Adsorption chromatographic separation of testosterone-3-(O-carboxymethyl)oxime tyrosine methyl ester and its $^{125}$I-labelled derivative

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As shown previously, iodine-substituted low-molecular-weight substances are retarded on Sephadex LH-20 dextran gel as compared with the parent unsubstituted molecules. The enhanced adsorption of radioiodine-labelled iodothyronines\(^1\), \(^2\), 2,3,5-triiodobenzoic acid\(^3\), prostaglandin tyrosine methyl esters\(^4\) and progesterone succinyl tyrosine methyl ester\(^5\) as compared with their parent molecules makes possible the separation of the radioiodine-labelled and unlabelled molecules.

Systematic investigations showed that the distribution coefficient, \(k\), of the radioiodine-labelled substances varies with the organic solvent concentration of the eluent according to

\[
\log k = \log k_0 - n \cdot \log S
\]  

(1)

where \(S\) is the concentration of the organic solvent in the binary eluent, \(k_0\) and \(n\) are constants for a given binary eluent and iodo compound. Eqn. 1 enables the adjustment of the distribution coefficient of the radioiodine-labelled compounds to any desired value, and removes the need for preliminary investigations aimed at the empirical selection of the optimum composition of binary or ternary eluents.

As anticipated, both radioiodine-labelled and unlabelled testosterone-3-(O-carboxymethyl)oxime tyrosine methyl ester (TCTME) are adsorbed on Sephadex LH-20 gel which enables their separation and validates eqn. 1.

EXPERIMENTAL

The labelling method, apparatus and adsorbent used were described previously\(^1\)–\(^5\). TCTME was labelled with $^{125}$I by the use of the chloramine-T method. To 25 \(\mu\)g (50 nmole) of TCTME (MW = 530.6) dissolved in 25 \(\mu\)l ethanol–100 \(\mu\)l phosphate buffer (pH 7.6) were added 1–2 mCi (0.5–1.0 nmole) of carrier-free $^{125}$I, followed by 50 \(\mu\)l solution containing 200–300 \(\mu\)g of chloramine-T. The labelling reaction was quenched after 30–60 sec with 700 \(\mu\)g of sodium metabisulphite in 100 \(\mu\)l. In the course of the chloramine-T labelling, $^{125}$I is introduced via electrophilic substitution in the 3- and/or 5-position of the tyrosine methyl ester residue (Fig. 1).
With such a high excess of TCTME as compared to the amount of radioiodine (i.e., 50 n mole to 0.5–1.0 n mole) the formation of diiodo-TCTME cannot be observed. So, in order to produce TCTME substituted with $^{125}$I in the 3 and 5 positions of the phenolic group, 4 μg (32 n mole) inactive iodide were added to the $^{125}$I$^-$ used for labelling.

Sephadex LH-20 dextran gel was swollen in distilled water prior to being packed in the column (130 x 10 mm I.D.). The height of the packing was 100 mm. In order to check the separation of the starting material (TCTME) from the $^{125}$I-labelled TCTME as well as from free radioiodine, tritium-labelled TCTME was also chromatographed separately from the chloramine-T labelling mixture.

In all 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 free radioiodine was eluted with 10–15 ml aqueous buffer (pH 4) which did not cause displacement of the adsorbed TCTME or $[^{125}\text{I}]$TCTME from the top of the gel. The elution of TCTME and $[^{125}\text{I}]$TCTME was performed with water–ethanol (1.5:1).

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

The distribution coefficient was calculated according to

$$k = (V_e - V_d)/W$$

where $V_e$, $V_d$ and $W$ are the elution volume, the dead volume and the weight of the adsorbent, respectively.
RESULTS

Fig. 2 shows the superimposed elution curve of TCTME, $[^{125}\text{I}]$TCTME and $[^{125}\text{I}_2]$TCTME when 40% ethanol–water was used as eluent. The elution volume increases in the order TCTME < $[^{125}\text{I}]$TCTME < $[^{125}\text{I}_2]$TCTME in full agreement with results for other iodine-substituted compounds$^{1-3}$. The distribution coefficients, $k$, of TCTME, $[^{125}\text{I}]$TCTME and $[^{125}\text{I}_2]$TCTME calculated according to Eqn. 2 as a function of the ethanol concentration of the eluent is presented in Fig. 3, which shows that $k$ increases monotonously with decreasing ethanol concentration. The experimental results in Fig. 3 are shown in Fig. 4 as a log $k$ vs. log $S$ plot. The linearity of these plots confirms the validity of eqn. 1.

It should be mentioned that eqn. 1 holds not only for mono- and diodo-
Fig. 4. The log k versus log S relationship obtained for TCTME (1), [\textsuperscript{125}I]TCTME (2) and [\textsuperscript{125}I\textsubscript{2}]TCTME (3).

TCTME, but also for unsubstituted TCTME. Thus, besides the iodo derivatives, the separation of TCTME itself can also be controlled by adjusting the ethanol concentration of the eluent to the value corresponding to the required distribution coefficient.

In radioimmunoassay the monosubstituted TCTME, i.e., [\textsuperscript{125}I]TCTME, is used as tracer. From a practical point of view, to produce [\textsuperscript{125}I]TCTME of high specific activity, radiochemical purity and stability, it is recommended that free radioiodine and unlabelled TCTME be eluted with 30\% ethanol followed by 40–50\% ethanol which results in considerable increase of the radiochemical concentration.

REFERENCES