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Note

Gel filtration of thyroxine-binding proteins

Systematic study of rat liver

The gel filtration of thyroxine (T_4) and its analogues had been of much interest for a number of years. Gel filtration was used for the separation of free and bound T_4 in blood plasma[1,2], for the separation of various iodinated amino acids[3–6], and in the determination of the binding of these compounds to proteins[7]. However, there are some problems that limit the use of gel filtration of iodinated amino acids on Sephadex, due mainly to the adsorption of the aromatic moieties of the above compounds on polydextran gel[8,9].

On the other hand, gel filtration is an excellent method for separation of proteins. It has been used for the separation of iodoproteins in serum[10] as well as in some steps in the isolation of thyroxine-binding serum proteins[11]. HAMADA *et al.*[12] used separation on Sephadex G-100 for the presentation of specific T_4 and triiodothyronine binding proteins in soluble fractions of rat-liver homogenates. The aim of the present work was to make a systematic study of the gel filtration behaviour of soluble thyroxine-binding proteins of rat-liver homogenates.

Materials and methods

The arteries of a rat liver were washed *in vivo* with an isotonic solution of saccharose so as to free the tissue from residual blood. The liver was then homogenized in four volumes of 0.9% saline solution at 0° and the homogenate was centrifuged at 4° for 30 min at 10 000 *g*. Portions of 0.5 ml of the clear supernatant were kept frozen at −10 to −15°, and before each experiment 0.5 μCi of I^{125}-labelled radiothyroxine (RT_4) (Radiochemical Centre, Amersham, Great Britain) was added to 0.5 ml of thawed homogenate and incubated for about 1 h in order to bind the thyroxine to its carrier proteins.

Chromatographic columns (95 cm × 1 cm I.D.) of Sephadex G-75, G-100, G-150 and G-200 (Pharmacia, Uppsala, Sweden) were prepared[13] in Tris–HCl buffer (pH 7.4; 0.01 *M*). Then 0.5 ml of the RT_4-labelled homogenate was applied to the top of the column and eluted with the same Tris–HCl buffer, and 1-ml fractions of the eluate were collected on a fraction collector (Fractiomat, Budapest, Hungary). This separation was performed in a cold room (4°), where the fractions obtained were stored until further processing.

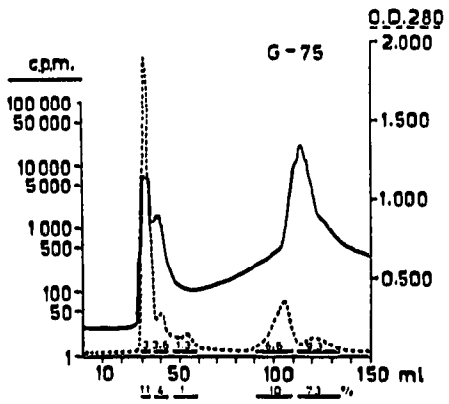
The radioactivity of each fraction was measured in an automatic gamma-counting system (Series 1185, Nuclear-Chicago, Chicago, Ill., U.S.A.) and the protein concentrations of the same fractions were determined by measuring their optical densities at 280 nm in a spectrophotometer (VSU 1, Zeiss, Jena, G.D.R.). The ratio of the radioactivity in counts per minute divided by the relative protein concentration

(1000 × O.D.$_{280}$) was calculated, and represents a relative measure of the amount of thyroxine bound to a unit amount of protein.

According to the peaks of radioactivity and optical density, some fractions were combined and freeze-dried. The radioactivity of each such peak was expressed as a percentage of the total radioactivity from all of the peaks in each chromatographic separation.

Results

Fig. 1 shows the separation of RT_4-labelled rat-liver homogenate on Sephadex G-75. The clear separation of proteins into two main peaks shows that the main T_4-binding proteins are in the second, low molecular weight, peak. The molecular weights of proteins and peptides in this peak should be below 50 000. Most of the other proteins and probably some residual constituents of the liver cells are in the first, high molecular weight, fraction. However, this fraction contains only an insignificant amount of bound thyroxine.



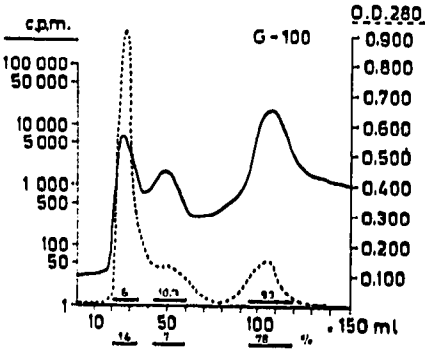


Fig. 1. Separation of radiothyroxine-labelled homogenate of rat liver on Sephadex G-75. Full line, radioactivity (counts per minute); broken line, optical density at 280 nm. Bars indicate collected fractions; the number above the bar represents the average relative specific radioactivity, and the number below the bar represents the percentage of radioactivity in each collected fraction.

Fig. 2. Separation of radiothyroxine-labelled rat-liver homogenate on Sephadex G-100. Details as in Fig. 1.

The residual free thyroxine that was not bound by the liver proteins remains adsorbed on the Sephadex gel[8] and is eluted only by prolonged washing[3]. The results of STERLING *et al.*[11] showed that the molecular weight of the thyroxine-binding globulin in human blood plasma is approximately 35 000. If the thyroxine-binding proteins of the rat liver are of approximately the same weight, it is reasonable to expect them to appear in the second fraction during gel filtration on Sephadex G-75.

A separation similar to that achieved on Sephadex G-75 is also obtained on Sephadex G-100 (Fig. 2). Again, most of the high molecular weight proteins in the first two peaks contain about one fifth of all of the added RT_4, while in the third,

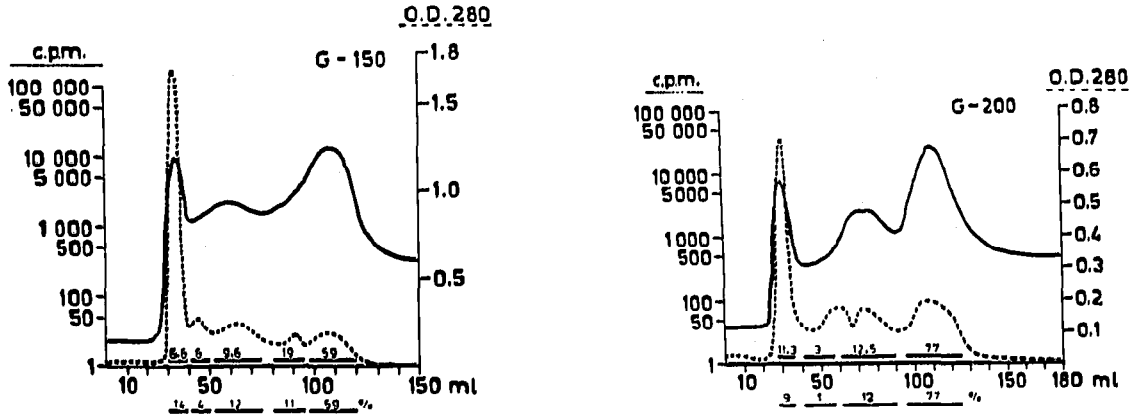


Fig. 3. Separation of radiothyroxine-labelled rat-liver homogenate on Sephadex G-150. Details as in Fig. 1.

Fig. 4. Separation of radiothyroxine-labelled rat liver homogenate on Sephadex G-200. Details as in Fig. 1.

low molecular weight peak, the thyroxine: protein ratio is approximately ten times higher than that in the first two peaks.

The same general pattern of distribution of T_4-binding proteins from rat-liver homogenate was also achieved on both Sephadex G-150 and G-200 (Figs. 3 and 4). The highest T_4: protein ratio and, therefore, the specific T_4-binding proteins were always found in the last peak, containing the lowest molecular weight proteins.

It could therefore be concluded that the total proteins in the low molecular weight peak obtained by separation on Sephadex G-75 could provide a fraction that is enriched with specific T_4-binding proteins. This fraction could be used as a starting material for further purification of the T_4-binding proteins of rat liver.

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