the case of high salt (600 mM NaCl) and low salt (40 mM NaCl). In high salt there is little or no concentration-dependence and the average M_w over the range employed, estimated as 1.65×10^6 daltons, does not differ significantly from an extrapolated M_w (Fig. 4). The concentration-dependence of M_w is wholly consistent with dynein being monodispersed in high salt, though the presence of a small degree of dimerisation, masked experimentally by the effects of thermodynamic non-ideality, cannot be excluded (see below). In contrast, at low salt there is a strong dependence of M_w upon c. At the higher concentrations (>1 mg ml^{-1}) the $M_{r,z}$ value tends asymptotically towards a value of about 3.3×10^6, which would correspond to a dimer. It is experimentally difficult to estimate point $M_{r,z}$ values at very low concentrations

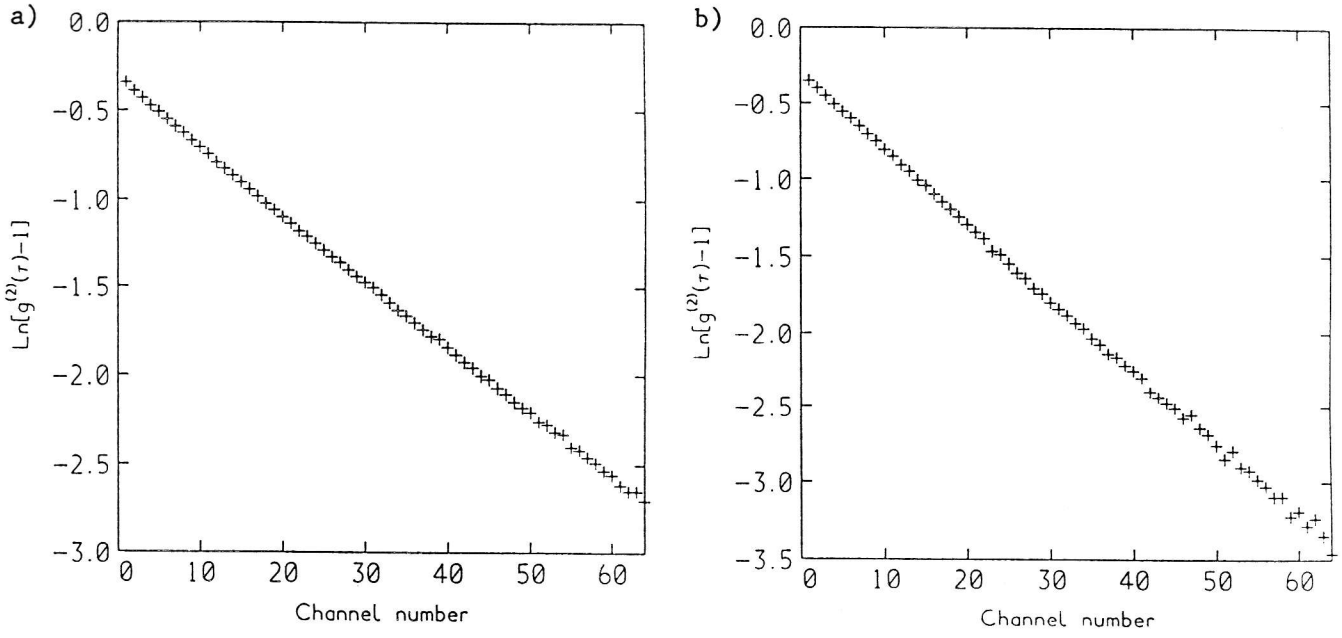


Fig. 3. Plot of $\ln[g^{(2)}(T)-1]$ as a function of channel number (time, t = channel number, b × sample time, T) for dynein (1.1 mg ml^{-1}) in (a) 40 mM NaCl and (b) 600 mM NaCl. The scattering angle was 90°, sample time was 5 μs, duration time was 360 s and temperature was 25° C.

Table 1. Effect of NaCl on the hydrodynamic properties and ATPase activity of dynein

	40 mM NaCl	*600 mM NaCl*
$s_{20,w}$ (s) $\times 10^{13}$	27.9	24.3
$D_{20,w}$ ($cm^2 s^{-1}$) $\times 10^7$	1.07	1.28
$M_{s,D} \times 10^{-6}$	2.50	1.64
ATPase (mmol min^{-1} mg^{-1})	0.14	0.38

(< 0.2 mg ml^{-1}), but the results obtained at these lower ranges would not be inconsistent with an approach towards the high-salt (presumptive monomer) value as $c \rightarrow 0$ (Fig. 4).

Computer simulation of the monomer-dimer interaction

The transition observed between presumptive monomer and dimer values in low salt is unexpectedly well defined, and shows a distinctly sigmoidal shape (Fig. 4). Computer modelling of the variation of M_z with concentration for assumed interaction constant K_a shows that it is not possible to model so sharp a transition with a single, concentration-invariant value of K_a.

In order to fit the data, it is necessary to assume that K_a itself varies with concentration. An effect of overlapping electrical double layers (Shaw, 1970) could well be responsible and would give rise to a strong concentration-dependence. Many functional relationships between the parameters would give an adequate fit: for example an assumption that K_a varies with c^3 has been found to be adequate (unpublished results).

Discussion

Dynein shows a reversible self-interaction to form dimers at low ionic strength

At 600 mM salt, we show that dynein behaves as a single monodispersed solute component by three criteria: (1) solute concentration and field invariance of M_r at sedimentation equilibrium; (2) linearity of the QLS plot for D_z; and (3) the presence of a single, symmetrical boundary in sedimentation velocity profiles. Using the partial specific volume computed from the amino-acid analysis, a value of $M_{w,r} = 1.64 \times 10^6$ is obtained, by both sedimentation equilibrium and by the combination of s & D. This is rather lower than the value previously published (1.95 megadaltons) on the basis of STEM analysis (Johnson and Wall, 1983). This latter method, however, is inherently less accurate than good solution technology, both by virtue of statistical effects and from the danger of minor salt contamination leading to significant overestimation. Our present value is consistent with preliminary hydrodynamic evidence previously published (Clutter *et al.*, 1983). The dynein monomer is thus described in solution as a hydrodynamic particle probably of low assymmetry (a low second virial coefficient is implicated by the lack of c-dependence of M_r values in 600 mM NaCl; Fig. 4). There is, however, a significant degree of solvent entrainment ($\bar{V}_s/\bar{v} = 9.1$; Rowe 1977; a plausible value for a large multi-subunit structure; Squire & Himmel 1979). This description is compatible with models derived from electron microscopy (Johnson and Wall, 1983).

At lower ionic strength, dynein self-associates. This we deduce from the fact that M_r values now show a strong tendency to increase with solute concentration, approach-

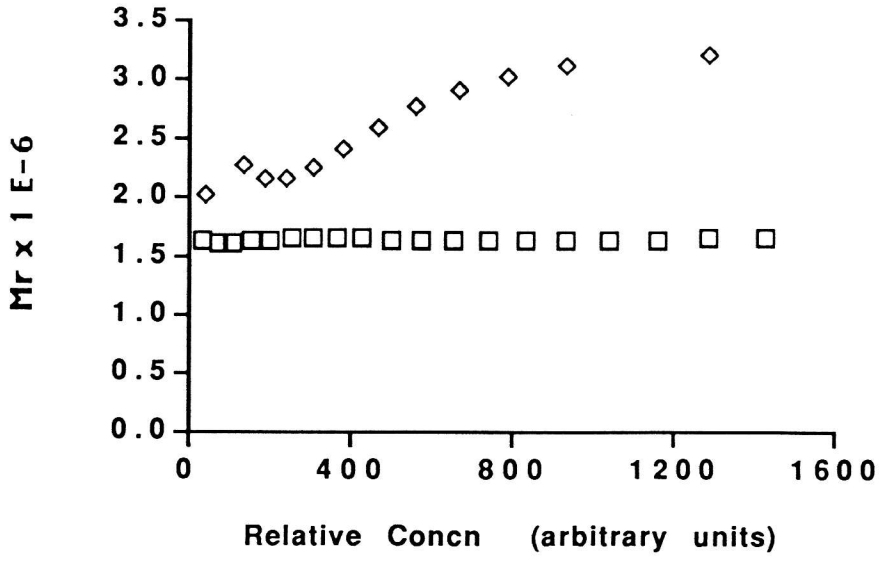


Fig. 4. Point average molecular weights as a function of concentration for dynein in 600 mM NaCl (open squares) M_w values plotted and 40 mM NaCl (open diamonds) M_z values plotted to emphasize the lack of species higher than a dimer. The cell loading concentration was 0.5 mg ml^{-1} corresponding approximately to 1300 arbitrary units as plotted. (Zero on the concentration axis displayed corresponds to 480 arbitrary units).

ing a limiting value of close to 3.3×10^6, i.e. a dimer value (Fig. 4), whilst QLS plots also indicate increased polydispersity with respect to D_z (Fig. 3). Under the conditions studied an average M_r of close to 2.5×10^6 is yielded, again by both methodologies employed. That this rise in average M_r is not due to an indefinite association is evidenced by the approach to a dimeric 'plateau' value (Fig. 4). The presence of dimers, but not of significant numbers of higher oligomers, has also been noted by electron microscopy (Johnson and Wall, 1983). The sedimentation velocity evidence likewise indicates that no significant quantity of higher oligomers is formed, and additionally makes it clear that the dimerisation must be reversible. This follows from the observation that under low salt conditions a *single* sedimenting boundary is observed which is only slightly broadened as compared to the monomer boundary (Fig. 1). This finding is incompatible with the presence of two non-interacting species, but wholly consistent with a monomer–dimer equilibrium (Gilbert & Gilbert, 1973).

The dimerisation of dynein is paralleled by a decrease in ATPase activity.

Data are presented which demonstrate that the ATPase activity of dynein decreases with decreasing ionic strength, in parallel with the increasing degree of self-interaction observed (Fig. 2). We cannot however state with any certainty that dimerisation *causes* the change in enzymic activity. Ionic strength changes might for example lead to conformation changes in the monomer of a type which would depress the ATPase activity even if dimerisation did not occur. Equally of course such conformation changes might be a necessary pre-condition for dimerisation.

In the case of dynein from sea-urchin sperm, Gibbons & Fronk (1979) have also shown an effect of monovalent salt on the ATPase activity of similar sign and magnitude to that obtained in the present study. This they described as an activation by salt of a 'latent' form of dynein; a process which they compared to similar activation shown by EDTA, detergents or heat. It is not known in this system whether dimerisation occurs as an function of ionic strength, although no ionic strength effect was observed on the s value (Evans *et al.*, 1986). However this evidence is not conclusive, as the s-values were obtained by the less accurate method of sucrose gradients, and probably at lower solute concentrations which would favour monomers.

Could a modulation of ATPase activity by self-interaction have physiological significance?

Dynein monomers are arranged in linear array along the length of the A-tubules, with a repeat spacing of 24 nm (Warner & Mitchell, 1978). They are packed essentially as a polymer, with the head region of one molecule riding on the basal region of the adjacent molecule, analogous to a row of circus elephants (Goodenough & Heuser, 1984).

Flexure of the axoneme (and hence the A-tubules) in ciliary beating will compress dynein polymers on the inside of the bend and therefore increase the possibility of self-interaction. Conversely, dynein on the outside will experience the opposite effect. This follows from simple geometry, since the outer arm dynein is located at a radial distance slightly greater than the centre of the A-tubule about which flexure occurs. It has been noted that sliding activity, as estimated by dynein attachment and re-attachment, is more pronounced in this plane than in the plane of the bend (Warner, 1979).

If we assume that self-interaction does modulate ATPase activity, then the result would be a relatively elevated sliding force on the outside of the bend, as compared to the force on the inside. This would be consistent with the requirements for the initiation and maintenance of bend formation in an initially linear axoneme.

An estimate of the extent of compression can be obtained. If we assume that the radial position of outer arm dynein monomers bridging an A-tubule to a B-tubule on the inside of the bend differs from the radial position which it would occupy were the same doublet interaction to be spatially located on the outside of the bend, by approximately 40 nm, then for axonemal bend radii of 1–5 μm, a relative compression of about 1.0–0.2 nm in axial spacing is calculated.

Hence the magnitude of the compression occurring is comparable to the movement observed between protein units during allosteric interactions. If intra- or intermolecular interactions of dynein molecules do modulate ATPase activity, the mechanism involved may be similar to that involved in allostery. Clearly a more precise knowledge of the self-interaction of dynein in solution is needed to ascertain whether it acts as a model for interaction within the axoneme and thus modulate ATPase activity. At the present juncture, we consider it to have some degree of plausibility and to be worthy of further study as a possible insight into the mechano–chemical coupling of this motile system.

Acknowledgements

We are grateful to Dr M. Davison & Ms. K. Lilley for carrying out amino acid analysis and Ms. E. Crossley for some experimental assistance. We acknowledge the Science and Engineering Research Council for financial support.

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