COMPARATIVE ANALYSIS OF DIFFERENT METHODS OF FIXATION OF UNICELLULAR CYANOBACTERIA FOR TRANSMISSION ELECTRON MICROSCOPY

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ABSTRACT

Cells of cyanobacteria were fixed using chemical fixative, with progressive lowering of temperature and cryo fixation, with high pressure and progressive increase in temperature. The high pressure freezing employed yeast cells as the cryoprotectant. No chemical fixatives were used. Although the inherent contrast of the cells fixed with high pressure was low as compared to chemically fixed cells, the samples retained intracellular organization and good immuno-reactivity.

Introduction

Sample fixation represents a critical step in the preparation of biological specimens, for Transmission Electron Microscopy (TEM). An ideal method should preserve the structure closest to in vivo. Traditional TEM techniques employ chemical cross linkers like gluteraldehyde and paraformaldehyde as fixatives. These methods apparently result in preservation of intracellular structures but also result in some fixation artifacts due to extensive protein cross linking. The serial dehydration and infiltration is also carried out at room temperatures, which further aggravates the artifacts. The advent of cryo-techniques such as cryo-fixation and cryo substitution have solved these problems to some extent. Currently cryo-fixation techniques like High Pressure Freezing and Metal Mirror Freezing are being used, in combination with chemical cross linkers, to achieve maximum preservation of the internal structure. Cryo fixation alone without the use of chemical crosslinkers has been rarely described. We report here the use of yeast cells and hexadecene as cryo-protectant during high pressure freezing of unicellular cyanobacteria. This method resulted in the effective preservation of internal structure as well as chemical reactivity of the epitopes as shown by immuno-electron microscopy.
**Materials and Methods**

**Chemical fixation**

Log Phase cells of both the Cyanobacteria viz. *Synechococcus* (7942) and *Synechocystis* (6803) were harvested and fixed with 0.5% gluteraldehyde and 2% paraformaldehyde in 0.05M cacodylate buffer pH 7.2 for 1h followed by 0.5% OsO₄ for 1 h at RT. The samples were serially dehydrated in graded ethanol, followed by propylene oxide at RT, infiltrated with and embedded in low viscosity resin for 48h at 60°C.

**Automatic freeze substitution**

Cells were fixed as described above and serially dehydrated with progressive lowering of temperature from 4°C to -35°C, infiltrated with HM20 resin at low temperature and UV polymerized for 48h at -35°C.

**High pressure freezing**

Log phase cells were taken in micro capillary tubes, coated with yeast cell paste or hexadecene or both and put between aluminium planchets. The cells were quickly frozen in a Leica high pressure freezer 2100 psi and freeze substituted with 100% acetone at low temperature from -90°C to +20°C over 7 days.

The samples were infiltrated with LR-white resin and embedded in the same resin.

**Sectioning and staining**

70 nm thin sections were cut with Leica ultamicrotome, stained using 2% Uranyl acetate for 30 min. and viewed.

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Fig. 1: Transmission electron micrographs of unicellular Cyanobacteria

A) Chemically fixed *Synechococcus* 7942 in low viscosity resin
B) Chemically fixed and PLT dehydrated *Synechococcus* 7942 in HM20 resin
C) HPF fixed *Synechococcus* 7942 in LR white resin
D) Chemically fixed *Synechocystis* 6803 in low viscosity resin
E) Chemically fixed and PLT dehydrated *Synechocystis* 6803 in HM20 resin and
F) HPF fixed *Synechocystis* 6803 in LR white resin
under Tecnai G² Sphera Transmission Electron Microscope at 120 KeV.

Results and Discussion

Fig. 1 shows the ultrastructure of cells fixed by chemical preservatives and by high pressure fixation. As seen in Fig. 1 (A, B, D and E), chemically fixed cells showed the typical ultra structure. The thylakoids had even diameter throughout and thylakoid lumen was visible. However, plasma membrane was seen to be receded from the outer envelope and the contrast was comparatively high probably due to extensive protein cross linking. This leads to formation of large complexes that take up large quantities of stain leading to higher contrast. Extensive linking resulted in loss of reactivity of the epitopes as seen by immunolabeling. In contrast, in cryo fixed cells, the thylakoid membranes appeared uneven in diameter and the luminal space was barely visible. The contrast was weaker probably due to non availability of protein aggregates. The plasma membrane and outer envelope were preserved and looked pressed against each other, which may be closer to the situation in vivo. Antibody reactivity data showed that epitopes were available in HPF fixed cells.

Recently transmission electron microscopy of cyanobacteria (cryo fixed in conjunction with chemical fixatives) was used for 3-D tomography (1, 2). The use of yeast as cryo-protectant not only preserves the structure in a better way, but also retains the immuno reactivity of the antigenic sites in our studies. 3-D tomography of these samples may reveal more accurate intracellular details.

References


ABOUT THE AUTHORS

Ms. Rachna Agarwal joined the Molecular Biology Division of BARC from the 47th Batch of OCES, BARC Training School and is currently working under Plant Biochemistry Section. Her area of research includes studies on molecular architecture and biogenesis of photosynthetic apparatus and effects of ionizing radiation on photosynthesis in unicellular cyanobacteria.

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