Chapter 15  Cryofixation of Diffusible Elements in Cells and Tissues for Electron Probe Microanalysis

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1 Introduction

The interaction of the electron beam with the specimen not only provides information on its ultrastructure, but also on its elemental composition. In principle, the irradiating electron beam (probe) is focused on the area of interest in the specimen, while the secondary radiation (e.g. X-rays) generated by the interaction with the specimen is processed through a spectrometer to provide information on the kind and amount of the elements present. Today, electron probe X-ray microanalysis (EPXMA) is the most widely used method in biological microanalysis. Also, electron energy loss spectrometry (EELS) acquires more and more attention. It would be beyond the scope of this book to explain the physical principles and the methodology of these analytical methods here, in particular, since there are numerous, well-written reviews on this subject (e.g. Chandler 1977; Hren et al. 1979; Hall and Gupta 1983; Morgan 1985). Rather, we want to discuss the particular importance of cryotechniques in this field and to point out the still existing problems of specimen preparation. Its crucial steps are not only relevant for EPXMA and EELS, but also for other microanalytical techniques such as proton probe X-ray microanalysis and laser probe mass spectrometry.

Indeed, the history of EPXMA surprisingly resembles that of electron microscopy in general, the development of instrumentation having been far in advance of that of specimen preparation. Being well established in the material sciences since the 1960s, biological specimens only gave meaningful results in those cases where strongly bound elements were analyzed, e.g. mineralized tissue (Hall and Hohling 1969) or metal-intoxicated tissue (Yárom et al. 1973). However, most elements of biological interest, such as electrolytes, are quantitatively extracted from the tissue by conventional specimen preparation, in particular, by chemical fixation, dehydration and sectioning on a water surface. Attempts to precipitate ions during chemical fixation only occasionally were successful and the results are qualitative at best.

The introduction of cryofixation changed the situation, as documented by the increasing number of laboratories producing meaningful results. Rapid freezing not only is the best approach to preserve the original composition of a specimen,

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it also allows one to arrest physiological processes under defined conditions with high time resolution (see Knoll et al., Chap. 14, this Vol.). Although cryofixation may preserve the original distribution of diffusible substances, this is by no means guaranteed, as it depends on the cooling rate like the preservation of structure (see Bachmann and Mayer, Chap. 1, this Vol.). Moreover, severe artefacts may be produced during sampling of the specimen before it is frozen, and likewise later during the various procedures following cryofixation. Even the processing of the raw analytical data is not an easy task and has many pitfalls. For the latter aspect, the reader again is referred to the reviews on electron probe microanalysis as given above (see also Hall and Gupta 1982).

In this chapter we want to compare the different cryotechniques in use and discuss their relative advantages and disadvantages for the in situ analysis of mobile elements. We will also give brief reference to some papers, which highlight the potential of microanalysis in giving physiologically relevant data on the intracellular and intercellular concentration of ions, which, with no other method so far, can be obtained with comparatively high accuracy of localization.

2 Specimen Preparation

Cryopreparation pathways as used for the quantitative analysis of diffusible ions and water in cells and tissues by electron microprobe methods are schematically outlined in Fig. 1. The preparation starts by specimen sampling, followed by cryofixation. Then there exist essentially three different ways of processing, which yield three different types of electron microscopic specimens:

1. Dehydration by freeze-drying or freeze-substitution with subsequent embedding in resin (see Steinbrecht and Muller, Chap. 7, this Vol.). The result is an ultrathin plastic section, which can be studied by STEM (scanning transmission electron microscopy) or TEM (transmission electron microscopy).

![Fig. 1. Pathways of cryopreparation as used for electron probe microanalysis of biological specimens](image-url)