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ScienceDirect
Journal of Structural Biology 157 (2007) 371–383
Journal of Structural Biology
www.elsevier.com/locate/yjsbi

Macromolecular mass spectrometry and electron microscopy as complementary tools for investigation of the heterogeneity of bacteriophage portal assemblies

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Received 19 July 2006; received in revised form 8 September 2006; accepted 8 September 2006
Available online 19 September 2006

Abstract

The success of electron-cryo microscopy (cryo-EM) and image reconstruction of cyclic oligomers, such as the viral and bacteriophage portals, depends on the accurate knowledge of their order of symmetry. A number of statistical methods of image analysis address this problem, but often do not provide unambiguous results. Direct measurement of the oligomeric state of multisubunit protein assemblies is difficult when the number of subunits is large and one subunit renders only a small increment to the full size of the oligomer. Moreover, when mixtures of different stochiometries are present techniques such as analytical centrifugation or size-exclusion chromatography are also less helpful. Here, we use electrospray ionization mass spectrometry to directly determine the oligomeric states of the *in vitro* assembled portal oligomers of the phages P22, Phi-29 and SPP1, which range in mass from 430 kDa to about 1 million Da. Our data unambiguously reveal that the oligomeric states of Phi-29 and SPP1 portals were 12 and 13, respectively, in good agreement with crystallographic and electron microscopy data. However, *in vitro* assembled P22 portals were a mixture of 11- and 12-mer species in an approximate ratio of 2:1, respectively. A subsequent reference-free alignment of electron microscopy images of the P22 portal confirmed this mixture of oligomeric states. We conclude that macromolecular mass spectrometry is a valuable tool in structural biology that can aide in the determination of oligomeric states and symmetry of assemblies, providing a good starting point for improved image analysis of cryo-EM data.

Keywords: Phage; Portal; Assembly; Stochiometry; Electrospray mass spectrometry; Non-covalent complexes

1. Introduction

The development of electrospray ionization coupled to mass spectrometry has enabled the analysis of very large intact protein complexes even when they are held together by weak non-covalent interactions. Together with equally spectacular advances in mass spectrometric instrumentation a new field has emerged, sometimes termed native protein mass spectrometry, that focuses on the structural and functional analysis of the dynamics and interactions occurring in protein complexes. In the past years, several important technological innovations have been reported that have enabled exciting applications ranging from the detailed study of equilibria between different quaternary structures as influenced by binding of substrates or cofactors to the

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doi:10.1016/j.jsb.2006.09.003

analysis of intact nano-machineries, such as whole virus particles, proteasomes and ribosomes (Benjamin et al., 1998; Videler et al., 2005; Bothner and Siuzdak, 2004; Loo et al., 2005). For the analysis of intact protein complexes by mass spectrometry the sample of interest is electrosprayed from an aqueous solution of a volatile buffer such as ammonium acetate. Desolvation of the protein assemblies in the ion source interface generates multiply charged ions of the intact complexes for analysis by the mass spectrometer. Mass spectrometric detection of the assemblies has made it possible to obtain accurate information about protein complex stoichiometry, stability and dynamics (Heck and Van den Heuvel, 2004; Loo, 1997; van den Heuvel and Heck, 2004; Ilag et al., 2005), through which, for instance, the folding cycle of the GroEL-gp31 machinery could be monitored while folding the bacteriophage gp23 capsid protein (van Duijn et al., 2005). The detectable mass range in electrospray ionization mass spectrometry exceeds several million Da, allowing the analysis of species as big as ribosomes and viruses (Benjamin et al., 1998; Bothner and Siuzdak, 2004). Therefore, macromolecular mass spectrometry lends itself as an excellent tool to study protein complex assembly and, in particular, virus assembly, where major questions focus on the early multi-protein intermediates of assembly, or the stoichiometries of subcomplexes, such as the portal. We have used macromolecular mass spectrometry to examine the stoichiometry of several bacteriophage portal complexes, which are the molecular motors that drive the DNA packaging.

dsDNA viruses of orders *Caudovirales* and *Herpesviridae* rely on an active packaging mechanism for encapsidation of their genomes (Hohn, 1976). Genomic DNA is packaged into a pre-formed icosahedral protein shell (termed a procapsid) against a large internal force in an ATP-dependent manner to near crystalline density. The molecular motors that perform this task constitute a distinct structural class (Moore and Prevelige, 2002b). The packaging motor of Phi-29 is the most powerful molecular motor known to-date and is capable of creating up to 57 pN of force (Smith et al., 2001).

The DNA packaging motors consist of two principal parts: the terminase complex and the portal complex or head-to-tail connector (Fujisawa and Morita, 1997). Terminase is an ATPase that cuts the concatameric DNA at a particular recognition sequence, targets it to portal and, possibly, constitutes an integral part of the packaging complex translating the energy of ATP hydrolysis into the inward movement of the DNA (Catalano, 2000; Guo et al., 1987; Mitchell and Rao, 2006; Grimes et al., 2002; Morita et al., 1993). The portal complex is a ring-like structure located at one icosahedral vertex of the capsid. It performs multiple tasks during the viral lifecycle: (a) it comprises the conduit through which DNA passes during packaging and infection (Simpson et al., 2000); (b) it acts as a sensor in controlling the amount of DNA packaged (Casjens et al., 1992; Lander et al., 2006; Tavares et al., 1992); (c) together with other structural proteins it prevents packaged DNA from leaking out of the capsid prior to infection (Strauss and King, 1984; Orlova et al., 2003); (d) it possibly plays an active role in DNA packaging and (e) it mediates the connection between the phage head and tail (Ackermann, 1998).

The portal occupies one of the 12 pentameric vertices of icosahedral viral capsid. All portals studied *in situ* or in complex with other viral structural proteins are dodecameric (12-mer) ring-like structures composed of the portal protein (Lurz et al., 2001; Carrascosa et al., 1985; Donate et al., 1988; Kochan et al., 1984; Jiang et al., 2006; Tang et al., 2005; Chang et al., 2006; Agirrezabala et al., 2005a), which share significant overall structural similarity despite the lack of any noticeable sequence similarity (Orlova et al., 2003; Badasso et al., 2000; Guasch et al., 2002; Simpson et al., 2000; Agirrezabala et al., 2005b; Trus et al., 2004). Structurally, portals can be divided into three domains: the stalk, the wings and the crown (Fig. 1), with the greatest morphological similarity between portals found in the stalk region. The narrowest part of the central channel has a diameter of 30 Å, slightly larger than the diameter of DNA.

The tail attaches to the narrow end of the portal (stalk region) after DNA packaging has been terminated. While interaction of the 12-fold portal with the 6-fold tail generates symmetry ambiguity, since the 6-fold can attach in one of two registers, the replacement of a pentameric icosahedral vertex by a dodecameric portal complex represents a more obvious symmetry mismatch, and implies that there can be no regular interactions between the capsid and the portal (Jiang et al., 2006; Lander et al., 2006). This observation led to the proposal that the portal sits in the capsid like a bearing and that it might function like one, i.e. the whole packaging assembly might work as a rotary motor (Hendrix, 1978). Supporting that model, the high resolution structure of phage Phi-29 portal has revealed that the surface opposing the capsid is hydrophobic and may form a greasy interface that would facilitate rotation (Guasch et al., 2002; Simpson et al., 2000).

The lack of regular portal/capsid interactions in the final structure raises the intriguing question of how portal and capsid subunits co-assemble into the well-defined capsid structure. Assembly occurs in a two-step process. In the first step the capsid protein co-polymerizes with a scaffolding protein to form a procapsid. In the second step, genomic DNA is packaged into the procapsid triggering a structural transformation known as maturation. The portal protein is not required for procapsid assembly, nor can the portal be incorporated into already assembled procapsids (Moore and Prevelige, 2002a; Newcomb et al., 2005), suggesting that important interactions between portal and capsid proteins are only formed during procapsid assembly.

Portal protein subunits readily assemble both *in vivo* and *in vitro*. Surprisingly, however, *in vitro* the portal protein subunits do not necessarily assemble into the same oligomeric state as they would *in situ*, i.e., as part of the complete bacteriophage structure. Whereas exclusively dodecameric