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MICROELECTROCHEMICAL RADIOIODINATION OF MONOCLONAL ANTIBODY: A Preliminary Study

Z.M. Wong(1), F.W. Teare(1), B.M. Bowen(1,2), S.K. Liao(3), C.S. Kwok(4), P.C. Kwong(3), and I. Boxen(5)
(1)University of Toronto, Faculty of Pharmacy, 19 Russell Street, Toronto, Ontario, Canada, M5S 1A1
(2)Chedoke-McMaster Hospitals, Department of Nuclear Medicine, Hamilton, Ontario
(3)McMaster University Medical Center, Departments of Pediatrics & Pathology, Hamilton, Ontario
(4)Ontario Cancer Research Foundation, Hamilton Clinic, Hamilton, Ontario
(5)Henderson General Hospital, Department of Nuclear Medicine, Hamilton, Ontario

SUMMARY

The optimal reaction conditions for the microelectrochemical iodination of immunoglobulins were determined with non-specific human serum immunoglobulins. These conditions were used for the efficient radioiodination of a monoclonal antibody, 140.240, in submilligram quantities. An approximately five-fold decrease in the titre of the antibody against melanoma cells, as determined by the miniaturized mixed hemadsorption assay, was observed after iodination with an average of 0.85 atoms of iodine per molecule of antibody.

KEY WORDS: Iodination Efficiency, Immunoglobulins, Electrochemical, Monoclonal Antibody, Iodine-125

INTRODUCTION

Electrochemical iodination has been used to prepare iodinated proteins of high specific activity and satisfactory quality for radioimmunoassay, radioimmunodetection and tracer studies. The use of platinum electrodes minimizes electrode reactions (1) so that there is minimal chemical damage to proteins, even though they come into contact with the electrode

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during iodination (2). Iodination procedures have been scaled down considerably to volumes as small as 17 ul (3) and to label as little as 2 ug of proteins (4). Several investigators have reported the successful labelling of submilligram quantities of polypeptide hormones with good efficiency and no detectable biological damage (5,6,7,8). Constant current electrolysis can yield metabolically, fully viable iodinated proteins but other proteins may suffer biological damage during electrochemical iodination (9,10).

This paper describes the electrochemical radioiodination of submilligram quantities of a monoclonal antibody, 140.240, which is specific against many human melanoma cells (13). Instant thin layer chromatography (11) showed that microelectrochemical radioiodination resulted in a high percentage of the available iodide in the reaction mixture being incorporated into the antibody molecules. Optimal reaction conditions were determined using non-specfic human serum immunoglobulins. The most critical parameters were the iodide concentration (40 uM to 100 uM) and the current flow through the microcell (2.5 uA to 3.5 uA) for a reaction volume of 60 ul. Maximum incorporation of iodine into protein was observed with prolonged reactions. Radioiodination of monoclonal antibody 140.240 with 0.85 atoms of iodine per molecule of antibody resulted in an approximate five-fold decrease in the titre of the antibody as determined by the miniaturized mixed hemadsorption assay (12).

MATERIALS AND METHODS

Determination of Optimal Reaction Conditions for Non-specific Immunoglobulions

The 110 ul capacity platinum microelectrochemical cell and its associated circuitry previously described by Teare and Rosenberg (8) was employed with minor modifications: the

reaction temperature was controlled by placing the microcell in a cooling/heating coil, supported by a nylon block, through which was pumped water from a controlled temperature water bath. The paddle-shaped rotating platinum wire cathode was fitted with a teflon sleeve so that only the lower tip of the cathode is in electrical contact with the reaction mixture. This reduced the surface of the cathode at which the reverse deiodination reaction can occur, and prevents the cathode from inadvertently contacting the anode during iodination.

Investigation of the reaction conditions was carried out using immunoglobulins prepared from human serum. Reaction volumes of 29 ul to 60 ul containing 4 uM to 143 uM immunoglobulins and 10 uM to 250 uM iodide in phosphate buffer (0.025M, pH 7.5) were allowed to react at current flows ranging from 1.5 to 4.0 uA for as long as 120 minutes at room temperature. Aliquots of 1 ul reaction mixture were sampled for monitoring the reaction by instant thin layer chromatography (ITLC) at predetermined time intervals.

Iodination of Monoclonal Antibody 140.240

Monoclonal antibody 140.240, an IgG2a mouse antibody raised against a cultured human melanoma cell line, is highly specific for an oncofetal antigenic determinant of approximately 87K daltons expressed by many melanoma cell lines. For the iodination of monoclonal antibody 140.240, a 54 ul reaction mixture containing 22 uM monoclonal antibody, 23 uM potassium iodide and 1.3 uM sodium iodide ^{125}I (17 mCi per mg) in 0.025M phosphate buffer pH 7.5 was stirred in the microcell. A current of 2.5 uA was allowed to flow through the cell for a predetermined reaction time. This current flow, corresponding to a current density of 3.7 uA/cm^2, was sufficient for gentle anodic oxidation of iodide to a positive iodine species for labelling but not for the formation of iodate (8). At predetermined time

intervals, 1 ul aliquots of the reaction mixture were removed using a flexible plastic tipped oxford pipette for monitoring by the modified instant thin layer chromatographic procedure (11). The electrochemical reaction was terminated by switching off the power supply to the microcell.

At the end of the reaction, the contents of the microcell were quantitatively transferred to an anion exchange column (50mm x 4mm diameter, Biorad AG1-X8 Chloride resins) followed by two 100 uL rinses with the buffer. The iodinated monoclonal antibody was eluted with the same phosphate buffer and the first 1 mL of the eluate was collected and stored at 4° C for further testing. To reduce adsorption of monoclonal antibody to the column, the resins were precoated with human serum albumin and washed with 40 mL of the eluting buffer before chromatographic purification of the antibody preparation.

Ten replicate experiments were performed to check the reproducibility of the results, and to obtain a statistically significant mean and deviation from the mean.

Immunoassay of Radioiodinated Monoclonal Antibody 140.240

The radioiodinated monoclonal antibody 140.240 was evaluated using in vitro immunological assays. The affinity of binding of the antibody to melanoma cells (CaCL 78-1) was determined by the miniaturized mixed hemadsorption assay (12). In brief, 100 target cells were plated in each of the 60 wells of the minimicrotitre plates 18 hours prior to the scheduled test. A 10 ul aliquot of a serial dilution of the sample was added to each well and the plates were incubated for 2 hours at room temperature. After washing each plate with three 20 mL volumes of phosphate buffered saline containing 0.2% heat-inactivated gelatin, 10 ul of indicator sheep red blood cells were added to each well and the plates were incubated for another hour at room

temperature. The indicator sheep red blood cells were prepared by incubating sheep red blood cells with appopriate quantities of mouse-antisheep red blood cell antibodies, washing and then following with polyvalent goat anti-mouse-immunoglobulin antibodies. After careful washing of the plates with three 20 mL volumes of phosphate buffered saline, 0.25% glutaraldehyde was added to the plates to stabilize the rosettes for scoring under the light microscope at 100x magnification. Target cells were registered as positive if at least five sheep red blood cells were adherent to the cell surface. The end-point titre is defined as the inverse of the highest dilution at which more than 10% of the target cells were positive.

RESULTS AND DISCUSSIONS

Table 1: The effect of iodide concentration on the incorporation iodine into human serum immunoglobulins.

Molar concentration of human serum immunoglobulins	Molar concentration of iodide in reaction mixture (60 uL)	Percent incorporation of iodine into human serum immunoglobulins after 15 minutes reaction at 2.5 uA
1.4×10^{-4}	2.5×10^{-4}	34.2
	1.0×10^{-4}	44.1
	5.0×10^{-5}	39.3
	2.5×10^{-5}	28.6
3.5×10^{-5}	6.0×10^{-5}	80.9
	5.0×10^{-5}	72.3
	4.0×10^{-5}	81.1
	3.0×10^{-5}	64.0
	2.5×10^{-5}	53.1
	2.0×10^{-5}	50.1
	1.0×10^{-5}	12.9
1.7×10^{-5}	3.0×10^{-5}	66.9
	2.0×10^{-5}	59.6
	1.0×10^{-5}	14.4

Table 2: The effect of reaction time on the incorporation of iodine into human serum immunoglobulins.

Reaction time in minutes for a current flow of 2.5 uA.	Percent incorporation of iodine into human serum immunoglobulins. 1.4 x 10^{-4} M IgG and 5 x 10^{-5} M iodide in a 60 ul volume.
5	85.5
10	85.5
15	83.0
20	84.5
30	84.0
45	79.0
60	78.5
90	74.0
120	69.0

Table 3: The effect of protein concentration on the incorporation of iodine into human serum immunogloubins.

Molar concentration of human serum immunoglobulins.	Percent incorporation of iodine into human serum immunoglobulins after 15 minutes reaction at 2.5 uA in a 60 ul volume containing 5 x 10^{-5} M iodide.
1.4 x 10^{-4}	85.5
7.1 x 10^{-5}	86.5
3.5 x 10^{-5}	73.1
8.5 x 10^{-6}	72.3
4.3 x 10^{-6}	81.2

Table 4: The effect of current flow on the incorporation of iodine into human serum immunoglobulins.

Current flow through microcell (uA)	Percent incorporation of iodine into human serum immunoglobulins after 15 minutes reaction of 5 x 10^{-5} M iodide and 1.4 x 10^{-4} M protein in a 60 ul volume.
1.5	32.7
2.0	60.2
2.5	64.8
3.0	67.4
3.5	70.0
4.0	64.4
4.5	58.2

The optimal reaction conditions determined using human serum immunoglobulins were: iodide concentrations from 40 to 100 uM, protein concentration from 40 to 140 uM, current flow through the cell ranging from 2.5 to 3.5 uA, corresponding to an applied voltage of 4.3 to 5.0V for a 5 to 10 minute reaction in a 60 ul volume at room temperature and a pH of 7.5. The maximum degree

of incorporation of iodide available in the reaction mixture into the human serum immunoglobulins, as determined by the instant thin layer chromatographic procedure exceeded 85%. The most important parameters were the iodide concentration and the current flow through the microcell. Scrupulous cleaning of the platinum microanodic cylinder with 6N nitric acid was found to be necessary to maintain the incorporation of iodine into immunoglobulins at their maximal levels. These criteria were in good agreement with the optimal reaction conditions found for human serum albumin.

Using these optimized reaction conditions for the microelectrochemical radioiodination of monoclonal antibody 140.240, a high degree of incorporation of available iodide into the antibody molecules was achieved.

Table 5: The effect of iodide and protein concentration on the incorporation of iodine into monoclonal antibody 140.240.

Molar concentration of monoclonal antibody 140.240	Molar concentration of iodide	% incorporation of iodine into monoclonal antibody after 15 minutes reaction at 2.5 uA
2.21×10^{-5}	2.7×10^{-4}	45.0
	9.1×10^{-5}	89.9
	6.8×10^{-5}	89.2
	4.6×10^{-5}	90.3
	4.6×10^{-5}	87.3
	3.4×10^{-5}	91.2
1.64×10^{-5}	3.4×10^{-5}	65.8
3.1×10^{-6}	6.0×10^{-6}	19.6

In order to attain a desired iodine to protein ratio of one, it was necessary to decrease the iodide concentration to 24 uM to match that of monoclonal antibody 140.240 (22 uM, 150,000 daltons). This resulted in a decrease in the efficiency of incorporation of iodine into the antibody molecules to 78.0 ± 3.6%. The product, therefore, contains an average of 0.85 atoms

of iodine per molecule of antibody. The monoclonal antibody 140.240 labelled with an average of 0.85 atoms of iodine per molecule of antibody showed a 5.4 ± 2.0 fold decrease in the titre as determined by the miniaturized mixed hemadsorption assay. This would indicate that microelectrochemical iodination affected the antigen-binding sites of the monoclonal antibody 140.240 molecules so that the affinity of binding between antibody and antigen was slightly diminished even at this low level of iodination.

The electrochemical technique has generally been considered the gentlest technique available for the iodination of proteins containing tyrosyl or histidyl residues. Generally, up to two atoms of iodine may be incorporated into each molecule of antibody without significantly affecting the immunological properties of the antibody in radioimmunoassays. However, these observations were for the antigenic properties of the molecules which makes them recognizable and specifically bound to the corresponding antibodies. The immunological activity studied in this paper refers to the ability of antibody molecules to recognize and bind specifically to the appropriate antigens expressed by melanoma cells.

The mixed hemadsorption assay is highly specific and sensitive, resulting in titres as much as 4000 times that obtained using immunofluorescence assays and about 500 times that obtained using cytotoxicity assays (14). This mixed hemadsorption technique was further improved by the development of the miniaturized mixed hemadsorption assay which resulted in another 4-8 fold increase in sensitivity (titre) without a concomitant decrease in specificity (12). A five-fold decrease in the titre of the radiolabelled antibody as determined by the miniaturized mixed hemadsorption assay is therefore a very minor effect and is unlikely to be reflected by either the original

mixed hemadsorption assay or conventional immunofluorescence assay. Thus, the immunological damage to monoclonal antibody 140.240, labelled with 0.85 atoms of iodine per molecule antibody, may merely reflect previously undetected detrimental effects of the microelectrochemical technique or the sensitivity of this specific antibody to iodination so that even lower levels of iodination would affect its immunological, antigen-binding properties. A comparison of the effect of microelectrochemical radioiodination on the functional integrity of the antibody with other methods of radioiodination will be the subject of a separate paper.

CONCLUSION

Submilligram quantities (175 ug) of monoclonal antibody 140.240 were successfully labelled with iodine with high (78%) efficiencies of incorporation of iodine into the antibody molecules. After radioiodination with 0.85 atoms of iodine per molecule of antibody by the microelectrochemical technique, the titre of monoclonal antibody 140.240, reflecting the affinity of specific binding to melanoma cells, was found to be decreased approximately five-fold from 44,800 to 8960 for the 1.2×10^{-6} M antibody preparation. The retention of antigen-binding properties of the labelled monoclonal antibody 140.240 is in close agreement with the results obtained with polypeptide hormones (6,8) where nearly full retention of the antigenic and biological properties were observed after iodination by the microelectrochemical technique.

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