The use of redox polymers in labelling procedures of proteins and peptides with ^{99m}Tc

II. Technique of preparation of kits for protein labelling by ^{99m}Tc and its effect on the stability and radiochemical purity

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Abstract

BACKGROUND: Our research into new methods of labelling biologically active substances with technetium (^{99m}Tc) to be used in nuclear medicine is focused on proteins and peptides whose labelling is difficult and continues to pose a problem. Use of redox polymers is the gentlest method as there is no need to use other compounds to modify reaction conditions, as is the way with the currently employed methods.

METHODS: Kits containing proteins, e.g., human immunoglobulin (IgG), human leukocytes dialysate (HLD), were labelled with ^{99m}Tc using a redox polymer (RP-IDA) and/or using reduction of pertechnetate (^{99m}Tc) with stannous chloride. The radiochemical purity was evaluated using paper and/or electrophoresis of the labelled proteins. The results were confirmed by column chromatography of the labelled IgG.

RESULTS: Use of a redox polymer for labelling raises the stability of ^{99m}Tc labelled proteins so that labelling efficiency remains virtually the same (96–98%) after 30 hours while it is about 45% with the routine method using stannous chloride. The stability of protein-containing kits prepared using redox polymers was improved; the labelling efficiency of HLD and IgG after 300 days of kit storage was over 97% and 87%, respectively.

CONCLUSIONS: The labelling procedure using redox polymers can be used in formulating protein- and peptide-based radiopharmaceuticals to improve their stability and ^{99m}Tc labelling efficiency.

Key words: redox polymers, technetium (^{99m}Tc), radioassay, labelled proteins

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Introduction

The issue of labelling of proteins and peptides of labile structures is very extensive and complex, and has not yet been fully explored. Currently, small proteins are often employed whose structures in preparation and labelling are sensitive to reaction conditions. Generally, the procedures for preparing and labelling of small proteins and peptides can be classified into two main groups (1):

1) methods of direct labelling (2, 3),

2) methods of indirect labelling (procedures with bifunctional chelates) (4).

This experimental study is focused on the methods of direct labelling. Generally speaking, they are methods based on the use of functional groups naturally occurring in proteins or peptides in the form of -NH_2, -OH, -COOH or -SH, which are either free in the native protein, or released during its modification. Under certain structural conditions, these groups are capable of binding reduced technetium. Two different binding sites have been suggested:

a) high affinity and low capacity, associated with SH groups (5),

b) low affinity and high binding site capacity, associated with free -NH_2, -OH and COOH groups.

A drawback of direct labelling procedures is that technetium may bind to these quite different binding sites with high and low affinity. This usually leads to variable stability of the labelled product. For this reason, we focused on the preparation of protein and peptide kits for labelling by technetium (^{99m}Tc) using redox polymers which, in our view, is the gentlest method. Use of redox polymers at pH values over 6.0 allows practically selective labelling of these compounds with technetium (^{99m}Tc) at end SH groups. Redox polymers do not require the use of other adjuvants to modify reaction conditions as is necessary with the currently used methods (6).

In nuclear medicine, proteins isolated from human blood, e.g., serum albumin, immunoglobulins, monoclonal antibodies (7), and peptides of varied molecular weight are employed as radiopharmaceuticals (8–10). Their labelling using redox polymers may eliminate the problems encountered in the preparation of these radiopharmaceuticals and raise the stability of labelled compounds (11, 12).

In this study we selected—for technetium (^{99m}Tc) labelling using redox polymers—human immunoglobulin and dialysate of human leukocytes.

Materials and methods

Chemicals

Human immunoglobulin (IgG-5% solution containing minimally 95% of monomer and dimer of IgG), NIGA-SEVAC a.s. Prague,
Human leukocytes dialysate (HLD), IMMODIN-SEVAC a.s. Prague,
Sodium pertechnetate(^{99m}Tc) — (Ultratechnecow FM generator), Mallinckrodt Medical,
Stannous chloride dihydrate (Merck),
Redox polymer (RP-IDA), FNKV Prague.

Preparation of the kits

5 ml of the protein solution was mixed with 500 mg of redox polymer RP-IDA. The synthesis of redox polymers with IDA functional groups from the dextran matrix is described in our previous paper (13). Alternatively, ligands of these proteins were labelled with ^{99m}Tc by reduction of pertechnetate (^{99m}Tc) without a redox polymer using kits prepared by mixing of 5 ml of the protein solution with 5 μl of stannous chloride dihydrate solution (0.25 mg). After stirring for 16 hr at room temperature, a mixture of the ligand with redox polymer or solution of protein with stannous chloride was filtered through a 0.22 μm pore size filter and the solution was filled per 0.2 ml into sterile vials. All steps were performed in nitrogen atmosphere. Kits were stored at –35° C.

Labelling with ^{99m}Tc

After reconstruction of the kit, 2 ml of sodium pertechnetate (^{99m}Tc) solution were added. The reaction was terminated after 15 min incubation at room temperature.

Radiochemical purity assessment

Our methods of control were limited to paper and column chromatography and electrophoresis.
Paper: Whatmann No. 3, ascending. 10 cm distance, solvent: Acetone 90%
Column: Sephadex G-50 (30x1 cm), solvent: Saline
Electrophoresis: Paper: Cellulose acetate, Solvent: Borate buffer pH 9, Current: 500 V/40 min

Results

Labelling efficiency

Samples of a solution of immunoglobulin and human leukocyte dialysate labelled with technetium (^{99m}Tc) with the help of a redox polymer (RP-IDA) showed a high labelling efficiency. Use of a redox polymer also has a positive effect on the stability of the labelled compounds. When not using redox polymers for protein labelling, the labelling efficiency declines by more than 50% after 30 hours. The results are shown in Table 1.

One month after kit preparation, proteins labelled using redox polymers exhibit high efficiency of ^{99m}Tc binding throughout the shelf life of the radionuclide.

As evident from Table 2, technetium (^{99m}Tc) tends to get released from compounds with proteins labelled not using redox polymers.

Table 1. Labelling efficiency of kits prepared using RP-IDA

Time since labelling (hrs)	Labelling efficiency (%) ^{99m}Tc-IgG RP-IDA	^{99m}Tc-IgG Sn^{2+}	^{99m}Tc-HLD RP-IDA	^{99m}Tc-HLD Sn^{2+}
0	97.0	95.2	98.9	95.8
4	98.9	94.2	98.8	94.3
6	97.6	93.9	98.4	94.2
8	98.2	92.4	98.2	93.6
24	98.7	63.2	98.0	55.7
30	98.3	42.0	95.6	46.4

Table 2. Stability of the labelled kits 1 month after their preparation expressed in terms ^{99m}Tc labelling efficiency

Time since labelling (hrs)	Labelling efficiency (%) ^{99m}Tc-IgG RP-IDA	^{99m}Tc-IgG Sn^{2+}	^{99m}Tc-HLD RP-IDA	^{99m}Tc-HLD Sn^{2+}
0	96.7	95.5	97.6	93.1
4	95.9	94.2	97.7	91.3
6	95.8	93.9	97.2	89.3
8	95.3	92.4	96.1	82.9
24	95.0	68.2	96.8	55.6

Table 3. Stability of the kits expressed as ^{99m}Tc labelling efficiency

Time since kit preparation (days)	Labelling efficiency (%) ^{99m}Tc-IgG RP-IDA	^{99m}Tc-IgG Sn^{2+}	^{99m}Tc-HLD RP-IDA	^{99m}Tc-HLD Sn^{2+}
0	97.1	95.5	98.2	96.1
1	97.0	95.2	98.2	96.3
2	95.4	93.2	98.0	92.8
7	95.2	89.5	98.7	91.4
30	95.0	72.3	97.6	93.1
50	92.7	63.4	98.1	88.9
100	90.9	turbidity	97.1	87.5
150	89.0	turbidity	98.0	87.2
300	87.0	turbidity	97.5	87.4

Another important finding is the long-term stability of kits prepared with HLD prepared using a redox polymer. As shown by data in Table 3, the labelling efficiency of HLD- and IgG-containing kits did not decrease below 97% and 87%, respectively, at 300 days; kits prepared without using a redox polymer for direct labelling with ^{99m}Tc are not stable.

Table 4. Radiochemical purity of ^{99m}Tc-IgG prepared using RP-IDA

Component	Labelling efficiency (%)
^{99m}Tc-IgG	96.0
^{99m}Tc-hydrolysed	2.5
$^{99m}TcO_4^-$	1.5

The efficiency of protein labelling with technetium (^{99m}Tc) and the radiochemical purity in labelled IgG was evaluated by electrophoresis on cellulose acetate in borate buffer. The course of the electrophoregram is shown in Figure 1.

As can be seen in Figure 1 and when compared with Table 4, the radiochemical purity of labelled protein is high (96 % ^{99m}Tc-IgG, 2.5 % hydrolysed form, 0.5 % $^{99m}TcO_4^-$).

These results were confirmed by column chromatography where ^{99m}Tc-IgG was eluted with a maximum peak between 12 to 22 ml. Free pertechnetate (^{99m}Tc) was found to elute between 38 and 44 ml. The fraction of ^{99m}Tc-IgG contained 96% of the activity, while 1.5% of the activity at most belonged to pertechnetate (^{99m}Tc). These results are in Table 4.

Discussion

When labelling protein-like substances with technetium (^{99m}Tc), the reductant employed is Sn^{2+} in the form of stannous chloride in acid medium (below pH 2.6). The drawbacks of this method include the possible inactivation of biologically active proteins as well as the formation of colloids when adjusting pH to optimal values. The method using various complexes of Sn^{2+} to transfer this ion to protein also leads to protein „contamination" with Sn^{2+} complexes with possible formation of colloids, which are practically unable to be removed from the reaction mixture. It is for these reasons that we employ, when using biologically active substances for labelling with ^{99m}Tc, insoluble redox polymers on the basis of cross-linked dextran.

Use of redox polymers is intended especially for the labelling of labile structures and substances sensitive to reaction conditions. In these, the possibility of incorporating of chelating groups anchoring to proteins that can make them inactive is virtually ruled out. As results, labelling of proteins with technetium (^{99m}Tc) using redox polymers for use in nuclear medicine is most promising. This will provide the opportunity to prepare radiopharmaceuticals in a kit form which can be readily labelled with technetium (^{99m}Tc) attaining high radiochemical purity and stability.

Electronic Res: Mod Bkgnd: POZADI.R01 Norm: NORMALA.R01 Base Correct: On
Run Time: 1:01 Total Counts: 239001 Total CPM: 235083.3

Total count region: –5mm –200mm

Name	Start (mm)	End (mm)	Max (mm)	RF	Region Counts	Region CPM	% of Total	% of ROIs
Rgn 1	0.4	17.1	8.1	0.041	5901	5804.0	2.47	2.57
Rgn 2	40.6	90.7	62.5	0.313	222203	218560.2	92.97	96.89
Rgn3	180.6	199.4	194.7	0.974	1227	1207.3	0.51	0.54
3 Peaks					229331	225571.5	95.95	100.0

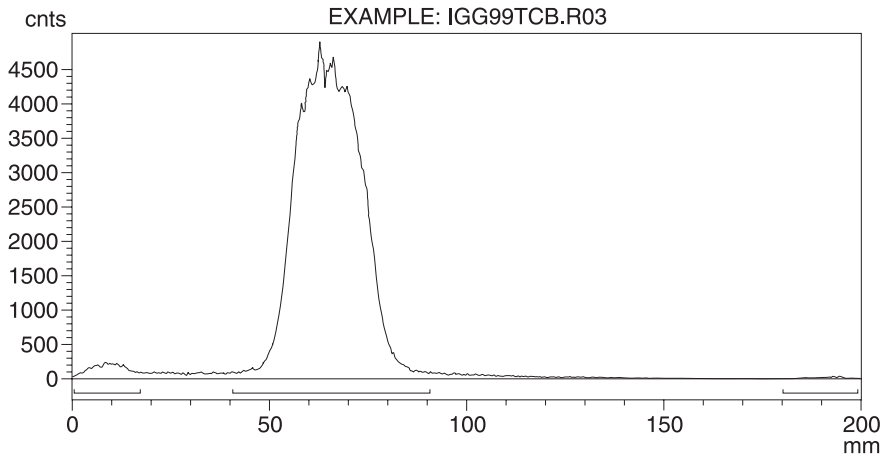

Figure 1. Electrophoresis of ^{99m}Tc-IgG prepared using RP-IDA (Rgn 1 — hydrolysed ^{99m}Tc, Rgn 2 — ^{99m}Tc-IgG 2, Rgn 3 — $^{99m}TcO_4^-$).

Conclusions

When using the method for direct protein and peptide labelling by ^{99m}Tc with the help of a redox polymer, the result is practically selective labelling at free end SH groups.

The radiochemical purity and stability of ^{99m}Tc labelled proteins depends on the degree of cross-linking of the redox polymer dextran matrix and on the chemical structure of end functional groups anchored in the dextran matrix.

After labelling with ^{99m}Tc, proteins and peptides prepared into kit form using this method show high radiochemical purity and stability. These kits also show high stability when stored frozen (300 days).

The method for preparing protein- and peptide-containing kits using RP-IDA is the gentlest technique developed to date and allows the use of single-compound kits without adjuvants while retaining high radiochemical purity and stability of the labelled proteins and peptides.

Acknowledgements

This study was supported by grant No. 4387-3, awarded by the Internal Grant Agency of the Ministry of Health, Czech Republic.

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