Journal of Labelled Compounds and Radiopharmaceuticals Vol. XXIX, No. 6

NOTE

PREPARATION OF BROMINE-77 LABELLED MONOCLONAL ANTI-hPLAP ANTIBODY USING CHLORAMINE-T

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SUMMARY

A tumor-associated monoclonal antibody, named 7E8 and raised against human placental alkaline phosphatase (hPLAP), is labelled with bromine-77 by means of chloramine-T. The paper describes optimum radiobromination conditions resulting in 34 % radiochemical yield of labelled antibody with more than 90 % immunoreactivity.

Key Words : Bromine-77, anti-hPLAP monoclonal antibody, chloramine-T, bromination.

INTRODUCTION

Monoclonal antibodies (MAbs) labelled with radioisotopes have great potential for specific radiolocalization of tumor cells by external imaging techniques. In most studies ^{125}I- or ^{131}I-labelled MAbs are used as immunoimaging agents (1). However iodinated proteins suffer from some major disadvantages. It has been shown (2,3) that extensive dehalogenation occurs at many sites in the body including the tumor, resulting

0362–4803/91/060729–09$05.00

Received 4 February, 1991
Revised 4 March, 1991

in decreased tumor to tissue uptake ratios and iodine accumulation in the thyroid and gastrointestinal system (4). Use of ^{125}I and ^{131}I is associated also with a high radiation dose. Radioisotopes of bromine represent more suitable radiolabels due to the stronger carbon-bromine bonds compared to carbon-iodine bonds (5). Bromine-77 decays mainly by electron capture and about 1 % by positron emission with major γ-rays at 238 (23.8 %), 300 (6 %), 521 (21.4 %) and 580 keV (7 %) (6). Its half-life of 56 h makes it particularly suitable to study antigen-antibody reactions by PET or by using gamma cameras.

The whole-body radiation dose delivered by ^{77}Br in the form of a plasma tracer is about 1/10 the dose delivered by either ^{125}I or ^{131}I (7) and bromide released does not accumulate in the thyroid (8).

Bromination of proteins is achieved by several methods (5,9-18). Our data describe a labelling procedure for anti-hPLAP monoclonal antibodies using chloramine-T. Although the latter is a very reactive substance and tends to easily damage the antibodies, the method is useful to label this MAb without significant loss of immunoreactivity by proper adjustment of the concentration of reagents, pH, temperature and time.

Selection of anti-hPLAP monoclonal antibodies to be labelled is chosen for several reasons. Tests have proven (19) that about 50 % of patients with ovarian tumors and the majority of patients with seminomas have increased serum levels of the enzyme. Tumor material of these patients is also positive. Thus hPLAP may be considered as a potentially valuable tumor marker for these malignancies and MAbs raised against hPLAP labelled with ^{77}Br could be useful for the early detection of these cancers (20). An enzyme linked immunosorbent assay (ELISA) is used to determine the immunoreactivity of the labelled monoclonal antibody.

EXPERIMENTAL

Materials

Chloramine-T was purchased from UCB (Belgium).

Anti-hPLAP monoclonal antibody (MW 160.000 ; IgG_1 subclass) was a generous gift of Dr. A. Van de Voorde of Innogenetics (Belgium). Goat anti-mouse IgG was purchased from Jackson Immuno Research Laboratories, Inc. (USA).

Bovine serum albumin (BSA), p-nitrophenylphosphate and human placental alkaline phosphatase (hPLAP) were from Sigma Chemical Company (USA). Tween 20 was obtained from Laboratoria Flandria (Belgium) while all other chemicals were of analytical grade from Merck (FRG). The multi-well microtiter plates were purchased from Nunc (Denmark).

Phosphate buffered saline (PBS) pH 7.4 was made as follows : 9.00 g NaCl ; 0.20 g KCl ; 1.42 g Na_2HPO_4 ; 0.20 g KH_2PO_4 in 1000 ml of distilled water.

Production of ^{77}Br. ^{77}Br is cyclotron produced by the ^{75}As $(\alpha,2n)^{77}Br$ reaction. The target material As_2O_5 (0.5 g) is irradiated with α-particles of 29 MeV at beam intensities of 5 to 10 µA yielding 0.3 $mCi.\mu A^{-1}.h^{-1}$ of ^{77}Br on the target. At the end of bombardment the target is left for at least 12 h to allow the decay of short lived contaminants e.g. ^{18}F from the ^{16}O $(\alpha,pn)^{18}F$ reaction.

After this decay period the arsenic pentoxide is washed from the target plate and transferred as a slurry to a distillation apparatus. Concentrated H_2SO_4 and $K_2Cr_2O_7$ are added, and on heating (90 °C, 0.5 to 1 h) the radiobromine is swept by a nitrogen stream into an ice-cooled water trap.

This bromine solution ($\leq$ 1 ml) is made alkaline with NaOH and 100 µl of a 2.5 mM hydroxylamine.hydrochloride solution is added to reduce the radiobromine to bromide. The

solution is evaporated to dryness to destroy the remaining hydroxylamine and the residue is dissolved in 500 µl water and the pH adjusted to 6-7 with H_2SO_4 (21).

Radiobromination. 62.5 µg of the anti-hPLAP MAb is radiobrominated at pH 6.5 using a 250-fold molar excess of chloramine-T in a total reaction volume of 125 µl. For tracer studies about 1 µCi of $^{77}Br^-$ is used and 5 µl chloramine-T solution containing the optimum amount to carry out the reaction. Bromination is allowed to proceed for 15 min at 20 °C. The labelling yield is determined by trichloroacetic acid (TCA) precipitation of the labelled protein. The bromination is stopped by adding bovine serum albumin subsequently followed by the addition of ice-cooled TCA. The final concentrations in 400 µl end volume of both BSA and TCA are 1 mg/ml and 25 % (w/v) respectively. The suspension is stored at 0 °C for at least 30 min and centrifuged for 10 min at 1500 g. The pellet is resuspended and centrifuged three times with 1 ml of ice-cooled 10 % (w/v) TCA. Precipitate, supernatant and wash fractions are counted on a NaI(Tl) one channel gammaspectrometer.

Enzyme linked immunosorbent assay. Wells of a polystyrene microtiter plate are coated at 37 °C with 100 µl of goat anti-mouse IgG antibodies per well diluted in 50 mM carbonate buffer pH 9.8. The liquid is discarded by flicking of the assay plate and wells are washed two times with PBS + 0.05 % Tween 20 (referred to as the washing buffer). Plate washing is performed by repeated aspirating and filling with buffer. Nonspecific binding sites of microtiter plates are blocked by exposure to a 0.5 % Tween 20 solution in PBS. After blocking the plate is washed and a series of labelled and unlabelled anti-hPLAP MAb dilutions made in incubation buffer (PBS, 0.01 % BSA, 0.02 % sodium azide and 0.05 % Tween 20) is then added and incubated for several hours at 37 °C with the immobilized

goat anti-mouse IgG antibodies. Any unbound MAb is washed away by rinsing with washing buffer. A known amount of human placental alkaline phosphatase (hPLAP) is next allowed to react with the bound MAb (overnight, 4 °C). Any excess unbound hPLAP is removed after the reaction by rinsing with washing buffer. The substrate (100 µl/well of a solution of p-nitrophenylphosphate) is added which results in a color change. The reaction is allowed to proceed at 37 °C for a suitable time interval and absorbancies are read at 405 nm.

RESULTS AND DISCUSSION

The effect of pH on the bromination using different buffer systems can be seen in Fig.1 where maximum incorporation is achieved between pH 5.5 and 6.5.

Tyrosyl residues provide the most stable radiolabel (7) and to achieve this a neutral pH is required (22,23). Thus pH 6.5 is chosen as the optimum pH for the radiobromination of this MAb.

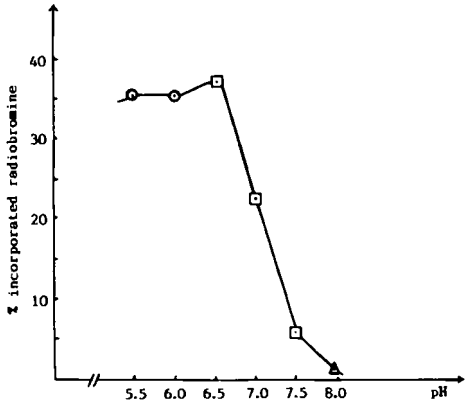

Fig.1. Effect of pH on the bromination yield.
(o) 0.1 M potassium acetate pH 5.5-6.0 ; (□) 0.1 M potassium phosphate pH 6.5-7.5 ; (Δ) 0.1 M potassium borate pH 8.0. Conc. of MAb : 0.1 mg/ml ; reaction vol. : 125 µl ; reaction temp. : 20°C ; reaction time : 15 min ; conc. of chloramine-T : 250-fold molar excess versus MAb conc.

The influence of reaction time on the bromination is shown in Table 1.

Table 1 : Effect of reaction time*

reaction time min	% incorporated radiobromine	% immuno-reactivity
5	13.8 ± 0.6	82.5 ± 2.9
10	14.3 ± 0.5	78.6 ± 2.8
15	34.8 ± 0.5	72.8 ± 2.9
30	36.2 ± 0.4	7.6 ± 3.7

* pH : 6.5 ; all the other reaction parameters are as described in Fig. 1.

Three reaction temperatures were tested : 0, 20 and 37 °C. The results are shown in Table 2.

Table 2: Effect of reaction temperature on the Mab bromination*

reaction temperature °C	% incorporated radiobromine	% immuno-reactivity
5	22.2 ± 0.4	78.2 ± 2.7
20	34.8 ± 0.5	72.8 ± 2.9
37	11.7 ± 0.5	66.3 ± 2.8

* pH : 6.5 ; the other reaction parameters are the same as those described in Fig. 1.

Using chloramine-T concentrations below 250-fold molar excess gave very low bromine incorporation in the immunoglobulin. Chloramine-T concentrations above 250-fold molar excess resulted in increasing bromine incorporation however with decreasing immunoreactivity. By applying a 500-fold molar excess of chloramine-T to antibody, only 2 % of the immunoreactivity remained.

The concentration of antibody (Table 3) seemed to have little effect on the radiochemical yield.

Table 3 : Effect of antibody-concentration*.

Conc. of MAb (mg/ml)	% incorporated radiobromine	% immuno-reactivity
0.1	34.8 ± 0.5	72.8 ± 2.9
0.5	34.4 ± 0.5	92.6 ± 2.9
0.75	26.2 ± 0.7	77.5 ± 2.7

* pH : 6.5 ; the other reaction parameters are the same as those described in Fig. 1.

CONCLUSION

The method described is suitable for labelling anti-hPLAP antibodies and by carefully monitoring the reaction conditions the immunoreactivity of labelled anti-hPLAP antibodies is sufficiently retained.

ACKNOWLEDGEMENT

This work was supported by a grant of the Belgian Government (Fonds voor Geneeskundig Wetenschappelijk Onderzoek).

REFERENCES

1. Thedrez Ph., Blottière H., Chatal J.F., Grzyb J. and Douillard J.Y. - Tumour Biology 7 : 137 (1986).
2. Krohn K.A., Sherman L., Welch M.J. - Biochim.Biophys. Acta 285 : 404 (1972).
3. Metzger J.M., Secker-Walker R.H., Krohn K.A. et al. - J.Lab.Clin.Med. 82 : 267 (1973).
4. Powe J. - Hybridoma 5 (1) : S39 (1986).
5. Stöcklin G. - Int.J.Appl.Radiat.Isotop. 28 : 131 (1977).
6. Blessing G., Weinreich R., Qaim S.M. and Stöcklin G. - Int.J.Appl.Radiat.Isot. 33 : 333 (1982).

7. McElvany K.D., Barnes J.W. and Welch M.J. - Int.J.Appl.Rad. Isot. 31 : 679 (1980).
8. Nicholson J.P. and Zilva F. - Clin.Sci. 19 : 391 (1960).
9. Lambrecht R.M., Mantescu C., Redvanly C.S. and Wolf A.P. - J.Nucl.Med. 13 : 266 (1972).
10. El-Garhy M. and Stöcklin G. - Radiochem.Radioanal.Letters 18 : 28 (1974).
11. Coenen H.H., Machulla H.-J. and Stöcklin G. - J.Labelled Compd.Radiopharm. 16 : 891 (1979).
12. Wilbur D.S., Bentley G.E. and O'Brien H.A. Jr. - J.Labelled Compd.Radiopharm. 18 : 1693 (1981).
13. Coenen H.H., El-Wetery A.S. and Stöcklin G. - J.Labelled Compd.Radiopharm. 18 : 114 (1981).
14. Katzenellenbogen J.A., Senderoff S.G., McElvany K.D., O'Brien H.A. Jr. and Welch M.J. - J.Nucl.Med. 22 : 42 (1981).
15. Petzold G. and Coenen H.H. - J.Labelled Compd.Radiopharm. 18 : 1319 (1981).
16. Knight L., Krohn K.A., Welch M.J., Spomer B. and Hager L.P. - "Radiopharmaceuticals", Society of Nuclear Medicine, New York, 1975, p. 149.
17. Mc.Elvany K.D. and Welch M.J. - J.Nucl.Med. 21 : 953 (1981).
18. Mc.Elvany K.D., Barnes J.W. and Welch M.J. - Int.J.Appl. Radiat.Isotop. 30 : 679 (1981).
19. Briers T.W., Nouwen E.J., Pollet D.E., Hendrix P.G., Martens E.J., Bogaerts J.B., De Weerdt D. and De Broe M.E. - Tijdschrift van de Belgische Vereniging van Laboratorium Technologen 13 (3) : 127 (1986).
20. Eerdekens M.W., Nouwen E.J., Pollet D.E., Briers T.W. and De Broe M.E. - Clin.Chem. 31 : 687 (1985).
21. Skrabal F., Arnot R.N., Helus F., Glass H.I. and Joplin

G.F. - Int.J.Appl.Radiat.Isotop. 21 : 183 (1970).

22. Krohn K.A., Knight L.C., Harwig J.F. and Welch M.J. - Biochim.Biophys.Acta 490 : 497 (1977).

23. Knight L.C. and Welch M.J. - Biochim.Biophys.Acta 534 : 185 (1978).