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Combined analytical ultracentrifugation, light scattering and fluorescence spectroscopy studies on the functional associations of the bacterial division FtsZ protein

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ABSTRACT

The combined application of different biophysical techniques – analytical ultracentrifugation, light scattering and fluorescence-based assays – to study the ligand-linked self-association and assembly properties of the cell division protein FtsZ from *Escherichia coli* is described. These reactions are thought to be important for the formation of the dynamic division ring that drives bacterial cytokinesis. In addition, the use of this orthogonal experimental approach to measure the interactions between FtsZ oligomers (GDP forms) and polymers (GTP forms) with two variants (a soluble form and a full-length protein incorporated in phospholipid bilayer nanodiscs) of the ZipA protein, which provides membrane tethering to FtsZ, is described as well. The power of a global analysis of the results obtained from complementary biophysical methods to discriminate among alternative self- and hetero-associating schemes and to propose a more robust description of the association reactions involved is emphasized. This orthogonal approach will contribute to complete our quantitative understanding of the initial events of bacterial division.

1. Introduction

Bacterial cell division is mediated by a multiprotein machinery whose components gather together at midcell towards the end of the cell cycle to form a dynamic ring that drives cytokinesis. The ring is formed by at least ten division specific proteins, most of them integral membrane proteins. In the case of *Escherichia coli*, the division process is thought to be initiated by the interaction of three proteins (the GTPase FtsZ, a tubulin ancestor; the amphitropic protein FtsA; and the membrane protein ZipA) at the cytoplasmic membrane to form the first molecular complex of the divisome, the proto-ring, that is followed by the incorporation of the rest of the essential division proteins (reviewed in [1,2]).

The functions of these assemblies depend upon the ability of the constituent macromolecules to dissociate and re-associate in a reversible manner, either to regulate the biochemical activity of the functional entity active in division or to facilitate function-linked structural reorganizations of the division machinery [3]. To fully describe the function of such complex assembly and to complete our understanding on how division works, it is necessary to know how changes in the concentrations of the division proteins and regulatory substances, as well as changes in environmental variables, such as pH and ionic strength or excluded-volume effects due to the natural crowding [4], affect the relative abundance of the various states of association of the interacting macromolecules involved in bacterial division. There exist a variety of powerful and versatile techniques for the quantitative characterization of reversible macromolecular associations, including protein–ligand, protein–protein, protein–nucleic acid and protein–membrane interactions [5–9].

In this work, we have concentrated in the application of a set of biophysical techniques (analytical ultracentrifugation, light scattering and fluorescence-based methods) to study the activity, interactions and assembly of the protein FtsZ (from *E. coli*), a central component of the division machinery. FtsZ (40 kDa) is widely conserved among prokaryotic organisms and it is taken to be the ancestor of tubulin, sharing the nucleotide-binding and GTPase activities and the capacity to form polymers [10,11]. The self-association of

Abbreviations: C_c, critical concentration of assembly; CG-SLS, composition gradient static light scattering; DLS, dynamic light scattering; FCS, fluorescence correlation spectroscopy; GDP-FtsZ, GDP bound FtsZ; GMPCPP, guanosine-5′-[(α,β)-methylene] triphosphate sodium salt; GTP-FtsZ and GMPCPP-FtsZ polymers, polymers of FtsZ where assembly was triggered by GTP or GMPCPP respectively; MALLS, multiangle laser light scattering; Nd-ZipA, wild type ZipA inserted into nanodiscs; Nd*-ZipA, labeled nanodiscs containing wild type ZipA; RS, GTP regeneration system; SE, sedimentation equilibrium; SLS, static light scattering; SV, sedimentation velocity; sZipA, soluble mutant of ZipA.

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FtsZ in the presence of GDP and its polymerization in the presence of GTP have been extensively studied ([10,11]; and references therein; see also section 4). While the mechanism of GDP-FtsZ oligomer formation is relatively well understood, that is not the case for the GTP-linked FtsZ polymerization. In the presence of GTP, FtsZ assembles in an apparent cooperative manner to form plastic polymers able to associate into multi-stranded flexible structures. FtsZ polymers have been described as very dynamic both *in vitro* and *in vivo*, with a rapid exchange of FtsZ between the Z-ring and the cytoplasm [12,13]. However, the implications of these processes on the kinetics governing fiber or Z-ring assembly are not at all clear. The dynamic character of FtsZ polymers, linked to the relatively high GTPase activity of the protein, constitutes an additional challenge to study FtsZ assembly in the presence of GTP as it requires special procedures to maintain polymer stability throughout the duration of a typical biochemical or biophysical experiment [14]. Consequently, the number of quantitative techniques applied to study FtsZ assembly is lower than that used to study other protein assembly systems [15,16]. Current research on FtsZ is aimed at determining the role of the Z-ring in division, describing the polymerization and force-generating mechanisms and evaluating the roles of nucleotide exchange and hydrolysis [10,11].

E. coli FtsZ is anchored to the cytoplasmic membrane through the interaction with the other proto-ring proteins, ZipA or FtsA [1]. FtsA (48 kDa) is thought to be a member of the actin family and its association to the membrane seems to be mediated by a short amphipathic helix [17]. ZipA (39 kDa) contains a short N-terminal region that is integrated in the membrane, and connected to the C-terminal FtsZ-interacting domain by a flexible, unstructured, linker region [18]. As the assembly of the proto-ring is a membrane-linked process, a considerable effort is being made to study the interactions and assembly properties of FtsZ under topologically restricted reconstructions of the proto-ring in biomimetic membrane systems, such as nanodiscs, coated microbeads, bilayers and vesicles [19–24]. They provide the opportunity to investigate complex membrane-associated reactions under defined experimental conditions, as the biochemical parameters can be controlled precisely [25,26].

As this review is focused in the biophysical analysis of FtsZ associations in solution, results have been structured as a first section regarding Mg^{2+}-linked self-association of GDP- and GTP-FtsZ (or its analog GMPCPP), which allowed estimating the polymer molar masses in close to physiological conditions, and a second section where we have limited our description of membrane-based studies to the analysis of the dynamic interactions between FtsZ oligomers/polymers and ZipA incorporated in nanodiscs. These structures, formed by a membrane scaffold protein encircling a phospholipid bilayer, are promising novel tools to study interactions of membrane proteins while the system under study remains soluble [27]. When a target membrane protein (e. g., ZipA) is included in the mixture, each nanodisc self-assembles and incorporates the target protein preserving its natural properties. A summary of the potential implications of the main findings of these studies - related with the features that FtsZ polymers do not grow indefinitely with protein concentration, and that ZipA in nanodiscs binds FtsZ oligomers and polymers with equal moderate affinity - is included.

2. Quantitative approaches used to study FtsZ-related associating reactions: summary of general principles

The methods used to characterize FtsZ homo- and hetero-associations are described below emphasizing the information derived from the individual techniques, and, more importantly, how their combination allows discriminating among alternative association schemes. Fig. 1 shows representative experimental data and main information derived from the biophysical methods employed. Fig. 2 schematically confronts the information obtained by each method (Fig. 2A) and relates the trend of hydrodynamic parameters with changes in particle properties for a given mass (Fig. 2B).

2.1. Analytical ultracentrifugation

Analytical ultracentrifugation (AUC) is a powerful and versatile technique, based on the application of a centrifugal force and the direct observation of the resulting spatial macromolecular redistribution, to characterize the size distribution and overall shape of individual macromolecular components in solution, and is specially well adapted to detect and measure macromolecular associations under physiological conditions [6,8,28]. Two complementary AUC approaches are available: sedimentation velocity (SV) and sedimentation equilibrium (SE) (Fig. 1).

2.1.1. Sedimentation velocity

SV is a hydrodynamic method in which the rate of transport is measured and the macromolecules are fractionated upon the application of a high centrifugal force according to their differences in buoyant mass and shape. SV is the method of choice to study strong associations that are very slowly reversible during the time scale of the experiment, and it is possible to separate the different states of association to characterize them individually. Estimates of the sedimentation coefficient and molar mass of the sedimenting species can be obtained from the analysis of the time dependent gradients [29,30].

The rate of transport of solute species i, centrifuged at angular velocity ω in a sector-shaped centrifuge cell, is described by the following relation:

$$J_i = s_i\omega^2 r S_i - D_i\frac{dS_i}{dr} \tag{1}$$

where J_i is the rate of transport of species i, ω is the rotor angular velocity, r the radial position, and S_i and D_i are the sedimentation and diffusion coefficients of species i, respectively. S_i is a measurable property or signal (S) of the solution that varies linearly with the weight/volume concentration of each species of sedimentable solute at radial position r:

$$S(r) = \sum_i S_i(r) = \sum\nolimits_i \alpha_i w_i(r) \tag{2}$$

where α_i denotes the signal proportionality constant of species i. Suitable signals are UV–VIS absorbance, refractive index at a given wavelength, or the radioactivity of a radiolabeled solute in AUC experiments with preparative ultracentrifuges coupled to microfractionation [31].

The sedimentation coefficient of species i is given by:

$$S_i = \frac{M_i^*}{N_A f_{sed,i}} \tag{3}$$

where M_i^* denotes the actual buoyant molar mass. $M_i^* = M_i d_i$, where M_i is the molar mass and d_i the specific density increment of species i [32]. N_A is the Avogadro's number and $f_{sed,i}$ is the frictional coefficient of species i.

The diffusion coefficient is given by:

$$D_i = \frac{RT}{N_A f_{diff,i}} \tag{4}$$

where R is the molar gas constant, T the temperature, and $f_{diff,i}$ the diffusion frictional coefficient of species i.

In the ideal limit the two frictional coefficients (which are size and shape-dependent macromolecular properties) are equal and eqs. (3) and (4) can be combined to yield the Svedberg relation: