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ANAIS - PROCEEDINGS

PREPARATION, PURIFICATION AND STABILITY OF HIGH SPECIFIC ACTIVITY ^{125}I - TRIIODOTHYRONINE (T_3).

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Abstract

A radioiodination of triiodothyronine (T_3) by modified chloramine-T method is described. Diiodothyronine (T_2) was employed as the starting material, which yields ^{125}I-T_3 with specific activities of 110.8 ± 23.6 TBq/g. Purification by Sephadex G-25 fine column, elution with phosphate buffer in high pH has shown to be convenient, for the separation of the desired products. Studies on the stability of ^{125}I-T_3 stored in 50% solution of propylene glycol showed that it is stable for at least 3 months. The T_3 labelled was analysed, using three types of RIA: a) The PEG solution, b) the double antiserum with PEG and c) the solid phase technics. The three RIA standard curves obtained, gave sensitive, precise and accurate assay.

Introduction

The development of high quality radioimmunoassay (RIA) for thyronines as T_3 and T_4 and their correlations with inumerous pathologies make them the most required clinical test for the RIA divisions labs[7].

Presentely a number of clinical laboratories have already achieved economical conditions to develop their own labelled RIA products [7].

In the RIA methodology the labelled radiotracer and the antiserum are the main reagents to be obtained.

In T_3 RIA methodology, good quality labelling at low cost is the first goal to be achieved. Labelling is necessary in the antiserum quality control procedures and, of course, in the ordinary RIA.

The domestic labelled T_3 must be analogue to the imported one available in the market, which is furnished with a specific activity in the order of 1.11×10^{12} Bq/g (3000 Ci/g)[4].

In the present communication we describe and evaluate the chloramine-T methodology for synthetizing ^{125}I-T_3, using low cost procedures, i.e., column chromatography on Sephadex G-25 fine (25 x 0.6 cm) eluted with phosphate buffer pH 11.9 in the separation step. A further study relating 30 radiolabells, include specific activity determinations by self displacement, according to Morris description,[5] and product stability along 105 days. We also discuss the use of T_3 labelled on three types of RIA a) The PEG solution, b) the double antiserum with PEG and c) the solid phase technics.

Methods

1. Labelling procedure: Radioiodination was made using the modified chloramine-T method, described by Hunter and Greenwood et al[3,4]. T_2 solution was prepared as a stock solution by dissolving 1 mg of T_2 in 5 ml of 50% aqueous propylene glycol adding 25 µl of 1M.NaOH.

   The iodination was carried at room temperature in small glass tubes (11X68mm). The reagents were added in the following order: a) 20 µl of 0·25M phosphate buffer pH 7·4, b) 1-3 µl of $Na^{125}I$ (37MBq), c) 5 µl of T_2 (1 µg/µl), d) 10 µl of chloramine-T, (52.5 mg/10ml phosphate buffer). After 45 sec. the reaction was stopped by adding 10 µl of sodium metabisulphite (105 mg/10ml phosphate buffer) and 50 µl of 50% aqueous propylene glycol.

2. Purification by sephadex G-25 fine column filtration:

   Sephadex G-25 fine was allowed to swell in 0.05M phosphate buffer pH 11.9 for 24 hr and packed in a 25x0.6 cm glass column. Immediately after iodination, the reaction misture from the above labelling procedure was applied on the column and eluted with 0.05 M phosphate buffer pH 11.9. The flow rate was adjusted to 40 ml/hr and 2 ml eluates fractions were collected. One drop of 6M HCl added to each fraction to bring the pH down at about 7·5. Fifty fraction were collected and 10 µl aliquots of each was counted. The fractions corresponding to the labelled T_3 were pooled and stored at -20ºC in 50% propylene glycol solution[4].

3. Estimation of specific activity (SA): The specific activity of the ^{125}I-T_3 was estimated by the self-displacement method according Morris discriptions.[5]

4. Stability: The stability of the ^{125}I-T_3 was analysed, every week, during 105 days by the electrophoresis technique and the immunoreactivity was verified by radioimmunoassay.

5. Standard curves for radioimmunoassay (RIA):

To verify the quality of the T_3 labelled, standard curves of T_3 were run, using three types of separation for the antigen-antibody complex: a) the PEG solution[1] b) the double antiserum with PEG [2,7] and c) the solid phase techinics [6]. The following RIA parameters were analysed: sensitivity, precision and correlation betweeen the above three techinics.

Results and Conclusion

1- Radionation: The yield of 30 labelling procedures, expressed as the percentage of the total radioactivity incorporated into the thyronine, averaged 75% (range,69-81%), wich 43.6% (range, 40-59%) corresponds to ^{125}I-T_3 and 26.4% (range, 21-30%) to T_4.

2- Purification of the labelled T_3: Fig. 1 shows a typical elution pattern on sephadex G-25 column. In our study this method has proved to be simple, rapid and reliable with good separation of the fractions.

3- Specific activity : The estimated specific activity of the ^{125}I-T_3 was 110.8 ± 23.6 TBq/g (2994 ± 638 Ci/g), which correspond to the addition of (0.9 ± 0.2) ^{125}I atom/molecule.

4- Stability: Fig 2 shows that the ^{125}I - T_3 solutions are stable during 3 months. The specific activity, B_o/T and NSB remained unchanged along this time.

5- Standard curves for RIA of T_3: Fig. 3 shows typical standard curves of the three types of RIA. The RIA parameters analysed are given in table 1. These experimental data are in agreement with imported products, so, we can optimistically conclude that our primary purpose was achieved, i.e., we obtain a good ^{125}I-T_3 for RIA use, with a low cost.

Table 1 - RIA of the labelled - T_3

		PRECISION (ERROR %)					
Assay	Sensitivity ng/dl	50 ng/dl	250 ng/dl	500 ng/dl	NSB %	Correlation Coeficient (r)	
Solid phase (S.Ph)	17	10	6	8	7	S.Ph / PEG	0.96
Double Antiserum (D.A.)	14	13	8	9	5	D.A. / S.Ph	0.97
PEG	22	12	7.5	10	110	PEG / D.A.	0.98

REFERENCES:

1- DESBUQUOIS,B.; AURBACH,G.D. - Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassay. J.Clin.Endocrinol., 33: 732-7, 1971.

2- HENLEY,R. & KISGYORGY,D. - Polyethylene glycol 6000 as an accelerator in a

double antibody radioimmunoassay system. Med.Lab.Sci.,37:367-9, 1980.

3- HUNTER,W.M.; GREENWOOD,F.C. - Preparation of iodine 131 labelled human growth hormone of high specific activity. Nature, 194: 495-6, 1962

4- KJELD,J.M.; KUKU,S.F.; DIAMANT,L.J.; FRASER,J.R.; JOPLIN,G.F.,MASHITER,K. Production and storage of ^{125}I thyroxine and ^{125}I triiodotyronine of high specific activity. Clin.Chim.Acta, 61:381-9, 1975.

5- MORRIS,B.J. - Specific radioactivity of radioimmunoassay tracer determined by self-displacement a re-evaluation. Clin.Chim.Acta: 73:213-16, 1976.

6- PARSENS,G.H. - Antibody-coated plastic tubes in radioimmunoassay. Meth. Enzymol., 72:224-39, 1981.

7- RUSSO, E.M.K.; VIEIRA,J.G.H.; MACIEL,M.R.B.; FONSECA,R.M.G. - Desenvolvimento e caracterização de métodos de radioimunoensaios para dosagem de iodotironinas (T_4, T_3 e rT_3). Arq. Bras. Endocrinol. Metabol., 26: 23-8, 1982.

Figures

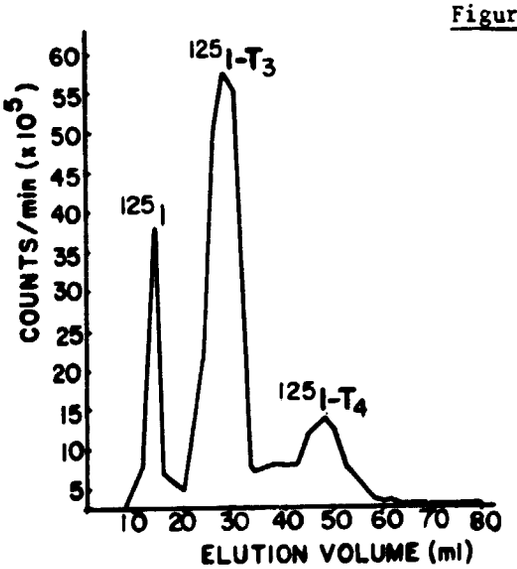

Fig.1 - Radiocromatogram of ^{125}I-T_3 on Sephadex G-25 column

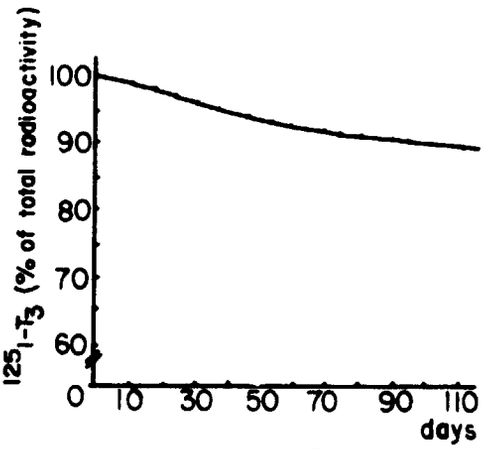

Fig.2- Analysis of the ^{125}I-T_3 stored in 50% propylene glicol solution

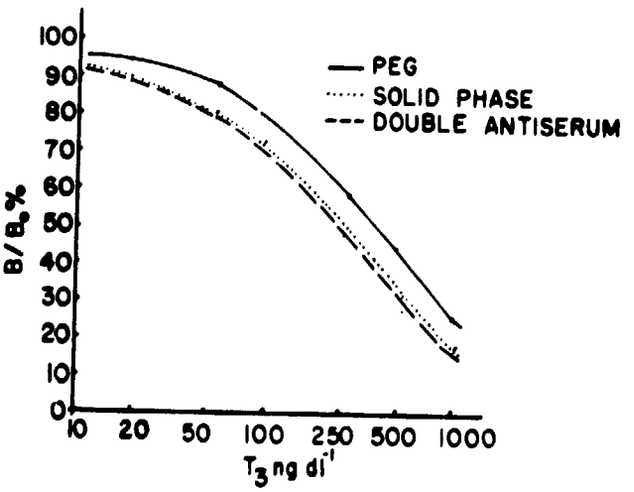

Fig.3 - Standard curves of the three types of RIA of T_3