RHENIUM-186 DIRECT LABELLED HIgG

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Abstract. The aim of this study is to develop and improve existing radiolabelling techniques of peptides and monoclonal antibodies with ^{186}Re for achievement of potential agents for cancer targeted radiotherapy. There were selected methods and techniques for the direct labelling of intact HIgG by studding chemical and radiochemical processes of —S—S— bridges prereduction, reduction of $^{186}ReO_4^-$ and coupling reaction of rhenium with HIgG. The —S—S— bridges prereduction of HIgG to sulfhydryls was effected using different reducing agents: ascorbic acid, 2,3 dimercaptopropanol, cysteine, active hydrogen. The prereduction reactions are controlled by masic ratios of HIgG/reduction agent, pH, temperature and time of incubation. A pH= 4.5 and a 24 hours incubation time are in the advantage of the prereduction yield. The labelling with ^{186}Re of prereduced HIgG with ascorbic acid or active hydrogen and 37^{0}C incubation in 22 hours releases 92% radiochemical purity.

1. INTRODUCTION

Rhenium-186 has been considered as an ideal radionuclide for radioimmunotherapy because its physical half-life of 90 hours and beta emission of 1.07 MeV. The 137 KeV (9%) gamma emission also allows simultaneous scintigraphic imaging. The protocols regarding the labelling of specific biomolecules with $^{186,188}Re$ allowed the formulation of an instant kit and the clinical and laboratory testing for both radioimmunotherapy and radiodiagnostic in the same time [1,2,3].

In our laboratory HIgG (G human immunoglobulin) has been labelled with ^{186}Re by direct method in three distinct steps: i) the —S—S— bridges prereduction to —SH groups; ii) the reduction of $^{186}ReO_4^-$ and iii) the coupling reaction of reduced ^{186}Re to —SH groups. The following reducing agents were used: ascorbic acid, cysteine, active hydrogen and 2,3 dimercaptopropanol. $^{186}ReO_4^-$ has been reduced with stannous chloride. The coupling reaction of ^{186}Re with HIgG calls incubation of mixture HIgG in thyol form with stannous chloride and $Na^{186}ReO_4$ at 37^{0}C during 22 hours.

2. EXPERIMENTAL

2.1. Materials and methods

The materials used in this study were: HIgG (from Cantacuzino Biological Institute, Bucharest, Romania), $SnCl_2 \times 2H_2O$, ascorbic acid and citric acid (from Sigma), all with high chemical purity. ^{186}Re was obtained by irradiation (n, γ) on TRIGA Reactor, Pitesti, Romania, 5×10^{13}n/cm^2.s flux by the nuclear reaction:

$$^{185}Re \xrightarrow[\sigma = 104b]{(n,\gamma)} {}^{186}Re \xrightarrow[t = 90h]{\beta^-} {}^{186}Os$$

2.2. $Na^{186}ReO_4$ preparation

The preparation of $Na^{186}ReO_4$ was been effected by the Eisenhut method [4]. An amount of 10 mg of metallic Re in powder with rhenium 185, 37.7% enrichment, was irradiated in reactor for a week at a 5×10^{13}n/cm^{2}s neutron flux (see FIG. 1). After 3 days cooling, the probe was transferred in a 10 mL vessel and 2 mL of H_2O_2 10% for the $H^{186}ReO_4$ formation were added. The reaction time for the complete oxidation was 2 hours. The vessel was covered during the reaction to avoid contamination. The pHwas adjusted to 5 with NaOH 0.1 N after $H^{186}ReO_4$ setting up. We obtained a solution with 62 mCi/mL (2.3 GBq) radioactive concentration and 98–99% radiochemical purity. The results of quality control effected by Watman 1 paper radiocromatography are presented in TABLE I.

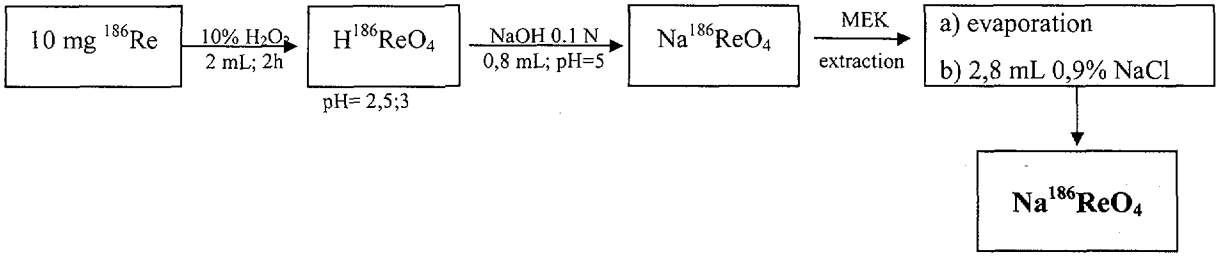

FIG. 1. The irradiated sample processing.

TABLE I. THE DETERMINATION OF RADIOCHEMICAL PURITY OF $Na^{186}ReO_4$

Solvent	R_f (ReO_4^-)	R_f (impurities)	% Radiochemical purity
Acetone	0.87–1.00	0.00–0.08	99.43
Ethanol	0.39–0.53	0.00	99.00
Ethanol: NH_3: H_20 (2:1:5)	0.77	0.00	99.15
0.9% NaCl	0.60–0.84	0.00	98.00

2.3. $Na^{186}ReO_4$ reduction

The stannous chloride was used as reducing agent for $Na^{186}ReO_4$. 200 μL solution containing 2 mg/mL $SnCl_2 \times 2H_2O$ and 20 mg/mL citric acid concentrations, were added to 100 μL $Na^{186}ReO_4$ (≈500 μCi). After 24 h of incubation time the reducing yield was 92%–96%, determinated by Watman 1 paper chromatography in acetone solvent (TABLE II).

TABLE II. REDUCTION SYSTEMS OF STANNOUS CHLORIDE

Reduction system	$SnCl_2 \times 2H_2O$: $^{186}ReO_4$	Reducing yield after 24 h incubation
$SnCl_2$:0,05 N HCl	200 μL:74 MBq/100 μL	96,35
$SnCl_2$:20 mg/mL citric acid	200 μL:74 MBq/100 μL	92,08

2.4. Prereduction of the bounds —S—S— of HIgG

For the prereduction of the bounds —S—S— to —SH groups we used different reduction agents: ascorbic acid, 2,3 dimercaptopropanol, cysteine and active hydrogen. The HIgG solution had been prepared as follows: 1 mg of HIgG was dissolved in 1 mL bidistillated water and divided in 100 μl portions. The reduction agent was added to each HIgG sample (TABLE III). The sample has been purged with pure N_2 for the elimination of air from the system.

After incubation the pHof samples (no 1,2,3,4) was adjusted to 5 by addition of sodium citrate buffer; than, in each sample were added 200 μL of $SnCl_2$ in citric acid solution, 20 mg/mL. After 30 minute of slow purged N_2 the samples were lyophilised.

TABLE III. THE REDUCTION OF THE DISULPHIDE GROUPS TO SULFHYDRYLS

Reducing agent (RA)	(HIgG:RA)/vol	pH	Incubation	
			t^0C	Time
Ascorbic acid	*sample no 1* 100 µg:500 µg/200 µL	4	37	21 h
2,3 Dimercaptopropanol	*sample no 2* 100 µg:64 µg/200 µL	3,5	22	22 h
Cysteine	*sample no 3* 100 µg:120 µg/300 µL	4,5	37	22 h
Sn + citric acid →(AH)*	*sample no 4* 100µg:/300µl	3,5	37	24 h

** Active hydrogen.*

2.5. The labelling of HIgG lyophilised samples

The lyophilised samples were reconstituted in 1 mL $Na^{186}ReO_4$ (1.2 mCi, pH= 5 for the final solution) and incubated at 37^0C. The pHprobes was always raised to 7 with 0.1 M sodium bicarbonate buffer. The best results of radiolabelling are presented in TABLE IV.

TABLE IV. THE RESULTS OF RADIOCHEMICAL PURITY OF HIGG-^{186}RE, OBTAINED BY INCUBATION FOR 1 HOUR AND RESPECTIVELY 22 HOURS.

HIgG samples	^{186}Re	Incubation time (h)	% Radiochemical purity	
			ethanol	ethanol:NH_3:H_2O (2:1:5)
AA* — HIgG	74 MBq ^{186}Re (red)	1	83	75
AH — HIgG	74 MBq ^{186}Re (red)	1	92	84
AA — HIgG + $SnCl_2$	74 MBq $^{186}ReO_4^-$	22	93	87
AH** — HIgG + $SnCl_2$	74 MBq $^{186}ReO_4^-$	22	95	96

**AA = ascorbic acid*
***AH = active hydrogen.*

2.6. Quality Control

The samples of HIgG-^{186}Re were analysed by Whatman 1 paper radiochromatography and colour reveal test (in 2% ninhydrine ethanol solution). The used solvents and the R_f values are inserted in TABLE V.

TABLE V. THE VALUES OF R_F (RADIOACTIVE AND NINHYDRINE SPOT TEST)

Sample	Solvent	
	ethanol	ethanol:NH_3:H_2O (2:1:5)
HIgG — ^{186}Re	0.00 -0.08	0.84–1.00
$^{186}ReO_4^-$	0.30–0.50	0.77–0.84
Re (red)	0.00	0.00–0.08

The labelled immunoglobulin was analysed by the UV gel chromatography method, too. The Sephadex G-25 columns (0.3 × 15 cm) and elution buffer 0.1 M $NaHCO_3$ in 0.15 M NaCl [2] were used. 100 µl HIgG-^{186}Re solution were loaded on each gel column and eluted with buffer. Fractions of 200 µl in volume were collected and monitored spectrophotometrically at 280 nm. Concomitant

radioactive measurements were effected to a gamma counter. FIG. 2 represents the counting speed (—— B curve) and the extinction (—•—C curve) for 14 HIgG-^{186}Re samples.

We see that for the same sample fraction (no 8 in FIG. 2) there are a spectrophotometrical absorption maximum and a radioactivity distribution maximum. The determined radioactive purity was 90–92% for HIgG prereduced samples (no 1 and no 4, TABLE III) with ascorbic acid and native hydrogen (active).

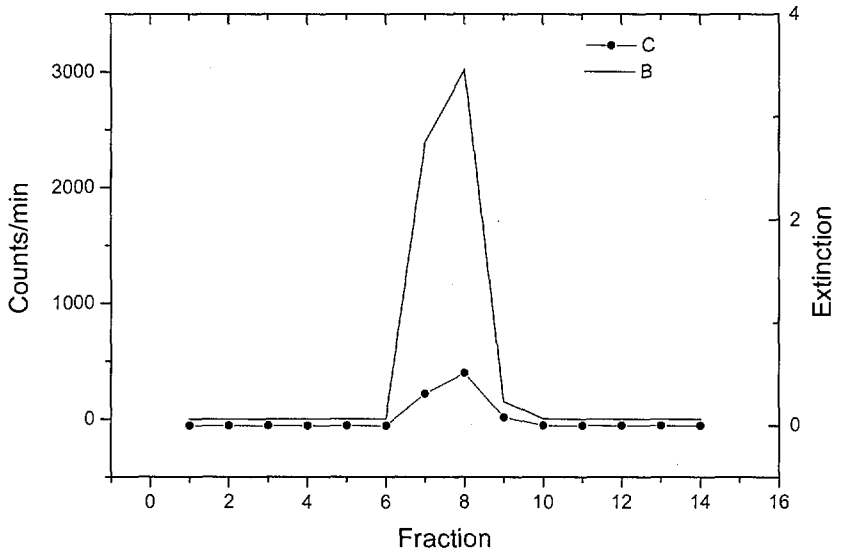

FIG. 2. The radioactive (——B) and UV absorbtion (—•—C) measurements of gel chromatography fractions.

3. DISCUSSION

The reducing agents used in HIgG prereduction are specific for the electronic transfer reactions —S—S— + $2H^+$ (low acid) → —SH + —SH. The parameters which have drastic influence on the labelling yield are: temperature and incubation time, masic ratio HIgG/reducing agent, self radiolyse effect.

For all the studied probes a difficulty in the obtaining of HIgG-^{186}Re was observed. The reducing of perrhenate to inferior oxidation states [5] necessities the increasing of Sn^{2+} quantity. The low acid pHencourages both the prereduction reaction of HIgG and the labelling reaction of ^{186}Re. After the forming and stabilisation of the HIgG-^{186}Re molecule, the increasing of pHto 7 doesn't influence the stability of labelled molecule.

4. CONCLUSIONS

HIgG can be labelled with ^{186}Re by direct method in prereduction conditions with ascorbic acid or active hydrogen. The molar ratio 1.6:1of ^{186}Re:HIgG used in labelling process is favourable for a high labelling yield. In all this study it doesn't observe aggregation phenomena of HIgG-^{186}Re.

That research work was accomplished with the intention of obtaining a kit for labelling with ^{186}Re. The direct labelling technique encourages the achievement of this kit.

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