An Improved Janus Green Technique for Detection of Mitochondrial Structure with Electron Microscope

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Abstract

Previous studies have shown that Janus Green have a role in mitochondrial localization in light microscopy. In this study, Janus Green have been used in electron microscopic technique to demonstrate fine mitochondrial structure. Tissue specimens from mice liver were processed for electron microscope examination according to Elayat method. Certain modification was performed to use Janus green with propylene oxide. The results have shown that Janus Green gives a good contrast to the membranous system not only to the mitochondria but also to other cytoplasmic organelles (rough endoplasmic reticulum and nuclear envelope). Using Janus Green in electron microscopic preparation reflected the interaction between Janus Green and uranyl acetate and gives a good contrast to many cell components.

Introduction

Numerous procedure have been used to demonstrate mitochondrial localization and their fine structure. Potentiometric dyes were used to demonstrate the oxidation-reduction level in the mitochondria of kidney tubule (1), while Jay and Small (2) found that using LDS-751 achieved a good procedure to demonstrate the mitochondria. Furthermore, triphenyltetrazolium chloride has been used to detect the activation of mitochondrial respiratory function (3). Since Janus Green was used to demonstrate mitochondria in light microscopic preparation (4, 5), the present study was designed as a modifying procedure in which the mitochondrial structure will be studied within the electron microscope by using Janus Green.

Materials and Methods

Tissue specimens from mice liver were used in this study. Tissue specimens were divided into two groups. Each group was processed for electron microscope examination according to Elayat, (6). One of the two groups was processed by using Janus Green as in the following:

1. Tissue specimens were primarly fixed with 2.5% glutaraldehyde in phosphate buffer pH 7.4.
2. The specimens were rinsed in 1% Janus Green dissolved in the same buffer solution for several times and left overnight in Janus Green-buffered solution.
3. The specimen were post fixed with 1% osmium tetroxide for one hour then, washed for ten minutes in distilled water.
4. Specimens were dehydrated through a series of ethanol with concentrations (50%, 50%, 70%, 80%, 90% and 100%).
5. Specimens were cleared in propylene oxide for 10 minutes (2 times).
6. Specimens were placed in a mixture of propylene oxide and embedding material (araldite) for 1 hour and then left in araldite for 24 hours at lab temperature.
7. Each specimen was cleaned from coherent araldite by a filter paper, then placed in plastic capsule and filled it with araldite, then left in the oven for 48 hours at 60°C.

All specimens were sectioned and stained with uranyl acetate & lead citrate, then examined with CV10 electron microscope.

Results

Janus Green gave good contrast to the membrane of all cytoplasmic organelle (Mitochondria, Rough endoplasmic reticulum (RER) & Nuclear envelope). The results are summarized in table (1) and Fig (1, 2, and 3).

Table 1: Showing the Cyto-architecture of liver cells processed with Janus Green.

<table>
<thead>
<tr>
<th>Cytoplasmic Organelle</th>
<th>Specimen treated with Janus Green</th>
<th>Specimen without Janus Green</th>
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</thead>
<tbody>
<tr>
<td>RER</td>
<td>Normal appearance</td>
<td>Normal appearance</td>
</tr>
<tr>
<td>Mitochondria</td>
<td></td>
<td></td>
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<tr>
<td>Nuclear envelope</td>
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<td>Ribosomes</td>
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Discussion

Mitochondria are cytoplasmic organelles found in variable numbers in all plant and animal cells. They are very large in size, and their large size makes them very visible by light microscopy and can be demonstrated by the following types of techniques:

1. Electron microscopy
2. Enzyme histochemistry
3. Histological methods

Since electron microscopy is the most satisfactory method used to demonstrate mitochondria even in cells where they are small and few, it is a technique that must be used to demonstrate the mitochondria in EM. The results have been shown that Janus Green gives more contrast to the mitochondrial structure and other cytoplasmic organelles. These results might reflect an interaction between Janus Green and nuclear acetyl, since dinitro acetyl is considered as a stabilizing agent to demonstrate various proteins including HAM, RB69, RB60, and RB60, which are the main constituents of the membrane system in all eukaryotic cells.

References