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## Rapid and simple microscale separation of proteins after radioiodination

The labeling of proteins with radioactive iodine, as used for example in radioimmunoassays, has become a routine procedure in many laboratories. Since the methods used for the radioiodination have become more refined during the past few years, any damage to the proteins occurring during the iodination reaction has been considerably reduced. As a consequence of this, tedious procedures for the separation of damaged from intact radioiodinated proteins has become unnecessary in many cases. Instead simple procedures for the removal of unreacted radioactive iodide from labeled proteins could be applied such as, for example, dialysis[1], gel filtration[2,3] and ion exchange[4]. The choice of method is largely dependent on the nature of the protein, its concentration and on the total amount of radioactivity used. The application of fast and simple methods is advantageous since in an iodination mixture, where the concentration of radioactivity is high and the concentration of protein is low, the protein is very susceptible to radiation damage[5]. Therefore exposure of proteins to the radioactive material should be as brief as possible between the addition of radioiodide and the separation. The method of gel filtration–centrifugation[6,7] offers a simple, rapid and practical solution especially when handling small volumes and high radioactivities, and is applicable to most proteins.

*Materials and methods*

The method used for labeling the proteins with radioactive iodine has been described elsewhere[8,9]. The concentrations of the main components present, after labeling, in the iodination mixtures which were used for these experiments were as follows: human serum albumin ~ 17 mg/ml; [$^{125}$I]-$\gamma$-globulin ~ 50 ng/ml; potassium iodide ~ 1.7 mg/ml; [$^{125}$I]iodide ~ 0.5 ng/ml, all in 0.4 *M* borate–carbonate buffer, pH 9.15.

Before the iodination mixtures were subjected to gel filtration–centrifugation, Tween-20 was added to a final concentration of 0.5 %. This addition assures high recoveries by avoiding non-specific adsorption of the proteins to Sephadex.

Sephadex G-25 coarse was swollen in 0.1 *M* Tris–HCl buffer, pH 7.7, containing 0.2 *M* NaCl, 0.002 *M* EDTA, and 0.02 % $NaN_3$. A small plexiglass tube, shown in Fig. 1, with a grid and a nylon filter disk, with a volume of 2.5 ml was filled with a thick slurry of the gel up to about 5 mm from the top edge of the tube. A supporting ring was screwed on top of the tube which then was suspended in a suitably sized centrifuge tube. Interstitial water was spun off at 1000 × *g* for 5 min resulting in a final gel bed volume of ~ 2.25 ml. The sample (iodination mixture) was carefully applied on the top of the gel surface followed by 5 min centrifugation at 1000 × *g*. The total volume recovered was determined by weight, corrected for the sample density.

Part of the original iodination mixture as well as part of the protein fraction obtained after gel filtration–centrifugation were chromatographed on conventional columns, in order to estimate the degree of "desalting" (*i.e.* the degree of separation from radioiodide) and the recovery of iodinated protein. (Column size, 0.9 × 15 cm; bed volume, 8 ml; linear flow rate, 17 cm/h; number of fractions collected, 40; material, Sephadex G-25 coarse; elution buffer, 0.1 *M* Tris–HCl, pH 7.7, containing 0.2 *M* NaCl

and 0.05 % Tween-20.) Calculations were based on the absolute radioactivities present in the protein-, and salt-peaks. The results are listed in Table I. The effect of increasing sample volume on the recovery and the "desalting" degree is summarized in Table II.

*Results and discussion*

As shown in Table I, high protein recoveries, high "desalting" degrees and small changes in volumes were obtained with good reproducibility when "desalting" 100 $\mu$l of an iodination mixture. With the device described sample volumes up to 300 $\mu$l can be applied with over 99 % degree of "desalting" and a 90 % "desalting" was still obtained with sample volumes up to 800 $\mu$l. Using larger volumes the sample may be

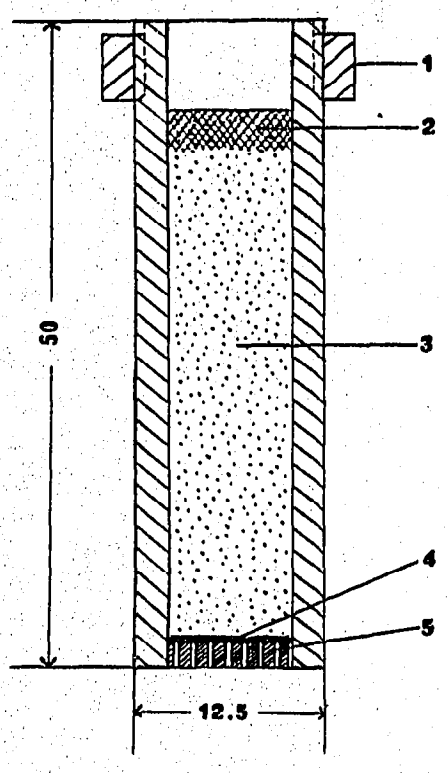


Fig. 1. Separation cell (dimensions in mm). (1) Supporting ring, holds the cell when suspended in a centrifuge tube; (2) protein solution; (3) Sephadex G-25 coarse; (4) nylon filter on the grid; (5) grid; diameter of holes 1 mm.

TABLE I

STUDY OF THE REPRODUCIBILITY OF SEPARATING 100 $\mu$l OF AN IODINATION MIXTURE

| *Experiment No.* | *Volume after separation* ($\mu$l) | *Recovery of protein* (%) | *Degree of "desalting"* (%) |
|---|---|---|---|
| 1 | 132.6 | 100.9 | 99.0 |
| 2 | 132.4 | 96.3 | 99.1 |
| 3 | 136.1 | 98.7 | 99.2 |
| 4 | 136.3 | 99.8 | 99.4 |
| 5 | 133.3 | 99.7 | 99.4 |
| 6 | 132.4 | 97.0 | 99.2 |

TABLE II

INFLUENCE OF THE SAMPLE VOLUME ON THE RECOVERY OF PROTEIN AND THE DEGREE OF "DESALTING" (*i.e.* THE DEGREE OF SEPARATION FROM RADIOIODIDE)

| *Experiment No.* | *Sample volume* ($\mu l$) | *Volume after separation* ($\mu l$) | *Recovery of protein* (%) | *Degree of "desalting"* (%) |
|---|---|---|---|---|
| 1 | 100 | 133.9 | 98.7 | 99.2 |
| 2 | 200 | 232.2 | 96.2 | 98.1 |
| 3 | 300 | 338.5 | 98.3 | 98.5 |
| 4 | 400 | 436.4 | 99.0 | 97.2 |
| 5 | 500 | 541.5 | 98.9 | 94.9 |
| 6 | 700 | 733.9 | 96.7 | 93.5 |
| 7 | 800 | 840.0 | 97.6 | 91.4 |
| 8 | 1000 | 1050.5 | 100.9 | 86.4 |

divided into smaller portions and separated on additional cells simultaneously or a correspondingly larger separation cell may be used.

The advantages of this method, for the removal of radioactive iodide from radioiodinated proteins, can be summarized as follows: (a) it is rapid and simple with good reproducibility (the "desalting" step takes less than 5 min); (b) it is suitable for very small volumes, but applicable to larger volumes as well; (c) there is little increase in sample volume after separation, *i.e.* little change in the protein concentration; (d) protein can be transferred into another buffer system during the same operation; (e) there is no risk of radioactive contamination; (f) the method can be carried out behind lead glass or lead shielding for remote control; (g) several samples can be "desalted" at once in a suitable centrifuge with a swing-out rotor by using more separation cells.

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