Polymer Bulletin 7, 77–81 (1982)

Polymer Bulletin

Radiation Grafted Polyethylene as Carrier for Protein Immobilization

1. Covalent Immobilization of Human Serum Albumin

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SUMMARY

Different vinyl monomers carrying carboxyl-, hydroxyl-, and aminogroups were grafted onto polyethylene foil with the aid of γ-radiation. The polymers served as carrier for a covalent coupling of human serum albumin. Best coupling results were obtained with a 1-hydroxybenzotriazole ester of acrylic acid grafted polymer. Adding tetrahydrofurane to the coupling medium increases the coupling yield over 2o $\mu g/cm^2$ foil.
Abundant functional groups, to which the albumin is bound, can effectively be reduced by grafting different monomers simultaneously.

INTRODUCTION

Immobilization of biological molecules to polymeric supports, has been widely used in the field of affinity chromatography, enzyme technology and immunoassay (ORTH and BRÜMMER 1972; PORATH and AXEN 1976). The polymer supports, to which the substance is attached, range from modified dextranes, cellulose to synthetic polymers.
In this paper a polymeric support, which is obtained by grafting vinyl monomers onto polyethylene (PE) foil with the aid of γ-radiation, is described. In addition to using only one monomer, two monomers were grafted simultaneously, too (co-grafting). This technique does not require additional preparation (oxydation or reduction) of the grafted substrate to make it susceptible to the covalent attachment as described elsewhere (ABDEL-HAY et al. 198o; ABDEL-HAY et al. 1981). Human serum albumin (HSA) was used for immobilization. A number of different coupling methods were applied in order to obtain suitable coupling conditions.

MATERIALS

A normal commercial low density PE foil (density: o.924 g/cm^3; thickness 85 μm) was used for grafting . Prior to the grafting tests, the foils were washed with methanol in an ultrasonic bath for a few minutes and then dried at room temperature (RT). The vinyl monomers and all other chemicals (obtained from Merck, Darmstadt, W-Germany) were of analytical grade and used without further purification. ^{125}J-labelled HSA (specific activity 5o $\mu Ci/2o$ mg) was supplied by the Radiocentre Amersham, England. 2 ml containing 4o mg HSA were diluted with water to 5o ml serving as stock solution.

0170-0839/82/0007/0077/$01.00

GRAFTING PROCEDURE

Grafting was conducted in a Cs (137) Gammacell-4o (Atomic Energy of Canada Ltd.) for 2o h in air at RT using a radiation dose of o,oo8 Mrad/h. The plastic foil was placed in a stoppered glass container. The monomer/solution composition and graft uptake are listed in Table I. After irradiation, the plastic was immersed in hot water and stirred over night in order to remove homopolymerized polymer.

TABLE I

Monomer/solution composition of the radiation induced grafting onto PE foils. The uptake level was calculated according to $(W-W_o)\cdot 1oo/W_o$, where W_o is the initial weight of the PE foil

specification	monomer	concentration (%)	solvent	graft-uptake (%)
foil I	acrylic acid	1o	methylene-chloride	3,5
foil II	acrylic acid	4o	water[a)]	1o,4
foil III	acrylic acid	1o	warer[a)]	3,9
foil IV	hydroxyethyl-methacrylate	2o	water	1o,2
foil V	maleic anhydride/acrylic acid	2o/2o	water[a)]	6,5
foil VI	acrylic acid/acrylamide	2o/1o	water[a)]	11,6

a) contained o,oo5 M $CuSO_4$

COUPLING PROCEDURES

Method a: The foil was washed 3 times with tetrahydrofurane (THF) before adding o,5 ml THF containing o,2 M 1-hydroxybenzotriazole (HBT) and o,2 M dicyclohexylcarbodiimide (DCC); the mixture was agitated for 3o min at 42 °C; after washing with THF for 5 min, 1oo µl HSA and 4oo µl of the following buffers (o,2 M sodium citrate pH 4,4; o,2 M sodium acetate pH 4,9; o,1 M sodium phosphate pH 6,5; o,o1 M phosphate-buffered saline pH 7,2; o,2 M borate pH 8,5) were added; the mixture was shaken for 16 h at RT.

Method b: (as a) instead of the buffer, 3oo µl THF was added. After each coupling procedure, the PE foils were treated with phosphate-buffered saline containing o,o5% v/v Tween 2o and 2% w/v bovine serum albumin for 1 h at RT, in order to remove non-covalently bound HSA. Other desorption agents were tried viz. o,5% v/v Tween 2o; o,1% v/v Tween 2o; o,1 M glycine-HCl buffer (pH 2,3). However, the above agent proved to be most effective.

Condensation with hexamethylenediamine (HMD)

Acrylic acid grafted foil was condensed with HMD to produce an aminogroup-containing carrier. 1 ml THF containing o,2 M DCC and o,2 M HBT and 5o mg HMD dissolved in 3oo µl methanol were added to the PE foil. The mixture was stirred for 3o min at 42 °C and then washed with THF and methanol (5 times each).

RESULTS AND DISCUSSION

For the radiation grafting, vinyl monomers containing either carboxyl-hydroxyl- or aminogroups, were used. These monomers are hydrophilic, and so the grafted PE surface becomes hydrophilic and can induce a hydrogel-like structure, the extent depending on the graft yield. Hydrogels normally show a high affinity for proteins which can be entrapped in the pores of the gel (YOSHIDA et al. 1981), rendering diffusion to/and/or reactions at the active sites more difficult. However, the grafted substrate should be of hydrophilic nature, in order to counteract hydrophobic interactions between the hydrophobic carrier PE and the protein, which can lead to a diminished biological activity (ORTH and BRÜMMER 1972). For a chemical coupling, tests showed that good results are obtained with graft uptakes of about 1o%; uptakes below 2% showed very little immobilization efficiency.

The yield of grafting is a function of the radiation dose, temperature and monomer concentration for a given substrate and monomer (SUNDARDY 1978). A number of grafting tests (the conditions of which are stated in Table I), were carried out varying these parameters. In some cases, the grafting was conducted under addition of copper salts, to prevent gelling and/or precipitation of homopolymer formed in the solution and to enhance the graft level (RATNER and HOFFMAN 1974).

For the covalent immobilization the following coupling agents and procedures were used: BrCN (MARCH et al. 1974); 1-cyclohexyl-(2-morpholinoethyl)-carbodiimide metho-p-toluene sulfonate (ABDEL-HAY et al. 198o), N-hydroxysuccinimide (CUATRECASAS and PARIKH 1972), glutardialdehyde (AVRAMEAS and TERNYNCK 1969), 1,5-difluoro-2,4-dinitrobenzene (REINERT 1972), hydrazide (INMAN and DINTZIS 1969). For the coupling using BrCN and hydrazide, respectively a hydroxyethylmethacrylate and acrylamide grafted foil (foil IV, VI) was used, whereas in case of glutardialdehyde and difluorodinitrobenzene foil II previously condensed with HMD was applied. These methods, although frequently used, revealed in all cases, low coupling yields which lay in the range of o,2 - 1,5 $\mu g/cm^2$. Varying the pH of the reaction medium towards the isoionic point of HSA (being 4,9) in order to overcome repulsion forces between the acidic protein and the negatively charged carrier (as demonstrated by Frost et al. 1981 for agarose derivatives), showed very little effect. These results show that grafted PE carriers necessitate different reaction conditions than other polymersystems.

The use of the HBT ester leads to a 4 - 2o fold coupling increase (Table II) compared to above mentioned methods.

Method b), in particular, which was conducted under addition of THF, revealed coupling yields which lay (based on surface area) in the same range as obtained with synthetic polymers or Sephadex in powder or microbead form (ABDEL-HAY et al. 198o; ABDEL-HAY et al. 1981; AXEN and ERNBACK 197o; MANECKE and VOGT 1976). The pronounced effect of THF might be attributed to a reduced hydrolysis of the activated ester and increased reactivity of the amino groups.

Comparing the coupling results as function of the differently grafted foils of method b) (Table II), the tests show that the immobilization efficiency is not always directly related to the graft uptake, thus indicating that not the quantity but rather a specific distribution of functional groups plays an essential role in immobilization.
In the second part of this investigation, tests with antibodies against thyroxine are described. The topic to be discussed is the influence of the immobilization on the biological activity of the protein.

TABLE II

Amount of HSA ($\mu g/cm^2$) covalently bound to radiation grafted PE foils. As comparison, the amount of HSA which is adsorptively bound to the different carriers, is also stated

coupling method a	pH of buffer	carrier	coupling method b	carrier	HSA adsorbed[c]	carrier
4,42	4,4		15,2o	foil V	o,o9	foil I
1,77	4,9		13,74	foil III	o,29	foil II
2,27	6,5	foil I	9,o2	foil VI	o,15	foil III
1,86	7,2		19,63	foil V	o,o8	foil IV
1,12	8,5		23,86[a]	foil V	o,19	foil V
			21,o5[b]	foil V	o,24	foil VI
			23,26	foil V		

a) activated with o,4 M DCC and o,4 M HBT

b) coupling medium contained 8o mM $CaCl_2$ according to Frost et al. 1981.

c) 1oo µl HSA in 3oo µl buffer (pH 4,9) were incubated for 16 h at RT

ACKNOWLEDGEMENT

The authors wish to thank Prof. H. Zahn and Dr. D.J. Saunders, German Wool Research Institute, Aachen, W-Germany, for critical remarks and for reading the paper.

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Received March 3, accepted March 10, 1982