ADSORPTION PROPERTIES OF IODOTYROSINES AND DERIVATIVES ON SEPHADEX*

FRANCESCO BLASI AND RICCARDO V. DE MASI
Impresa Endocrinologia del C.N.R., Istituto di Patologia Generale dell'Università, Naples (Italy)

Separation of iodotyrosines and derivatives can be achieved by many techniques, however no rapid preparative procedure is so far available. During our studies on the mechanism of the synthesis of thyroxine from diiodotyrosine and its ketoacid derivative, 4-hydroxy-3,5-diiodo-phenylpyruvic acid (DIHPPA)[1,2], it became evident that a method was needed by which the separation of the three components (diiodotyrosine, DIHPPA and thyroxine) and measurement of their concentration could be achieved.

Dextran gel chromatography had already been employed in other laboratories to separate iodotyrosines and thyroxine[3,4]. We have improved and extended this method, finding conditions in which separation of diiodotyrosine, DIHPPA and thyroxine is achieved in a single step by elution of a Sephadex G-25 column with 0.02 *N* NaOH. The behaviour of other iodotyrosines and tyrosines, as well as that of their acetic acid derivatives, has been studied under the same conditions.

MATERIALS AND METHODS

The following pure reagents have been used: 3-iodotyrosine, 3,5-diiodotyrosine, 3,3',5-triiodothyronine and thyroxine (Sigma); 3,5-diiodothyronine and 3-iodothyronine (Warner-Chilcott); thyronine, 3',5'-diiodothyronine and 3,3',5'-triiodothyronine (Calbiochem); 3,5-diiodothyroacetic acid and 3,3',5-triiodothyroacetic acid (Aldrich Chem. Co.). DIHPPA (Osaka Synthetic Chem. Labs.) was recrystallized twice before use.

^{125}I-diiodotyrosine was obtained from New England Nuclear Co. Sephadex G-25 was purchased from Pharmacia.

Column chromatography

A column (1.5 × 18 cm) of Sephadex G-25 was used, and eluted at room temperature with 0.02 *N* NaOH at a flow rate of 0.55 ml/min. Fraction volumes of 3.25 ml or smaller have been collected using an automatic fraction collector.

RESULTS AND DISCUSSION

Thyroxine synthesis from ^{125}I-diiodotyrosine and DIHPPA was achieved with a yield of 43 %[3]. The reaction mixture, containing diiodotyrosine, DIHPPA and the newly synthesized thyroxine, was put on a G-25 column at the end of the incubation

* Supported in part by the PHS research grant AM-06860/03.

time. The column was eluted with 0.02 *N* NaOH. The elution profile, followed by measuring the optical density at 325 mμ and the ^{125}I-radioactivity of each fraction, is shown in Fig. 1. Three peaks are clearly visible. These peaks, analyzed by paper

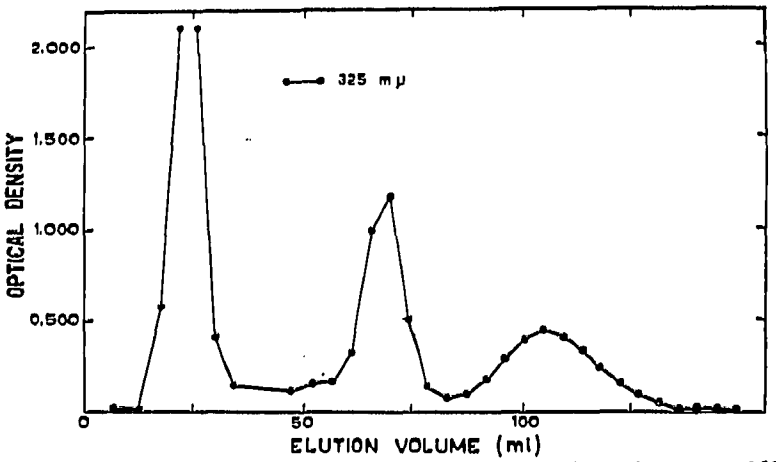

Fig. 1. Fractionation on Sephadex G-25 of a mixture of ^{125}I-diiodotyrosine and DIHPPA incubated in the conditions for the synthesis of thyrosine. Elution medium: 0.02 *N* NaOH.

chromatography in *n*-butanol–ethanol–0.5 *N* ammonia (5:1:2) and by U.V. spectrophotometry, have been identified as diiodotyrosine (first peak), thyroxine (third peak) and diiodobenzaldehyde (DIBA) (middle peak); this aldehyde derivative of DIHPPA is quantitatively produced from DIHPPA[5,6] under our elution conditions. Table I shows the values of the partition coefficients (K_d) for the pure compounds diiodotyrosine, DIBA and thyroxine.

This method has the advantage that purified thyroxine can be obtained very easily in a single step. It can be used for both micro- and macro-scale preparation of pure thyroxine. Furthermore, kinetic studies, which so far could not be easily per-

TABLE I

PARTITION COEFFICIENTS OF TYROSINE AND THYRONINE DERIVATIVES

*Compound**	K_d
Tyrosine	0.32
3-Iodotyrosine (MIT)	0.36
3,5-Diiodotyrosine (DIT)	0.52
3,5-Diiodobenzaldehyde (DIBA)	3.10
Thyronine (T_0)	0.52
3-Iodothyronine	0.93
3,5-Diiodothyronine	1.13
3,5,3′-Triiodothyronine (T_3)	2.35
3,5,3′,5′-Tetraiodothyronine (thyroxine)	5.20
3′,5′-Diiodothyronine	1.95
3,3′,5′-Triiodothyronine (reverse T_3)	4.40
3,5-Diodothyroacetic acid	1.54
3,5,3′-Triiodothyroacetic acid	2.60

* 1.5 mg in 0.5 ml 0.02 *N* NaOH.

formed, can now be made because the amount of thyroxine synthesized, as well as the concentration of unreacted diiodotyrosine and DIHPPA, can be determined very easily.

The K_d values of DIBA and thyroxine clearly show that they are adsorbed on the dextran. In order to get a clearer picture of the mechanism of adsorption of these compounds, other related iodoamino acids have been eluted under the same conditions. Table I shows that the partition coefficients of tyrosine and its iodinated derivatives (MIT and DIT) are not very different.

The iodothyronines, however, behave differently. The elution volume is greatly increased with the increasing number of iodine atoms per molecule. Also, the position of the iodine atoms on the two phenyl rings influences the elution volume and the K_d (see Fig. 2 and Table I). With regard to the dependence of elution volume on the

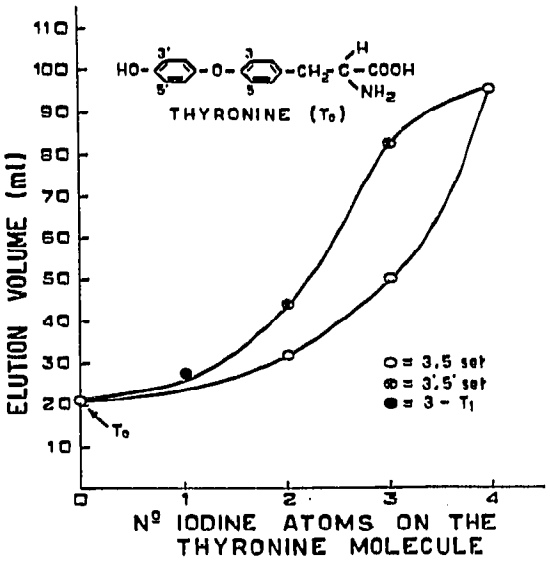

Fig. 2. Dependence of the elution volume of thyronine derivatives on the number of iodine atoms on the molecule.

number of iodine atoms per molecule, two groups of iodothyronines can be distinguished: (1) the 3,5 and (2) the 3′,5′ group, in which the 3 and 5 positions or the 3′ and 5′ positions, respectively, are always occupied by iodine (see Fig. 2). The 3′,5′ group shows a larger increase in the elution volume with increase of the number of iodine atoms on the molecule. This indicates that adsorption is probably effected somehow through the phenolic hydroxyl group.

From the results reported in Fig. 1, *i.e.* the separation between diiodotyrosine and DIBA, a further possibility appears, namely that the nature of the side chain residue modifies the degree of adsorption of the two substances (DIT and DIBA). The situation seems to be similar in the thyronines. In Table I, the K_d values of diiodo and triiodothyroacetic acids can be compared with those of diiodo and triiodothyronine. The K_d values of the acetic acid derivatives in both cases slightly exceed those of their alanine analogs. The presence of the amino group on the side chain of the thyronines seems to decrease slightly the adsorption of the iodothyronines to dextran.

SUMMARY

Iodinated derivatives of tyrosine and thyronine have been eluted through a Sephadex G-25 column with 0.02 *N* NaOH as eluent. Under these conditions these compounds become reversibly adsorbed to the dextran matrix, and this, in most cases, allows their separation.

A list of the partition coefficients (K_d) of the substances tested is presented. Furthermore, a tentative relationship between the structure of the compound and its elution volume on Sephadex has shown that the presence of iodine on the hydroxyl-carrying phenol of the thyronine derivatives strongly influences the degree of adsorption, more than the presence of iodine on the alanine-carrying phenol. Side-chain effects have been studied by means of the acetic acid analogues of diiodo- and triiodothyronine; the amino group slightly decreases the degree of adsorption of these substances.

REFERENCES

1 F. BLASI, *Biochim. Biophys. Acta*, 121 (1966) 204.
2 F. BLASI, *Compt. Rend. Soc. Biol.*, 160 (1966) 1403.
3 E. H. MOUGEY AND J. W. MASON, *Anal. Biochem.*, 6 (1963) 223.
4 S. LISSITZKY AND J. BISMUTH, *Clin. Chim. Acta*, 8 (1963) 269.
5 F. BLASI, unpublished results.
6 T. MATSURA, personal communication.