Note

Adsorption chromatographic separation of ^{125}I-labelled derivatives of 3'-azido-3'-deoxythymidine

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A sensitive radioimmunoassay of 3'-azido-3'deoxythymidine (AZT) requires a tracer of high specific activity. When ^{125}I is used for labelling, the maximum specific activity is 2200 Ci/mmol, provided that one radioiodine atom is introduced into the AZT molecule. Despite the fact that a higher specific activity of the tracer gives a higher sensitivity of the radioimmunoassay in question, the incorporation of two radioiodine atoms in the starting material, which would result in a specific activity of 4400 Ci/mmol, should be avoided as doubly labelled tracers usually exhibit poor binding to the antibody. As AZT cannot be directly labelled with radioiodine, a tyrosine methyl ester (TME) side-chain was coupled through either a succinyl or a carboxymethyl group at the 5'- position and ^{125}I was introduced into the 3- and/or 5-position of TME (Fig. 1) via electrophilic substitition.

In order to suppress the formation of the doubly labelled 3,5-diiodo-TME derivative, the inactive compounds to be labelled should be applied in large excess relative to radioiodine and care should be taken to ensure complete separation of the ^{125}I-labelled monoiodo derivatives, otherwise the specific activity is drastically decreased.

A decrease in the specific activity (expressed as radioactivity of the labelled molecule per unit mass, *e.g.*, Ci/g or Ci/mmol) results in a decrease in the sensitivity of the radioimmunoassay, *i.e.*, an increase in the detection limit.

Fig. 1. 3'-Azido-3'-deoxythymidine-5'-succinyl-TME (AZT-S-TME) and 3'-azido-3'-deoxythymidine-5'-O-carboxymethyl oxime-TME (AZT-C-TME).

Previously, it was shown for several small molecules that the introduction of iodine substitutent(s) into the phenolic ring gives rise to a considerable increase in the adsorption affinity towards Sephadex LH-20 dextran gel compared with the parent molecules[1-4]. Based on this finding, we report an adsorption chromatographic separation of ^{125}I-labelled AZT from the inactive parent compounds using Sephadex LH-20 as adsorbent.

EXPERIMENTAL

Sample preparation

In order to examine the chromatographic behaviour of AZT-S-TME and AZT-C-TME (Fig. 1), used for radioiodination, these compounds were synthesized using tritium-labelled AZT. The latter was prepared from AZT (Sigma, St. Louis, MO, U.S.A.) according to the method of Hill and Freeman[5]. From tritium-labelled AZT the carboxymethyl oxime or hemisuccinate derivative was produced by the use of aminooxyacetic acid (Sigma) and succinic anhydride (Sigma). Tyrosine methyl ester (Sigma) was coupled to these derivatives by the use of the carbodiimide method.

Labelling with [125]I

The labelling of AZT-S-TME and AZT-C-TME with ^{125}I was performed by the use of the chloramine T method[6]. To 10–20 μg of AZT-S-TME or AZT-C-TME, 1–2 mCi of ^{125}I in slightly alkaline solution were added, followed by 200–300 μg of chloramine T in 50 μl of phosphate buffer (pH 7.4). After 30–60 s, the labelling reaction was quenched with 700 μg of sodium metabisulphite in 100 μl.

Chromatography

Sephadex LH-20 dextran gel (Pharmacia, Uppsala, Sweden) was swollen in distilled water prior to being packed in the column (130 × 10 mm I.D.). The height of the packing was 100 mm. The sample (0.1–0.2 ml) was placed on the top of the column and allowed to soak in and, 10–20 min later, *i.e.*, when adsorption equilibrium had been attained, elution was performed with ethanol–water (flow-rate 22–24 ml/h).

The pH of the eluent, when not indicated otherwise, was adjusted to 4 with 0.1 M citrate buffer so as to suppress the dissociation of the phenolic hydroxyl group of the tyrosine methyl ester residue. At higher pH ionization of the OH group may take place, which decreases or cancels the adsorption of the TME residue[1-4].

Radioactivity measurement

To measure the elution volume of tritium-labelled derivatives, the effluent was collected with a fraction collector (LKB 2211) in 0.5-ml fractions and its radioactivity was determined by liquid scintillation counting (LKB 1214).

In the case of chromatography of ^{125}I-labelled compounds, the effluent was passed over a NaI(Tl) scintillation crystal and the count rate was 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 the equation

$$k = \frac{V_e - V_0}{W} = \frac{V_e - 5.44}{1.46} \tag{1}$$

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

RESULTS

The elution volume of tritium-labelled AZT-S-TME and AZT-C-TME was 10–11 ml and proved to be independent of the ethanol concentration. For ^{125}I-labelled AZT-S-TME and AZT-C-TME the elution volumes obtained for different ethanol concentrations are given in Table I.

From the data in Table I, the conclusion can be drawn that at any ethanol concentration investigated the ^{3}H-labelled compounds are eluted first, followed by the ^{125}I-labelled compounds. With ^{125}I-labelled AZT-S-TME and AZT-C-TME the elution volume decreases with increasing ethanol concentration. With the exception of dilute eluents (10 and 20% ethanol), the distribution coefficient depends on the ethanol concentration of the eluent as follows:

$$\log k = \log k_0 - n \log X \tag{2}$$

where k is the distribution coefficient, X is the concentration of the organic solvent expressed as a molar fraction in the binary eluent and k_0 and n are constants for a given binary eluent and iodo compound.

$$\log k = 1.33 \log X \ (^{125}\text{I-AZT-S-TME}) \tag{3}$$

$$\log k = 0.004 - 1.24 \log X \ (^{125}\text{I-AZT-C-TME}) \tag{4}$$

The distribution coefficient as a function of the ethanol concentration expressed as a molar fraction is shown in Fig. 2. Comparison of the data in Table I and the elution volume of the ^{3}H-labelled AZT-S-TME and AZT-C-TME reveals that the introduction of the radioiodine atom into position 3 of the TME residue considerably increases the elution volume. Consequently, the adsorption affinity of the ^{125}I-labelled molecules towards the LH-20 gel can mainly be attributed to the ^{125}I-labelled TME

TABLE I

ELUTION VOLUME OF ^{125}I-LABELLED AZT-S-TME AND AZT-C-TME

Eluent: aqueous ethanol (pH 4)

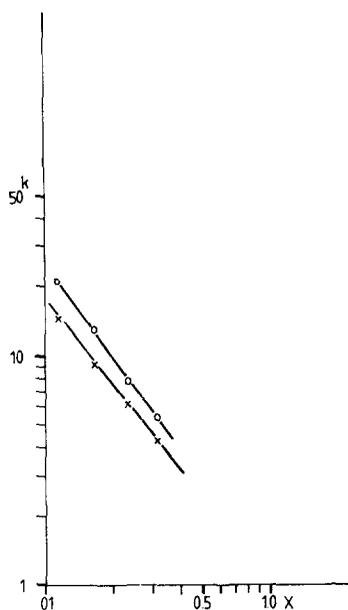| Ethanol concentration		Elution volume (ml)	
% (v/v)	Molar fraction, X	[125I]AZT-S-TME	[125I]AZT-C-TME
10	0.032	35	52
20	0.07	33	44
30	0.115	28	36
40	0.166	19	25
50	0.23	14	17
60	0.312	12	14

Fig. 2. Distribution coefficient as a function of ethanol concentration. Eluent: aqueous ethanol (pH 4). × = AZT-S-TME; ○ = AZT-C-TME.

residue and only to a negligible extent to the AZT itself. On the other hand, the linear $\log k$ vs. $\log X$ relationship which proved to be valid in the ethanol concentration range 30–60% (v/v) (molar fraction 0.115–0.312) makes possible the adjustment of the optimum distribution coefficient and the complete separation of ^{125}I-labelled AZT-S-TME and AZT-C-TME from the parent molecule.

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