Journal of Chromatography, 213 (1981) 511–514
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,893

Note

Adsorption chromatographic separation of [^{125}I]progesterone-succinyl-tyrosine methyl ester

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(Received April 14th, 1981)

Radioiodine-labelled steroids, *e.g.* progesterone, are frequently used as tracers in radioimmunoassay. Their high specific activity compared with the tritium-labelled compounds makes possible a considerable increase of the sensitivity, *i.e.* a decrease of the minimum detectable amount.

There are two ways to produce radioiodine-labelled steroids. One consists of coupling tyrosine methyl ester or histamine through a succinyl group to the steroid molecule[1]; the other one is the so-called direct labelling, in the course of which radioiodine is introduced into the steroid skeleton itself[2,3].

So as to make use of the theoretically high specific activity of the ^{125}I-labelled steroids, it is of vital importance to separate the ^{125}I-labelled and the unlabelled molecules because the latter would decrease the specific activity to an extent depending on the amount of starting material used in the labelling procedure.

The aim of this paper is to report a method for the separation of the starting material used in the chloramine-T labelling procedure, *i.e.*, progesterone-11-succinyl-tyrosine methyl ester (PSTME), shown in Fig. 1, mono- and diiodo-PSTME, and unreacted free radioiodine. The method is based on a special feature of the Sephadex LH-20 dextran gel: it adsorbs low-molecular-weight substances with iodine substituents, and the adsorption affinity increases with the increasing number of iodine

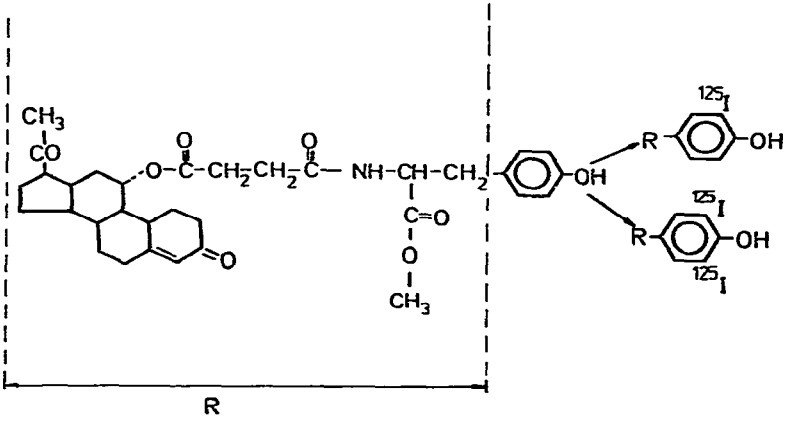

Fig. 1. Mono- and diiodo derivatives formed in the course of the chloramine-T labelling.

0021-9673/81/0000–0000/$02.50

atoms per molecule[4–6]. On the other hand, by variation of the composition of water–organic solvent binary eluent, the distribution coefficient of the target molecule can be adjusted to any desired value, which enables the optimum balance to be achieved between a high radioactive concentration and good purity of the separated products.

MATERIALS AND METHODS

PSTME was labelled with ^{125}I by the use of the chloramine-T method. To 10 μg (0.01 μmole) of PSTME dissolved in 50 μl of phosphate buffer (pH 7.4), 1–2 mCi (0.5–0.1 nmole) of carrier-free ^{125}I was added in slightly alkaline solution, followed by the addition of 24υ μg of chloramine-T in 50 μl of phosphate buffer. The labelling reaction was quenched after 30–60 sec with 700 μg of sodium metabisulphite in 100 μl.

Sephadex LH-20 dextran gel, swollen in distilled water for 12–24 h, was poured into a glass tube (130 × 10 mm I.D.), the bottom of which was equipped with a porous disc. So as to check the efficiency of the separation of the starting material (PSTME) from the labelled products as well as free radioiodine, tritium-labelled PSTME was also chromatographed separately from the chromatography of the chloramine-T labelling mixture. In both cases the sample (0.1–0.2 ml) was placed on the top of the column and was allowed to soak in. After 10–20 min, *i.e.* when adsorption equilibrium had been attained, the elution was started with aqueous citrate buffer (pH 2) or water–ethanol binary eluent (pH 2).

When tritium-labelled PSTME was chromatographed the effluent was collected with a fraction collector, and its radioactivity was determined by liquid scintillation counting. In case of the ^{125}I-labelling mixture, the effluent was passed over a NaI (Tl) scintillation crystal and the count rate was monitored by a ratemeter and registered on an x–y plotter. A peristaltic pump, flow-rate 22–24 ml/h, was used.

RESULTS

Fig. 2 shows the superimposed elution curves obtained when tritium-labelled PSTME and the labelling mixture from the chloramine-T labelling procedure were chromatographed. Tritium- and ^{125}I-labelled PSTME were separately chromatographed because of the difficulties arising when tritium and ^{125}I are counted simultaneously.

According to the elution pattern shown in Fig. 2 for free radioiodine, the elution volume (V_e = 6 ml) is practically equal to the dead volume; for tritium-labelled PSTME, V_e = 23 ml, and for [^{125}I]PSTME, 56 ml. Thus if the fraction between 35 and 45 ml is discarded, [^{125}I]PSTME practically free from unlabelled PSTME can be produced. It means that the specific activity is close to the theoretical maximum, *i.e.* 2900 Ci/g.

Although free radioiodine, PSTME and [^{125}I]PSTME can be separated using water as eluent, it is advisable to replace water by aqueous ethanol solution after elution of PSTME has been completed. Considerably higher radioactive concentrations can thus be achieved, and also the radiochemical stability of [^{125}I]PSTHME is increased enormously.

Fig. 3 shows a few elution patterns obtained when 20, 40 and 60% (v/v) aqueous ethanol mixture was used as eluent.

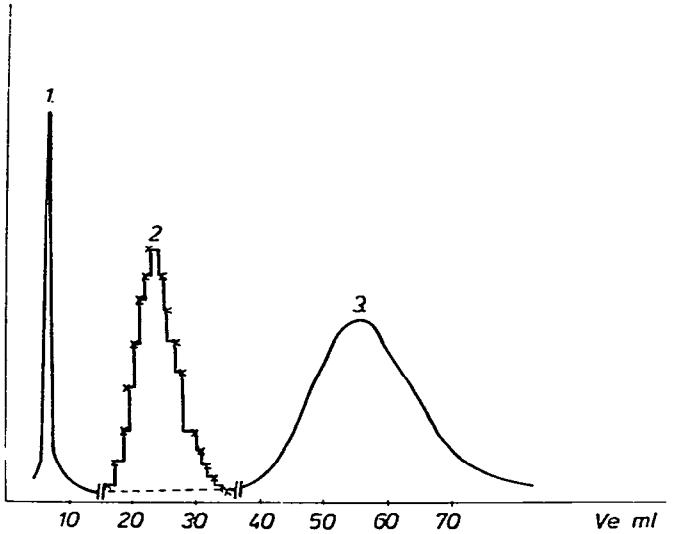

Fig. 2. Elution pattern obtained when the labelling mixture from the chloramine-T procedure and tritium-labelled PSTME were chromatographed. Peaks: 1 = free radioiodine; 2 = tritium-labelled PSTME; 3 = ^{125}I-labelled PSTME.

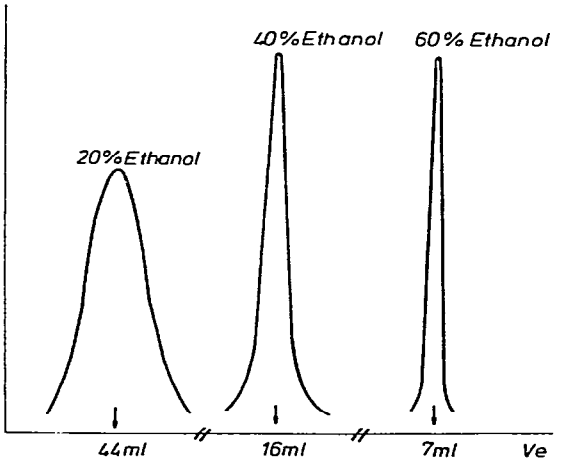

Fig. 3. Elution pattern obtained when [^{125}I]PSTME was chromatographed using ethanol–water binary eluent of different concentrations.

Radiochemical stability of [^{125}I]PSTME

Decomposition to yield free radioiodine is a common feature of radioiodine-labelled substances. The rate of decomposition increases with increasing number of iodine substituents per molecule, *e.g.* thyroxine decomposes more rapidly than diiodothyronines. On the other hand, the decomposition can be suppressed when the labelled compound is stored in an organic solvent–water mixture instead of in pure water. As a general rule, the lower the dielectric constant of the solvent–water mixture, the lower the decomposition rate of radioiodothyronines[7]. Similar results were found for [^{125}I]PSTME.

The radiochemical purity of [^{125}I]PSTME as a function of the time elapsed since the labelling procedure was checked by the use of thin-layer chromatography. The chromatograms on Macherey-Nagel SILNR-HR plates were developed at ambient temperature with chloroform–methanol–water (9:1:0.1). Free radioiodine and [^{125}I]PSTME were localized by counting the radioactivity between the start and the solvent front. The R_F values were 0.1 for $^{125}I^-$ and 0.85 for [^{125}I]PSTME; thus practically complete separation of radioiodine and [^{125}I]PSTME could be achieved.

TABLE I

RADIOCHEMICAL STABILITY OF [^{125}I]PSTME

Time elapsed since labelling (days)	*Free radioiodine content (%) of the [^{125}I]PSTME stored in*		
	Aqueous solution	*Aqueous buffer solution, pH 7.4*	*Ethanol–water, 1:1*
0	1	1	<1
6	12	–	<1
12	27	12	<1
18	30	17	<1
24	46	20	<1
33	56	26	<1

The free radioiodine contents of the [^{125}I]PSTME preparations stored in water, aqueous buffer and 1:1 ethanol–water mixture are listed in Table I. This shows that [^{125}I]PSTME can be stored in 1:1 ethanol–water mixture for a long time without any significant damage. On the contrary, in aqueous solution the breakdown of the [^{125}I]PSTME after 1 week makes its application for radioimmunoassay impossible.

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