Title

Cryo-Electron Microscopy and Image Analysis of SV40

Authors

Baker, TS
Drak, J
M. Bina

Publication Date

1985-08-01

DOI

10.1017/s042482010011845x

Peer reviewed

G. W. Bailey, Ed., *Proceedings of the 43rd Annual Meeting of the Electron Microscopy Society of America*

CRYO-ELECTRON MICROSCOPY AND IMAGE ANALYSIS OF SV40

T. S. Baker, J. Drak* and M. Bina*

Departments of Biological Sciences and Chemistry,* Purdue University, West Lafayette, Indiana 47907

The discovery that the T=7 icosahedral capsid of polyoma virus is composed of 72 pentameric capsomers[1] rather than 12 pentamers and 60 hexamers as predicted by constraints of quasi-equivalence[2] has prompted an examination of SV40 virus by electron microscopy to determine whether the capsids of other members of the papovavirus family are similarly constructed.

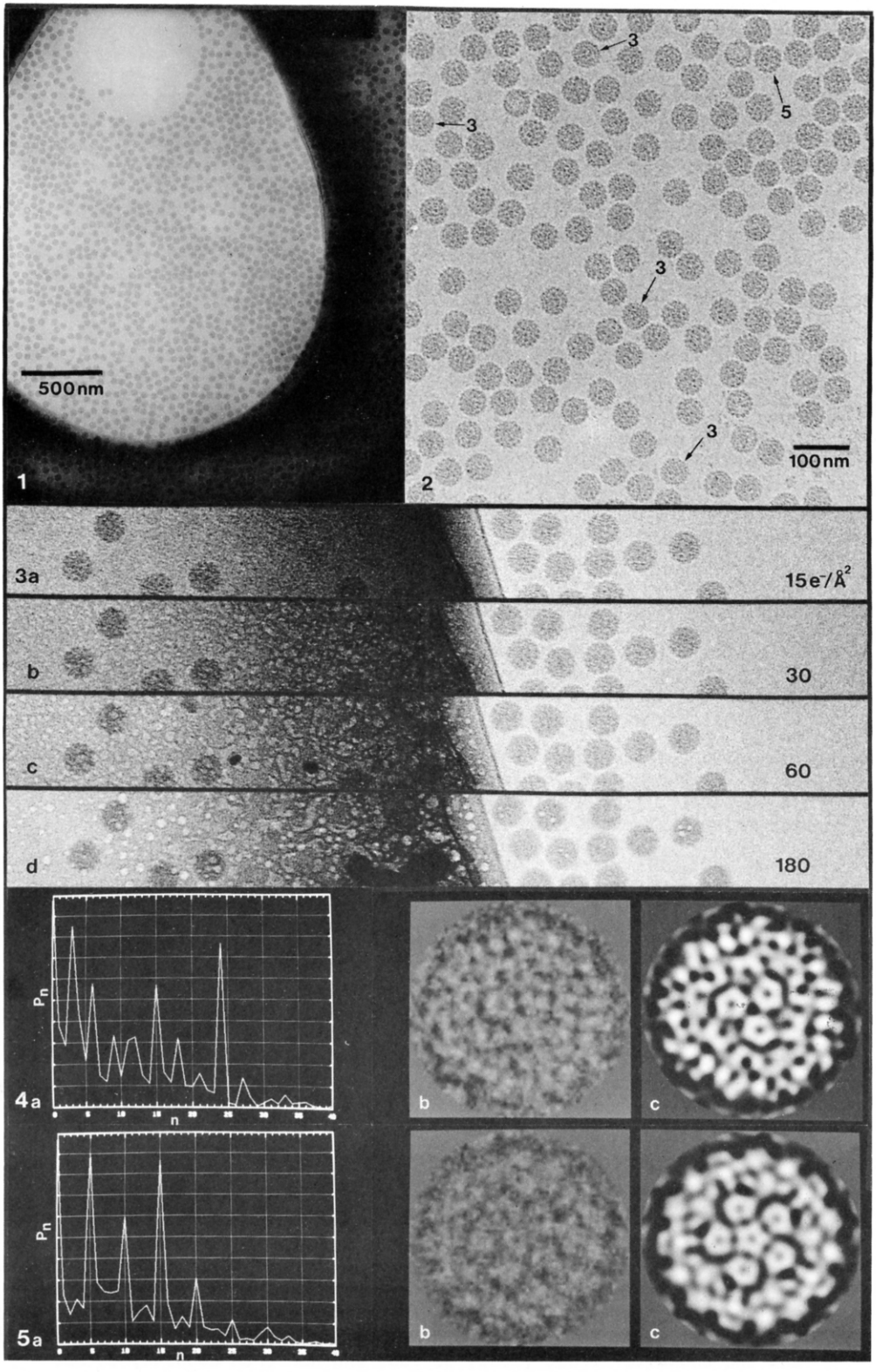
Thin layers of buffered aqueous solutions (∿4 mg/ml) of Simian virus 40 (strain WT776) were prepared for cryo-microscopy using recently developed procedures.[3,4] Images of virus particles suspended in thin layers of vitreous ice over holes in the carbon support film and maintained at -170°C were recorded using minimal irradiation conditions.[5] Figure 1 shows a typical field in which the frozen solution is similar in thickness to the virus particles (∿49 nm diameter). Particles appear to be excluded or squeezed away from the thinnest regions of solution (e.g., the clear region bordered by particles at the top of Fig. 1).

Virus particles are denser than ice, therefore they scatter electrons more strongly and appear with contrast opposite that in conventional negatively stained specimens. Micrographs were recorded 1-3 μm underfocus[6] to enhance phase contrast in the unstained specimen in the 25-35 Å resolution range, allowing features of the capsomer substructure to be resolved. At higher magnification (Fig. 2), many particles show characteristic two-sided superposition patterns from which the view orientation can be estimated to within a few degrees.[7] Arrows in Fig. 2 identify particles viewed close to icosahedral symmetry axes.

The radiation sensitivity of the frozen-hydrated specimen is demonstrated in Figure 3. Although more dramatic changes are observed in regions over the carbon substrate, fine details of the virus structure rapidly disappear after the accumulated electron dose exceeds ∿20-30 $e^-/Å^2$.

Images of particles viewed close to icosahedral three-fold (Fig. 4b; reverse contrast) and five-fold (Fig. 5b; reverse contrast) symmetry axes were examined using rotational image analysis procedures.[8] Power spectra (4a,5a) and rotational symmetry averages (4c,5c; reverse contrast) clearly demonstrate that the SV40 structure is well preserved in ice with minimal irradiation conditions. The three-dimensional structure of SV40 will be reconstructed by combining several images of particles viewed in different orientations[9] and compared to the polyoma capsid structure studied by x-ray crystallography[1,10].

1. I. Rayment et al., Nature 295(1982)110.
2. D. L. D. Caspar and A. Klug, Cold Spring Harbor Symp. Quant. Biol. 27(1962)1.
3. R. A. Milligan et al., Ultramicrosc. 13(1984)1.
4. M. Adrian et al., Nature 308(1984)32.
5. T. S. Baker and L. A. Amos, J. Mol. Biol. 123(1978)89.
6. H. P. Erickson and A. Klug, Phil. Trans. Roy. Soc. London B261(1971)105.
7. A. Klug and J. T. Finch, J. Mol. Biol. 31(1968)1.
8. R. A. Crowther and L. A. Amos, J. Mol. Biol. 60(1971)123.
9. R. A. Crowther et al., Nature 226(1970)421.
10. We thank Drs. N. Unwin and P. Flicker for invaluable guidance with cryo-microscopy and for the use of the Stanford University Cell Biology Philips EM400 microscope and Gatan cold stage. Supported by grants from NIH (GM33050-02), American Cancer Society (IN-17), Indiana Elks (SUB0133) and the Showalter Trust (6056) to TSB, and NSF (PCM-8303944) to MB.


500nm
1
3
3
5
3
3
100nm
2
3a
15e⁻/Å²
b
30
c
60
d
180
Pn
n
4a
Pn
n
5a
b
c
b
c