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ABSTRACT

Stoichiometric iodination of porcine insulin was performed to the general method of Hunter and Greenwood with modifications recommended by Roth. These method was compared with radioiodination using Iodogen. Films of Iodogen react rapidly in the solid phase with aqueous mixtures of I^- and proteins. For two methods satisfactory activity of the labeled porcine insulin was obtained and characteristics of the radioimmunoassay were studied.

RADIOIODAÇÃO DA INSULINA PORCINA EM CONDIÇÕES MODERADAS PARA RADIOIMMUNOENSAIO: COMPARAÇÃO ENTRE OS MÉTODOS DA CLORAMINA-T E EM FASE SOLIDA COMO IODOGEN

RESUMO

Padronizamos 2 métodos de radioiodação da insulina porcina: Método controlado da Cloramina-T utilizando o método clássico de Hunter e Greenwood, modificado por Roth e método do Iodogen descrito por Fraker e Speck em que o Iodogen, ligado à fase sólida orgânica é empregado como aceptor de elétrons no processo de marcação radioisotópica de proteínas. Nos 2 métodos evitou-se excessiva oxidação proporcionando porém atividade específica suficiente para a manutenção da estabilidade e capacidade imunorreativa.

INTRODUCTION

The chloramine T method which yields iodinated peptides of very high specific radioactivity may have reduced the immunoreactivity or increased affinity for nonspecific sites, presumably due to a contact with to an excess of the oxidizing agent (Chloramine T) or reducing agent (Metabisulfite).

Adding sufficient ammounts of chloramine T to the reaction tube, it doesn't affect the molecular integrity of the hormone. It is performed to the general method of Hunter and Greenwood[3], classical chloramine T, with the modifications recommended by Roth[2], chloramine T is added in limiting amounts in multiple small additions.

In this report we compare the chloramine T modified with Iodogen iodination methods of insulin for the production of radioinsulin of high specific activity suitable for radioimmunoassay.

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The Iodogen, 1, 3, 4, 6-tetrachloro-3a, 6a-diphenylglycouril, it was first described by Fraker and Speck[1] as a reagent for the iodination of proteins and cell membranes. Films of 1, 3, 4, 6-tetrachloro 3a, 6a-diphenylglycouril (conveniently "plated" in the reaction tube) react rapidly in the solid phase with aqueous mistures of I^- and proteins. Reaction tubes coated with the reagent can be prepared in advance and stored indefinitely.

This noval method for radioiodination of proteins is rapid, gentle, efficient and reproducible.

PURIFICATION

For the fact that of insulin exhibits strong adsorptive affinities to cellulose, purification is fairly simple. The contents of the iodination tube is applied to a small cellulose column (4). Unreacted iodide and components damaged during preparation as well as other heterogeneous fractions are not adsorbed for the most part and are washed through the column. The adsorbed hormone is then eluted from the adsorbent.

MATERIAL AND METHODS

Radioiodination: Both iodinations were performed at the same time using the same amount of porcine insulin (4μg) and the same lot of 125I (1mCi) at room temperature.

Stoichiometric iodination: To a reaction tube the following reagents were added in the order given: 35 μl of 0.3M phosphate buffer pH7.5; 1mCi of 125I; 4μg of porcine insulin and 15μl of chloramine T (0.6μg in 0.3M phosphate buffer). After that the percentual of radioactivity was determined by precipitation with trichloroacetic T.C.A. 10%. In our laboratory, generally about 40% was T.C.A. -precipitable at this stage of the procedure. The addition of 10μl of chloramine T (0,4μg) was usually required in order to achieve 80 per cent T.C.A. precipitable radioactivity. Following 5μl of sodium metabisulfite (1μg) was added, followed by 100μl of a 2.5 per cent solution of bovine serum albumin in 0.3M phosphate buffer.

Iodogen Iodination: To a reaction tube coated with 2μg of iodogen, prepared in advance, the reagents were added as follows: 10μl of 125I (1mCi) in 0.5M phosphate buffer pH 7.5 and 10μl of porcine insulin (4μg) in 0.05M Borate buffer pH 8.5. The reaction is usually processed in 10 minutes and finished by decanting the mixture from the residual iodogen.

The efficiency of iodination was determined by TCA 10% precipitable.

PURIFICATION: In both iodination method the purification of the labeled insulin were performed by adsorption to, and elution from, a column of Whatman cellulose powder. The iodination mixture was transferred to the column and forced into the body of the column with air pressure. The major fraction of the labeled insulin adsorbs to the cellulose and was washed in order to get free of unreacted iodide and damaged components, with 0.5ml of distilled water. The label insulin was eluted with four sucessive 0.5ml portions of alcoholacid solution (ethylic alcohol, concentrated chloridric acid and distilled water (7.50, 0.15, 2.35ml).

The radioactivity of the four eluates was determined by TCA precipitable.

STABILITY AND IMMUNOREACTIVITY OF THE LABELED INSULIN

The four eluates collected in the cellulose column for each labeled preparations were tested for integrity and immunoreactivity.

The labeled insulin of the second eluate, for two methods, was employed in the radioimmunoassay. Its stability and immunoreactivity was tested for 60 days.

RESULTS AND CONCLUSIONS

Radioiodination: The efficiency of six labeling procedures (3 for each iodination method) expressed as the percentage of the total radioactivity incorporated into the intact radioiodinated porcine insulin average 78% with chloramine T and 79% with iodogen. Satisfactory specific activity of the two labeled insulin were obtained (Table I).

The second eluate, from the cellulose column for two labeled preparations, with greater purity (Table II) and greater specific binding (Table III) was tested (at the same time and the same way) for integrity and immunoreactivity during 60 days (Table IV – Figure 1).

The nonspecific binding (NBS) was stable (± 5%) independently of the method used and the immunoreactivity decrease during a storage.

Specific binding was greater for radioinsulin from chloramine T in the 1º and 30º day after preparation but the percentual relation between maximum and inicial binding was the same for two methods 60º day after preparations.

Both methods offered reproducible iodination with greater stability and adequated immunoreactivity.

Iodogen method, for radioiodination of proteins, as effective as chloramine T method, showed easy to perform.

Table I

Efficiency of Iodination

Samples	Chloramine T		Iodogen	
	TCA	Sp. Act.	TCA	Sp. Act.
Nº	%	μCi/μg	%	μCi/μg
1	81	205	79	197
2	78	195	77	192
3	76	190	80	200
X	78	195	79	197

Table II

Percentual Values of Purities in the 4 Eluates from Cellulose Column by TCA Precipitable

	ELUATES							
Samples	**Chloramine T**				Iodogen			
Nº	**1**	**2**	**3**	**4**	1	2	3	4
1	**98**	**97**	**95**	91	96	**96**	**92**	**90**
2	**97**	**97**	**94**	**90**	97	**95**	**94**	**90**
3	**96**	**97**	**94**	**90**	95	**96**	**93**	**90**
X	**97**	**97**	94	**90**	96	**96**	**93**	**90**

Table III

Percentage of Specific Binding (BO/T) in the 4 Evaluates from Cellulose Column by TCA Precipitable

Samples	ELUATES							
	Chloramine T				Iodogen			
Nº	1	2	3	4	1	2	3	4
1	**44**	**46**	**38**	**34**	**40**	**42**	**35**	**31**
2	**43**	**45**	**36**	**32**	**37**	**40**	**33**	**29**
3	**44**	**47**	**35**	**33**	**36**	**39**	**31**	**28**
X	**44**	**46**	**36**	**33**	37	**40**	**33**	**29**

Table IV

Percentage of Specific Binding (BO/T) in the Second Eluate from Cellulose Column During Storage

Samples	DAYS					
	Chloramine T			Iodogen		
Nº	1	30	60	1	30	60
1	**43**	**40**	29	37	36	22
2	**46**	**41**	**38**	32	31	**30**
3	**48**	**43**	**40**	40	37	**35**
X	**46**	**41**	40	37	36	**30**

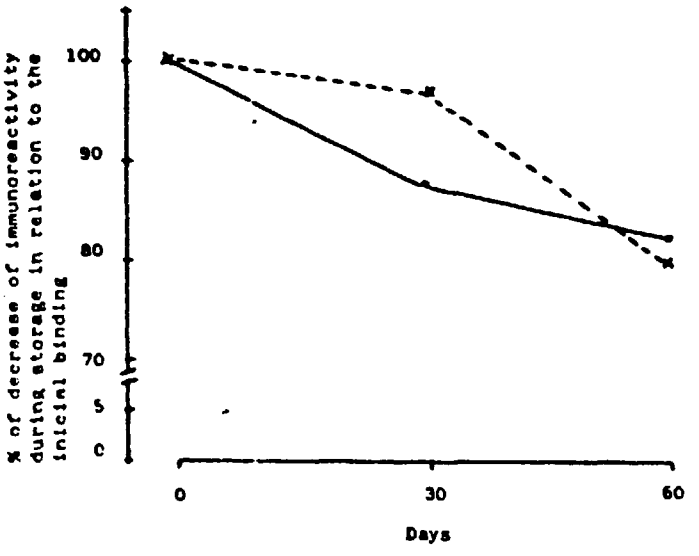

Figure 1 – Percentage of decrease of maximum specific binding during storage (30 and 60 days) with chloramine T (.——.) and Iodogen (x– – –x).

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