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

HELENA OKADA
IRACELIA TORRES DE TOLEDO E SOUZA
ROSANA SIMÕES
COMISSÃO NACIONAL DE ENERGIA NUCLEAR - CNEN/SP

ABSTRACT

Our objective was the development of an experimental protocol to optimize: 1.Immunochemical procedures: the purification of Anti-CEA monoclonal antibody 4C11 belonging IgG_{2a} subclass from mouse ascitis, donated by Ludwig Institute/Brazil; the fragmentation of purified IgG_{2a} by pepsin digestion and analytical studies by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate as preliminary assessment for their specific application in immunoscintigraphy. 2.Radioiodination of purified IgG_{2a}: labelling of intact IgG_{2a} with 131-I carried out using Iodogen method.

INTRODUCTION

Since of the development of Hybridoma Technology (Köhler and Milstein, 1975) monoclonal antibodies have found an increasing number of applications and uses. The availability of monoclonal antibodies to tumour 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 purpose of this study were the development of immunochemical procedures to obtain an adequate immunoglobulin reagent from ascitic fluid from BALB/c mouse carrying the Anti-CEA 4C11 monoclonal antibody IgG_{2a} subclass,generously provided by Ludwig Institute -Brazil, and the conditions to radioiodination of purified IgG_{2a}.

MATERIAL AND METHODS

1.IMMUNOCHEMICAL PROCEDURES:

Purification of IgG_{2a} from ascitic fluid (Ey 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 $\epsilon^{1\%}_{1cm} = 14$.

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 OD_{280} as well as whole IgG_{2a} (Demingnot et al,1989).

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 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 diphenylglycoluril).Films of Iodogen (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. This method is rapid, gentle, efficient, reproducible and can be accomplished in most radiopharmaceutical laboratories.

Iodogen iodination. Labelling of intact IgG_{2a} with 131-I carried out using the Iodogen method.

To a reaction tube coated with 10µg of Iodogen, the reagents were added as follows: 40µl of 0,5M phosphate buffer pH 7.5; 10µl of 131-I (2mCi) and 20µl (37µg) of IgG_{2a}. The reaction is usually processed in 10 minutes and finished by the addition of 300µl of 0,05M phosphate buffer ph 7.5.

Purification of iodinated IgG_{2a} (Wong et al, 1988). For the purification we utilized an anion exchange column prepared from 1ml 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 1ml of eluate (pure 131-I-IgG_{2a}) was collected and stored.

The miniature chromatographic system (Colombeti 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 differents 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 1ml of each solvent . The chromatogram was developed

for distance of 5cm (~ 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

Purification of IgG_{2a} from ascitic fluid. At a concentration of 1.4mg/ml the OD_{280} 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.

Fragmentation of IgG_{2a} (Fraction 5). At a concentration of 1.4mg/ml the OD_{280} 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 0.159mg/ml respectively.

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

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

2. RADIOIODINATION OF PURIFIED IgG_{2a}

Iodogen Method for radioiodination of IgG_{2a} offered reproducible iodination and showed to be easy to perform. Efficienty incorporation of iodine into monoclonal antibody 4C11 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 45uCi/mg).

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

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 131-I monoclonal antibody preparation.

REFERENCES

[1] Colombeti,L.G.; Moerlien,S.; Pate,G.C.; Pinsky,S. M., Rapid determination of oxidation state of unbound 99m-Tc and labelling yield in 99m-Tc radiopharmaceuticals. J.Nucl. Med. 17:805-9,1976.

[2] Demignot,S.; Garnett,M.C.; Baldwin,R.W., Mouse IgG_{2b} monoclonal antibody fragmentation: preparations and fragmentation of Fab, Fc and Fab/c fragment. J.Immunol. Methods. 121:209-17,1989.

[3] Ey,P.L.; Prowse,S.J.; Jenkin,C.R., Isolation of pure IgG_{2a} and IgG_{2b} immunoglobulins from mouse serum using Protein A-Sepharose. Immunochemistry. 15:429-36,1978.

[4] Fraker,P.; Speck,J., Protein and cell membrane iodination with sparingly soluble chloramide 1,3,4, 6 tetrachloro 3a-6a diphenylglycoluril. Biochem. Biophys. Commum. 80: 849-57,1978.

[5] Köhler,G.; Milstein,C., Continous cultures of fused cells secreting antibody of predefined specificity. Nature. 256:495-5,1975.

[6] Laemmili,V.K., Cleavage of structural proteins during the assembly of the bead of bacteriophage. Nature (London). 227:680-4,1970.

[7] Lamoyi,E., Preparation of $F(ab')_2$ fragments from mouse of various sub-classes. In: Langone,J.J. ; Van Vunakis,H. eds. Methods in Enzymology. N.Y. Academic Press. 121:652-63,1986.

[8] Wong,Z.M.; Teare,F.W.; Bowen,B.M.; Liao,S.K.; Knok, C.S.; Boxen,I., Comparison of the iodogen and the microelectrochemical techniques for the radioiodination of monoclonal antibody. Nucl. Med. Biol. 15:505-9,1988.