Transmission Electron Microscopy of Semi-thin Sections of Cyanobacterium - *Anacystis nidulans* at 160kV

D. N. Dani and J.K. Sainis  
Molecular Biology Division  
Bhabha Atomic Research Centre

and

G.K. Dey  
Material Science Division  
Bhabha Atomic Research Centre

**CONVENTIONALLY TRANSMISSION**  
electron microscopy for biological samples is done with TEM operating at 80-100kV using ultra-thin sections of 70-100 nm. Since higher voltage electron microscopes are not traditionally used for biological samples, semi-thin sections (over 100 nm) are rarely used for electron microscopy. In this report we present our results on use of Transmission Electron microscope at 160 kV to investigate the structural details of *Anacystis nidulans* cells using semi-thin sections.

*Anacystis nidulans* is a unicellular cyanobacterium having a specialized system of internal membranes, called thylakoid membranes. We are interested in understanding supramolecular organization of photosynthetic enzymes around thylakoid membranes. Cells of *Anacystis nidulans* (strain BD1) were fixed and embedded in araldite by conventional protocols. Thin and semi-thin sections (70 nm, 200 nm, 300 nm and 500 nm) were cut using Leica Ultracut UCT. The sections were contrasted with uranium acetate. Thin sections were observed under 80 kV TEM (Jeol JEM 1010) and semi-thin sections were observed under 160kV TEM (Jeol JEM 2000 FX).

Figure 1 shows the conventional ultra thin sections (70 nm) of *Anacystis* seen under 80kV Jeol Transmission Electron Microscope. The outer cell wall and inner thylakoid membranes are seen clearly. These images were similar to those observed earlier (Allen, 1988). Figures 2-4 show sections of *Anacystis* cells which are 200, 300 and 500 nm in thickness. The sections show typical cell wall, 3-4 layers of thylakoid membranes running parallel to each other, enclosing nucleoplasm, carboxysomes and polyphosphate granules as described by Stanier (1988). The electron transparent thylakoid membranes appear to be around 50-60 nm thick and the electron-opaque layers around thylakoid membranes are 20-30 nm thick. The electron opaque region shows regular globular bodies along the thylakoid membranes. Interconnections between the thylakoid membrane systems and cytoplasmic membranes are clearly visible in 500 nm thick sections.
These results are similar to those observed in the case of an unicellular halotolerant cyanobacterium *Agemenellum quadruplicatum* with 250 nm thick sections using 1.0 MV High Voltage Electron Microscope (Nierwicki-Bauer, et al., 1984).

The results indicate that, TEM operating at 160kV can be used to investigate structural details of biological samples using section thickness up to 500nm. Since *Anacystis* cells are simple prokaryotic cells without any complex intracellular structural features, thin sections at low voltage and semi-thin sections at higher voltage were comparable. There was no loss of information. However, the thylakoid membranes show interconnections and continuity, which can be easily observed in 500 nm sections (figure 4) which is not seen in thin sections. Thus, TEM at 160kV will be useful to observe semi thin sections (up to 500nm) of biological sample which can give 3-7 times more structural information as compared to conventional 80kV TEM. Besides semi thin sections will be useful for 3D reconstruction of images as well as immuno-labeling.

**References**

2. Nierwicki-Bauer et al., 1984, The use of high-voltage electron microscopy and
semi-thick sections for examination of cyanobacterial thylakoid arrangements, *Journal of Microscopy*, 133, 55-60


**Acknowledgements**

We wish to thank Dr. S. K. Mahajan, Head, Molecular Biology and Agriculture Division, BARC, and Dr. S. Banerjee, Director, Material Sciences Group, BARC, for the encouragement. We also want to thank Dr. A. R. Chitale, Head, Electron Microscopy Facility, Jaslok Hospital and Mr. D. B. Kanaskar and Mr. S. S. Bhosale, Electron Microscopy Section, Jaslok Hospital, for the help in taking images with 80 kV TEM.

---

**This paper received the Best Poster Presentation award at the Silver Jubilee Conference of the Electron Microscope Society of India on Electron Microscopy and Allied Fields, held at IIT, Bombay, during February 20-22, 2002**

---

**About the authors ...**

**Ms Diksha N. Dani** has done M. Sc in Botany from Mumbai University and joined Molecular Biology Division in 1999. She holds Senior Research Fellowship for Ph.D from Department of Atomic Energy and is studying the supramolecular organization among photosynthetic enzymes and thylakoid membranes.

---

**Dr Jayashree Krishna Sainis** is working in Molecular Biology Division. She has done M.Sc in Biochemistry from Nagpur University in 1971. She joined BARC in 1972 after completing one year Training Course in Biology-Radiobiology (15th batch). She did her Ph. D from Gujarat University in Biochemistry. Her research deals with various aspects of Plant Biochemistry and Photosynthesis. She has over 50 papers in journals and symposia proceedings.

---

**Dr Gautam Kumar Dey** obtained his B.Tech in Metallurgical Engineering in 1979 from the Institute of Technology, Banaras Hindu University. He joined the 23rd batch of Training School and was awarded the Homi Bhabha prize for standing first in his discipline. On completion of training, he joined the Metallurgy Division. He obtained his Ph.D. degree from Banaras Hindu University in 1988. He was a postdoctoral fellow at University of Cincinnati from 1994 to 1996. He has won several awards for his scientific contributions. Of these mention can be made of the Young Scientist award of Indian National Science Academy, Young Metallurgist Award given by Ministry of Steel and Mines and MRSI Medal given by Materials Research Society of India. Areas of his research interest are Phase Transformation in Zirconium and Nickel Base Alloys, Amorphous Alloys, Rapidly Solidified Crystalline and Quasicrystalline Alloys, Electron Microscopy and Defect Characterization and High Resolution Electron Microscopy. He has more than 150 scientific publications to his credit.