Radioiodination of purified monoclonal antibody

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We have tried to set up experimental protocol to optimize: 1. The immunochemical procedures: purification of monoclonal antibody, reduction of the purified immunoglobulin size to fragments and analytical studies by SDS-PAGE electrophoresis; 2. The conditions to radioiodination of purified immunoglobulin. Ascitic fluid from BALB/c mouse carrying the anti-CEA 4C11 monoclonal antibody IgG_{2a} subclass, generously provided by Ludwig Institute/Brazil, was used as model to evaluate these procedures.

Uniterms: Monoclonal antibodies — electrophoresis. Radiopharmaceuticals — iodination. Immunology — scintiscanning.

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INTRODUCTION

Since of the development of "Hibridoma Technology" (Köhler and Milstein, 1975) monoclonal antibodies have found an increasing number of applications and uses. The availability of monoclonal antibodies to tumor associated antigens having a high degree of specificity has contributed to more extensive *in vivo* use of radiopharmaceuticals. It was soon obvious that prior of their specific application in immunoscintigraphy a lot of immunochemical work has to be done for each monoclonal antibody. It is important to remember that antibodies are sensitive biochemicals, subject to losses of the activity that is essential to their mode of action, namely the ability to bind specific antigen.

The aim of this study was develop affinity chromatographic methods in order to isolate the specific IgG_{2a} fraction from anti-CEA monoclonal antibody 4C11 belonging to IgG_{2a} subclass; fragmentation of the purified digestion; analytical studies by SDS-PAGE electrophoresis and radioiodination of purified immunoglobulin by "Iodogen Method".

MATERIAL AND METHODS

1. Immunochemical procedures

1.1. Purification of anti-CEA 4C11 IgG_{2a}

The intact IgG_{2a} was isolated from ascitic fluid by affinity chromatography Protein A-Sepharose, according to Ey et al.[3]. 1.5 g of Protein A-Sepharose (Pharmacia Fine Chemicals) was ressuspended in 5.0 ml 10 mM phosphate buffered saline (PBS) pH 8.0 and packed into a small column. The column was washed with pH 3.0 buffer to free bound material and then equilibrated at pH 8.0. The sample at pH 8.0 was added to the column. In these conditions all IgG_{2a} are retained and all other proteins were eluted. IgG_{2a} was eluted from the column at pH 4.5. Fractions of approximately 3.0 ml were collected of flow rate of 0.46 ml/min. The concentration of IgG_{2a} was determined by optical density at 280 nm using an extinction coefficient $\varepsilon^{1\%}_{1cm} = 14$.

1.2. Fragmentation of monoclonal antibody[6]

The bivalent $F(ab')_2$ fragments were prepared by incubating the purified IgG_{2a} with pepsin at pH 4.2 for 24 hours at 37°C. The protein to enzyme ratio was 20:1.

The $F(ab')_2$ fragments were separated from indigested IgG_{2a} and proteolytic fragments by application to Protein A-Sepharose column equilibrated at pH 8.0. The $F(ab')_2$ fragments appeared in the unbound fraction. Its concentrations were estimated from OD_{280} as well as whole IgG_{2a}[2].

1.3. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The purity of IgG_{2a} and their $F(ab')_2$ fragments were monitored by polyacrylamide gel electrophoresis (SDS-PAGE).

The SDS-PAGE electrophoresis was carried out in 10% gels by the method of Laemmili[5].

2. Radioiodination of purified IgG_{2a} (Iodogen Method)[4]

Films of Iodogen (1,3,4,6 tetrachloro 3a-6a diphenylglycoluril) conveniently "plated" in the reaction tube react rapidly in the solid phase

with aqueous mixtures of I^- and proteins. Reaction tubes coated with the reagent can be prepared in advance and stored.

2.1. Iodogen Method

To a reaction tube coated with 10 μg of Iodogen, the reagents were added as follows: 40 μl of 0.5 M phosphate buffer pH 7.5; 10 μl of ^{131}I (2 mCi) and 20 μl (37 μg) of IgG_{2a}. The reaction was processed in 10 minutes and finished by the addition of 300 μl of 0.05 M phosphate buffer pH 7.5.

2.2. Purification of iodinated IgG_{2a}[9]

The iodinated protein was purified by an analytical grade anion exchange Resin Ag 1-X8, 100-200 mesh, chloride form (Bio-Rad) filled plastic disposable syringe (1 ml). The column was washed with 40 ml of 0.125 N phosphate buffer pH 7.5 and loaded with 25 mg of human serum albumin. The column was, finally, washed with 40 ml of the same buffer to remove excess albumin. The iodination mixture was transferred to the column. The ^{131}I-anti-CEA 4C11 IgG_{2a} was eluted with 0.125 M phosphate buffer pH 7.5. The first eluate (1 ml) was collected and stored (Figure 1).

2.3. The miniature chromatographic system[1]

This system was elaborated to determine the labelling efficiency incorporation of iodine into immunoglobulin and the radiochemical purity of the preparation.

The miniaturized chromatographic procedures were performed using Whatman 3MM paper (1.0 cm × 6.5 cm) as support with three different solvents: sodium chloride 0.9%; trichloroacetic acid (TCA 10%) and methanol 85%. The paper was spotted at 1 cm from the bottom. The strips were placed in a vial containing approximately 1 ml of each solvent. The chromatogram was developed for a distance of 5 cm. The elapsed developing time was approximately 10 min. The advantage of this method is that the radiochromatographic systems are chosen such that in one the impurities move with the solvent front (Rf = 0.8—1.0) while the radiopharmaceuticals remain near the origin (Rf = 0.0—3.0) or vice-versa. This permits one to cut the strips at Rf = 0.5 (midway) and to assay the two segments (section 1 and section 2). The activity of each portion was compared with the total radioactivity of the strip (Figure 2).

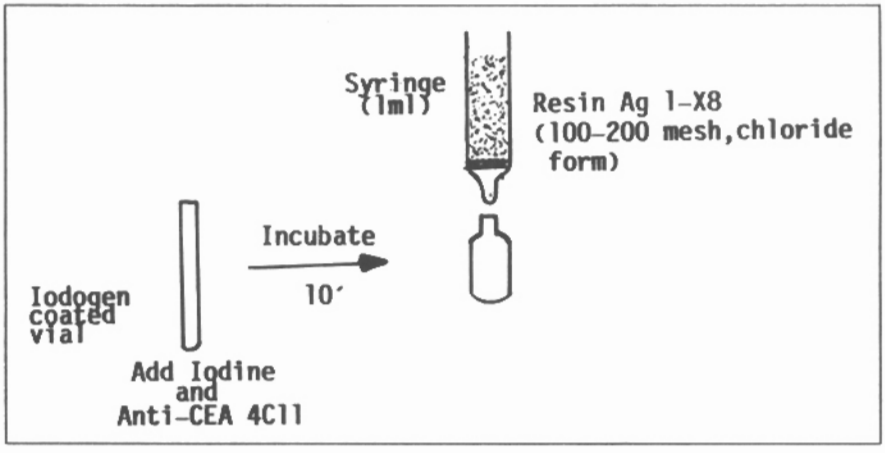

Figure 1 — Schematic diagram of Iodogen Method.

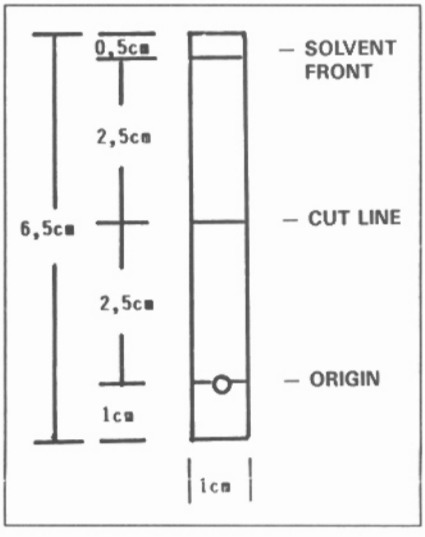

Figure 2 — Whatman 3MM miniaturized chromatographic strip used for evaluating ^{131}I-anti-CEA 4C11 IgG_{2a}.

RESULTS

1. Immunochemical procedures

1.1. Purification of anti-CEA 4C11 IgG_{2a}

Ascite containing 4C11 monoclonal antibody IgG_{2a} subclass was adjusted to pH 8.0 and passaged through the Protein A-Sepharose column which was washed sequentially at pH 8.0 and pH 4.5. At pH 4.5 amounts of IgG_{2a} were collected in 4-5-6- effluent fractions. The optical density at 280 nm was used to determine IgG_{2a} concentrations, using the extinction coefficient $\epsilon^{1\%}_{1cm} = 14$. At a concentration of 1.4 mg/ml, the OD_{280} of the fractions was 3.09, 3.10 and 3.00, representing a IgG_{2a} concentration of 2.20, 2.20 and 2.14, respectively. The fraction size was 3.0 ml.

The OD_{280} profile of this elution is depicted in Figure 3.

1.2. Fragmentation of monoclonal antibody

The bivalent $F(ab')_2$ fragments were prepared by incubating the purified IgG_{2a} with pepsin at pH 4.2 for 24 hours at 37°C. The protein to enzyme ratio was 20:1.

The $F(ab')_2$ fragments were separated from IgG_{2a} and proteolytic fragments by application to a Protein A-Sepharose column equilibrated at pH 8.0. The $F(ab')_2$ fragments appeared in the unbound fractions. At a concentration of 1.4 mg/ml, the

OD_{280} of the fractions was 0.228, 0.320 and 0.222, representing a $F(ab')_2$ fragments concentration of 0.163, 0.215 and 0.159 mg/ml, respectively. Fraction size was 3.0 ml.

The OD_{280} profile of this elution is depicted in Figure 4.

The final yield from purified IgG_{2a} to purified $F(ab')_2$ fragments (fraction 5) was approximately 10% of the starting material.

1.3. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The purity of IgG_{2a} and their $F(ab')_2$ fragments were monitored by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE). The SDS-PAGE was carried out in 10% gels.

The purified IgG_{2a} and $F(ab')_2$ fragments were shown to be homogeneous with SDS-PAGE.

2. Radioiodination of purified IgG_{2a}

2.1. Iodogen Method for radioiodination of IgG_{2a} offered reproducible iodination and showed easy to perform. Efficiently incorporation of iodine into monoclonal antibody 4C11 IgG_{2a} was achieved by this technique. The efficiency of two labelling procedures, expressed as the percentage of the total radioactivity incorporated into the intact IgG_{2a} (average 70%). Satisfactory specific activity was obtained (average 45 $\mu Ci/\mu g$).

2.2. Purification of iodinated IgG_{2a}

The radiochemical purity of IgG_{2a} was 98% for both preparations.

2.3. The miniature chromatographic system

The results of labelling efficiency of ^{131}I-anti-CEA 4C11 IgG_{2a} and radiochemical purity of preparations are shown in Table 1 and Table 2, respectively.

As indicated in Table 1 and Table 2 the ^{131}I-anti-CEA 4C11 IgG_{2a} remained at the origin (section 1) where as free ^{131}I migrated with the solvent front (section 2).

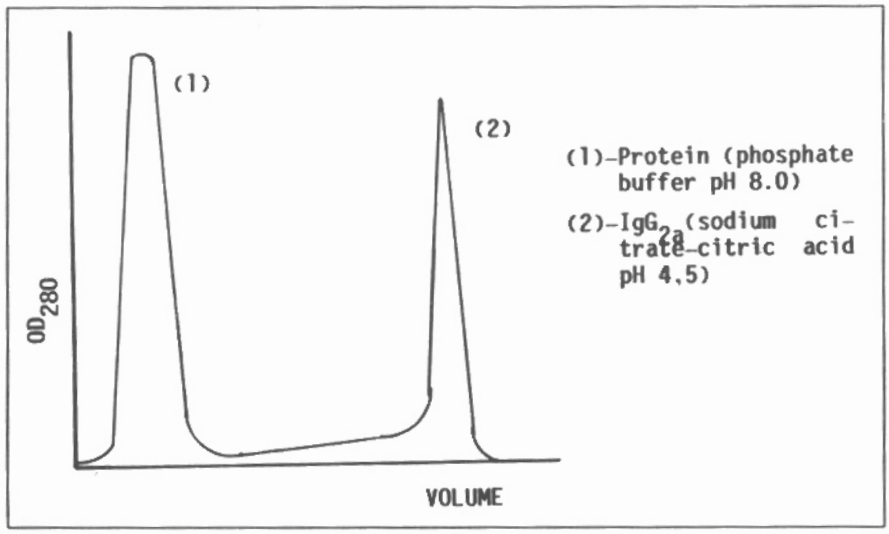

Figure 3 — Elution of IgG_{2a} from Protein A-Sepharose (ascitic fluid 1 ml was mixed with buffer pH 8.0). Fractions eluted at pH 4.5 were collected.

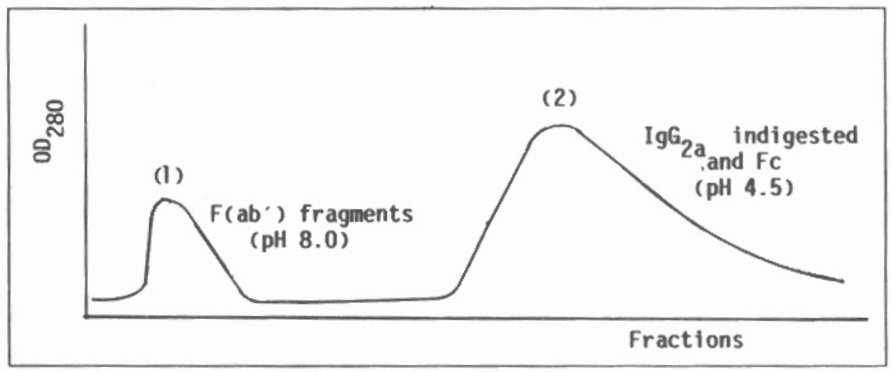

Figure 4 — Elution of $F(ab')_2$ fragments from Protein A-Sepharose. The $F(ab')_2$ fragments were eluted with void volume at pH 8.0 while indigested IgG_{2a} and Fc were eluted after equilibrated of the column at pH 4.5.

Table 1
LABELLING EFFICIENCY OF IgG ESTABLISHED BY MINIATURIZED CHROMATOGRAPHIC SYSTEM.

	Labelling n.º 1*		
	Counts (cpm)		
Solvents	Section 1	Section 2	Efficiency (%)
TCA 10%	1 201 210	429 489	73
	1 217 470	435 814	75
Methanol 85%	1 090 320	440 399	71
	1 463 990	668 992	69
NaCl 0.9%	392 758	165 997	70
	249 457	118 706	68
	Labelling n.º 2**		
TCA 10%	527 021	213 740	71
	468 416	198 350	70
Methanol 85%	636 233	311 591	67
	592 989	268 643	69
NaCl 0.9%	599 474	328 580	65
	666 359	322 612	66

* Specific activity 50 $\mu Ci/\mu g$. ** Specific activity 40 $\mu Ci/\mu g$.

This system provides a rapid and easy method to evaluate the labelling and radiochemical purity of ^{131}I-monoclonal antibody preparations.

Resumo. Radioiodação de anticorpo monoclonal purificado.

Objetivamos o desenvolvimento de um protocolo experimental para otimizar: 1. Procedimentos imunoquímicos: purificação de anticorpo monoclonal, redução da imunoglobulina purificada em fragmentos e estudos analíticos por eletroforese SDS-PAGE; 2. Condições de radioiodação da imunoglobulina purificada. O fluido ascítico de camundongo BALB/c isogênico contendo o anticorpo monoclonal anti-CEA 4C11 subclasse IgG$_{2a}$, doado pelo Instituto Ludwig/Brasil, foi usado como modelo para avaliar estes procedimentos.

Table 2
PERCENTUAL VALUES OF PURITIES IN THE FIRST ELUATE FROM ANION EXCHANGE COLUMN Ag 1-X8 (BIO-RAD) ESTABLISHED BY MINIATURIZED CHROMATOGRAPHIC SYSTEM.

	Counts (cpm)		
Solvents	Section 1	Section 2	Efficiency (%)
Labelling n.° 1*			
TCA 10%	109 499	1 610	98
	123 359	1 844	98
Methanol 85%	107 507	1 523	98
	86 309	1 181	98
NaCl 0.9%	74 270	1 442	98
	92 801	1 775	98
Labelling n.° 2**			
TCA 10%	68 221	859	98
	372 898	4 654	98
Methanol 85%	2 983	78	98
	286 381	3 823	98
NaCl 0.9%	296 089	3 088	98
	195 187	1 625	98

* Specific activity 50 μCi/μg. ** Specific activity 40 μCi/μg.

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