Tissue Fixation Prevents Contamination of Tritium-Sensitive Storage Phosphor Imaging Plates

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The use of storage phosphor imaging plates is becoming an increasingly popular alternative to X-ray film when detection speed rather than spatial resolution is the prime concern for detection of radioactive decay during in situ hybridization, northern/Southern blotting and radioligand binding assays. Because the photo-stimulable imaging plates are approximately 5 times more sensitive than traditional film, exposure times can be dramatically shortened (2). The imaging plates are placed in contact with the sample in exactly the same way as film (but without the need for darkroom conditions), and an image is captured in the coating of fine crystals of halogenated barium phosphors containing traces of Europium rather than the silver halides of X-ray film. The last image is retrieved by instruments such as the Fuji BAS™ 3000 (Fuji Photofilm Ltd., Tokyo, Japan), which uses a photomultiplier to measure the light emitted from an irradiated plate as an He/Ne laser scans across its surface.

Imaging plates come in two formats. The standard plates, used to detect most radiolabels, are covered by a robust protective coating and are thus readily reusable, keeping costs low. However, to detect tritiated probes, plates without protective coating and allow repeated use of the tritium-sensitive imaging plates, we have examined the ability of fixation by immersion in 10% formalin, 4% paraformaldehyde, 3% gluteraldehyde or vapor fixation in paraformaldehyde, to fix the radioligand [3H]-SCH-23390 into coronal sections of mouse brain after binding to striatal dopamine D1 receptors.

Tissue for this experiment was obtained from five male C-57 Black/6J mice (17-25 g and 5-6 weeks of age) that were killed by nembutal overdose (600 mg/kg, i.p.). Then, the brains were removed and snap-frozen in dry ice-cold isopentane for storage at -80°C. Twenty-micron serial coronal sections were cut through the striatum using a Model 5030 Cryostat (Bright Instruments, Huntingdon, Cambs, England, UK), with the chamber and blade maintained at -20°C and thaw-mounted onto gelatin-chrome, alum-coated glass slides, which were then desiccated and stored at -80°C until needed for the assay. Dopamine D1 receptor density was measured using the method of Cortes et al. (1). Briefly, sections were washed at room temperature in 50 mM Tris-HCl buffer at 0°C. After the assay, the slides were sequentially divided into five series, each providing a representative sample of the striatum from each mouse. Sections from the control (unfixed) series were stored overnight under vacuum at 4°C. For fixation, three of the other series of slides were incubated overnight at 4°C in 10% formalin, 4% paraformaldehyde or 3% gluteraldehyde, respectively, dissolved in phosphate-buffered saline (PBS), pH 7.4. The fifth series of slides were fixed by placing the slides in a

Figure 1. Contamination of tritium-sensitive I.P. plates by [3H]-SCH-23390 bound to striatal D1 receptors is prevented by fixation. All values are mean ± standard error of the mean (SEM).
desiccator containing paraformaldehyde powder at the base. After evacuation of the air, the slides were left overnight in the paraformaldehyde vapor at 4°C. After fixation, the slides were air-dried and then desiccated at room temperature for 3 h before exposure to a new tritium-sensitive imaging plate that had been pre-erased by exposure to bright light in an I.P. Eraser (Fuji Photo Film Ltd.) for 10 min. After a 2-day exposure, the plates were processed on a Fuji BAS 3000 (scan parameters: sensitivity 10 000, latitude 4, grey scales 4095), and the digitized images stored on an IBM-compatible personal computer. Once each imaging plate had been read, the latent image it contained was erased, and the plate was placed back in a clean autoradiography cassette and exposed for another 2 days in the absence of tissue to assess the effects of radionuclide contamination of the plate. After a 2-day exposure, the plate was read as before. Densitometry was then performed on the stored images with the Microcomputer Imaging Device (MCID) Version 4.12 suite of software (Brock University, St. Catherines, ONT, Canada) using an Autoradiographic [3H] Microscale (Amersham Pharmacia Biotech, Little Chalfont, Bucks, England, UK) exposed with each plate for calibration. Ligand binding was assessed in three coronal sections from each animal by measuring the density of binding within the boundary of the striatum visible in each hemisphere. The striatum is the major basal ganglia target of dopamine neurons from the substantia nigra in the midbrain.

In the unfixed tissue, storing plates previously exposed to [3H]-SCH-23390-labeled sections for another 2 days after erasing the primary image produced a significant secondary image (Figure 1). There are two potential sources of this secondary image. Proteins or lipids from the tissue itself might contaminate the imaging plate by a process analogous to the transfer of protein to nitrocellulose membranes during western blotting. Alternatively, [3H]-SCH-23390 might leach from the sample onto the plates. Two simple observations eliminate simple transfer of brain matter as the source of contamination: (i) the secondary image matches exactly the distribution of specific radioligand binding to its target receptors; and (ii) in control incubations to determine the extent of nonspecific ligand binding, no secondary image is seen. In these incubations, the tissue handling is identical to those that do cause contamination; the only difference being that radioligand binding has been competitively inhibited by the presence of an excess of unlabeled ligand. Thus, the presence of receptor-bound radioligand appears to be the source of the contamination. Of course, it is possible that the underlying mechanism for transfer of [3H]-SCH-23390 is movement of the protein to which the ligand is bound. However, this seems unlikely because other ligand-receptor complexes (for example, dopamine D2 receptors and the ligand [3H]- sulpiride) do not cause similar contamination. Also, it seems unlikely that the mechanism of contamination involves free diffusion of radioligand within the tissue sample. If this occurred, we would expect that autoradiograms on standard film would become fuzzy after long exposures, but they do not. Therefore, it would seem that the imaging plates have a particularly high affinity for the radioligand that facilitates movement out of the tissue.

Fixation by immersion in 10% formalin, 4% paraformaldehyde or 3% gluteraldehyde prevented the contamination, but at the expense of the initial signal strength, which was reduced to below that of the secondary image seen with unfixed tissue. Thus, it seems likely that most [3H]-SCH-23390 was displaced from its receptor by each of these fixatives. Whether this occurred as a direct result of the fixative itself or because the ligand was displaced by the PBS used as a diluent is unclear. It is unlikely that the effect is due to pH changes, since both radioligand binding and fixation were performed at pH 7.4. If this loss of signal is a result of the composition of the diluent rather than the fixatives used, it would seem likely that better recovery of the ligand might be achieved under the right conditions. However, this would probably entail adopting fixation conditions specifically tailored for each ligand to be studied.

Vapor fixation in a sealed vessel containing paraformaldehyde powder
gave the best result. The signal detected after fixation upon initial exposure of the plates was essentially the same as in unfixed tissue (Figure 1). Moreover, after storing the plates for another two days, no residual signal was seen, indicating that the fixation had successfully locked the ligand into the tissue. Thus, paraformaldehyde vapor fixation represents a simple and effective means of preventing radioligand contamination of tritium-sensitive imaging plates, and it allows the safe reuse of the plates.

REFERENCES


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Elimination of Background Due to Discharge of Static Electricity from X-Ray Film

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The use of nonisotopic nucleic acid labeling and detection systems provides numerous advantages over techniques performed with radioactively labeled probes. However, when performing chemiluminescent detection of bound probes, I am periodically plagued by background exposure on X-ray film that is apparently due to the discharge of static electricity. Following the addition of the chemiluminescent substrate, membranes must be kept wet for the remainder of the analytical process. Therefore, the membranes must be sealed in leakproof pouches, not only to prevent drying but also to avoid wetting the film. A buildup of static electricity sometimes occurs on the surface of these pouches, which creates sparks when the film is removed. This often causes a pinpoint or tree branch background pattern on the film. Since its location changes with each exposure, and since it occurs in areas of the film corresponding to membrane both with and without bound DNA, this phenomenon appears to be random. The end result is film that is often unsuitable for publication or presentation, especially when the background obscures or interferes with the interpretation of results. Attempts to solve the problem by using alternative types of heat-sealable plastic pouches or standard plastic page protectors sealed with tape were unsuccessful. Here, I demonstrate that an antistatic device that was originally intended for the elimination of static electricity from the surface of vinyl LP record albums also eliminates static-induced background from film exposed to membranes sealed in plastic pouches. The device consists of two piezoelectric crystals, contained in a small portable plastic housing that has a compression trigger. When the trigger is slowly squeezed and then released, ionization of the air results in neutralization of static charge from nearby surfaces.

Southern blots were used to demonstrate the utility of the antistatic device. Genomic DNA used for these blots was purified from Bordetella bronchiseptica using a Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) and was digested with either PvuII or NotI. Restriction fragments were separated by electrophoresis in a 0.8% agarose gel and then transferred to a charged nylon membrane (Boehringer Mannheim, Indianapolis, IN, USA) using the standard procedure (1). The membrane was hybridized to a 2.9-kb fragment of the Bordetella pertussis gene encoding filamentous hemagglutinin (fha) that was random-prime labeled with digoxigenin as described (1). Hybridization conditions, post-hybridization washes and detection using the chemiluminescent substrate disodium 3-(4-methoxyxpyrilo[1,2-dioxetane-3,2′-(5′-chloro) tricyclo [3.3.3]decane]-4-yl)phenyl phosphate (CSPD®) were performed according to the manufacturer’s recommendations (1). Membranes were heat-sealed in plastic pouches and exposed to X-OMAT® AR-5 Film (Scientific Imaging Systems, Eastman Kodak, New Haven, CT, USA) for approximately 5 min. In some cases, a Discwasher Zerotstat® 3 Anti-Static Instrument (available at music stores or from Sigma-Aldrich, Milwaukie, WI, USA) was used to neutralize static electricity before removing film from the surface of the plastic pouch.

The chemilumigraph in Figure 1A

![Figure 1](image-url)