Published in final edited form as:

Science. 2012 January 13; 335(6065): 182. doi:10.1126/science.1214120.

Bubblegrams Reveal the Inner Body of Bacteriophage ϕ KZ

Weimin Wu¹, Julie A. Thomas², Naiqian Cheng¹, Lindsay W. Black², and Alasdair C. Steven^{1,*}

¹ Laboratory of Structural Biology, National Institute for Arthritis, Musculoskeletal and Skin Disease, National Institutes of Health, Bethesda MD 20892

² Department of Biochemistry & Molecular Biology, University of Maryland School of Medicine, 108 N. Greene St., Baltimore MD 21201

Dense packing of macromolecules in cellular compartments and higher-order assemblies makes it difficult to pick out even quite large components in electron micrographs, despite nominally high resolution. Immuno-gold labelling and histochemical procedures offer ways to map certain components but are limited in their applicability. Here we present a differential mapping procedure based on the physical principle of protein's greater sensitivity to radiation damage, compared to nucleic acid.

ϕ KZ is a large and complex virus that infects the Gram-negative bacterium *Pseudomonas aeruginosa* and has long-term potential for phage therapy against this pathogen. The virion has a long contractile tail and a large icosahedral capsid containing densely packed DNA (280kb)(1). Observations of disrupted virions (2) have shown that it also contains a cylindrical structure called the "inner body". However, the inner body is invisible in conventional cryo-electron micrographs of intact virions because it cannot be distinguished from the surrounding DNA (Figure 1A).

It has been found, serendipitously, that the inner body is exceptionally sensitive to radiation damage and explodes into bubbles of gaseous radiation products at electron doses that leave most protein complexes, including the surrounding capsid, only slightly blurred (Figure 1B). We have been able to determine its structure by using these "bubblegrams" to locate the inner body in individual nucleocapsids; then, knowing these locations and orientations, we could calculate a three-dimensional reconstruction of the inner body from previously recorded, low-dose, micrographs depicting the same virions in a relatively undamaged state.

The inner body is ~24 nm wide, ~105 nm long, and tilted at ~22° relative to the portal axis (Figure 1C,D). It consists of multiple stacked tiers (Figure 1E) and some regions have evident 6-fold symmetry, as confirmed by their angular power spectra (Suppl. Material). The two ends, which are structurally distinct, are anchored on opposing hexons on either side of the capsid. Mass spectrometry and SDS-PAGE indicate that the inner body has five major proteins, present in 100–200 copies each, as well as a number of minor proteins ((3) and J.A.T., L.W.B. - unpublished results). The shape and position of the inner body suggest that it plays a role of organizer in the DNA packaging process. Consistent with this assignment, its tilt relative to the portal axis matches that of the ϕ KZ DNA spool (4). The inner body is dismantled when the DNA is ejected from the capsid, during infection, (2). It is likely that inner body proteins are also injected into the host cell, based on the precedent of phage T7 (5) which also has a multi-tiered internal protein structure (6).

*Correspondence: stevena@mail.nih.gov.

Bubbling is the end-point to damage induced by electron irradiation of ice-embedded proteins (7). While a detailed understanding of the radiation chemistry is lacking, this phenomenon appears to represent the formation of hydrogen-containing bubbles at high pressure (8). Why do inner body proteins bubble at relatively low electron doses (~ 50 electrons/Å² in a 0.5 sec exposure)? As there is no evidence to suggest that inner body proteins are chemically distinct from other proteins, we posit that they have a propensity to bubble because they are embedded in DNA. This impedes the diffusion of radiation products from their site of origin and promotes their build-up to concentrations at which bubbles nucleate. Support for this interpretation comes from absence of bubbling in DNA-free particles containing the same inner body proteins. We suggest that bubblegram imaging may be productively applied to map proteins in other DNA-rich contexts such as cryo-sections of the cell nucleus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Intramural Research program of NIAMS and by NIH grant AI11676 to L.W.B.

References and Notes

1. Mesyanzhinov VV, et al. J. Mol. Biol. 2002; 317:1. [PubMed: 11916376]
2. Krylov VN, et al. Can. J. Microbiol. 1984; 30:758. [PubMed: 6435847]
3. Black, LW.; Thomas, JA. Viral Molecular Machines. Rossmann, MG.; Rao, V., editors. Springer; New York: 2011. p. 469-488.
4. Fokine A, et al. Structure. 2007; 15:1099. [PubMed: 17850749]
5. Chang CY, et al. Virology. 2010; 398:176. [PubMed: 20036409]
6. Agirrezabala X, et al. EMBO J. 2005; 24:3820. [PubMed: 16211007]
7. Conway JF, et al. J. Struct. Biol. 1993; 111:222. [PubMed: 8003383]
8. Leapman RD, Sun S. Ultramicroscopy. 1995; 59:71. [PubMed: 7571121]

Figure 1.

Cryo-electron micrographs of purified ϕ KZ virions: (A) initial low-dose exposure; (B) subsequent exposure of the same field, with bubbling in radiation-damaged virions. (C) 3D reconstruction of the ϕ KZ capsid viewed along the axis of 5-fold symmetry that passes through the portal. The capsid has T=27 icosahedral symmetry (4). The centers of all hexons on one icosahedral facet are marked with red dots. Hexons in the ring of five, on one of which the inner body (in magenta) is anchored, are colored green. The symmetry-related hexons on the other side of the capsid are in pale green. Each of these hexons lie on a facet edge, connecting two vertices. The central axis of the inner body passes through the center of capsid. (D) Central section of the ϕ KZ head sampled in the plane in which the inner body axis lies. This view illustrates the tilt of the inner body relative to the portal axis and the offsetting of its point of contact with the capsid from the portal vertex. (E) Multi-tiered structure of the inner body shown in surface rendering (left, magenta) and central grayscale section (right). The structures shown in (C) and (E) were 6-fold rotationally averaged (justified by the angular power spectra analysis in Figure 3s); the structure shown in (D) was not.