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Radiation Grafted Polyethylene as Carrier for Protein Immobilization

2. Covalent Immobilization of Antibodies Against Thyroxine

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SUMMARY

Radiation grafting onto polyethylene tubes was carried out using vinyl monomers carrying functional groups to which antibodies against thyroxine were covalently bound. The grafting tests were carried out in different solvents varying the dose rate and irradiation time so that the grafting yield could be effectively controlled. Coupling via an active ester using 1-hydroxybenzotriazole shows optimal results with a retention of biological activity of over 70%.

INTRODUCTION

Radiation grafting has been widely used for biomedical polymers (HOFFMAN et al. 1972), mainly to improve biocompatibility. Yet this modification method is rarely used as carrier for biological molecules (ABDEL-HAY et al. 198o and ABDEL-HAY et al. 1981). Low immobilization yields on bulk polymers, cumbersome radiation procedures and/or unavailability of suitable radiation sources, might be the reason for this. In a previous work (MÜLLER-SCHULTE and HORSTER 1982) human serum albumin could effectively be bound to a radiation grafted polyethylene (PE) film. The immobilization capacity achieved lay in the range similarly obtained with Sephadex or synthetic polymers in bead or powder form.
In this paper, radiation grafting test on PE tubes and immobilization of antibodies against thyroxine to these tubes are described. The purpose is to assess the extent to which the coupling procedure affects the biological activity of the protein.

MATERIALS

Commercial low density PE tubes (thickness 1 mm, length 7 cm, diameter 1 cm) were used as grafting substrate. Prior to the grafting, the tubes were washed with methanol. The vinylmonomers and the other chemicals were supplied by the Merck Company, W-Germany. These were of analytical grade and used without further purification. Antiserum (AS) antigen and tracer, obtained from the

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Henning Company, W-Berlin, were the same as those used for the radio-immune-assay 'T_4-RIA Henning'.

EXPERIMENTAL

GRAFTING PROCEDURE

Two radiation sources viz. a Co(60)AECL Gamma beam-650 and a Cs(137) Gammacell-40 (both of the Atomic Energy of Canada Ltd.) with a radiation dose of 0.3 respectively 0.008 Mrad/hour, were used. The tubes were immersed in the solvent-monomer solution at various ratios and irradiated in stoppered glass containers. Irradiation was carried out in air at room temperature (RT). After irradiation the tubes were treated with hot water over night in order to remove homopolymerized polymer.

ANTIBODY COUPLING PROCEDURES

Method a) 0.2 ml AS in 0.3 ml 0.05 M phosphate-buffered saline pH 7.2 (PBS) was added to the tube and the pH adjusted to 9.0 with 0.05 M NaOH. The solution was shaken for 3 h at 4 °C (BRÜMMER et al. 1972). Method b) 0.3 ml phosphate buffer (pH 6.6) containing 0.1% bovine serum albumin (BSA) and 0.3 ml 0.01 M 1-cyclohexyl-3-(-2-morpholinoethyl)carbodiimide metho-p-toluene sulphonate (CMC) was incubated for 16 h at 4 °C. Method c) The tubes were washed three times with tetrahydrofuran (THF). 0.4 ml THF containing 0.2 M 1-hydroxybenzotriazole (HBT) and 0.2 M dicyclohexylcarbodiimide (DCC) was incubated for 2 h at RT. The tube was washed with THF (three times) and 0.2 ml AS and 0.3 ml THF were added and shaken for 16 h at RT. Method d) The tube was washed with THF; 0.4 ml THF containing 0.2 M HBT and 0.2 M DCC were incubated and shaken for 2 h at RT. The tube was washed with THF (three times) and 0.2 ml PBS containing 0.1% BSA and 0.3 ml THF were incubated for 16 h at RT. The tube was treated with PBS containing 0.05 v/v Tween 20 and 2% w/v BSA for 0.5 h. After washing with PBS and THF, procedure described in method c then followed.
After each AS coupling, the tube was treated with a glycine-HCl buffer (pH 2,3) for 0.5 h to remove unreacted antibody and to block off unreacted active groups of the carrier. Then followed a 0.5 h treatment with a stabilizing agent (PBS containing 2% w/v polyvinylalcohol and 0.01% sodium azide) to protect the coupled substance against denaturation (BROWN et al. 1979).

ASSAY

After the final washing, 0.2 ml ^{125}J-labelled thyroxine (T_4) and 0.2 ml barbital buffer (pH 8.2) was incubated for 1 h. The solution was aspirated and the tube washed with cold PBS. Counting was performed in a Gammascint BF 3000 for one minute (CPM).

RESULTS AND DISCUSSION

Immobilization carriers for biological molecules are almost exclusively hydrophilic in origin. It has been shown that hydrophilic polymers help to preserve biological activity of the coupled substance (ORTH and BRÜMMER 1972), whereas hydrophobic carriers seem to cause the reverse effect. For the modification of PE (a hydrophobic polymer), hydrophilic monomers were used to counteract the hydrophobic forces. In addition, the grafted chain represents an excellent molecular spacer which keeps the coupled substrate in a quasi dissolved state, contributing to a retention of the biological activity (CUATRECASAS and ANFINSEN 1971).
The results of the grafting tests are stated in Table I. Apart from the irradiation condition and monomer concentration, a careful selection of the solvent has a pronounced effect on the grafting process. Normally, solvents are used to swell the upper polymer layer, thus making the polymer sides more accessible to the monomers, resulting in an increased graft uptake (MEMETEA and STANNETT 1979). This effect is, however, not applicable to PE, as it practically does not swell in these solvents at RT. The solvent, therefore, rather influences the chain transfer and thus the polymerization kinetics. Apart from using only one monomer, a number of tests were carried out using two monomers simultaneously (co-grafting). The grafting tests were concipated in such a manner that the graft yield lay in the range of 1o%. For the immobilization of the antibodies, different coupling procedures were conducted (Table II). Best results were obtained with acrylic acid/maleic anhydride co-grafted tubes, using HBT to transfer the acrylic acid into an activated ester. The subsequent reaction with the antibody should preferably be conducted in an aqueous THF solution which is superior to a plane buffer solution or other organic solvents such as DMF or DMSO. This confirms results of above mentioned experiments with human serum albumin with regards to grafting and coupling conditions (MÜLLER-SCHULTE and HORSTER 1982).
For the purpose of an immunoassay, uniformity of the carrier surface is an important factor for precision and reproducibility. It has been suggested (SMITH and GEHLE 198o and BROWN et al. 1979) to precoat the surface of the plastic with an inert protein such as BSA or gelatine, which has a stabilizing effect on the coupled substance. In the present work, these precoating tests were done with BSA, showing, however, no visible improvement of the biological activity (Table II, method 2 and 4). Therefore, the latter argument might only apply to bulk polymers. As regards the uniformity, this has, as yet, to be elucidated.

TABLE I

Monomer solvent compositions and irradiation conditions of the grafting experiments on low density PE tubes

tube specification	monomer (conc. %)	solvent	radiation-dose (Mrad/h)	radiation-time (h)	grafting yield (%)
1	acrylic acid (1o)	water[a)]	o.3	o.5	4.4
2	acrylic acid (1o)	methylen-chloride	o.3	o.5	6.6
3	hydroxyethyl-methacrylate (2o)	water	o.3	1	11.9
4	acrylic acid (3o)	water[a)]	o.oo8	15	6.5
5	acrylic acid/ vinylpyrrolidone (2)/(48)	methanol	o.3	o.5	7.o
6	acrylic acid/ maleic anhydride (2o)/(2o)	water[a)]	o.oo8	15	8.1
7	acrylic acid/ acrylamide (2o)/(1o)	water[a)]	o.oo8	15	11.6
8	acrylic acid/ vinylpyrrolidone (1)/(49)	methanol	o.oo8	15	14.8

a) solution containing o.oo5 M $Cu(NO_3)$ (RATNER and HOFFMAN 1974)

TABLE II

Amount of T_4 bound to radiation grafted PE tubes covalently coated with T_4-antibodies via different coupling methods.

tube number	coupling method	CPM	T_4 bound[a] (%)
6	1	12389	5o
6	2	65o4	23
6	3	18128	76
1	3	11o61	44
6	4	15672	65
2	2	542o	18
8	3	8695	33
7	3	11oo2	44

a) The amount of T_4 adsorptively bound lies (depending on tube modification) between 12oo - 14oo CPM; this can be reduced to ca 6oo CPM by adding o.o5 ml o.o5% Tween 2o in PBS to the tracer solution.

CONCLUSION

Radiation grafted PE carrier exhibit remarkable immobilization properties. A careful selection of the coupling method leads to high coupling yields combined with high retention of biological activities. This method, therefore, could well be used for further developments of RIA kits.

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