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Note

Separation of radioiodine-labelled 3,3'5'-triiodothyronine (reverse T3) by adsorption chromatography

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Dextran gels such as the highly crosslinked Sephadex LH-20 reversibly adsorb organic acids and bases, especially those which contain an aromatic ring and iodine[1]. The separation of organic acids, including iodothyronines, by adsorption chromatography can be performed by making use of the fact that organic ions are partially excluded from the gel[2]. Fractionations of thyroxine and 3,3',5-triiodothyronine can easily be achieved by adjusting the pH of the eluent between the two pK_{OH} values[3].

Separation near the pK_{OH} value is governed by the different adsorption affinities of the ionized and un-ionized forms towards the gel. At low pH values, however, where dissociation of the phenolic hydroxyl group is negligible, segregation of iodothyronines can be carried out by utilizing the fact that the adsorption affinity increases with increasing number of iodine atoms in the molecule[4,5]. Although no convincing experimental results concerning the nature of the interaction between dextran gel and iodothyronines are available, it seems plausible that the hydroxypropyl groups of the gel on the one hand and the iodine substituents, the phenolic hydroxyl group and, to a lesser extent, the aromatic ring on the other are responsible for the interaction[6].

The aim of this paper is to present an example of the separation of 3,3'-diiodothyronine (T-2), 3,3',5-triiodothyronine (T3) and 3,3',5'-triiodothyronine (rT3) on the basis of the pK_{OH} value and the iodine content.

EXPERIMENTAL

The chloramine-T labelling method was used to produce rT3 and T3 labelled with I-131 and/or I-125[3,7].

Sephadex LH-20 dextran gel, previously swollen for 12–24 h in distilled water, was used as an adsorbent. The gel was poured into a glass tube (length 130 mm, I.D. 10 mm), in the bottom of which was a porous disc. A volume of 0.1–0.3 ml of the reaction mixture from the chloramine-T labelling procedure was added to the column. After adsorption equilibrium had been attained (15–30 min), the free iodine was washed out with distilled water. This procedure did not result in any displacement of the iodothyronines from the top of the gel.

The effluent was passed over a sodium iodide (thallium) scintillation crystal and the count rate was recorded with an X-ray plotter. The flow-rate was adjusted

to 20–25 ml/h. The pH of the eluent was adjusted to the required value by means of citrate and phosphate buffer.

RESULTS

Fig. 1 shows the elution curve obtained when the reaction mixture from the chloramine-T labelling procedure was chromatographed at pH 4 with 30% ethanol as the eluent. Co-chromatography of T3 and rT3 at pH 4 failed to effect any separation (Fig. 2).

The elution pattern of T3 and rT3 obtained when using an eluent of the same ethanol concentration (30 V%) but of pH 8.4 is shown in Fig. 3.

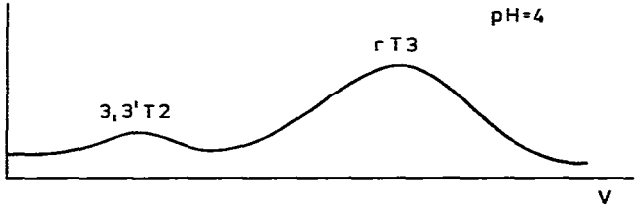

Fig. 1. Elution pattern obtained when the reaction mixture from the chloramine-T labelling of rT3 was chromatographed. Eluent, 30% ethanol; pH = 4.

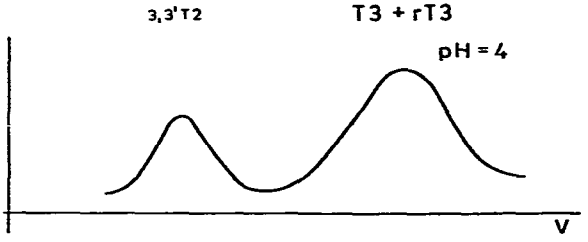

Fig. 2. Elution pattern obtained when the reaction mixture from the chloramine-T labelling of rT3 and labelled T3 was co-chromatographed. Eluent, 30% ethanol; pH = 4.

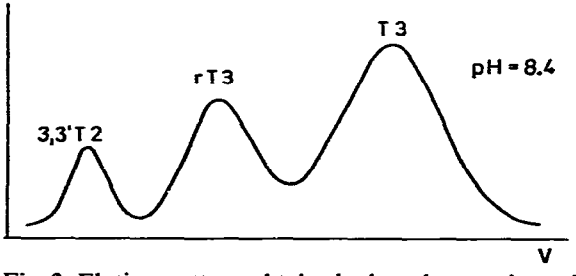

Fig. 3. Elution pattern obtained when the reaction mixture from the chloramine-T labelling of rT3 and labelled T3 was co-chromatographed. Eluent, 30% ethanol; pH = 8.4.

From the elution curves in Figs. 1–3, the conclusion can be drawn that at pH 4, *i.e.* where the ionization of the phenolic hydroxyl group is negligible, the two iodothyronines that contain three iodine atoms (*i.e.*, rT3 and T3) exhibit the same distribution coefficient. Splitting of the adsorption affinity and thus the distribution coef-

ficient, which makes separation possible, becomes considerable above pH 6.5, where ionization of the phenolic hydroxyl group of rT3 has to be taken into account.

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