High pressure freezing and freeze substitution on Biological electron microscopy

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Combining fine structural preservation with potential of molecular characterization of the cell's native state is one goal of modern cell biology. Considering the increasing necessity for improved preparation techniques in biological electron microscopy as a basis for the identification and localization of cellular substances within the compartments of the cell, this work is focused on the method of high pressure freezing (ultra rapid freezing) and freeze substitution as an important tools and resin embedding of biological specimens. The theories of high pressure freezing and freeze substitution are summarized with particular interest in the physical and thermodynamic as well as in the chemical basis of this technique. The main advantage of high pressure freezing versus conventional chemical fixation is seen in the maintenance of the hydration shell of molecules and macromolecular structures. These were proven in an improved fine structure preservation, superior retention of the antigenicity of proteins and decreased loss of unbound, diffusible cellular components. Examples of excellent visualization of the ultrastructure of small organisms (bacteria, algae, cyanobacteria, and fungi) and large biological samples such as plant and animal tissue as well as the plant-pathogen (fungus) interface and infection structures are presented. Recent data on the molecular characterization of freeze-substituted biological tissues are exemplified with special emphasis on the subcellular detection of soluble components (proteins) and the inter-/intra cellular localization of proteins. The molecular analysis of freeze substituted specimens is acheived by the combination of low temperature preparation technique in biological electron microscopy with immunocytochemistry