Minireview

Mass spectrometry of intact ribosomes

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Abstract The ability to maintain intact ribosomes in the mass spectrometer has enabled research into their changes in conformation and interactions. In the mass spectrometer, it is possible to induce dissociation of proteins from the intact ribosome and, in conjunction with atomic structures, to understand the factors governing their release. We have applied this knowledge to interpret the structural basis for release of proteins from ribosomes for which no high resolution structures are available, such as complexes with elongation factor G and ribosomes from yeast. We also describe how improvements in technology and understanding have widened the scope of our research and lead to dramatic improvements in quality and information available from spectra of intact ribosomes.

Keywords: Mass spectrometry; Nanoflow electrospray; Ribosome; Yeast; *Thermus thermophilus*; *E. coli*

1. Introduction

Ribosomes are the universal translators of the genetic code and in bacteria are composed of two subunits, the 30S and 50S, that together constitute the 70S particle. The 54 different proteins and three large RNA molecules have represented formidable challenges for structural biologists but recent high resolution images [1] have paved the way for application of other approaches, including mass spectrometry (MS). As MS is routinely applied to peptides and individual proteins, one might wonder what contribution it could make to our understanding of their structure and function. The sheer size (2.3 MDa and an estimated 4.5 MDa for prokaryotic and eukaryotic ribosomes, respectively) make these particles daunting targets. To meet this challenge, we have developed two different MS approaches: first to monitor dissociation of individual proteins from the intact particle, second to maintain interactions within the intact particle, its subunits and associated complexes and then to subject these species to tandem MS (see below). The two approaches arise from developments that we have made to our MS instrumentation and from our increased understanding of the properties of gas phase macromolecular complexes.

In the first approach, dissociation of the complex occurs after the electrospray process. On projection into the vacuum of the MS, ions gain in translational energy which is converted to internal energy by collisions with residual gas molecules. This induces dissociation of individual proteins, which can be identified by comparison of their unique masses with theoretical masses calculated from sequence information from databases. The second approach, in which interactions between the proteins and rRNA are maintained, requires introduction of inert gas at various stages during the flight path of the ions to dampen excess translational energy by inducing multiple low energy collisions.

2. Mass spectrometry

A mass spectrometer measures the mass-to-charge ratio (m/z) of ions in the gas phase. During the electrospray process, formation of ions takes place in solution and for most applications acidified organic solvents yield the highest sensitivity and quality in mass spectra. Electrospray ionization produces multiply charged bio-macromolecules, giving rise to a series of peaks that each result from a different charge state. The ions so formed travel through differential pumping and focusing stages before analysis in either a quadrupole or time-of-flight (TOF) mass analyzer. A quadrupole mass analyzer consists of four parallel rods and transmits ions through simultaneously applied d.c. and radio frequency fields. For TOF analysis, ions are separated by virtue of their different flight times over a known distance after pulsing and acceleration into an orthogonal flight tube. Historically, the quadrupole preceded the TOF analyzer for coupling with electrospray ionization. Recently, quadrupoles have been used in conjunction with TOF analysis in Q-TOF mass spectrometers enabling tandem MS experiments. For such experiments, ions at a discrete m/z range are isolated by setting a specific frequency on the quadrupole, the selected ions are activated by collisions in the gas cell to induce dissociation and then the products analyzed in the TOF.

3. Mass spectrometry of ribosomes – challenges to overcome

Obtaining mass spectra from solutions of intact ribosomes is more challenging than for individual denatured proteins for several reasons. Complexes containing only protein subunits

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are more straightforward than those containing both proteins and RNA, since the latter binds numerous positively charged ions. This phenomenon coupled with many post translational modifications and the possibility that not all proteins are present make ribosomes considerably more heterogeneous than say a virus capsid of similar magnitude to intact ribosome but containing 180 identical protein subunits [2]. To maintain interactions between proteins and RNA, it is necessary to employ an aqueous buffer at neutral pH. In addition, magnesium ions are required to retain association of the subunits but interfere with the electrospray process. Exchange into highly concentrated ammonium acetate buffer immediately prior to recording a spectrum removes all but tightly associated ions. The viscosity of ribosome-containing solutions is also deleterious to electrospray; the increased surface tension makes forming sufficiently small droplets to yield individual ribosomes problematic (see schematic in Fig. 1). To produce a stable electrospray from solutions containing ribosomes, it is necessary to dilute samples in the appropriate buffer to the point where individual scans show very few ions. The spectrum is obtained by summing multiple acquisitions, often over time periods approaching one hour.

4. Mass spectrometry as a probe of protein – RNA interactions

Our first report of *Escherichia coli* ribosomes in 1998 [3] described spectra recorded for proteins that had dissociated from the 70S particle and the 50S (large) and 30S (small) subunits [4]. Peaks were assigned to individual ribosomal proteins and to a non-covalent complex of five proteins that corresponded to the protuberance known as the stalk. At this time atomic structures of ribosomes were not available, so it was not possible to examine why only a small subset of the 54 proteins dissociated from the ribosome. We noted, however, that the proteins that most readily dissociated tended to be amongst the most acidic of the ribosomal proteins.

The atomic resolution structures of ribosomal subunits allowed a thorough investigation into the factors that govern the loss of proteins from *E. coli* ribosomes [4]. Assuming the universally accepted extensive structural conservation between *E. coli* ribosomes and high resolution structures of 30S [5,6] and 50S [7] subunits from *Thermus thermophilus* and *Haloarcula marismortui* ribosomes, respectively, we examined the extent of protein RNA interactions for all large subunit proteins. A plot of surface area of interaction of each protein against the extent of dissociation calculated from spectra recorded under a variety of different conditions allowed us to establish a strong negative correlation: the greater the surface area of interaction between the protein and RNA the less likely the protein is to be released in the mass spectrometer (see Fig. 2). We used this established correlation to examine dissociation of proteins from ribosome – EF-G complexes, trapped in two different conformational states.

It is known from cryo-EM studies that thiostrepton and fusidic acid trap complexes in either pre- or post-translocational states, respectively [8]. Close examination of the proteins that dissociate, recorded in the spectra of the two complexes, reveals that they are markedly different. In the presence of fusidic acid, the spectrum is similar to that observed for ribosomes in the absence of EF-G, indicating that the extent of protein-RNA contacts is not affected by binding of EF-G. By contrast, the complex inhibited by thiostrepton is noticeable for the absence of peaks corresponding to L7/L12, which normally dominate the spectra, and the presence of additional peaks corresponding to proteins L1, L5, L6, and L18. L1 is at the periphery of the ribosome. Loss of L6 can be interpreted as being due to a conformational change in 23S rRNA caused by EF-G binding as it is close to the EF-G binding site. The proteins L5 and L18 have the largest surface area of interaction with 5S rRNA. Their dissociation is therefore consistent with a conformational change in 5S rRNA, leading to a reduction in surface area with L5 and L18. The absence of peaks assigned to L7/L12 on the other hand implies that their highly

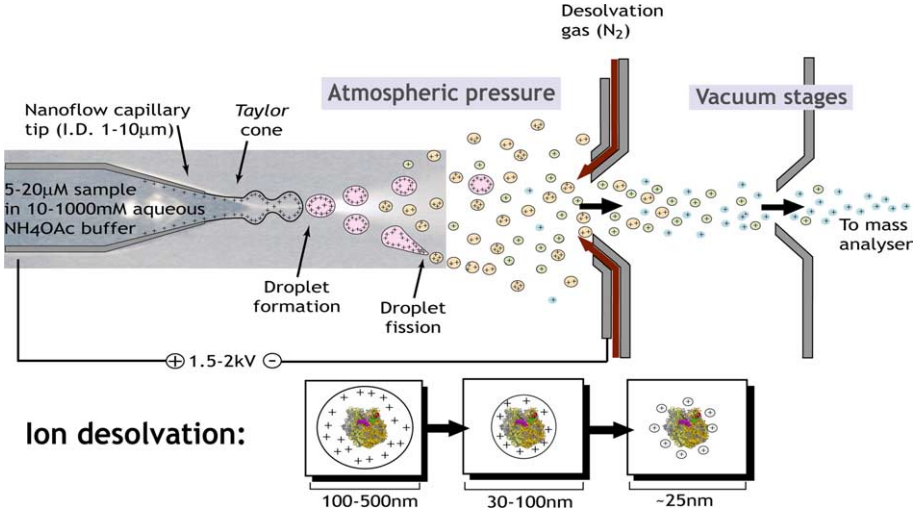

Fig. 1. Nano-electrospray of solutions of ribosomes. Schematic representation of the nanoflow electrospray process showing individual positively charged ions of ribosomes within each droplet. Desolvation of the electrosprayed droplets is aided by a counter current flow of gas until naked ribosome ions are achieved and transmitted through successive vacuum stages of the mass spectrometer. Typical solution conditions for analysis would be 1–2 μl of sample at 1–2 μmol concentration in an ammonium acetate buffer concentration of 1 mM.