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Note

Simple separation of iodide from radioiodinated proteins by ion-exchange chromatography–centrifugation

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The labelling of proteins with radioactive iodine has been used increasingly during the past few years because radioimmunoassay gives improved simplification and accuracy in many routine analytical methods, not only in immunology but also in endocrinology and biochemistry. The methods used for the radioiodination of proteins have become so refined that no substantial damage to the molecules is generally involved. In order to prevent the risk of radiation injury due to a high concentration of radioactivity, it is necessary, however, to separate unreacted iodide from labelled proteins in a simple and rapid manner. This is generally performed by means of dialysis[1,2], column gel filtration[3,4], column ion-exchange chromatography[5] or batch ion-exchange chromatography[6]. In 1971, Ceska *et al.*[7] discussed the possibility of utilizing gel filtration–centrifugation for the rapid separation of iodide from the iodination mixture. They used a 40×10 mm column of Sephadex G-25 Coarse from which interstitial water had been spun off at 1000 *g* for 5 min before a 300-μl sample was applied. After subsequent centrifugation under the same conditions, they obtained radioiodinated proteins in nearly the same volume with recoveries of about 98% and only trace amounts of unreacted iodide. This procedure is highly convenient in many respects: it is rapid and simple, causes little change in the protein concentration, offers the advantages of gel filtration, such as the possibility of transfering the sample into another buffer, and at the same time permits the simultaneous separation of a number of samples of small volume, which is a distinct advantage over the column technique. This paper describes an attempt to separate unreacted iodide from labelled proteins by means of centrifugation in combination with ion-exchange chromatography.

MATERIALS AND METHODS

Bovine IgG-2 was prepared by ammonium sulphate precipitation and lyophilized after chromatography on DEAE-cellulose. Then 10 mg of IgG-2 were dissolved in 0.05 *M* phosphate buffer of pH 7.0 and labelled with carrier-free ^{131}I by the method of McConahey and Dixon[1].

For the separation of unreacted iodide from the labelled proteins, Dowex 1-X4 (Cl^-) (100–200 mesh; Fluka, Buchs, Switzerland) was employed. It was added in an amount of 28 g (wet weight) to 100 ml of 0.15 *M* sodium chloride with or without

Tween 80 or human serum albumin (30 μl/ml and 10 mg/ml, respectively). After stirring for 1 h at room temperature, the suspension was applied to columns prepared from the barrels of 2-ml disposable syringes with Silon tissue at the bottom and centrifuged in a Media 415 (Chirana) centrifuge at 1000 rpm (*ca.* 80 *g*) for 4 min, resulting in a final bed volume of *ca.* 2.5 ml. After the solution, applied in 100-μl amounts, had been taken up, the columns were centrifuged for a second time and the centrifugates were weighed. In assessing the volume of the centrifugate, the specific gravity was taken to be 1.000.

The radioactivity of aliquots of the centrifugate was measured in an NK 101 apparatus with a well-type crystal (Gamma, Hungary). The results were corrected for changes in volume after the second centrifugation.

Checks on the completeness of removal of ^{131}I from the proteins were made by thin-layer gel filtration on Sephadex G-200 Superfine[8].

RESULTS AND DISCUSSION

Changes in the protein volume and recovery after application of 100-μl aliquots of radioiodinated proteins without unreacted iodide are presented in Table I. The results suggest that the sample became only slightly diluted during separation but that a considerable loss in activity occurred on equilibration of Dowex in 0.15 *M* sodium chloride solution. This prompted our attempt to reduce the adsorption of radioiodinated protein on to Dowex by equilibrating it in 0.15 *M* sodium chloride solution containing Tween 80 or albumin. Under these conditions, the protein recoveries were increased to 61.3 and 59.8% and the sample volume increased to 107.1 and 120% for Tween 80 and albumin, respectively.

TABLE I

INFLUENCE OF DOWEX EQUILIBRATION WITH TWEEN 80 AND ALBUMIN ON THE RECOVERY OF [^{131}I]IgG (100 μl)

Results are mean values ± standard errors of the means from four parallel determinations.

Equilibration	*Centrifugate volume (μl)*	*% of initial activity*
0.15 *M* NaCl	123.2 ± 2.23	11.13 ± 0.9
0.15 *M* NaCl with Tween 80	107.08 ± 1.78	61.30 ± 6.23
0.15 *M* NaCl with albumin	120.13 ± 2.36	59.80 ± 2.48

When the volume of protein applied was increased, the recovery also increased; the application of sample volumes ranging from 600 to 3000 μl resulted in a small decrease in the centrifugate volumes (Table II).

When Tween 80 was added to the solution of radioiodinated proteins (30 μl of Tween 80 per 4 ml of protein) and the Tween-treated proteins were applied in increasing volumes, there was a progressive increase in recovery rate and a small increase in centrifugate volume (Table III).

Addition of protein carrier (100 mg of IgG per 12 ml of [^{131}I]IgG) was also

TABLE II

INFLUENCE OF INCREASING [^{131}I]IgG VOLUME ON THE RECOVERY

Volume applied to the column (μl)	*% of initial activity*	*Centrifugate volume (μl)*	*% of initial volume*
100	49.34	126.3	126.30
300	68.79	304.3	101.33
600	68.75	555.9	92.65
900	71.12	854.9	94.98
1500	67.75	1388.5	92.56
3000	86.74	2841.5	94.71

TABLE III

INFLUENCE OF ADDITION OF TWEEN 80 TO [^{131}I]IgG ON THE RECOVERY

Volume applied to the column (μl)	*% of initial activity*	*Centrifugate volume (μl)*	*% of initial volume*
100	31.43	173.8	173.80
300	77.80	373.2	124.40
600	89.66	661.3	110.21
900	102.21	1175.0	130.55

TABLE IV

INFLUENCE OF PROTEIN CARRIER ON THE RECOVERY OF [^{131}I]IgG

Volume applied to the column (μl)	*% of initial activity*	*Centrifugate volume (μl)*	*% of initial volume*
100	52.95	132.0	132.00
300	65.05	321.4	107.13
600	78.68	627.2	104 53
900	76.94	916.7	101.85
1500	85.31	1476.5	98.43

found to result in a higher recovery rate and small changes in centrifugate volumes with increasing sample volumes (Table IV). In order to determine the recovery of the solution containing protein carrier, Dowex was equilibrated in 0.15 *M* sodium chloride solution without Tween 80.

An investigation into the effects of the speed of centrifugation using a sample volume of 300 μl showed that centrifugation at 1500 and 2000 rpm yielded higher protein recoveries than did centrifugation at 1000 rpm (Table V).

The column of Dowex equilibrated in 0.15 *M* sodium chloride solution with Tween 80 took up almost 100% of iodide in sample volumes up to 3000 μl. A small increase in the centrifugate radioactivity with a sample volume of 3000 μl occurred only in the case when carrier potassium iodide was added (Table VI).

TABLE V

INFLUENCE OF THE SPEED OF CENTRIFUGATION ON THE RECOVERY OF [^{131}I]IgG

The results are mean values from two measurements.

Volume applied to the column (μl)	*rpm*	*g value (approx.)*	*% of initial activity*	*Centrifugate volume (μl)*	*% of initial volume*
300	1000	80	53.91 ± 7.95	276.25 ± 0.65	92.08
300	1500	180	74.77 ± 0.52	277.85 ± 9.25	92.62
300	2000	325	74.99 ± 0.05	260.1 ± 9.30	86.70

TABLE VI

UPTAKE OF ^{125}I-LABELLED POTASSIUM IODIDE BY DOWEX (EQUILIBRATED IN 0.15 *M* SODIUM CHLORIDE SOLUTION WITH TWEEN 80)

K^{125}I	*Volume applied to the column (μl)*	*% of initial activity*
With carrier (0.25 ml of 1% KI per 12 ml of K^{125}I)	200	0.25
	600	0.24
	1500	0.21
	3000	0.91
Without carrier	200	0.15
	600	0.24
	1500	0.11
	3000	0.12

From the results obtained, it appears that ion-exchange chromatography–centrifugation is a very suitable method for the removal of unreacted iodide from an iodination mixture. It offers advantages similar to those of gel filtration–centrifugation, *i.e.*, it is rapid and simple, causes little change in sample volume after separation and little change in the protein concentration, permits the simultaneous separation of several samples and, in addition, permits the separation of larger volumes of iodination mixtures with complete removal of unreacted iodide.

REFERENCES

1 P. J. McConahey and F. J. Dixon, *Int. Arch. Allergy Appl. Immunol.*, 29 (1966) 185.
2 M. Morrison, G. S. Bayse and R. G. Webster, *Immunochemistry*, 8 (1971) 289.
3 W. M. Hunter and F. C. Greenwood, *Nature (London)*, 194 (1962) 495.
4 J. I. Thorell and B. G. Johansson, *Biochim. Biophys. Acta*, 251 (1971) 363.
5 A. S. McFarlane, *J. Clin. Invest.*, 42 (1963) 346.
6 T. Chard, M. J. Kitau and J. Landon, *J. Endocrinol.*, 46 (1970) 269.
7 M. Ceška, P. Pihl, L. Holmqvist and F. Grossmüller, *J. Chromatogr.*, 57 (1971) 145.
8 K. J. Hruška and M. Fránek, *J. Chromatogr.*, 93 (1974) 475.