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The intensity of immunogold labeling of deplasticized acrylic sections compared to deplasticized epoxy sections—Theoretical deductions and experimental data

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Abstract

The purpose of this study was to compare the level of immunogold labeling of deplasticized acrylic sections and deplasticized epoxy sections. Pure protein gels of IgG, albumin and thyroglobulin were produced by glutaraldehyde fixation and embedded in non-crosslinked acrylic resin (Technovit 9100) and epoxy resin (Epon 812), respectively. Ultrathin sections of acrylic and epoxy resin were separately deplasticized in 2-methoxyethyl acetate (MEA) and sodium ethoxide. Quantitative immunogold labeling was performed with anti-IgG, anti-albumin and anti-thyroglobulin antibodies on sections of the corresponding protein gels. For all antibodies tested, the intensity of labeling for deplasticized acrylic sections was significantly higher (two to four times) than for the corresponding deplasticized epoxy sections. The results fit with a theoretically deduced relation: the quotient of the labeling of two deplasticized sections of different resins is equivalent to the square root of the quotient of the labeling of the similar sections not exposed to any kind of pre-treatment. The practical significance of the results is that immunolabeling of deplasticized non-crosslinked acrylic resin results in more intense immunogold labeling than deplasticized epoxy sections. Deplasticizing is most useful when the requirements for ultrastructural preservation according to conventional criteria are moderate. Our theoretically deduced results also indicate that deplasticized Technovit (or other non-crosslinked acrylic resins) sections will be significantly better suited for immunolabeling at the light microscopic level than deplasticized epoxy sections.

Keywords: Antigen retrieval; Acrylic resin; Epoxy resin; Immunogold; Immunoelectron microscopy; Electron microscopy

1. Introduction

Conventional epoxy resins give significantly lower efficiency of immunogold labeling than acrylic resins in most cases (Newman, 1989; Brorson and Skjørten, 1996a). Therefore, it is often preferable to manipulate the epoxy sections before immunolabeling. Complete deplasticizing is one method for improving the intensity of immunogold labeling of epoxy sections (Mar and Wight, 1988; D'Alessandro et al., 2004); another method is heating the sections in aqueous solution (Yano et al., 2003; Brorson and Nguyen, 2001; Groos et al., 2001; Fossmark et al., 2005). Acrylic resins normally used for electron microscopy are Lowicryls, LR-White, LR-Gold and Unicryl. All these acrylic resins are crosslinked (Newman, 1989). Consequently, it is not possible to perform deplasticizing on such sections. However, the acrylic resin Technovit 9100 is not crosslinked, and can be deplasticized with 2-methoxyethyl acetate (MEA). At the first glance it seems reasonable to assume that the intensity of immunolabeling of deplasticized epoxy sections and deplasticized acrylic sections would be the same. The rationale behind this is that when a section has been deplasticized, only the tissue is left, and thus it does not matter what kind of resin was initially used.

However, acrylic resin and epoxy resin behave differently during the sectioning process: epoxies form covalent bonds with biological material, particularly with proteins. Co-polymerization of epoxies with the embedded tissues occurs,

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while polymerized acrylic resins permeate the embedded tissues without binding to them. Accordingly, during sectioning the behavior of epoxies differs from that of acrylics. Without co-polymerization (acrylics), the knife edge tends to follow the areas of least resistance, e.g., the interfaces between resin and proteins. In epoxy-embedded material, however, the resistance in these interfaces is not much less than in the proteins, and when cutting epoxy-embedded tissue, the knife edge has greater tendency to divide (and thereby destroy) the proteins. This is the reason why untreated acrylic sections give higher efficiency of immunolabeling than untreated epoxy sections (Kellenberger et al., 1987; Brorson and Skjørten, 1996a).

According to the above reasoning, a large number of destroyed antigens are present on the surface of an epoxy section. These destroyed antigens are also present in the section after deplasticizing (Brorson and Skjørten, 1996b). In contrast, the number of destroyed antigens is significantly lower on the surface of an acrylic section, while the number of undamaged antigens is high. Many antigens originally located at a level just below the surface will be exposed for immunolabeling after deplasticizing (Brorson and Skjørten, 1996b), but this is similar for both epoxy and acrylic sections. The current study was performed to test the hypothesis that the intensity of immunolabeling will be significantly higher on deplasticized acrylic sections than on deplasticized epoxy sections, but the ratio $\text{labeling}_{\text{depl-acrylic}}/\text{labeling}_{\text{depl-epoxy}}$ will be lower than the ratio $\text{labeling}_{\text{acrylic}}/\text{labeling}_{\text{epoxy}}$. Additionally, from theoretical considerations we wanted to deduce a mathematical expression describing the quotient $\text{labeling}_{\text{depl-acrylic}}/\text{labeling}_{\text{depl-epoxy}}$ as a function of $\text{labeling}_{\text{acrylic}}/\text{labeling}_{\text{epoxy}}$ taking issue with theories presented previously (Brorson and Skjørten, 1996a,b).

2. Material and methods

2.1. Fixation and embedding

Ten milligrams human IgG powder (Sigma–Aldrich Corp., St. Louis, MO, USA) was dissolved in 100 μl phosphate buffer (pH 7.3), and mixed with 100 μl 2% glutaraldehyde in the same buffer (50 mg/ml final IgG concentration). The mixture was incubated for 2 h to become a gel. Gel pieces were postfixed in 2% glutaraldehyde overnight and rinsed in phosphate buffer. Human albumin powder (Sigma–Aldrich Corp.) was gelled and fixed in the same way. Human thyroglobulin was gelled by solving 2 mg of powder (Fitzgerald Industries International Inc., Concord, MA, USA) in 20 μl phosphate buffer and then mixed with 5 μl 2% glutaraldehyde. The gels were embedded in epoxy resin (Epon 812) according to Brorson and Nguyen (2001), using 2% accelerator. The embedding in Technovit 9100 was performed according to the procedure of Yang et al. (2003), except for the polymerization that took place for 1 day at room temperature.

2.2. Sectioning

Ultrathin Technovit and epoxy sections of the gels were mounted onto 200-mesh nickel grids that had been coated with Formvar that was covered with carbon on both sides to protect the Formvar film against the deplasticizing solutions.

2.3. Deplasticizing

Epoxy sections were incubated in 50% saturated solution of sodium ethoxide for 10 min (Brorson and Skjørten, 1995), and then they were incubated in MEA for 15 min (Yang et al., 2003). Technovit sections were subjected to incubation in MEA for 15 min and then incubated in 50% saturated solution of sodium ethoxide for 10 min.

2.4. Immunogold labeling

Immunogold labeling was performed according to Brorson and Nguyen (2001) on deplasticized sections of Tecnovit and Epon, respectively, and on untreated sections of the same resins. The primary antibodies used were anti-IgG (rabbit anti-human IgG (1:800) [Sigma]), anti-albumin (rabbit anti-human albumin (1:800) [Dako, Glostrup Denmark]) and anti-thyroglobulin (rabbit anti-human thyroglobulin (1:2000) [Dako]). The secondary immunoreagent was antibodies coupled to 15 nm immunogold particles (Goat anti-rabbit IgG, Auroprobe EM Gar G15 [Amersham, Bucks, UK]). All sections were incubated in the primary antibody at 4 °C overnight and in the secondary immunoreagent for 75 min. Finally, the sections were stained with uranyl acetate and Reynolds lead citrate.

2.5. Examination and counting of gold particles

Immunostained sections were examined and images recorded in a transmission electron microscope (Phillips, Tecnai 12) at an instrumental magnification of 20,000×.

Table 1
The intensity of immunolabeling (gold particles per μm^2) of deplasticized epoxy and Technovit sections for IgG, albumin, and thyroglobulin in two different experiments

	L_{Td}	L_{Ed}	L_T	L_E
IgG (experiment 1)	160.9 ± 10.7	53.1 ± 6.2	43.9 ± 6.5	4.8 ± 0.9
IgG (experiment 2)	164.9 ± 14.6	59.3 ± 7.1	44.2 ± 4.3	5.4 ± 1.3
Albumin (experiment 1)	42.5 ± 3.4	11.5 ± 0.8	34.0 ± 3.1	2.5 ± 0.5
Albumin (experiment 2)	58.0 ± 9.1	18.1 ± 2.0	37.7 ± 5.9	2.7 ± 0.4
Thyroglobulin (experiment 1)	166.6 ± 12.9	65.2 ± 7.1	31.8 ± 4.2	3.0 ± 0.7
Thyroglobulin (experiment 2)	101.0 ± 8.2	49.8 ± 4.4	16.9 ± 2.5	3.0 ± 0.5

Td, deplasticized Technovit; Ed, deplasticized epoxy; T, non-deplasticized Technovit, E, non-deplasticized epoxy.