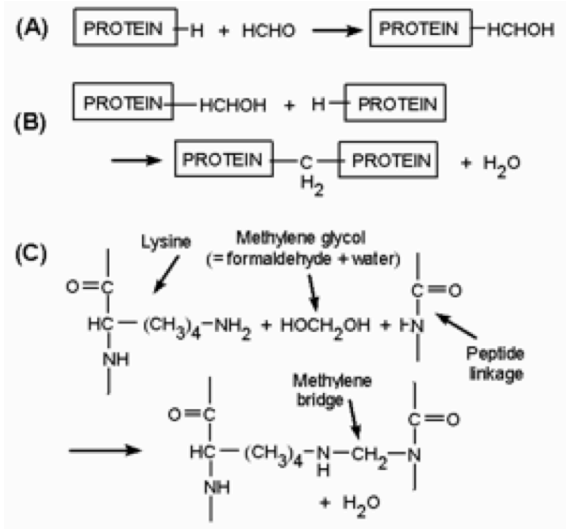
Fixing tissue for microscopy using formaldehyde

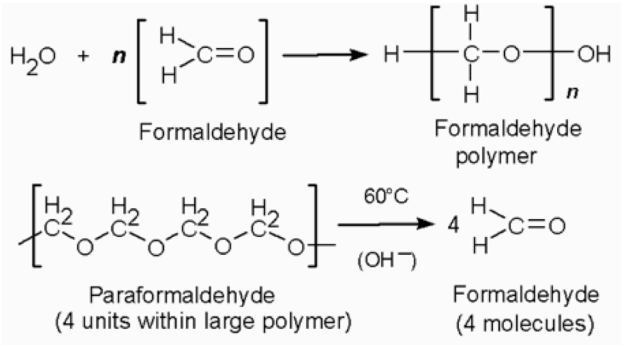
A. Background

Safety: The vast majority of fixation procedures employ formaldehyde. This is a toxin and possible carcinogen. Please read the MSDS before working with this chemical. Gloves and safety glasses should be worn and solutions made inside a fume hood.

Chemistry: Aldehydes combine with nitrogen and some other atoms of proteins, forming a "methylene bridge" between adjacent proteins. The most common cross-link is between the nitrogen at the end of the side-chain of lysine and the nitrogen of a peptide linkage. The fixative action of formaldehyde is probably due entirely to its reactions with proteins. Initial binding of formaldehyde to protein is largely completed in 24 hours, but methylene bridges form much more slowly. Substances such as carbohydrates, lipids and nucleic acids are trapped in a matrix of insolubilized and cross-linked protein molecules but are not chemically changed by formaldehyde.



Paraformaldehyde, formaldehyde, and formalin. Formaldehyde itself is a gas. Paraformaldehyde (polyoxymethylene) is a powder of polymerized formaldehyde. To be usable as a fixative, paraformaldehyde must be dissolved in hot water to become a formaldehyde solution. Formalin is a saturated formaldehyde solution in water (37% by weight, 40% by volume) containing 10-15% methanol. Methanol is often added to slow down the polymerization of formaldehyde, which would result in a paraformaldehyde precipitate. If a precipitate is present in your formalin or formaldehyde, its fixing power has been reduced.



For best results, make a solution of formaldehyde from paraformaldehyde powder, or use a sealed vial of paraformaldehyde (usually 16%). Once if vial is opened, store it in an opaque container at 4°C for a maximum of a week; after that time, the level of formaldehyde may have dropped significantly.

Protocol

1. Preparing the formaldehyde. Make a 1:1 dilution of 16% paraformaldehyde in 1x PIPES buffer (10 mM NaCl, 10 mM PIPES pH 6.8, 1 mM $MgCl_2$).

Recipe for PIPES buffer: (100 ml of 10X)
Dissolve 0.58 g NaCl in 50 ml H_20.
Dissolve 3 g PIPES in a few ml of 1 M NaOH.
Mix the PIPES with the NaCl
Add 0.2 g MgCl2•$6H_2O$.
Adjust the final volume to 100 ml with H_2O.
Filter through a 0.45 µm sterile filter.
Store in the dark at 4°C (or freeze).

2. Protocol for fixing mycelia.

a. Transfer a tuft of mycelia to ca. 200 µl clarified rye broth.
b. Add equal amount of 8% formaldehyde in 1x PIPES (from step 1).
c. Incubate at room temperature for 30 min.
d. Transfer the tuft of mycelia to 200 µl of 1x PIPES, and wait 5 min.
e. Repeat steps c and d (i.e. wash the mycelia one more time).
f. Transfer the tuft of mycelia to distilled water.
g. You are now ready to prepare slides for microscopy.

3. Protocol for fixing zoospores.

a. Make zoospores in cold sterile deionized water. A typical protocol is to:
   - add 7 ml water to each of three 60 mm plates (or 20 ml to a 100 mm plate).
   - rub with a bent rod.
   - pass the fluid through 40 micron nylon mesh.
   - put the flow-through (about 14 ml) into a prechilled glass plate.
   - wait about 90 minutes and check for zoospore release under the microscope.
   - If release is poor (<20%) wait another 40 min.
b. Carefully transfer about 7 ml of the zoospore suspension to a 15 ml orange cap tube. This is best done by tilting the glass plate, and removing the zoospores from the surface of the liquid, leaving most sporangia behind.
c. Slowly add 7 ml of 8% formaldehyde in 1x PIPES (see above), and invert twice.
d. Incubate at room temperature for 30 min.
e. Centrifuge at 1500 x *g* for 5 min.
f. Discard the supernatant by aspiration (be careful not to suck out the pellet).
g. Resuspend the pellet in 3 ml of 1x PIPES buffer (slowly with gentle agitation).
h. After 5 min, centrifuge again at 1500 x *g* for 5 min.
i. Again discard the supernatant, and repeat steps g and h.
j. Again discard the supernatant, and resuspend in about 0.5 ml water. It is a good idea to check under the light microscope to ensure that you have intact zoospores.