

IPEN-DOC-4047

ANTI-CEA MONOCLONAL ANTIBODY 4C11 IgG_{2a}

1st Research

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Co-ordination meeting

COMISSÃO NACIONAL DE ENERGIA NUCLEAR

INSTITUTO DE PESQUISAS ENERGÉTICAS E NUCLEARES

Caixa Postal 11049 - Pinheiros

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on labelling, quality
control and clinical
evaluation of
monoclonal antibodies
for scintigraphy, 1991

Sept.

9-13

INTRODUCTION:

Our study initiated with the development of an "experimental protocol" to optimize:

1. The immunochemical procedures to obtain an adequate immunological reagent from biologic preparations.
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.

MATERIAL AND METHODS:

1. Immunochemical procedures:

1.1 - Purification of IgG_{2a} from ascitic fluid (Ky et al, 1978).

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 fraction volume was 3.0ml. The optical density at 280nm was used to determine IgG_{2a} concentrations using the extinction coefficient $\epsilon_{1\%}^{1\text{cm}} = 14$.

1.2 - Fragmentation of IgG_{2a} (Lamoyi, 1986)

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. Its concentrations were estimated from OD₂₈₀ as well as whole IgG_{2a} (Demingnot et al, 1989).

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

The purity of IgG_{2a} and their F(ab')₂ fragments were monitored by

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Kuala Lumpur, Malaysia, Sept. 9-13, 1991

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polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE).

The SDS-PAGE was carried out in 10% gels by the method of Laemmli, 1970.

2. Radioiodination of purified IgG_{2a} (Iodogen Method-Fraker and Speck, 1978).

The advent of solid phase iodination agents has greatly expanded the range of gentle iodination techniques available for iodination sensitive biological materials, such as monoclonal antibodies.

The agent most widely used is the Iodogen (1,3,4,6 tetrachloro 3a-6a di-phenylglycoluril) films of Iodogen (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. This method is rapid, gentle, efficient, reproducible and can be accomplished in most radiopharmaceutical laboratories.

2.1 - Iodogen iodination.

Labelling of intact IgG_{2a} with ¹³¹I carried out using the 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 ¹³¹I (2 mCi) and 20 µl (37 µg) of IgG_{2a}. The reaction is usually processed in 10 minutes and finished by the addition of 300 µl of 0.05M phosphate buffer pH 7.5.

2.2 - Purification of iodinated IgG_{2a} (Wong et al, 1988).

For the purification we utilized an anion exchange column prepared from 1 ml plastic disposable syringe with an analytical grade anion exchange resin Dowex 1X8, 100-200 mesh chloride form. The product obtained from the iodination of IgG_{2a} was loaded onto the column. The iodinated protein was eluted with 0.125M phosphate buffer pH 7.5. The first 1 ml of eluate (pure ¹³¹I-IgG_{2a}) was collected and stored.

2.3 - The miniature chromatographic system (Colombetti et al, 1976).

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

The miniaturized chromatographic procedures were performed using Whatmann 3MM paper (1cmx6,5cm) as support with three different solvents: sodium chloride 0.9%; trichloroacetic acid 10% and methanol 85%.

The paper was spotted at 1cm from the bottom. The strips were placed in a vial containing approximately 1 ml of each solvent. The chromatogram was developed for distance of 5 cm (~10 minutes).

The advantage of this method is that radiochromatographic systems are chosen such that in one the impurities move with the solvent front ($R_f = 0.8-1.0$) while the radiopharmaceuticals remain near the origin ($R_f = 0.0-0.03$) or vice-versa. This permits one to cut the strips at $R_f = 0.5$ (midway) and to assay the two segments. The activity of each portion was compared with the total radioactivity of the strip.

RESULTS:**1. Immunochemical procedures****1.1 - Purification of IgG_{2a} from ascitic fluid.**

At a concentration of 1.4 mg/ml the OD₂₈₀ of the fractions (4-5-6 pH 4.5) were: 3.09; 3.10 and 3.00 representing a concentration of 2.20; 2.20 and 2.14 mg/ml respectively.

1.2 - Fragmentation of IgG_{2a} (Fraction 5).

At a concentration of 1.4 mg/ml the OD₂₈₀ of the fractions (4'-5'-6' pH 8.0) were: 0.228; 0.302 and 0.222 representing a F(ab')₂ fragments concentration of 0.163; 0.215 and 0.159 mg/ml respectively. The final yield from purified IgG_{2a} to purified F(ab')₂ fragments (fraction 5') was approximately 10% of the starting material.

1.3 - 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 to be easy to perform. Efficient incorporation of iodine into monoclonal antibody 4 C11 IgG_{2a} was achieved by the Iodogen 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 µCi/mg).

2.2 - Purification of iodinated purified IgG_{2a}.

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

2.3 - The miniature chromatographic system.

All solvents tested gave good separation and produced comparable values.

The miniaturized chromatography system provided a rapid and easy method to evaluate the labelling efficiency and radiochemical purity of ¹³¹I monoclonal antibody preparation.

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