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ARTICLE

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Oligomerization and kinetic mechanism of the dynamin GTPase

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Abstract Dynamin is a large molecular weight GTPase. Amongst other biological processes, it is involved in clathrin-dependent endocytosis. It can self-assemble or assemble on other macromolecular structures that result in an increase in its GTPase activity. Its role in endocytosis has been variously attributed to being a force-generating enzyme or a signalling protein. Here we review evidence for the oligomeric state of dynamin at high and low ionic strength conditions. We also review work on the elementary processes of the dynamin GTPase at high ionic strength and compare these to the ATPase of the force-generating protein myosin and the GTPase of the signalling protein Ras. New data on the interaction of dynamin with a fluorescent derivative of GTPγS are also presented. The possible mechanism by which assembly of dynamin leads to an increase in its GTPase activity is discussed.

Keywords Dynamin · GTPase · Kinetic mechanism · Oligomerization

Abbreviations *mantdGT(D)P*: 2′-deoxy-3′-*O*-(*N*-methylanthraniloyl)guanosine-5′-tri(di)phosphate · *mantGTPγS*: 2′(3′)-*O*-(*N*-methylanthraniloyl)-GTPγS

Dedicated to Professor H. Gutfreund on the occasion of his 80th birthday

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Introduction

Dynamin is a GTPase which is an important component of a wide variety of biological processes (reviewed by Hinshaw 2000). Its involvement in clathrin-dependent endocytosis has been the most studied, although it is also known to be involved in processes such as vesicle trafficking from the Golgi, clathrin-independent rapid endocytosis and phagocytosis. In clathrin-mediated endocytosis, endophilin and cholesterol are necessary to convert the shallow clathrin-coated pits into invaginated pits. Dynamin then associates with the neck region of the invaginated pits and GTP hyrolysis occurs. This process is followed by fusion of the membrane to release the vesicle into the cell. At the present time, there are two possible mechanisms proposed to explain this fusion process.

First, dynamin may be a force-generating enzyme that acts as a "pinchase" to cause membrane fission. Evidence for this possibility comes from the fact that incubation of lipid nanotubes with dynamin in the presence of GTP results in a helical arrangement of dynamin around the nanotubes and the pitch of this helix increases from 11 nm to 20 nm on GTP hydrolysis (Stowell et al. 1999). These observations suggest a mechanism of membrane lipid fusion and internalization of the vesicle. It has also been reported that, under certain conditions, lipid fusion by dynamin in the presence of GTP occurs (Sweitzer and Hinshaw 1998). The structure of dynamin bound in a helical array to lipid nanotubes, obtained from reconstruction of cryoelectron microscope images, has also been interpreted as showing that a rearrangement of the dynamin structure generates a force on the lipid bilayer and causes membrane constriction (Zhang and Hinshaw 2001).

Second, it may be that dynamin is not a force-generating enzyme but a signal-transducing protein, which recruits other proteins to the membrane. For example, Sever et al. (1999) have shown that over-expression of mutant dynamin with impaired GTPase activity leads to acceleration of receptor-mediated endocytosis. One such

candidate for recruitment is endophilin, a lysophosphatidic acid acyl transferase (Schmidt et al. 1999), which binds to the PRD domain of dynamin (Ringstad et al. 1997). It has been proposed that endophilin may induce the high degree of bilayer curvature found at the necks of budding vesicles by catalysing the conversion of lysophosphatidic acid to phosphatidic acid. This process essentially converts an ''inverted cone-shaped'' lipid into a ''cone-shaped'' lipid (Scales and Scheller 1999). Dynamin I is expressed in neuronal tissue whereas dynamin II is ubiquitously expressed.

Mechanisms of nucleoside triphosphatase activity

Prior to this work, little was known about the mechanism of hydrolysis of GTP by dynamin. Most measurements were limited to steady-state measurements of the GTPase. However, extensive work has been done on the ATPase mechanisms of force-generating proteins such as myosin and kinesin and on the GTPase mechanisms of the Ras superfamily of signal-transducing proteins.

The mechanism of hydrolysis in both the myosin ATPase and Ras GTPase involves a direct, in-line nucleophilic attack on the γ-phosphate of the nucleotide by water (Webb and Trentham 1980; Feuerstein et al. 1989). Thus the simplest reaction scheme to describe the kinetic mechanism for these proteins is:

$$\mathrm{P + XTP \rightarrow P.XTP \rightarrow P.XDP.P_i \rightarrow \begin{matrix} \mathrm{P.XDP} \\ + \\ \mathrm{P_i} \end{matrix} \rightarrow P + XDP} \quad (1)$$

where P is the nucleoside triphosphatase and XTP and XDP are ATP or ADP and GTP or GDP. Although more complex schemes have been described involving protein isomerization steps, this scheme is sufficient to illustrate the differences in the mechanisms of the myosin ATPase and Ras GTPase and how they are affected by other proteins or macromolecular structures which accelerate the hydrolysis and whose interaction is a crucial part of their biological function.

In the case of myosin (M), the cleavage step is fast and rapidly reversible and the release of P_i is the rate-limiting step (reviewed by Geeves and Holmes 1999). As a consequence of these rates the steady-state intermediate is a mixture of M.ATP and M.ADP.P_i in rapid equilibrium. Actin has only a small effect on the cleavage step but activates the release of P_i and ADP, thus activating the overall ATP hydrolysis rate by 200-fold. A similar situation exists with kinesin, where microtubules stimulate the rate of ADP release; although this is the main cause of activation, the cleavage step is also accelerated in this case (Gilbert et al. 1995; Ma and Taylor 1995a, 1995b).

With the Ras superfamily GTPases, there are two rate-limiting steps: the cleavage step and the release of GDP (Neal et al. 1989). In contrast to myosin, no evidence exists for reversibility of the cleavage step. The rate of cleavage can be accelerated by factors of up to 10^5 by GTPase activating proteins (GAPs). X-ray diffraction and site-directed mutagenesis studies have revealed the mechanism by which GAPs for Ras and Rho activate the GTPase. GAP binding orientates the nucleophile with respect to the γ-phosphate to stabilize the transition state and also introduces a catalytic arginine of GAP into the active site of Ras or Rho (Ahmadian et al. 1997; Rittinger et al. 1997; Hoffman et al. 1998; Nassar et al. 1998; Scheffzek et al. 1998; Graham et al. 1999).

GDP release is promoted by guanine nucleotide exchange factors (GEFs), which cause exchange of GTP for GDP. GEFs are generally considered to operate by a substituted enzyme mechanism where binding the exchange factor to Ras.GDP causes weakening of the binding of GDP. This facilitates dissociation of the nucleotide to form the Ras.exchange factor nucleotide-free complex (Klebe et al. 1995; Lenzen et al. 1998; Hutchinson and Eccleston 2000):

$$\mathrm{R.GDP + GEF \rightleftarrows R.GDP.GEF \rightleftarrows R.GEF + GDP} \quad (2)$$

Ras.GTP is then formed by a reversal of this process which is favoured by the higher concentration of GTP than GDP in vivo. By analogy to these GTPases, actin can be considered to be a GEF for myosin and microtubules can be considered to be a GAP and GEF for kinesin.

The intrinsic rate of hydrolysis of GTP by the Ras superfamily of proteins is generally very slow, typically having half-times of ~40 min, whereas the motor proteins hydrolyse ATP much faster. This difference has been attributed to the different biological contexts involving the two classes of proteins (Vale 1996). The motor proteins are usually in ordered structures, or at least operate in a processive manner and diffusion of the proteins during the ATPase reaction is not important. In contrast, the long lifetimes of the Ras.GTP complexes allows them to change location within the cell. For example, the Ran protein which is involved in nuclear transport has mainly GTP bound in the nucleus but mainly GDP bound in the cytoplasm (reviewed by Gorlich 1998). There is a nucleotide exchange factor in the nucleus which generates Ran.GTP and a Ran-GAP in the cytoplasm which generates Ran.GDP. This distribution of two different nucleotide states of Ran allows directionality to be imparted to the process.

Structure and oligomerization of dynamin

Members of the dynamin family of proteins are much larger than the Ras superfamily of proteins. Dynamin monomer itself has a molecular weight of 98 kDa, although other dynamin proteins have molecular weights between 70 kDa and 100 kDa (Hinshaw 2000). The

primary sequence of dynamin can be organized into five domains:

1. An N-terminal GTPase domain. This domain contains three guanine nucleotide binding motifs. The N-terminal domain of the dynamin-related human guanylate binding protein 1 (hGBP1) has a similar structure to Ras but with added insertions and a different conformation of the bound nucleotide, resulting in different protein-nucleotide interactions (Prakash et al. 2000).
2. The middle domain, which has a coiled-coil region thought to be involved in dynamin-dynamin assembly.
3. A PH domain which results in binding to phosphoinositides, so causing membrane localization of the dynamin.
4. A GTPase effector domain (GED). This domain also has a coiled-coil region and is involved in dynamin oligomerization. It has also been suggested that this is an internal GAP for dynamin since the isolated domain causes activation of dynamin GTPase (see later).
5. A proline-rich domain. This domain contains many SH3 binding sites which are responsible for binding to Grb2, amphiphysin, PLCγ and endophilin. These proteins are all implicated in the biological roles of dynamin such as clathrin-mediated endocytosis.

The oligomeric state of dynamin was first investigated by Muhlberg et al. (1997). They showed that dynamin eluted from a gel-filtration column with a molecular weight of ~500 kDa. Sedimentation equilibrium data showed that the dynamin was a tetrameric structure. We repeated these studies using dynamin I. Our data could not be fitted to a tetrameric model but were best fitted to a monomer-tetramer equilibrium model with an association constant of 1.7×10^{17} M^{-3} (Binns et al. 1999). This equilibrium results in dynamin existing as 50% monomer and 50% tetramer at 10^{-5} M. We repeated these measurements with dynamin II (Binns et al. 2000). Again the data could not be fitted to a single tetrameric species, but we could not distinguish between a monomer-tetramer model and a dimer-tetramer model. Based on the dynamin I results, we suggested that dynamin II also exists as a monomer-tetramer equilibrium with an equilibrium association constant of 1.9×10^{18} M^{-3}. This equilibrium results in dynamin II existing as 50% monomer and 50% tetramer at 10^{-6} M, based on monomer concentration. It should be noted that the dynamin I data are more definitive since measurements were made at concentrations of dynamin where a significant amount of monomer was present, whereas given the tighter association of dynamin II, only a small fraction of monomer was present and so the tetramer contributed most to the signal. These measurements were made at 4 °C because of the instability of the protein at 20 °C over the time course of a sedimentation equilibrium experiment. Low temperatures may be the cause of dissociation of tetramer to monomer, as may be pressure effects of ultracentrifugation although the speeds used were relatively low. However, we have made preliminary studies in which dynamin II was labelled with 1-pyrenemethyl iodoacetate (1-PMIA), a long-lived thiol-reactive fluorophore. This labelling did not affect the GTPase activity of the dynamin. The fluorescence anisotropy of the pyrene fluorescence was measured over the range of 0.05–15 μM dynamin at 20 °C and was seen to increase over this range with a half-point at ~3 μM. This result is consistent with the equilibrium sedimentation data at 4 °C showing a monomer-tetramer equilibrium. 1-PMIA was chosen since its relatively long lifetime (average lifetime >100 ns) (Hamman et al. 1996) was appropriate for the size of the molecular species involved.

All of the above measurements showing a monomer-tetramer equilibrium for dynamin were made at high ionic strength (300 mM). However, it is known that dynamin assembles into larger structures in low ionic strength. This aggregation is discussed in more detail later.

Kinetic mechanism of the dynamin II GTPase

The kinetic mechanism of dynamin GTPase was investigated using the fluorescent *N*-methylanthraniloyl (mant) derivatives of 2′-deoxyguanine nucleotides (Binns et al. 2000), using the same ionic strength conditions used for the analytical ultracentrifuge experiments. The use of the 2′-deoxy derivatives overcame any problems of a mixture of the 2′-*O*- and 3′-*O*-substituted mant derivatives (Jameson and Eccleston 1997). The 2′-deoxy derivatives have given smaller signals on binding to some proteins compared to the mixed isomers such as when mantGDP binds to Rho (Hutchinson et al. 2000). However, in the present case they gave larger signals based on preliminary experiments with mantGTP and dynamin I (Binns et al. 1999). This difference could be explained by the recent structure of hGBP1, which shows that a mant group in the 2′-*O*-position would be sterically blocked when bound to this protein (Prakash et al. 2000).

The binding of nucleotides to dynamin was first investigated (Binns et al. 2000). Dynamin II was mixed with excess mant*d*GTP in a stopped-flow fluorimeter. Excitation was at 280 nm and energy transfer between tryptophan and mant was observed (Woodward et al. 1991). This approach gave better signal-to-noise ratios than with direct excitation of the mant group (at 366 nm) and allowed higher concentrations of mant*d*GTP to be used. Over the range 5–70 μM mant*d*GTP there was an increase in fluorescence which could be well fitted to a single exponential. The rate constant for this process at low concentrations of mant*d*GTP was linearly dependent on [mant*d*GTP] giving a second-order rate constant of 3.0×10^{6} M^{-1} s^{-1}. However, at higher concentrations of mant*d*GTP there is evidence of a hyperbolic dependence of rate constant on [mant*d*GTP]. This result was interpreted on the basis

of a two-step binding mechanism with an initial rapid formation of a dynamin.mant*d*GTP complex with a K_d of 91 μM followed by a first-order isomerization rate constant of 280 s^{-1}. However, since the highest concentration of mant*d*GTP that could be used was 70 μM, these values should be viewed with caution. Similar experiments with mant*d*GDP gave a linear relationship of rate constant with [mant*d*GDP] with a slope of 3.3×10^6 M^{-1} s^{-1} and intercept of 64 s^{-1}, which correspond to the second-order association rate constant and first-order dissociation rate constant, respectively. This interpretation of the data was confirmed by a displacement experiment in which mant*d*GDP was displaced from dynamin II by excess GTP and an exponential decrease in fluorescence was observed with a rate constant of 88 s^{-1}. Therefore, dissociation of mant*d*GDP is very fast compared to the cleavage step (see later) and is not a rate-limiting step of the hydrolysis mechanism, obviating the need for a nucleotide exchange factor.

The above results are similar to the situation with the interaction of myosin with adenine nucleotides and Ras proteins with guanine nucleotides. However, the displacement of mant*d*GTP from dynamin by excess GTP gave unexpected results. Instead of a single exponential process, the data were better fitted to a double exponential with rate constants of 3.3 s^{-1} (amplitude 63%) and 0.64 s^{-1} (amplitude 37%). It could not be correlated with the monomer-tetramer equilibrium since the ratio of the two processes remained constant over a change in the concentration of dynamin.mant*d*GTP from 5 μM to 0.5 μM when the calculated concentration of monomer changes from 49% to 11%. Neither could it be correlated with one phase being the dissociation from a hydrolysis product of dynamin.mant*d*GTP, since the amplitudes of the two processes both decayed with time after mixing dynamin with mantdGTP with the same rate constants. If the two processes represent dissociation of mant*d*GTP from two different species of dynamin.mant*d*GTP complex, the calculated K_d values for the two species based on the association and dissociation rate constants are 1.0 μM and 0.2 μM. However, the effect of changing the concentration of dynamin.mant*d*GTP described above is not consistent with this and the effect remains unknown.

In order to investigate further the elementary processes of the dynamin GTPase, single turnover experiments were made in which excess dynamin II was mixed with mant*d*GTP. The reaction was followed in three ways. First, in a stopped-flow apparatus, both the fluorescence intensity and anisotropy of the mant fluorophore were measured. For intensity measurements, excitation was at 280 nm as described above. For anisotropy measurements, excitation was at 366 nm so that depolarization did not occur during energy transfer from tryptophan to the mant fluorophore. From the intensity measurements, there was an initial rapid three-fold increase in intensity accompanied by an increase in anisotropy from 0.05 to 0.23. These increases were followed by an exponential decrease in intensity to a value of 1.3-fold of the fluorescence before mixing with a rate constant of 0.0056 s^{-1} and an anisotropy decrease of 0.13 with a rate constant of 0.0046 s^{-1}. Within experimental error, these rate constants are identical. The same reaction was performed but instead of monitoring fluorescence, the reaction was stopped at increasing time intervals and the percentage of mant*d*GTP and mant*d*GDP determined by HPLC. The results showed that mant*d*GDP was formed in an exponential process with a rate constant of 0.0062 s^{-1}. All of the above data can be explained by a mechanism in which following two-step binding of mant*d*GTP to dynamin, cleavage of mant*d*GTP to mant*d*GDP occurs with a rate constant of 0.006 s^{-1} which is rate limiting, followed by the fast release of P_i and mant*d*GDP.

A single turnover experiment was also done using GTP itself in the presence of a phosphate binding protein covalently labelled with a fluorophore. This binds phosphate, which results in a large increase in fluorescence (Brune et al. 1994). A single exponential process was observed with a rate constant of 0.0061 s^{-1}, showing that mant*d*GTP behaved very similar in its interaction with dynamin as GTP.

Interaction of dynamin II with mantGTPγS

GTPγS has proved to be a valuable analogue for studies of G-proteins, including dynamin. It is sometimes described as a non-hydrolysable analogue of GTP (Sweitzer and Hinshaw 1998; Smirnova et al. 1999; Takei et al. 1999), but in fact it has been shown to be hydrolysed by the G-proteins EF-G (Webb and Eccleston 1981), EF-Tu (Eccleston and Webb 1982) and Ras (Feuerstein et al. 1989). In order to know whether it was also hydrolysed by dynamin, mantGTPγS was incubated with excess dynamin II and the hydrolysis of mantGTPγS to mantGDP was followed by HPLC analysis of the solution with respect to time. It can be seen in Fig. 1 that mantGTPγS was hydrolysed with a rate constant of

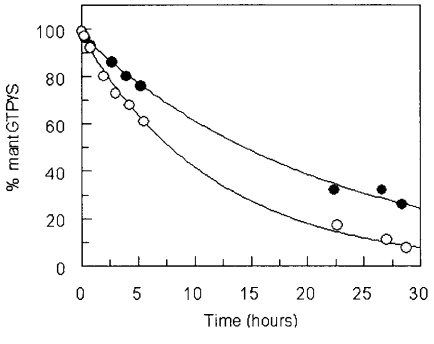

Fig. 1. Hydrolysis of mantGTPγS by dynamin II. A solution of 1 μM mantGTPγS and 10 μM dynamin II in 20 mM HEPES (pH 7.4), 300 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA and 0.5 mM dithiothreitol was incubated at 20 °C (*filled circles*) or 30 °C (*open circles*). Samples were analysed for mantGTPγS and mantGDP by HPLC. The *lines* are the best fits of the data to single exponentials with rate constants of 1.33×10^{-5} s^{-1} at 20 °C and 2.33×10^{-5} s^{-1} at 30 °C

1.33×10^{-5} s^{-1} at 20 °C and 2.3×10^{-5} s^{-1} at 30 °C. In the absence of dynamin II, less than 10% hydrolysis of mantGTPγS occurred over 30 hours, and less than 5% at 20 °C, showing that the hydrolysis was mediated by dynamin II. The rate constant of hydrolysis of mant*d*GTP by dynamin II is 0.0062 s^{-1} at 20 °C. Therefore, mantGTPγS is hydrolysed at 0.2% of the rate of mant*d*GTP hydrolysis.

The association and dissociation kinetics of mantGTPγS with dynamin II was also investigated. Figure 2a shows that on mixing 1 μM dynamin II with 10 μM mantGTPγS an exponential increase in fluorescence occurred with a rate constant of 10.8 s^{-1}. The effect of mantGTPγS concentration on this observed rate constant is shown in Fig. 2b. It can be seen that, as with mant*d*GTP, there is a hyperbolic dependence of rate constant on mantGTPγS concentration. Fitting the data to a model of an initial rapidly reversible initial binding step followed by an isomerization step as described by Binns et al. (2000) gives a dissociation equilibrium constant for the first step of 34 μM and a rate constant of the isomerization step of 55 s^{-1}.

The dissociation of mantGTPγS from dynamin II was measured by rapidly mixing 1 μM dynamin II and 2 μM mantGTPγS with 100 μM GTP (Fig. 2c). A decrease in fluorescence occurred. The data could not be fitted to a single exponential but was well fitted to a double exponential with rate constants of 2.77 s^{-1} (amplitude 63%) and 0.56 s^{-1} (37%). Again, this is similar behaviour to that seen with mant*d*GTP (Binns et al. 2000). However, with mantGTPγS it cannot be ruled out that it results from dissociation of the 2′-*O*- and 3′-*O*-mantGTPγS isomers, but is unlikely since the same biphasic process occurs with mant*d*GTP which is a single isomer. The biphasic behaviour with mantGTPγS does lend support to the proposal that the biphasic displacement of mant*d*GTP is not due to the formation of a hydrolysis product as discussed above.

Association of dynamin GTPase

The GTPase activity of dynamin falls between the very low GTPase rate of most of the small G-protein GTPases and the motor protein ATPases, but it is activated under certain conditions. As stated above, at low ionic strength, dynamin forms higher order aggregates which are accompanied by an increase in GTPase activity. Warnock et al. (1997) made steady-state measurements of the GTPase at low ionic strength with varying dynamin I concentrations and showed that there was a cooperative increase in activity from 0.03 s^{-1} to 0.13 s^{-1} on going from 0.5 to 2.0 μM dynamin I, which correlates with dynamin aggregation. With dynamin II, there is a

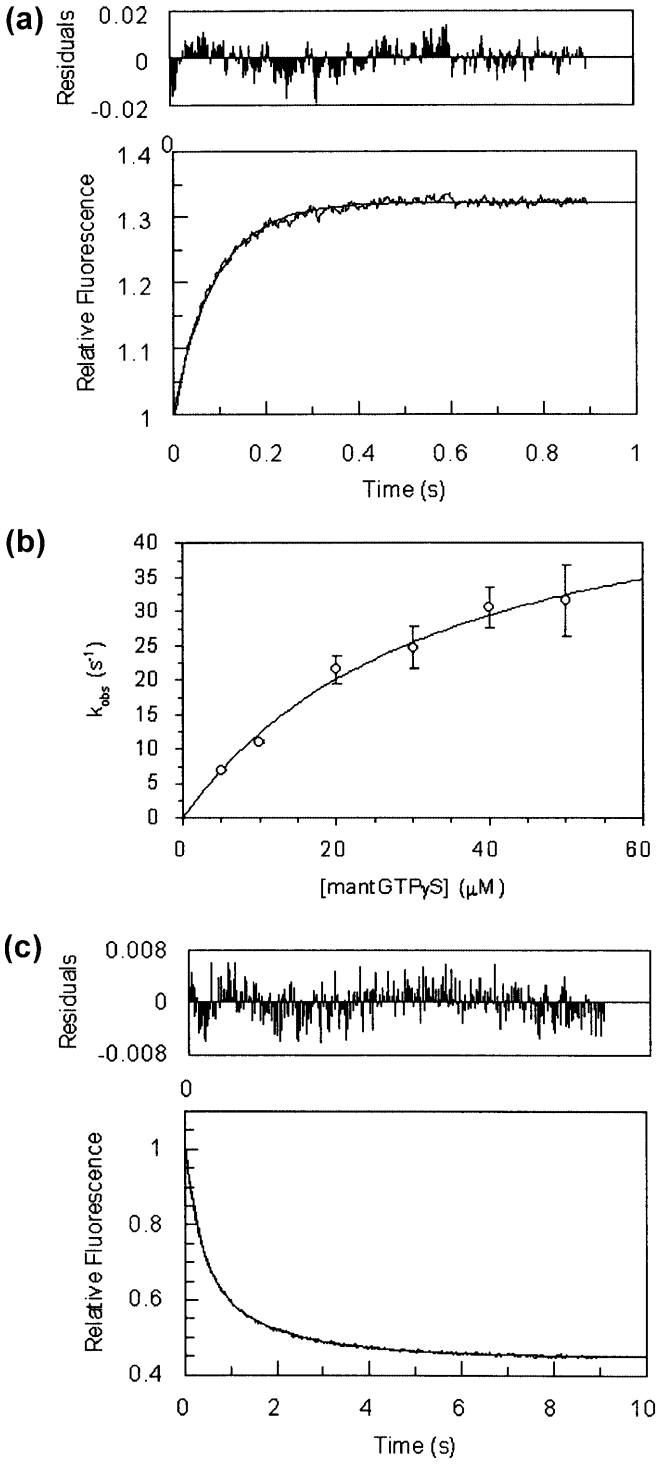

Fig. 2a–c. Interaction of mantGTPγS with dynamin II. (**a**) Stopped-flow fluorescence record of the binding of mantGTPγS to dynamin II. 1 μM dynamin II was rapidly mixed with 10 μM mantGTPγS at 20 °C. The *solid line* is the best fit of the data to a single exponential with a rate constant of 10.8 s^{-1}. (**b**) Dependence of the rate constant on the concentration of mantGTPγS. Data were obtained from experiments as in (**a**). The *solid line* is the best fit to a two-step binding model described by Binns et al. (2000). (**c**) Stopped-flow fluorescence record of the displacement of mantGTPγS from dynamin II. 1 μM dynamin II and 2 μM mantGTPγS was rapidly mixed with 100 μM GTP at 20 °C. The *solid line* is the best fit to a single exponential with rate constants of 2.77 s^{-1} (amplitude 63%) and 0.56 s^{-1} (amplitude 37%)

more marked increase in activation to 1.3 s^{-1} at 0.5 μM dynamin II. This has been suggested to be the result of the greater propensity for dynamin II to self-assemble (Warnock et al. 1997).

However, dynamin aggregation on its own is not sufficient for maximal activation of its GTPase. Dynamin assembled on microtubules or acidic phopholipids show a greater than 50-fold activation (Tuma et al. 1993; Barylko et al. 1998; Stowell et al. 1999). With microtubule-activated GTPase the activity depends on dynamin concentration but also on microtubule concentration. As the microtubule concentration is increased, a higher concentration of dynamin is needed for maximal activation (Binns et al. 1999). We have suggested that the explanation for this observation is that, at higher microtubule concentrations, the dynamin can distribute amongst more potential binding sites with a resulting decrease in the number of contiguous dynamin molecules. This activation is greatly reduced at higher ionic strengths, showing the ionic nature of microtubule-dynamin interaction.

The dynamin-microtubule system is a good model system to study the effect of assembly of dynamin on GTPase activation, although it may not be biologically relevant since there is no direct evidence that microtubules are involved in clathrin-mediated endocytosis. However, many other factors involved in endocytosis also promote assembly and activation, such as lipids and SH3 domain containing proteins.

The GED domain of dynamin

The region between amino acids 610 and 750 has been termed the GTPase effector domain (GED). It has the ability to self-assemble. Also it has been shown that addition of the GED domain to the GTPase domain of dynamin activates its GTPase by six-fold, whereas addition of the GED to full length dynamin results in a 50-fold activation, comparable to the activation by microtubules (Sever et al. 1999). These observations led to the proposal that the GED domain acted as a GAP, analogous to the GAPs for the Ras superfamily proteins. Sever et al. (1999) then prepared mutants of dynamin I in which potential catalytic residues in the GED were changed to alanine residues and gave evidence that the GED is an intramolecular GAP and that the essential catalytic residue in the GED was arginine-725. However, Marks et al. (2001) have also prepared the R725A mutant of dynamin I and showed that it was highly activated by microtubules. They concluded that it is not a GAP in the Ras superfamily sense in that it does not contribute a catalytic residue to the active site of the GTPase domain of dynamin. We have made the equivalent mutation in dynamin II (R719A) and shown that, at low ionic strength in the presence of microtubules, there is a cooperative increase in activity from 0.3 s^{-1} at 0.1 μM dynamin to 2.7 s^{-1} at 2 μM dynamin (Fig. 3). This result is therefore in agreement with the work of

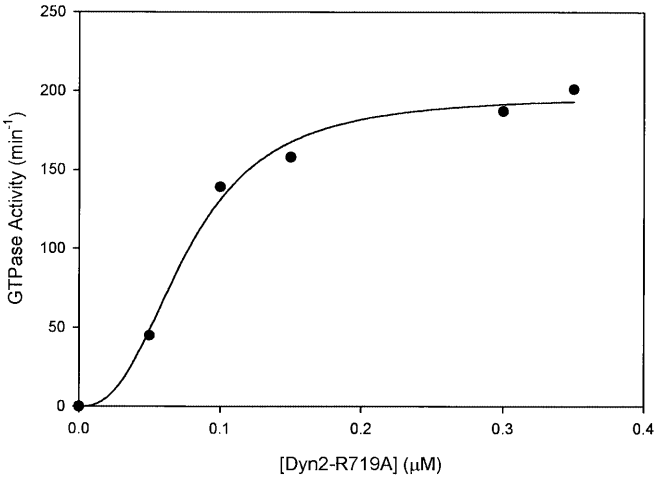

Fig. 3. Activation of dynamin II R719A. The steady-state GTPase activity was measured over the range of concentrations of dynamin II R719A shown. The solution also contained 20 mM HEPES (pH 7.4), 25 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.5 mM dithiothreitol, 1 mM GTP and microtubules stabilized with taxol (10 μM tubulin monomer)

Marks et al. (2001), showing that the GED does not contribute to the catalytic site of dynamin.

Support for the absence of a catalytic residue in GED comes from the recent structure of the GMP.PNP-bound form of the related protein hGBP1 (Prakash et al. 2000). Although this protein has a GTPase domain similar to that of the Ras superfamily of proteins, there are several differences. The angle of the glyosidic bond of the nucleotide is different from that in Ras proteins, with the result that there are differences in the protein-nucleotide interactions. Also, unlike with Ras or Rho, the bound nucleotide in hGBP1 is shielded from bulk water by a "phosphate cap" which would prevent the introduction of additional catalytic domains unless there was a major structural rearrangement of the protein on oligomerization. However, from this structure it is not possible to determine whether residues of the helical domain of hGBP participate in activation of its GTPase domain. Neither is it possible to answer this question from the recent three-dimensional reconstruction of cryoelectron micrographs of dynamin lipid tubes (Zhang and Hinshaw 2001), which only give a structure to a resolution of about 20 Å. However, it does show that the GED domain is spatially close the GTPase domain.

Conclusions

The sedimentation equilibrium data show that, at high ionic strength, both dynamin I and II exist as a monomer-tetramer equilibrium, although a dimer-tetramer equilibrium cannot be ruled out for dynamin II.

Under these conditions, the rate-limiting step of the GTPase is the cleavage step. Therefore, the steady-state intermediate is dynamin.GTP. The rate constant of cleavage is 0.006 s^{-1}, giving a half-life of this

intermediate of ~100 s. If this is accelerated 100-fold on activation, then its half-life would be ~1 s. This would be too short a time for it to act as a protein which recruits other macromolecules involved in endocytosis. Therefore, it is more likely that dynamin is an energy-transducing protein than a signalling protein.

GDP release is very much faster than the cleavage step; hence there is no requirement for an exchange factor.

MantGTPγS shows similar binding behaviour to dynamin II as mant*d*GTP, but is hydrolysed by dynamin II at 0.2% of the rate of mant*d*GTP.

Although the mechanism of activation of dynamin on assembly is unknown, most evidence points to it not being due to an arginine finger inserted into the active site from GED. However, in the Rho/rhoGAP system, activation is not solely due to the introduction of an arginine finger. A mutant GAP without this arginine (Graham et al. 1999) can still accelerate hydrolysis by a factor of 160. This has been attributed to the fact that rhoGAP, in addition to providing the catalytic arginine, also stabilizes the transition state of the cleavage step. This level of activation of Rho by mutant rhoGAP is similar to that seen for activated dynamin and it is probable that this activation is also the result of transition state stabilization.

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