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Achievable resolution from images of biological specimens acquired from a 4k × 4k CCD camera in a 300-kV electron cryomicroscope

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ABSTRACT

Bacteriorhodopsin and ε 15 bacteriophage were used as biological test specimens to evaluate the potential structural resolution with images captured from a 4k × 4k charge-coupled device (CCD) camera in a 300-kV electron cryomicroscope. The phase residuals computed from the bacteriorhodopsin CCD images taken at 84,000× effective magnification averaged 15.7° out to 5.8-Å resolution relative to Henderson's published values. Using a single-particle reconstruction technique, we obtained an 8.2-Å icosahedral structure of ε 15 bacteriophage with the CCD images collected at an effective magnification of 56,000×. These results demonstrate that it is feasible to retrieve biological structures to a resolution close to 2/3 of the Nyquist frequency from the CCD images recorded in a 300-kV electron cryomicroscope at a moderately high but practically acceptable microscope magnification.

1. Introduction

Charge-coupled device (CCD) cameras have gradually replaced photographic film as the image-recording medium in modern transmission electron microscopy. Their advantages over film are numerous (see reviews: Faruqi and Subramaniam, 2000; Mooney, 2007): first, the CCD camera makes the recorded images available for instant processing and analysis to provide a rapid feedback for specimen and image quality; second, it has a larger dynamic range and higher linearity relative to film well suited for electron diffraction (Brink and Chiu, 1994); third, it can provide slightly higher signal to noise ratio (SNR) at low resolution than photographic film (Booth et al., 2006, 2004; Sander et al., 2005); and lastly, it can facilitate automatic and high-throughput data collection (Dierksen et al., 1995; Koster et al., 1992; Stagg et al., 2006; Zhang et al., 2003). Due to these advantages, CCD cameras have been widely used in structural studies from cells to macromolecules at a broad range of resolutions.

The retrieval of structural details based on a CCD camera depends partially on the quantum detection efficiency (DQE) and the modulation transfer function (MTF) of the CCD, which may vary according to the electron microscope accelerating voltages and the characteristics of the camera itself (Mooney, 2007). In theory, one could use a higher magnification to overcome the limited resolution of the CCD camera (Sherman et al., 1996). However, even given the same total specimen dose, the usefulness of this approach is diminished by the small number of specimen particles recorded per CCD frame and the relatively large number of frames needed to build up sufficient statistics for the three-dimensional (3D) reconstruction. Furthermore, the accuracy in the contrast transfer function (CTF) parameter determination may be affected due to the small number of particles per CCD frame if no continuous carbon support film is used. Nevertheless, there have been several successful macromolecular applications using 4k × 4k CCD cameras in which 3D reconstructions at subnanometer resolutions have been obtained in 120, 200 or 300-kV electron microscopes (e.g., Booth et al., 2004; Chiu et al., 2006; Ludtke et al., 2005; Saban et al., 2006, 2005; Stagg et al., 2006; Yonekura et al., 2006; Yu et al., 2008) (see Table 1). However, the magnifications used in those studies were higher than those for photographic film at equivalent resolutions. The resolutions in those studies based on a Fourier shell correlation (FSC) (Harauz and van Heel, 1986) of 0.5 from two half datasets as a criterion ranged from 0.4× Nyquist frequency or less at 200 kV (Booth et al., 2004; Ludtke et al., 2005) and 300 kV (Saban et al., 2006, 2005) to 0.58× Nyquist frequency at 120 kV (Stagg et al., 2006).

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Table 1
Examples of cryo-EM maps of various specimens studied at different electron accelerating voltages achieved at subnanometer resolutions expressed in terms of a fraction of Nyquist frequency using the Gatan 4k × 4k CCD camera

Specimen	Voltage (kV)	Effective magnification	Å/pixel	Nominal map resolution (Å)	Resolution as a fraction of Nyquist frequency
Adenovirus (Saban et al., 2005)	300	253,654×	0.59	8.9	0.13
Adenovirus (Saban et al., 2006)	300	⩾253,654×	0.59	6.9	0.17
Cytoplasmic polyhedrosis virus (Booth et al., 2004)	200	~83,100×	1.81	9.0	0.40
Flagellar filament (Yonekura et al., 2006)	300	~110,00×	1.35	8.0	0.34
GroEL (Chiu et al., 2006)	200	~110,800×	1.34	9.0	0.30
GroEL (Stagg et al., 2006)	120	66,400×	2.26	7.8	0.58
Pyruvate dehydrogenase complex (Yu et al., 2008)	200	69,220×	2.17	8.8	0.49
Ryanodine receptor (Ludtke et al., 2005)	200	~83,100×	1.81	9.6	0.38

Recent characterization of a Gatan Ultrascan 4k × 4k CCD (model US4000, 15 μm/pixel) with amorphous carbon film images suggested that this latest generation of the Gatan CCD camera has a SNR comparable to photographic film on a 300-kV electron microscope for data out to at least 0.5× Nyquist frequency (Booth et al., 2006). Here we report the use of trehalose-embedded two-dimensional (2D) crystalline bacteriorhodopsin (bR) and ice-embedded ε 15 bacteriophage as biological test specimens to further evaluate the performance of the same Gatan Ultrascan 4k × 4k CCD in a 300-kV (JEM3000SFF) electron cryomicroscope. Structures for both of these specimens have already been determined by electron cryomicroscopy (cryo-EM) using film data (Henderson et al., 1986; Jiang et al., 2006), thus our results can be evaluated. This study shows the feasibility of retrieving biological structures from both specimens close to 0.67 of the Nyquist frequency with CCD images recorded at a moderately high but practically acceptable magnification.

2. Materials and methods

2.1. Preparation of specimens

2.1.1. Preparation of bR grids

The bR was prepared from *Halobacterium halobium*. Large 2D crystals with 2D space group of p3 were then obtained through the fusion and annealing of the smaller native patches of bR by mixing bR solution with octyl glucoside (OG) and dodecyl trimethylammonium chloride (DTAC) according to the established procedure (Baldwin and Henderson, 1984). Locally-produced 400-mesh holey carbon grids with holes around 3 μm in diameter were prepared according to the established procedure (Fukami and Adachi, 1965). A thin carbon film deposited onto mica by evaporation (EMITECH K950 vacuum evaporator) was transferred to the holey grid by floating it onto a water bath to cover the holes. A 5 μl bR solution was placed on the grid followed by the application of a droplet (~5 μl) of 3% trehalose solution. The extra solution mixture was removed slowly by filter paper with a sharp tip.

2.1.2. Preparation of ε 15 bacteriophage grids

The procedure for the purification of ε 15 bacteriophage was described previously (Jiang et al., 2006). The frozen-hydrated grids of ε 15 bacteriophage were prepared according to the procedure described previously (Chen et al., 2007). Briefly, the R 1.2/1.3 400-mesh copper quantifoil grids (Quantifoil Micro Tools GmbH, Jena, Germany) were chemically cleaned with acetone and rinsed with water several times. The dry cleaned grids were flood-beam irradiated for more than 4 h at 10 k× magnification in a JEOL microscope operated at 100 kV (Miyazawa et al., 1999) and then were kept in a desiccator until sample freezing. The treated grids were first glow discharged for 10 s, then covered with a thin layer of fresh continuous carbon film floated on a water bath, and dried out naturally at room temperature. A droplet (~3 μl) of ε 15 bacteriophage solution was applied onto the carbon film side of the treated quantifoil grid, blotted and quickly plunged into liquid ethane cooled by liquid nitrogen at 95–100% humidity and room temperature using a Vitrobot (FEI Company, http://fei.com/Vitrobot). The frozen grids were stored in liquid nitrogen for subsequent cryomicroscopy.

2.2. Cryo-EM and data processing

The crystalline bR was imaged on the Gatan Ultrascan 4k × 4k CCD at an effective magnification of 84,000× (equivalent to 1.79 Å/pixel on the specimen) in the JEM3000SFF electron cryomicroscope operated at 300 kV with specimen at liquid helium temperature (~4 K) and with 25–36 e/Å^2 specimen electron dose for each CCD frame. The exposure time was 1.4 or 2 s. The data were processed using the MRC 2D crystal processing package (Crowther et al., 1996). The Fourier transforms of all the bR CCD images were indexed manually using *Ximdisp* program (Smith, 1999) from the MRC package, and the reciprocal lattice refinement was executed to calculate the unit cell parameters in Fourier space, which were used as the basis for further unbending of the 2D crystal image (Henderson et al., 1986). The defocus for each of the crystal images was determined using *CTFFIND* program in the MRC package.

Theimages of ε 15 bacteriophage were collected on the same CCD in the same JEM3000SFF microscope, but with ~36 e/Å^2 specimen dose per image frame and an effective magnification of 56,000×, equivalent to 2.68 Å/pixel on the specimen. The exposure time for each CCD frame was 2.0 s. The virus particles from CCD frames were boxed out automatically using the program *ethan* (Kivioja et al., 2000), then screened manually by the EMAN (Ludtke et al., 1999) program *boxer*. The CTF fitting for all CCD images of ε 15 bacteriophage was done automatically using *fitctf.py* (Yang et al., unpublished) and then was fine-tuned manually with the EMAN program *ctfit*. The alignment parameters of each particle were determined by a multi-path simulated annealing (multi-path SA) optimization algorithm (Liu et al., 2007). This Monte Carlo type of algorithm is able to search for both center and orientation parameters simultaneously. Each particle's alignment parameters are checked by a consistency criterion. In each iteration of the refinement procedure, any particle that fails to pass the consistency criterion is not considered in the current 3D reconstruction, but it may be considered in the next iteration. The resolution of the finally converged density map was determined using a criterion of FSC = 0.5 from two independent sets of half data. The segmentation for the ε 15 bacteriophage subunit was performed with *Chimera* (Goddard et al., 2005), which was also used for the surface representations of the cryo-EM density maps. The secondary structure element assignment from the segmented subunit was made using *SSEhunter* (Baker et al., 2007).

3. Results

3.1. bR as a test specimen

Good CCD images of bR with isotropic Fourier transforms were selected for further data processing. Fig. 1 is a Fourier transform of a good CCD image frame taken at an effective magnification of