

SEPARATION OF RADIOIODINE LABELLED
3,3',5'-L-TRIODOETHYRONINE (rT3) BY ADSORPTION CHROMATOGRAPHY

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Received 25 June 1977

Accepted 29 June 1977

rT3 was labelled with ^{131}I and ^{125}I by the use of the chloramine-T method^{1,2}. The reaction products were separated by adsorption chromatography using Sephadex LH-20 as adsorbent and aqueous solution of ethyl alcohol as eluent. The relationship between ethanol concentration $/S/$ and distribution coefficient $\log k = \log k^0 - n \cdot \log /S/$ can be derived on theoretical basis and can be used to predict the optimum solvent concentration.

INTRODUCTION

Since it has been demonstrated that T3 is converted in the tissues partly into T3 and partly into rT3 the need of production and separation of radioiodine labelled rT3 arose³.

The difficulty of producing labelled rT3 of proper radiochemical purity and sufficient radioactive concentration lies in the facts that the labelling may lead to the formation of several by-products on one hand, and that the separation causes a decrease of the radioactive concentration, on the other one.

Since the increase or decrease of resolution of separation affects the radiochemical purity and radioactive concentration of the separated component adversely, an optimum resolution value is to be adjusted. To do so, the most promising way is to control the distribution coefficient of the

products to be separated and those of the by-products. In liquid-solid chromatography this is normally accomplished by varying the solvent according to the "eluting power". The main disadvantage of this method is that it requires stepwise elution and that the adjusting the proper distribution coefficient may require a solvent which influences the radiochemical stability of the separated iodoaminoacid in a disadvantageous manner.

The aim of this paper is to show that by choosing the proper concentration of an organic solvent the distribution coefficient and the selectivity of the separation can be varied over a wide range.

EXPERIMENTAL

Reagents

3, 3', 5' -L-triiodothyronine (rT3) (Henning GmbH) was used to produce ^{131}I and/or ^{125}I labelled rT3 according to the chloramine T method^{1,2}.

Eluent was aqueous solution of ethyl alcohol the pH of which was adjusted to pH=4 so as to prevent dissociation of the phenolic OH group and thus the exclusion of the labelled molecule from the gel.

Column

A 10 x 150 mm glass tube equipped with a porous disc at the bottom was filled with Sephadex LH-20 to a height of 100 mm. The radioactivity of the effluent was monitored continuously using a NaI(Tl) scintillation crystal and an automatic gamma counter. The count rate was recorded by an x-y plotter.

0.1-0.4 ml of the reaction mixture obtained after carrying out the chloramine T labelling process was placed on the top of the bed and the sample was allowed to soak into the gel. After 10-15 minutes, i.e. when adsorption of the labelled thyronine derivatives has already been completed, free radiiodine was washed out with three-four column volume of distilled

water, which did not result in any displacement of the adsorbed thyronines from the top of the gel. After removal of free iodine elution was started with aqueous solution of ethanol.

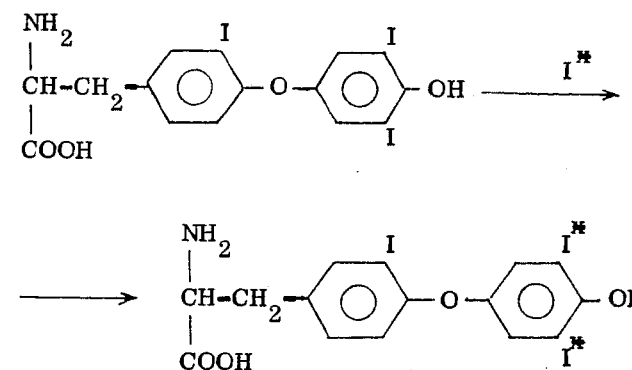
The volumetric distribution coefficient (k) was calculated according to Eq. (1):

$$k = \frac{V_e - V_o}{V_t - V_o} \quad (1)$$

where V_e , V_o and V_t stand for the elution volume, the dead volume and the total volume, respectively.

RESULTS AND DISCUSSION

Since electrophile substitution or isotope exchange of radiiodine (I^*) may take place only at the 3' and/or 5' positions, when using rT3 as starting material, the chloramine T labelling method results in the formation of labelled rT3 only:



Nevertheless either because of the oxidizing effect of the chloramine T, or because of the gross impurities present almost in every thyronine derivatives the formation of other radiiodine labelled thyronine derivatives like T3, T4 or T2 can not be excluded either.

Fig. 1 shows three elution curves obtained when water-ethanol of different concentrations were used for the elution.

The elution curves clearly indicate that the chloramine T method resulted in the formation of two labelled molecules, (rT3 and another thyronine derivative of lower iodine content than that of rT3, probably 3,3' T2 or 3',5', T2) on one hand, and that the resolution of the separation increases with decreasing ethanol concentration, on the other one.*

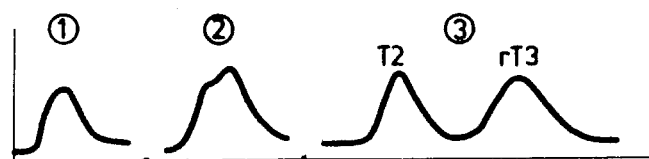


Fig. 1. Elution curves of the mixture of T2 + rT3 at pH=4. 1-Eluent 60% ethanol, 2-eluent 50% ethanol, 3-eluent 35% ethanol

The distribution coefficient of rT3 and that of T2 calculated from the elution volumes according to Eq. (1) depends on the solvent concentration as follows⁴:

$$\log k = \log k^0 - n \cdot \log /S/ \quad (2)$$

The linear log k vs. log /S/ relationship for T2, rT3 and for T4 is shown in Fig. 2.

*It should be noted that at pH=4, i.e. where the dissociation of phenolic OH group is negligible, the adsorption affinity of a thyronine derivative towards the dextrane gel depends only on the number of iodine atoms in the molecule. Consequently rT3 and T3 exhibit the same elution volume and distribution coefficient and so they can be separated only at higher pH values where the dissociation of rT3 and thus its exclusion from the gel takes place. The identification of the second elution peak in Fig. 1 as rT3 was also done at pH=8.5.

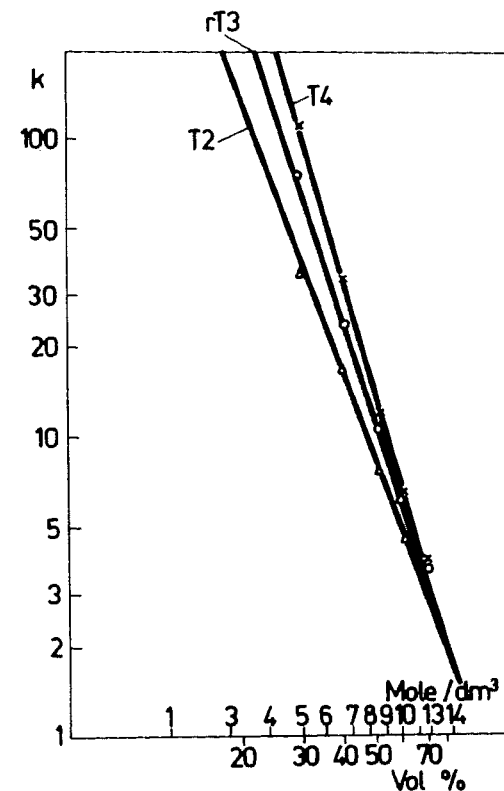


Fig. 2. The log k vs. log /S/ plots of T4, rT3 and T2

TABLE 1

Ethanol concentration		V_e^{T4}	V_e^{rT3}	V_e^{rT2}	$\alpha = k_{rT3}/k_{rT2}$
Vol%	Mole/dm ³	ml			
20	3.4	273	174	66	2.9
30	5.0	118	76	50	2.0
40	6.7	40	33.9	22.1	1.5
50	8.4	18	17.3	14	1.4
60	10	12.6	11.8	10.2	1.3
70	11.8	10.4	10	8.4	-

By the use of Eq. (1) the dependence of the elution volume and that of the selectivity on the solvent concentration can be calculated:

$$V_e = (V_t - V_o) k^o / S^{-n} + V_o \quad (3)$$

$$\alpha = \frac{k_2}{k_1} = \frac{k_2^o}{k_1^o} \cdot /S^{n_1 - n_2} \quad (4)$$

and the solvent concentration yielding the most proper separation predicted.

The data given in Table 1 demonstrate how elution volume and selectivity of separation can be varied by changing the solvent concentration.

The data of Table 1 show that by changing the ethanol concentration any required elution volume and/or selectivity can be obtained. The dependence of the resolution of separation on solvent concentration is beyond the scope of this paper, and will be discussed elsewhere.

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ÜBER DIE γ -BESTRAHLUNG VON CYCLITEN IV. BESTRAHLUNG VON D-INOSIT IN WÄSSRIGER LÖSUNG UNTER SAUERSTOFF*

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Eingegangen am 12 Juli 1977

Angenommen am 14 Juli 1977

After irradiation of an aqueous solution of D-inositol (1% w/v) in the presence of oxygen the following radiolysis products could be detected: L-3, 5, 6/2, 4-pentahydroxycyclohexanone (L-myoinosose-1), L-3, 5/4, 6-tetrahydroxycyclohexanedione, DL-xylitol-4, 5, 6-trihydroxycyclohexane-1, 2, 3-trione, glyoxylic acid, formic acid, and carbon dioxide. As an interesting matter of fact it should be noted that the triketone has been formed as racemate.

EINLEITUNG

Myo-Inositol in wässriger 1%iger Lösung wird unter Sauerstoff bei einer γ -⁶⁰Co-Bestrahlung in verschiedene Radiolyseprodukte umgewandelt¹, während bei einer Bestrahlung unter Stickstoff der myo-Inositol nur bis zum Monoketon (myo-Inosose-2) oxidiert wird; ausserdem tritt ein Desoxyderivat, nämlich der 1, 3, 5/2, 4-Cyclohexanpentit (scyllo-Quercit) auf und ebenso konnte auch die Bildung einer polymeren Verbindung, welche bei Bestrahlung unter Sauerstoff nicht entsteht, beobachtet werden². Es soll

*Für die Ermöglichung der Durchführung der Untersuchungen danken wir der Österreichischen Studiengesellschaft für Atomenergie, Forschungszentrum Seibersdorf.