Abstract

Preparation of biological samples for transmission electron microscopy is not a trivial task. The samples must withstand a vacuum environment present inside a microscope, and it is often necessary to use non-physiological procedures for their processing. These procedures usually involve aldehyde-based fixation, replacing water with alcohol (i.e. dehydration/substitution), and embedding into a resin, which creates support for the subsequent preparation of thin sections that can be placed into the microscope. In the last decade, the method of cryo-fixation (vitrification) using ultra-fast high-pressure freezing followed by freeze substitution and low-temperature resin embedding gained a dominant position in the cell biology research. In this way, a range of biological samples with a thicknesses up to several hundreds of micrometers was successfully vitrified to a state that was closely related to their *in vivo* structures. The cryo-fixation of isolated biological objects (with a limited thickness up to several micrometers) is possible in a thin layer of vitrified water by plunge freezing at ambient pressure. In combination with electron cryo-microscopy, this method has become the most effective and fundamental principle for the high-resolution studies and image analysis of fully hydrated samples breaking the sub-nanometer limit. These methods are presented in this thesis. The budding yeast *Saccharomyces cerevisiae* processed by high-presure freezing and freeze substitition was selected as a biological object for both fine ultrastructural and immunocytochemical studies using conventional transmission electron microscopy; the liposomes and other types of vesicular structures vitrified by plunge freezing were selected as nanoobjects for electron cryo-miscroscopic studies.

Keywords: cryo-fixation · electron cryo-microscopy · freeze substitution · high-pressure freezing · plunge freezing · transmission electron microscopy · vitrification