Technetium-99m labelling of the anti-tumour antibody PR1A3 by photoactivation

Maria A. Stalteri, Stephen J. Mather

Imperial Cancer Research Fund, Department of Nuclear Medicine, St. Bartholomew's Hospital, West Smithfield, London EC1A 7BE, UK

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Abstract. Irradiation of antibody with ultraviolet light leads to reduction of disulphide bonds. Thus irradiation can be used to generate free thiols prior to direct labelling of antibody with technetium-99m, and has a potential advantage over methods using chemical reducing agents such as mercaptotoethanol or tin, in that no purification step is needed to remove excess reducing agent. We have used the photoactivation method developed by Sykes et al. to label the anti-tumour antibody PR1A3 with $^{99m}$Tc. The antibody was irradiated at 300 nm using a Rayonet photochemical reactor with eight RMR3000 lamps. In a typical experiment, the antibody solution was injected into a nitrogen-filled borosilicate glass vial and purged with nitrogen. A degassed solution containing stannous fluoride and methylene diphosphonate was then added to the antibody and the vial was irradiated. Following the irradiation, $[^{99m}Tc]$pertechnetate was injected into the vial and the reaction mixture was incubated for 30 min at room temperature before being analysed by size-exclusion high-pressure liquid chromatography and instant thin-layer chromatography. Labelling yields greater than 95% were obtained using antibody concentrations ranging from 0.5 mg/ml to 5 mg/ml. Irradiation times as short as 5 min and tin to antibody ratios in the range between 11 and 32 $\mu$g tin per mg antibody gave high labelling yields. Labelling yields greater than 95% were obtained after storage of the photoactivated antibody at -70°C for several weeks. The stability of the $^{99m}$Tc-labelled photoactivated PR1A3 was similar to that of $^{99m}$Tc-labelled mercaptotoethanol-reduced PR1A3. The mean immunoreactive fraction was 77% for the photoactivation-labelled PR1A3, compared to 93% for PR1A3 labelled by mercaptotoethanol reduction. Biodistribution studies were carried out using $^{99m}$Tc-photoactivation-labelled PR1A3 or PR1A3 labelled by mercaptotoethanol reduction in Balb/c mice and in nude mice with MKN-45 human tumour xenografts. There was no significant difference in tumour uptake between the mice that received photoactivated PR1A3 and those that received mercaptotoethanol-reduced PR1A3. There was also no significant difference in uptake in most organs in Balb/c mice; however, the photoactivated antibody cleared more rapidly from the blood, and whole-body clearance was also faster for the photoactivated PR1A3. In conclusion, the photoactivation technique provides a very convenient “one-pot” method for labelling antibodies with $^{99m}$Tc.

Key words: Photoactivation - Monoclonal antibody - Radiolabelling - Technetium-99m


Introduction

After some years of reluctance and a tendency towards the use of longer-lived radionuclides, technetium-99m has now become established as the radionuclide of choice for imaging with monoclonal antibodies [1–3]. Methods used for radiolabelling antibodies with this radionuclide can be clearly divided between “direct” approaches in which the technetium is co-ordinated by the amino acid side chains of the protein itself and “indirect” methods in which the technetium is co-ordinated by a synthetic chelating agent which may be conjugated to the immunoglobulin either before or after the radiolabelling process [4]. The principal advantage of the indirect methods is that they are applicable to a wide range of proteins, in contrast to direct methods which can be used only for antibodies and some antibody fragments. However, the main advantage of the direct approach is its simplicity. While many chelating agents require complex synthetic routes and post-labelling purification of the radioconjugate is normally necessary, the most popular of the direct methods can be carried out in any competent radiopharmacy using reagents which are both inexpensive and commercially available and radiolabelling efficiencies are high, 95% or better, which enables the development of simple one-step “kit” formulations.

The first step in all direct antibody-labelling methods is the reduction of some of the disulphide bridges which stabilise the immunoglobulin molecule. This results in the generation of free thiol groups which can subsequently
form a stable bond with \(^{99m}\text{Tc}\). Depending upon the choice of reducing agent, a subsequent purification step may be required in which excess reductant is removed from the preparation in order to either prevent damage to the antibody structure or to remove potentially toxic compounds prior to administration to patients. While the use of non-toxic reducing agents which may safely be administered to patients has been proposed [5], results obtained with some of these reagents have been more variable and less reliable [6] than those obtained with the most widely used reductants, mercaptoethanol [7] and tin [8].

Recently Sykes and colleagues have described a novel approach to antibody labelling which also removes the need for post-reduction purification [9, 10]. This is based upon the use of long-wavelength ultraviolet (UV) irradiation to selectively cleave disulphide bonds. Following irradiation, the reducing agent is simply removed by switching off the UV lamp.

The aim of this study was to evaluate this new approach and compare it directly with our well-established mercaptoethanol reduction technique [11].

**Materials and methods**

**Photochemical reactor**

A Rayonet RMR 3000 photochemical reactor with eight UV lamps was used. This equipment was purchased from Southern New England Ultraviolet Company (Branford, CT) at a cost of approx. U.S. $ 600.

**Radioisotope**

Sodium \(^{99m}\text{Tc}\) pertechnetate was obtained from an Ameritron II technetium-99m generator (Amersham, UK).

**Antibodies**

PR1A3, a mouse IgG1 monoclonal antibody used in the study of colorectal cancer, was obtained from the Hybridoma Development Unit, Imperial Cancer Research Fund, London. PR1A3 is used in radioimmunoscintigraphy of patients with colorectal cancers [2]. The epitope recognised by this antibody is a cell-associated form of carcinoembryonic antigen (CEA) which is lost when the molecule is shed [12]. SM3 is a mouse IgG1 monoclonal antibody that recognises an epitope on stripped epithelial mucin [13], and was obtained from the Hybridoma Development Unit. Polyclonal human IgG (Endobulin) was obtained from Immuno Ltd., Dunton Green, Kent, UK.

**Cell Line**

MKN-45 cells were obtained from the Director’s Laboratory, ICRF, London. MKN-45 is a human gastric cancer cell line which expresses CEA on the surface [14]. The cells were grown in vitro in Dulbecco’s Modified Eagle’s Medium containing 10% foetal calf serum.

**High-performance liquid chromatography (HPLC)**

Size-exclusion HPLC was performed using a Beckman 114M pump with a Beckman 160 UV detector at 254 nm connected to a Spectra-Physics SP4290 integrator. A Beckman SEC 3000 column (optimal separation range 5-700 kDa), a 2.0-ml injection loop and flow rates of 0.5 ml per min or 1.0 ml per min were used. The mobile phase used was 0.2 M sodium phosphate pH 7.0, 2 mM ethylenediaminetetraacetic acid (EDTA). Radioactivity was measured using a NaI(Tl) flow through detector. The recovery was determined by collecting the eluate from the HPLC column for 20 min (at 1.0 ml/min) or 40 min (at 0.5 ml/min) from the time of injection and counting 1-ml aliquots in a gamma counter together with 1-ml aliquots of a suitable dilution of the injectate.

**Instant thin-layer chromatography (ITLC)**

Silica impregnated glass fibre ITLC SG strips (Gelman Sciences, Ann Arbor, MI) were developed in 0.9% saline. In this system, labelled antibody and colloids remain at the origin while technetium-methylene diphosphonate (MDP) and pertechnetate move with the solvent front. Typically 0.5–2.0 \(\mu\)l of labelled antibody solution was spotted onto an ITLC strip; the strip was developed and then cut in half and counted in an LKB Wallac CompuGamma CS gamma counter after 24 h.

**Labelling of photoactivated PR1A3**

A typical labelling experiment was performed as follows. Five ml of 50 mM phosphate buffered saline (PBS) pH 7.0 was injected into a sterile 10-ml nitrogen-filled borosilicate glass vial with a rubber septum (Instar Ltd., Wokingham, Berks., UK) using a sterile disposable syringe and the solution was purged with nitrogen for 15 min. One hundred \(\mu\)l of 4 mg/ml PR1A3 antibody in PBS was injected into another 10-ml vial and the vial was purged with nitrogen for 15 min. An Ameriscan Medronate II kit (containing 5 mg sodium medronate, 0.34 mg stannous fluoride and 2 mg sodium \(p\)-aminobenzoate) was reconstituted with 2.0 ml of the nitrogen-purged PBS. One hundred \(\mu\)l of this medronate solution was injected into the vial containing the antibody and the vial was irradiated for 20 min using a Rayonet RMR 3000 photochemical reactor. One hundred \(\mu\)l of sodium \(^{99m}\text{Tc}\) pertechnetate in saline, containing 500 MBq \(^{99m}\text{Tc}\), was added to the vial and the solution was incubated for 30 min at room temperature before analysis by size-exclusion HPLC and ITLC.

**Effect of irradiation time on labelling efficiency**

Vials containing 100 \(\mu\)l of 4 mg/ml PR1A3 in PBS and 100 \(\mu\)l of an Ameriscan Medronate II kit reconstituted in 2 ml of 50 mM PBS pH 7 (32 \(\mu\)g Sn/mg Ab) were irradiated for 0, 5, 10, 15, 20, 30, 40 or 60 min. One hundred \(\mu\)l of sodium \(^{99m}\text{Tc}\) pertechnetate in saline, containing 450 MBq \(^{99m}\text{Tc}\), was added to each vial. After incubation for 30 min at room temperature the samples were analysed by size-exclusion HPLC using UV and radiometric detection.

**Effect of amount of tin on labelling efficiency**

Vials containing 100 \(\mu\)l of 4 mg/ml PR1A3 in PBS and 50, 100, 150, 200 or 250 \(\mu\)l of an Ameriscan Medronate 11 kit reconstituted with 3 ml of 50 mM PBS pH 7 were irradiated for 20 min. One hundred \(\mu\)l of sodium \(^{99m}\text{Tc}\) pertechnetate in saline, containing 650 MBq \(^{99m}\text{Tc}\), was added to each vial. After incubation for 30 min at room temperature the samples were analysed by size-exclusion HPLC using UV and radiometric detection.