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Note

Adsorption chromatographic behaviour of ^{125}I-labelled progesterone-11α- and -12α-succinyltyrosine methyl ester

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Sephadex LH-20 dextran gel is frequently used to separate small molecules according to the number of iodine substituents. The gel can distinguish small molecules and their mono- and polyiodo-substituted derivatives, and enables the separation of ^{125}I-labelled iodothyronines[1] and steroids to which a tyrosine methyl ester prosthetic group is coupled[2–5]. It was considered that Sephadex LH-20 gel cannot distinguish isomers like 3,3′,5-triiodothyronine and 3,3′,5′-triiodothyronine (rT3) which are eluted as a single peak provided the phenolic hydroxyl group is not ionized[5].

This paper reports on the chromatographic behaviour of progesterone-11α-succinyltyrosine methyl ester (11-PTME) and progesterone-12α-succinyltyrosine methyl ester (12-PTME) labelled with ^{125}I at position 3 of the tyrosine methyl ester (TME) residue.

EXPERIMENTAL

The labelling method, apparatus and adsorbent used were described previously[1–5]. 11-PTME or 12-PTME was labelled with ^{125}I by the chloramine T method. To 10–20 μg (2–4 μmol) of 11-PTME or 12-PTME, 1–2 mCi (0.5–1.0 nmol) of ^{125}I in slightly alkaline solution were added followed by 200–300 μg of chloramine T in 50 μl of phosphate buffer. After 30–60 s the labelling reaction was quenched with 700 μg of sodium metabisulphite in 100 μl.

Sephadex dextran gel, swollen in distilled water for 12–24 h, was poured into a glass tube (130 mm × 10 mm I.D.) the bottom of which was equipped with a porous disc. The sample (0.1–0.2 ml) was placed on the top of the column and allowed to soak in; 10–20 min later, *i.e.*, when adsorption equilibrium had been attained, elution was performed with ethanol–water. The pH of the eluent was adjusted to the required value with citrate and borate buffers. The effluent was passed over a NaI/Tl scintillation crystal and the count rate was monitored by a rate meter and registered by an x-y plotter. The eluent was delivered by a peristaltic pump at a flow-rate of 22–24 ml/h.

The distribution coefficient was calculated according to

$$k = \frac{V_e - V_0}{W} \qquad (1)$$

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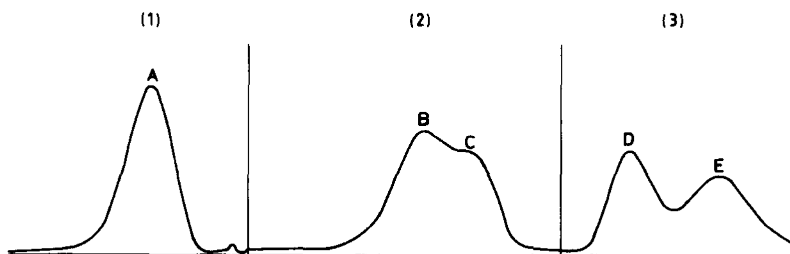

Fig. 1. Elution patterns obtained when ^{125}I-11-PTME and ^{125}I-12-PTME were co-chromatographed. (1) 30% ethanol, A = ^{125}I-11-PTME and ^{125}I-12-PTME. (2) 20% ethanol, B = ^{125}I-11-PTME, C = ^{125}I-12-PTME. (3) 10% ethanol, D = ^{125}I-11-PTME, E = ^{125}I-12-PTME. pH 4.

where V_e, V_0 and W are the elution volume, the dead volume and the weight of the adsorbent, respectively.

RESULTS

Fig. 1 shows the elution pattern obtained when aqueous ethanol of different concentrations was used as the eluent, the pH being adjusted to 4 so as to prevent dissociation of the phenolic hydroxyl group of the TME residue. Two conclusions can be drawn. On the one hand, with decreasing ethanol concentration the elution volumes of ^{125}I-11-PTME and ^{125}I-12-PTME increase, on the other hand the difference between the elution volumes of the two progesterone derivatives increases. While, *e.g.*, with 30% ethanol, both ^{125}I-11-PTME and ^{125}I-12-PTME are eluted as a single peak, when 10% aqueous ethanol is used as the eluent almost complete separation can be achieved.

As was anticipated on the basis of previous experiments[1], an increase in pH, which results in dissociation of the phenolic hydroxyl group at around pH 9, drastically decreases the elution volume and distribution coefficient. This is clearly demonstrated by the plots in Fig. 2 of the distribution coefficient of ^{125}I-11-PTME and ^{125}I-12-PTME as a function of the pH of the eluent.

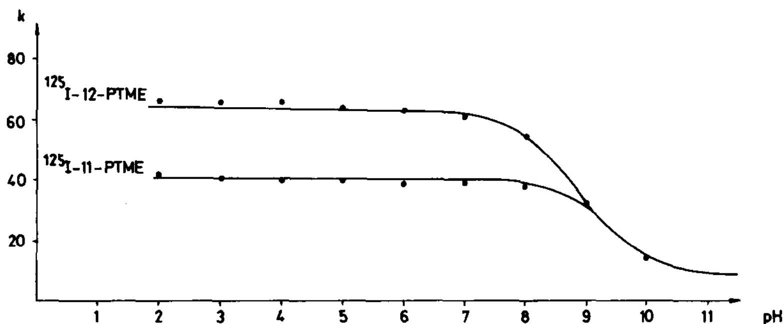

Fig. 2. The distribution coefficients of ^{125}I-11-PTME and ^{125}I-12-PTME as a function of the pH. Eluent: 10% ethanol.

Assuming that k reflects the free energy of adsorption of a solute[6]

$$\log k = \log V_a + \frac{G}{2.3\ RT} \tag{2}$$

the free energy change due to the ionization of the phenolic hydroxyl group corresponds is 1100 cal/mol in the case of ^{125}I-12-PTME and 820 cal/mol in the case of ^{125}I-11-PTME. It should be noted that at pH 9, *i.e.*, where the ionization of the OH group is practically complete, the distribution coefficients of ^{125}I-11-PTME and ^{125}I-12-PTME are the same. The finding that, at pH 7, ^{125}I-11-PTME and ^{125}I-12-PTME exhibit different elution volumes may be explained by the difference in stereochemistries of the two derivatives.

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