Iodination of EGF

Materials

1. 1 mCi ^{125}I (from Amersham, IMS 30)
2. 0.25 M PO_4 buffer, pH 7.5
3. 2 μg EGF
4. 0.5 mg/ml chloramine T in 50 mM PO_4 buffer
5. 0.17 mg/ml cysteine in 50 mM PO_4 buffer
6. 50 mM PO_4 buffer with 1% BSA (~100 ml)
7. 2 mg/ml KI in 50 mM PO_4 buffer + BSA
8. PD10 column
9. 12 x 55 iodination tube

Procedure

Do the iodination in the fume hood with tube clamped to ring stand behind lead shielding.

1. Add:

1 20 μl EGF (stock soln is 100 μg/ml so this is 2 μg)
2 10 μl 0.25 M PO_4 buffer, pH 7.5
3 7.5-8 μl ^{125}I (0.8 mCi) (can use 1 mCi if desired)
4 2.5 μl chloramine T

2. Mix by vortexing.

3. Let reaction proceed approximately 45 sec. Go longer if the last batch had a low specific activity. Stop by adding:

5 100 μl cysteine
6 860 μl KI or NaI in PO_4/BSA buffer

4. Save 5 μl of this starting material for counting.

5. Have ready a PD10 column equilibrated with the 50 mM PO_4/BSA buffer (~25 ml buffer through it).

6. Apply iodination mixture (1 ml) to top. Collect 1 ml flow through at bottom as fraction 1.

7. Apply 0.5 ml of PO_4/BSA buffer and collect a total of 25 – 0.5 ml fractions into Eppendorf tubes.

8. Take 5 μl from each fraction and dilute into a tube containing 495 μl PO_4/BSA buffer. Vortex and count 5 μl.

9. The first peak of counts contains the iodinated EGF. The second peak is the free ^{125}I.

10. Calculate the % of original ^{125}I incorporated into the EGF. Knowing the mCi of ^{125}I and the μg of EGF, you can calculate the specific activity of the iodinated EGF. Aim for 200 μCi/μg. Higher than that and the EGF becomes biologically inactive.

Example of Calculations

Original counts = 58,371 x 100 (5 μl/500 μl dilution) x 200 (fraction of SM counted)
= 1.16×10^9

Counts in protein = fractions 6 –14 (depends on column peaks) x 100 (dilution factor) x 100 (5 μl of total 500 μl fraction)
= 5.9×10^8

Counts in free iodine = fraction 16-26 x 100 x 100 (as above)
= 6.0×10^8

% iodination yield = fraction in protein/total counts
= 51% (in example)

Specific activity = (800 μCi (or however much ^{125}I added)/2 μg EGF) x 0.50 (iodination yield)
= 200 μCi/μg

Pooled fractions 7,8, 9 = 4.17×10^8 cpm 1.4 μg (70% of the protein peak) in 1.5 ml
= 154 nM

Counts (for example)

1	59
2	31
3	36
4	29
5	56
6	**3396**
7	**14889**
8	**18182**
9	**8637**
10	**5220**
11	**3250**
12	**2419**
13	**1715**
14	**1186**
15	1827
16	*4224*
17	*9304*
18	*13454*
19	*13690*
20	*9240*
21	*5927*
22	*1883*
23	*681*
24	*319*
25	*201*
26	*156*
27	130
28	58371 (starting material)
29	66 (background)

Protein peak in bold

Free ^{125}I peak in italics