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Note

Adsorption chromatographic behaviour of ^{125}I -labelled diethylstilbestrol

G. TÓTH

Institute of Isotopes of the Hungarian Academy of Sciences, H-1525 Budapest (Hungary)

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Previously we reported studies of the effect of the composition of water-organic solvent binary eluents on the elution volume and distribution coefficients of iodine-substituted phenol derivatives when Sephadex LH-20 dextran gel was used as the adsorbent¹⁻⁵. It was shown that for iodotyrosines, iodothyronines and prostaglandins and steroids to which a tyrosine methyl ester side-chain was coupled, the logarithm of the distribution coefficient (k) was linearly dependent on the logarithm of the organic solvent concentration in the eluent (X)

$$\log k = \log k_0 - n \log X \quad (1)$$

where k_0 and n are constants. On the other hand, it was also shown that iodine substituents increase the adsorption affinity, *i.e.*, the greater the number of iodine atoms per molecule the higher is the slope of the $\log k$ vs. $\log X$ plot.

Eqn. 1 holds only when the phenolic hydroxyl group is not ionized, as the dissociation of the hydroxyl group drastically diminishes the adsorption affinity even of iodine-substituted molecules. On the other hand, dissociation of the carboxyl group or protonation of the amine group in the alanine residue may also influence the adsorption affinity, although to a lesser extent.

The symmetry of the diethylstilbestrol (DES) molecule (Fig. 1) and the fact that in [^{125}I]DES there is an iodophenyl and a phenyl residue the $\text{p}K_{\text{OH}}$ values of which are different allows one to establish whether the adsorption model proposed by us for iodine-substituted tyrosine and thyronine derivatives can be applied in this instance also.

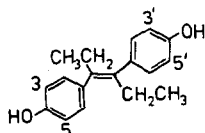


Fig. 1. DES molecule showing the four positions (3-, 5-, 3'- and 5'-) in which radioiodine can be incorporated via aromatic electrophilic substitution.

EXPERIMENTAL

The labelling method, apparatus and adsorbent used were as described previously¹⁻⁴. DES (MW 268) was labelled with ¹²⁵I by the use of the chloramine-T method. To 10–25 μg (40–100 nmole) of DES dissolved in 25–50 μl of ethanol were added 100 μl of phosphate buffer (pH 7.6) and 1–2 mCi (0.5–1.0 nmole) of carrier-free ¹²⁵I, followed by 50 μl of solution containing 200–300 μg of chloramine-T. Owing to the 10–50-fold excess of DES relative to ¹²⁵I, only the formation of monoiodine-substituted DES need be taken into account. In some experiments, aimed at the formation of polysubstituted DES, the molar ratio of iodine to DES was increased to 20 by the use of potassium iodide. The labelling reaction was quenched after 30–60 s with 700 μg of sodium metabisulphite per 100 μl. In the course of the chloramine-T labelling, ¹²⁵I is introduced into the 3- and/or 5-position of DES via aromatic electrophilic substitution.

Sephadex LH-20 dextran gel was swollen in distilled water prior to being packed in a column (130 × 10 mm I.D.). The height of the packing was 100 mm. The sample (0.1–0.2 ml) from the labelling reaction mixture 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 effluent was passed over an NaI/Tl scintillation crystal and the count rate was monitored by a rate meter and registered by an *x-y* plotter. A peristaltic pump delivered the eluent at a flow-rate of 22–24 ml/h.

The distribution coefficient was calculated according to

$$k = \frac{V_e - V_0}{W} \quad (2)$$

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

RESULTS AND DISCUSSION

In contrast to expectations, the chloramine-T labelling of DES with ¹²⁵I resulted in the formation of two labelled products even when ¹²⁵I was applied in a sub-stoichiometric amount relative to DES. Nevertheless, the ratio of the two labelled products varied from labelling to labelling; one of them, identified as [¹²⁵I]DES, proved always to be the major labelled product, the elution volume of which could be controlled by the ethanol concentration of the eluent.

The log k vs. log X plot for [¹²⁵I]DES shown in Fig. 2 shows a linear relationship. The straight line in Fig. 2 was obtained by fitting a line to the experimental data by the use of the linear least-squares method. Inserting the actual value of n and k_0 in eqn. 1, we obtain

$$\log k = -0.04 - 4 \log X \quad (3)$$

According to the simple molecular adsorption model proposed by Soczewiński and Golkiewicz⁶ for silica and by us for Sephadex LH-20 dextran gel adsorbent, n (*i.e.*,

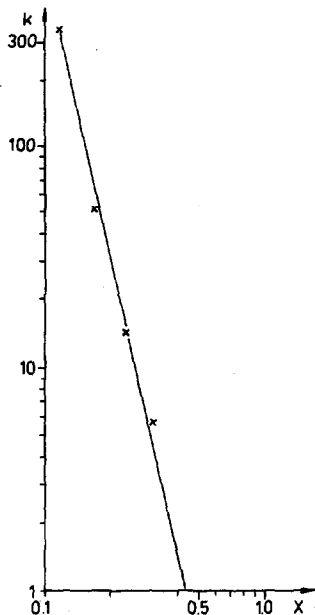


Fig. 2. Log k vs. log X plot for $[^{125}\text{I}]\text{DES}$ (pH 4; 25°C).

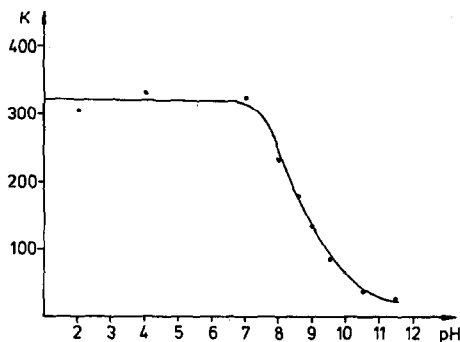


Fig. 3. k . vs. pH plot for $[^{125}\text{I}]\text{DES}$ (eluent, 30% aqueous ethanol; 25°C).

the slope of the log k vs. log X plot) is equal to the number of solvent molecules that displace one solute molecule. Thus a slope larger than unity indicates multipoint adsorption of the solute; in ideal cases n would be equal to the number of functional groups of the solute that interact with the gel.

In disagreement with expectation in most instances, n is not an integer. In addition, for iodothyronines n is smaller³ and for steroid tyrosine methyl ester (TME) derivatives larger⁴ than the number of functional groups (*i.e.*, phenolic hydroxyl and iodine substituents).

With iodothyronine the non-collinearity of the diphenyl ether carbon-oxygen bond may account for the finding that in the series mono-, di-, tri- and tetraiodothyronine the increment of n per iodine atom is less than unity³. From this finding, it may be concluded that both phenyl rings of the iodothyronines cannot interact simultaneously and completely with the dextran gel. Nevertheless, monosubstituted thyronines (*e.g.*, 3'-iodothyronine) has about a 23–30-fold larger k than thyronine⁷, from which the conclusion can be drawn that the contribution of the phenolic hydroxyl group to the adsorption is almost negligible in comparison with the iodine substituent(s). On the other hand, when comparing the retention of iodothyronines² and iodothyronines³ it seems evident that the two rings result in an increase in retention over the corresponding molecule with one ring. Taking into account that in the $[^{125}\text{I}]\text{DES}$ molecule there are only two phenolic hydroxyl groups and one iodine substituent, it is difficult to explain why n in eqn. 3 is higher than with thyroxine, which possesses four iodine atoms and one phenolic hydroxyl group³.

In addition, as DES exists as an equilibrium mixture of *cis* and *trans* isomers

and the relative amounts of the two isomers depend on the chemical nature of the solvent⁸, it is difficult to decide whether only the iodine substituent or both phenyl groups can interact with the gel. Nevertheless, the k vs. pH plot (Fig. 3) can provide an argument for the simultaneous but not equivalent contribution of both phenyl rings to the adsorption.

Brook and Housley⁹ reported that the dissociation of the phenolic hydroxyl group drastically decreases the distribution coefficient of phenols. Later it has also been shown that although iodine substituent(s) in the *o/o'*-position relative to the hydroxyl group considerably increase the adsorption affinity of the phenyl group, the dissociation of the phenolic hydroxyl group cancels the adsorption affinity of the whole phenyl ring, even if it is substituted with iodine³.

Fig. 3 shows that k decreases from 330 to 20 in the pH range 7.5–11.5. Accepting that $\log k$ reflects the free energy of adsorption of the solute¹⁰:

$$\log k = \log V_a + \frac{\Delta G}{2.3RT} \quad (4)$$

where V_a is the adsorption volume of the solvent and ΔG the free energy of adsorption of the solute, $\Delta k = 310$ corresponds to 1670 cal/mol. This is the difference between the free energies of adsorption of the solute at pH 4 and 11.5, *i.e.*, the dissociation of the phenolic hydroxyl groups almost completely cancels the adsorption energy. The change in free energy of adsorption may be ascribed partly to the dissociation of the iodophenyl and partly to that of the phenyl group. Nevertheless, the contribution of the phenolic group to the adsorption of the [¹²⁵I]DES molecule seems to be negligible compared with that of the iodophenyl residue.

It is worth mentioning that in spite of the four positions (*i.e.*, 3-, 5-, 3'- and 5'-) suitable for the incorporation of radioiodine atoms into the DES molecule, an increase in the iodine to DES molar ratio from 0.02–0.1 to 20 did not result in the formation of labelled products that could have been identified as di-, tri- or tetra-iodo-DES.

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