Electron Diffraction on Biological Samples

Ing. Vendula Hrabalová[1], Ing. Ph.D. Jaromír Bačovský[1], Ing. Radka Martínková[1], Helmut Gnaegi[2], Miloslav Klinger[3]
[1]Delong Instruments, Brno, Czech Republic, [2]Diatome Ltd., Port, Switzerland, [3]Institute of Physics, Czech Academy of Sciences, Czech Republic

Background incl. aims
Animals have the ability to generate a diverse array of minerals at various locations within or surrounding their cells. An example of this mineralization process is evident in the development of rigid skeletal structures. Especially skeletal hard parts like bones, teeth, shells, and exoskeletons are prime examples of biomineralization [1]. Carbonate apatite crystals constitute a significant portion of various mineralized tissues in vertebrates. In the mentioned structures, like bones and mineralized tendons, these crystals typically manifest as thin, irregularly shaped plates [2].

Electron diffraction experiments provide valuable insights into lattice parameters and symmetries and sometimes help uncover hidden crystals. Directing electron beams at these samples can explain their crystal arrangements, which helps to understand the mechanical properties of the materials, mineral composition, and integrity. A comprehensive understanding of the composition and characteristics of biomineralizations can be invaluable in diagnosing pathological calcifications, which are implicated in several severe diseases. Identifying and analyzing these crystals in tissues can provide crucial insights into underlying mechanisms and progression of such conditions, aiding in early detection, accurate diagnosis, and effective treatment strategies. Electron diffraction proves instrumental in diagnosing conditions such as Alzheimer's disease, which is characterized by abnormal accumulation of metals in the brain [3].On the bone samples, electron diffraction aids in identifying hard tissue calcification, which compromises elasticity, hardens these tissues and disrupts their function. Electron diffraction offers a significant advantage in its ability to analyze a much smaller area, typically at the nanometre scale [4], compared to X-ray diffraction, making it feasible to detect locally ordered structures (nanocrystals).

This technique is also generally suitable for examining thin layers, such as 2D crystals. Consequently, this method proves particularly valuable for studying membrane proteins that readily form 2D crystals but face challenges in forming 3D structures [5].

Methods
Low Voltage Transmission Electron Microscopy (LVEM) is a valuable technique in life science and materials research. LVEM operates at lower voltages, typically below 25 kV. This lower voltage regime offers distinct advantages, especially enhanced contrast. When assessing micrograph quality, spatial resolution and image contrast are key criteria. Low voltage improves contrast mechanisms, which is particularly beneficial for samples containing light elements, such as biological specimens. Enhanced image contrast helps to identify suspicious crystal structures in tissues. Moreover, low-voltage electron diffraction has a distinct specific advantage yielding from the dimensions of the Ewald sphere, providing more information [6].
The Low Voltage Electron Microscopes (LVEMs) by Delong Instruments integrate TEM and STEM (including dark fields in both regimes) and SEM (BSE) imaging modes. The LVEMs offer Electron Diffraction (ED) and eventually Energy Dispersive X-ray Spectroscopy (EDS), which makes them altogether highly versatile tools for material analysis.

The samples were sectioned with 35° diamond knives at a feed of 20-30nm using ultramicrotomy method.

Results
This study performed electron diffraction on selected biological samples, namely manganese-bound bacteria, bones, shells, and urea, and explored the advantages of analyzing biological samples using low voltage electron diffraction. The experiments were carried out using low-voltage electron microscopes LVEM 25E with an accelerating voltage of 25 kV and LVEM 5 operating at approximately 5 kV.

Conclusion
In summary, electron diffraction proves invaluable for analyzing biological materials, offering insights into crystal structures and tissue properties. Particularly, it aids in studying thin layers and 2D crystals, contributing to understanding membrane proteins and the structural complexity of hard biological materials like bones and shells. Additionally, it shows potential in diagnosing diseases such as Alzheimer's. This study highlights the utility of low-voltage transmission electron microscopy in such analyses, providing enhanced contrast and analytical precision for diverse biological samples.

Keywords:

Electron Diffraction, LVEM, biological samples

Reference:

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