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Note

Application of thin-layer gel filtration in the microanalysis of radioiodinated proteins

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The increased use of iodine-labelled proteins in biology has prompted attempts to obtain radioiodinated proteins that remain basically unchanged and has given rise to the need for their reliable characterization. It is well established, for example, that the degree of iodine substitution in the protein influences antibody activity[1], which suggests that changes in the characteristics and biological activity of proteins may be conditioned by specific radioactivity. Before using a radioiodinated protein, it is convenient, if not even necessary, to analyse the iodination mixture, to remove unreacted iodide and to check the purity of the protein. This is generally performed by means of dialysis[2], precipitation with trichloroacetic acid[3,4], paper electrophoresis or chromatography[5], high-voltage chromato-electrophoresis[6,7], filter-paper diffusion in combination with trichloroacetic acid precipitation[8] or column gel filtration[9].

This paper describes the application of thin-layer gel filtration on Sephadex in the analysis of radioiodinated proteins. The method permits the relatively rapid microanalysis of the iodination mixture as well as of radioiodinated proteins after removal of free radioactivity and indicates the presence of by-products in the iodination mixture on the basis of their different molecular weights.

MATERIALS AND METHODS

Human serum albumin and bovine IgG were labelled with ^{131}I using carrier-free potassium iodide according to McConahey and Dixon[10]. Bovine IgG-2 was prepared by ammonium sulphate precipitation and lyophilized after gradient chromatography on DEAE-cellulose. Lyophilized human serum albumin (supplied by Imuna, Šarišské Michalany, Czechoslovakia) had an electrophoretic purity of 97.5%. Unreacted and protein-bound iodine was separated by ion-exchange chromatography–centrifugation[11].

In preparing Sephadex G-200 Superfine or Sephadex G-75 Superfine layers, an apparatus for thin-layer chromatography (Shandon Southern Instruments, Camberley, Great Britain) has proved very useful, although good results can also be obtained with other devices or using the following procedure. Glass plates (20×5 cm) are overlaid with agar, of which margins of only 1 cm in width are allowed to remain after solidification in order to serve as guide bars for removing excess of Sephadex by means of a plastic spatula. Separation can be performed in any moist chamber. In our

experiments; the angle of slope of the layers was 15° and elution was carried out with 0.2 *M* Tris buffer of pH 8.0 containing Tween 80 (60 μl per 100 ml of buffer), which is known to reduce the adsorption of the proteins on to Sephadex. The protein solution to be analysed was applied in amounts of 5–10 μl at a distance of 1 cm from the filter-paper wedge. The separations took 45–60 min on Sephadex G-75 and 3–4 h on Sephadex G-200. Sephadex G-200 layers were dried at 60° before detection but Sephadex G-75 layers, on the other hand, fail to adhere to glass surfaces after drying and were therefore dried only partially.

The radioactivity was detected and recorded using (a) an Actigraph III apparatus (Nuclear Chicago, Des Plaines, Ill., U.S.A.), (b) autoradiography (Medix-Rapid R3 X-ray film, Fotochema, Hradec Králové, Czechoslovakia; exposure for 2 days) with subsequent densitometric evaluation of the spots in a Vitatron (UFD) apparatus

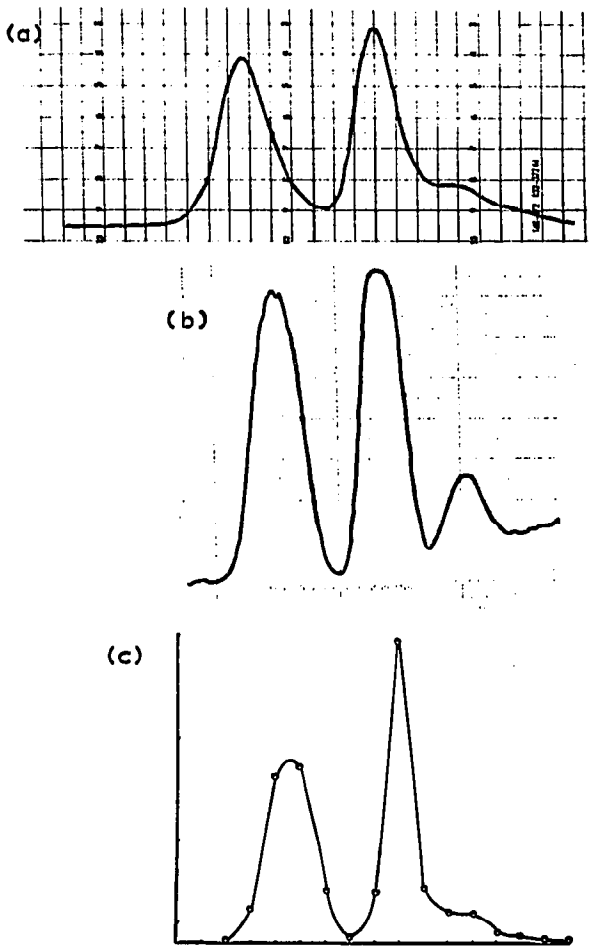

Fig. 1. Detection of radioactive fractions of an albumin iodination mixture after separation on Sephadex G-200 Superfine (separation period 4 h). (a) Detection by Actigraph; (b) densitometric evaluation of autoradiograph in a Vitatron apparatus (only of qualitative value owing to the slit employed); (c) radioactivity of strips 1 cm wide.

(Vitatron, Dieren, The Netherlands) or, (c) simple evaluation of the separated fractions by direct measurement. For these experiments, a Sephadex layer was prepared on plastic foil, which was cut up into strips 1 cm wide after the separation had been completed. The Sephadex was then washed into test-tubes with 2 ml of water and the radioactivity was measured in a Tesla NZQ 112 apparatus with a well-type crystal.

RESULTS AND DISCUSSION

The pattern of fractionation of the iodination mixture on Sephadex G-200 as evaluated by the Actigraph is shown in Fig. 1a. The first peak represents the radioactivity of iodide, the second peak labelled protein and the third peak a polymeric by-product which could develop either as a result of protein lyophilization and storage or during the labelling process. The same layer was also evaluated by autoradiography on X-ray film (Fig. 1b). It can be seen that this procedure, in conjunction with densitometric evaluation, also yields good results but requires more time. The result

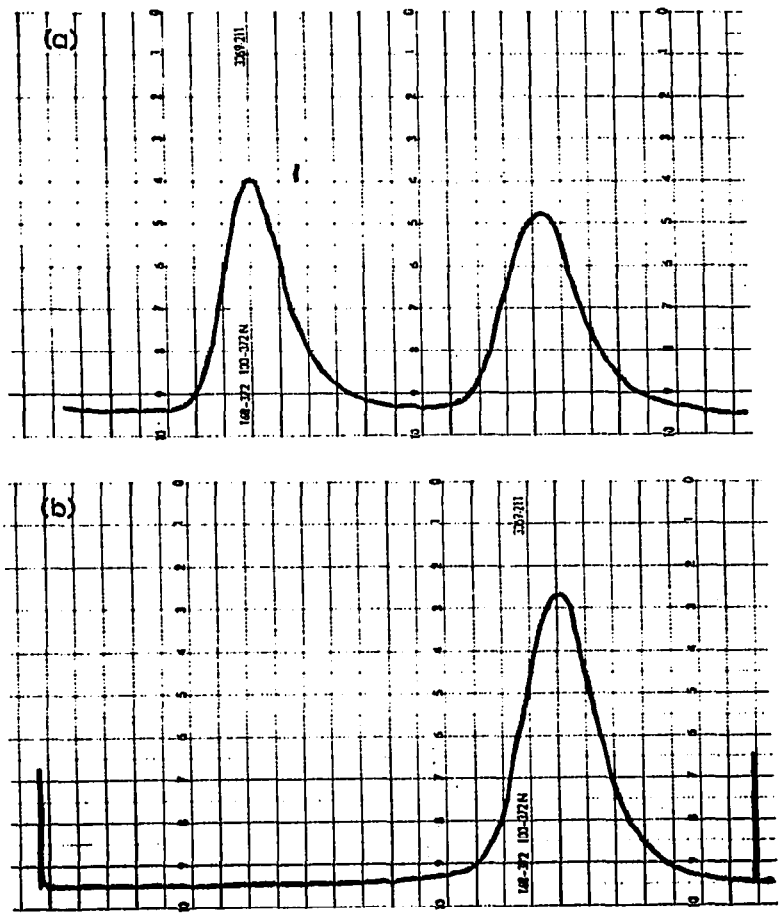

Fig. 2. Separation of an iodination mixture and [^{131}I]albumin on Sephadex G-75 Superfine (separation period 45 min; detection by Actigraph). (a) Albumin iodination mixture; (b) [^{131}I]albumin.

of the separation of the same iodination mixture after evaluation by measurement of the radioactivity on 1-cm strips is shown in Fig. 1c. This evaluation can be performed even in laboratories without elaborate equipment.

Fig. 2 shows the results of the separation of the same albumin iodination mixture and of labelled albumin alone on Sephadex G-75. In this case, the fractionation required less time (45 min) and there was a good separation of the free and protein-bound radioactivity, but the presence of the polymeric by-product could be demonstrated only by separation on Sephadex G-200, which took 4 h (Fig. 1).

There is evidence to indicate that the presence of degraded products in the initial preparation may reduce the extent of iodination. It can be seen from Fig. 3 that the iodination mixture (Fig. 3-Ia) contained a proportion of low-molecular-weight products capable of binding iodine because separation on Sephadex after ion-exchange chromatography–centrifugation yielded two peaks of which only part of the first was taken up by Dowex (Fig. 3-Ib). Before further labelling, the low-molecular-weight products were therefore eliminated by gel filtration on columns of Sephadex G-75 and this purification of the protein resulted in a considerable increase in protein-bound radioactivity (Fig. 3-IIa). After the unreacted iodide had been removed by ion-exchange chromatography–centrifugation, the separation on the Sephadex G-200 layer yielded the characteristic peak of labelled protein (Fig. 3-IIb).

From our experiments, it appears that the application of thin-layer gel filtration

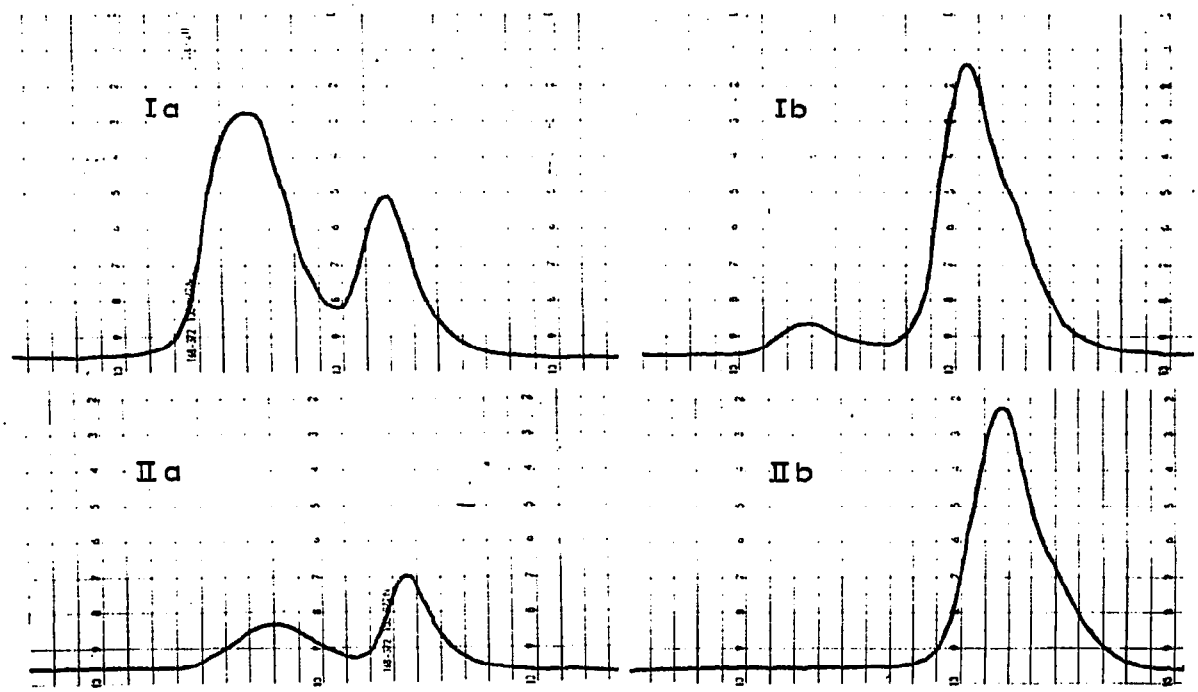

Fig. 3. Demonstration of degraded IgG after iodination (Sephadex G-200 Superfine; separation period 3 h; detection by Actigraph). Degraded IgG (I) and purified IgG (II), iodination mixture (a) and radioiodinated protein after removal of unreacted iodide by ion-exchange chromatography–centrifugation (b).

in microradioanalysis is very useful. Its advantages include a small consumption of the sample (5–10 μl are sufficient for analysis), rapid separation and the possibility of the simultaneous analysis of several samples. Using any of the procedures for detecting radioactivity as described here, the method provides information not only on the ratio of free to protein-bound radioactivity but also on the possible protein degradation or polymerization incurred during iodination. It is generally applicable to the separation of low- and high-molecular-weight components of iodination mixtures and can be employed, for example, for the separation of radioiodinated low-molecular-weight ligands and of their respective binding proteins.

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