Radioiodination (by Jun Takagi,6/16/2000)

Purpose and backgrounds

Principle of radioiodination

Addition of oxidizing reagents (such as chloramine-T or peroxidase+H_2O_2) converts I^- to I+ or I^{3-}. This highly reactive molecule attacks o-position of tyrosine (or in some case, histidine is also labeled).

About nuclide...

125-I

decay: electron capture (g-ray, 0.035 MeV)

half life: 60 days

requires >3 mm lead plate to block >90% emission. Glass, plastic, or water cannot block.

Chemical properties...

Usually shipped as $Na^{125}I$ in alkaline solution at 1 mCi(37 MBq)/10μl.

Easily evaporates when converted into molecular gas (I2) form. Therefore, you MUST NOT freeze the vial. Upon freezing, I_2 will sublimate.

*Always stabilize ionized form (I^-) and avoid low pH and oxidizing condition for waste solution containing inorganic I^-. To accomplish this, add 1/10 vol of stabilizer (see below) to the waste solution.

Handling of $Na^{125}I$ soln in v-vial

IODINE-125(Amersham; IMS30) will be shipped in v-vial with rubber septum. NEVER remove the septum! The solution must be taken out from the vial using Hamilton microsyringe with sharp needle.

Materials

For procedure 1

- 0.1 M phosphate buffer, pH 7.0
- Tris-buffered saline (TBS)
- 5% BSA in TBS
- stabilizer soln (10% sodium thiosulfate+0.1N NaOH)
- IODO-BEAD™ (PIERCE)
- Hamilton microsyringe (model 702, 25μl, needle gauge 22S, point style #2)
- desalting column (Bio-rad DG-10 etc.)
- column stand

For procedure 2

- PBS containing 20 mM glucose
- Hepes-Tyrode buffer (or any buffer compatible with your subsequent experiments with labeled cells)
- lactoperoxidase (Sigma L-8257) dissolved in PBS at 1 mg/ml
- glucose oxidase (Sigma G-7016) dissolved in PBS at 5 U/ml

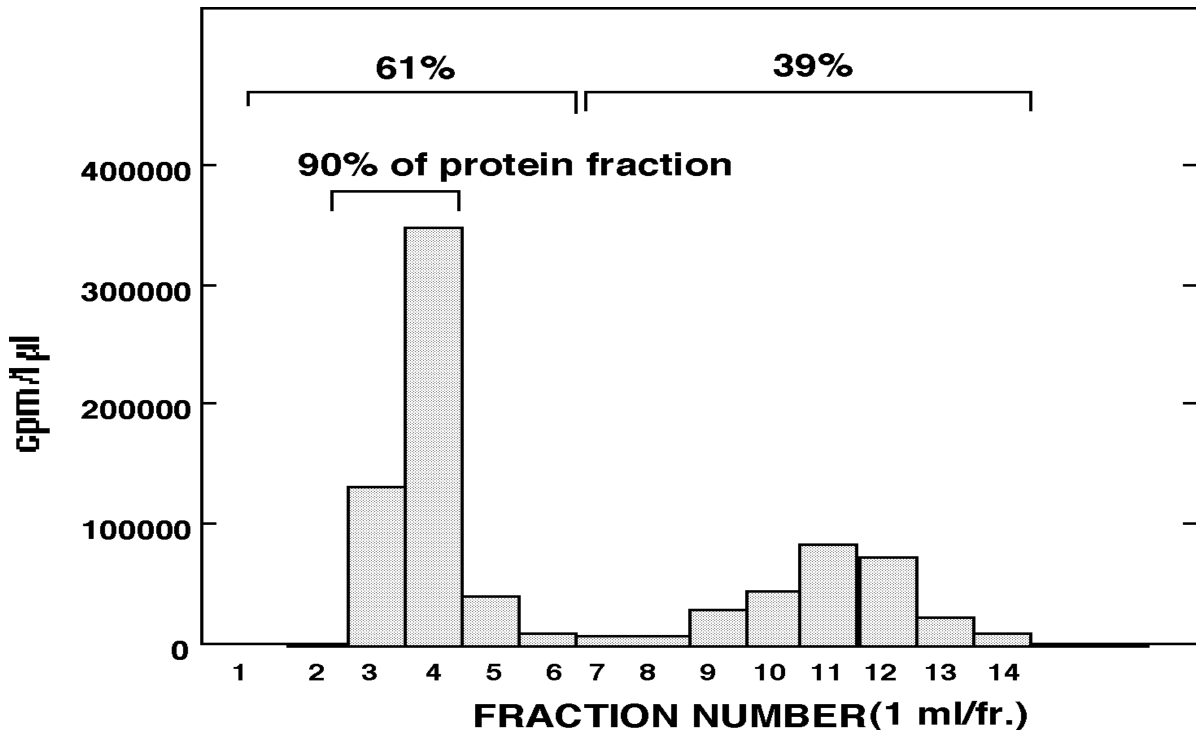
Procedure

i) Radioiodination of protein in solution

*Reading a free booklet available from Amersham ("Guide to radioiodination techniques") is recommended.

1. In clear plastic tube, add 200μl of 0.1M phosphate buffer, pH 7
2. Add 5μl (500μCi) of 125-I using microsyringe
3. Add 1-2 pieces of IODO-BEADs¶
4. incubate at room temp, 5 min
5. Add your protein(1-100μg, 25 μg is good for IgGs or Fab)
6. Incubateat room temp, 10-25 min
7. Take reaction mixture (leave beads) and apply on a desalting column§
8. Collect fractions (0.5 - 1 ml/fr.)

¶Prior to use, washed three times with phosphate buffer to remove debris on the beads)
§Block nonspecific binding site by applying 1ml of 5% BSA-TBS followed by equilibration with TBS.



Typical result with Fab fragment labeling (50μg used)

Labeling efficiency (incorporation) should be 10-90% (depends on amount of protein added). Collect appropriate fraction (in this case, fr #3+4), add 1/4 vol of 5%BSA*, and store either at 4°C or -80°C in aliquots.

*addition of high conc. BSA will guard your dilute protein from decomposition by radiation, but should not added if it interfere with subsequent experiments. However, labeled and dilute protein without carrier protein tends to flocculate and easily loses its activity.

ii).Radioiodination of cell surface proteins

1. Wash cells at least three times with PBS and resuspend in 1 ml of PBS cntg. glucose at 0.5 - 2 x 10^7 cells/ml) in 15 ml conical tube, hold on ice

2. Add 5 - 50 μl (0.5 to 5 mCi) 125I using microsyringe

3. Add 50 μl of lactoperoxidase (1 mg/ml)

4. Add 10 μl of glucose oxidase (5 U/ml)

5. on ice, 5 min (occasionally agitate)

6. Add additional 10 μl of glucose oxidase (5 U/ml)

7. on ice, 5 min (occasionally agitate)

8. Add additional 10 μl of glucose oxidase (5 U/ml)

9. on ice, 5 min (occasionally agitate)

10. Add 6 ml of PBS (termination)

11. spin down the cells (1500 rpm, 3 min)*

*transfer the supernatant to waste bottle containing stabilizing solution

12. Wash 2 more times with PBS

13. Suspend the cells in appropriate buffer for solubilization of cell surface proteins

(successful experiments will result in 10 - 30% incorporation of 125I into cells)

References

1. "Guide to radioiodination techniques"free booklet available from Amersham
2. Marchalonis, Biochem. J., 113, 299 (1969)
3. Kramer et al, Cancer Res., 49, 393 (1989)