An efficient method for labelling antibodies with ^{111}In

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Using a new method, rabbit IgG and a monoclonal antibody have been conjugated with the chelating agent DTPA. This was accomplished with reaction conditions that should entail lower antibody damage than existing methods. Gel filtration of the ^{111}In-labelled antibody conjugate indicated minimal damage to the antibody and radioimmunoassay showed no significant change in its immunological activity.

Isotope labelling *Indium* *Radionuclide imaging*

1. INTRODUCTION

Radioiodinated antibodies have been employed for some time in the detection of tumours [1,2] but recently the use of ^{111}In as a label has been investigated [3–5]. Its advantages are that it has gamma energies favourable for imaging, a convenient half-life and it does not emit high energy beta radiation [6,7]. Labelling with ^{111}In entails the attachment of a chelating agent to the antibody; the conjugate is then able to bind $^{111}In^{3+}$. Some methods involving multi-step chemical syntheses [8,9] would present problems for many clinical laboratories. Simpler procedures employing the chelating agent DTPA have been devised but these generally use high molar ratios of chelating reagent or involve other experimental conditions that could damage or aggregate fragile antibodies [3,5,10,11]. Here we report a new and efficient method for attaching DTPA which we believe avoids these difficulties.

Abbreviations: DTPA, diethylenetriaminepentaacetic acid; a.HCG, antibody to human chorionic gonadotrophin; DMSO, dimethyl sulphoxide

2. MATERIALS AND METHODS

Preliminary experiments used rabbit IgG (Miles), those involving a specific antibody used a mouse monoclonal a.HCG [12]. All water was distilled using glass apparatus and the DMSO was carefully dried. Reagents used were of the highest grade available.

2.1. *Preparation of the DTPA-IgG conjugate*

DTPA (197 mg, 0.5 mmol) was dissolved in DMSO (9.5 ml) at 90°C and the solution allowed to cool. To the stirring solution was added, dropwise, a solution of *N*-hydroxysuccinimide (57.6 mg, 0.5 mmol) in DMSO (2.0 ml) and then, dropwise, a solution of dicyclohexylcarbodiimide (92.9 mg, 0.45 mmol) in DMSO (2.5 ml). After stirring overnight the mixture was filtered. The volume of filtrate required to give the desired molar ratio of DTPA was then slowly added to a solution of the IgG in 0.1 M sodium phosphate, 0.1 M NaCl buffer (pH 8.0), IgG concentration 2.6–3.4 mg/ml. After stirring for 2 h at room temperature the solution was applied to a Bio-Gel P30 column (27 × 1.5 cm) and eluted using 0.9% NaCl, 0.02% NaN_3. The IgG fraction was dialysed for 24 h at 4°C against 3 changes of 800 ml of 0.9% NaCl, 0.02% NaN_3 and then for 20 h at 4°C against 3 changes

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of 800 ml of 0.1 M sodium citrate buffer (pH 5.0). The solution was then aliquoted and stored at −70°C.

2.2. *Labelling of the conjugate with* ^{111}In

A solution (0.2 ml) of $^{111}InCl_3$ in 0.04 M HCl (607 MBq/ml) (Amersham International plc, product code INS IP) was added dropwise to 0.3 ml of the stirring conjugate solution. The pH was kept at 5.0 by the addition of 0.05 M NaOH. After stirring for 40 min the solution was applied to a Bio-Gel P30 column (27 × 1.5 cm) and eluted with 0.1 M sodium citrate buffer (pH 5.0), the radioactivity of the fractions being monitored. The IgG fraction was rechromatographed on a Bio-Gel P30 column (25 × 1.5 cm) eluting with 0.9% NaCl. The product was then filtered (Microflow 25, pore size 0.2 μm).

2.2.1. Radioimmunoassay and gel filtration

The ^{111}In–DTPA–a.HCG conjugate used for these studies was prepared using a 12 molar excess of DTPA over the antibody. It had a specific activity of 170 MBq/mg. A radioimmunoassay [12] was conducted both on the conjugate and on a sample of the a.HCG used in its preparation. For the gel filtration (fig.2) samples of the conjugate and of the a.HCG were applied to a Sephacryl S-300 column (86 × 1.5 cm) and eluted with a 0.05 M sodium phosphate, 0.15 M NaCl, 0.02% NaN_3 buffer (pH 7.0) at a flow-rate of 9.6 ml/h at 4°C.

2.3. *Mass spectrometry*

This was conducted at M-Scan Ltd. (Ascot) by Dr N.J. Haskins. A VG Analytical ZAB-HF mass spectrometer equipped with a FAB source was used.

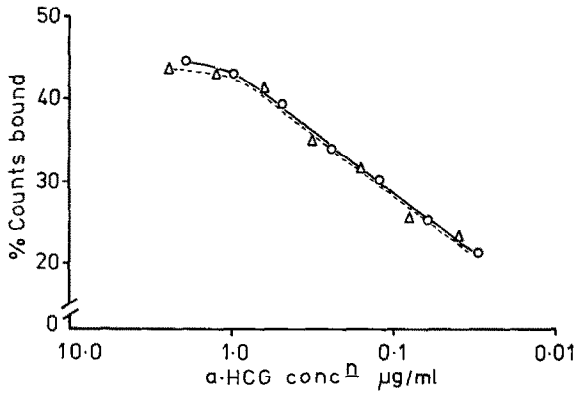

Fig.1. Radioimmunoassay of the ^{111}In–DTPA–a.HCG conjugate (○—○) and of the a.HCG used for its preparation (△—△).

3. RESULTS AND DISCUSSION

The intention of the study was to label a.HCG with ^{111}In via a DTPA–a.HCG conjugate formed by reaction of the antibody with the *N*-succinimidyl ester of DTPA. The preparation of the ester was based on previous methods for making similar derivatives [13,14]. Although the ester was not isolated, a mass spectrometric investigation of its DMSO solution showed a signal at m/z 489 corresponding to the ester anion. The maximum specific activities obtained from labelling a series of DTPA–IgG conjugates are given in table 1.

It could be argued that dicyclohexylcarbodiimide is capable of linking DTPA to IgG either directly or via the formation of an anhydride species. Thus the reaction was simulated in the absence of *N*-hydroxysuccinimide in order to demonstrate the necessity of using this reagent. The resulting product had a lower ability to bind ^{111}In (table 1). The use of the carbodiimide in substoichiometric quantities in the ester preparation was not only to discourage the possibility of the above reactions but also to avoid unreacted reagent causing antibody dimer formation.

The radioimmunoassay of the ^{111}In–DTPA–a.HCG conjugate (fig.1) showed that the coupling and labelling procedure had not significantly altered the immunological action of the antibody. Gel filtration (fig.2) indicated little protein damage

Table 1

Maximum specific activities achieved on labelling a series of DTPA–IgG conjugates

Molar ratio DTPA–IgG used in preparation of conjugate	Spec. act. (MBq/mg)
5	107
10	172
15	269
20	270
10[a]	68

[a]No *N*-hydroxysuccinimide was used during the preparation of this conjugate

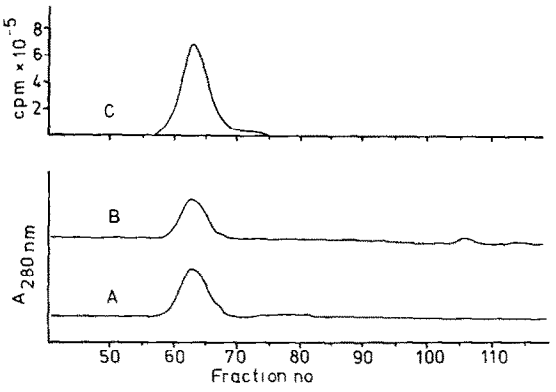

Fig.2. Gel filtration of the ^{111}In–DTPA–a.HCG conjugate and of the a.HCG used for its preparation. (A) Absorbance trace obtained for the a.HCG. (B) Trace for the conjugate. (C) Radioactivity of the same conjugate fractions.

and also illustrates the firm binding of the ^{111}In to the conjugate. Weakly bound ^{111}In would be removed as a citrate complex during the post-labelling gel filtrations.

We believe that this procedure affords a reliable way of labelling antibodies with ^{111}In and that because of its ease, efficiency and mild reaction conditions, it offers an advantage over existing methods.

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