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 tetrahydrofuran to the coupling medium increases the coupling yield over 20 μg/cm² 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; FORATH 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 (oxidation or reduction) of the grafted substrate to make it susceptible to the covalent attachment as described elsewhere (ABDEL-HAY et al. 1980; 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: 0.924 g/cm³; 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. ¹²⁵I-labelled HSA (specific activity 50 μCi/20 mg) was supplied by the Radiocentre Amersham, England. 2 ml containing 40 mg HSA were diluted with water to 50 ml serving as stock solution.
**GRAFTING PROCEDURE**

Grafting was conducted in a Cs (137) Gammacell-40 (Atomic Energy of Canada Ltd.) for 20 h in air at RT using a radiation dose of 0.008 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 
\[(W-W_0) \cdot 100/W_0\], where \(W_0\) is the initial weight of the PE foil.

<table>
<thead>
<tr>
<th>foil</th>
<th>monomer</th>
<th>concentration (%)</th>
<th>solvent</th>
<th>graft-uptake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>acrylic acid</td>
<td>10</td>
<td>methylene-chloride</td>
<td>3.5</td>
</tr>
<tr>
<td>II</td>
<td>acrylic acid</td>
<td>40</td>
<td>water(^a)</td>
<td>10.4</td>
</tr>
<tr>
<td>III</td>
<td>acrylic acid</td>
<td>10</td>
<td>water(^a)</td>
<td>3.9</td>
</tr>
<tr>
<td>IV</td>
<td>hydroxyethyl-methacrylate</td>
<td>20</td>
<td>water</td>
<td>10.2</td>
</tr>
<tr>
<td>V</td>
<td>maleic anhydride/acrylic acid</td>
<td>20/20</td>
<td>water(^a)</td>
<td>6.5</td>
</tr>
<tr>
<td>VI</td>
<td>acrylic acid/acrylamide</td>
<td>20/10</td>
<td>water(^a)</td>
<td>11.6</td>
</tr>
</tbody>
</table>

\(^a\) contained 0.005 M CuSO\(_4\)

**COUPLING PROCEDURES**

Method a: The foil was washed 3 times with tetrahydrofurane (THF) before adding 0.5 ml THF containing 0.2 M l-hydroxybenzotriazole (HBT) and 0.2 M dicyclohexylcarbodiimide (DCC); the mixture was agitated for 30 min at 42 °C; after washing with THF for 5 min, 100 µl HSA and 400 µl of the following buffers (0.2 M sodium citrate pH 4.4; 0.2 M sodium acetate pH 4.9; 0.1 M sodium phosphate pH 6.5; 0.01 M phosphate-buffered saline pH 7.2; 0.2 M borate pH 8.5) were added; the mixture was shaken for 16 h at RT.

Method b: (as a) instead of the buffer, 300 µl THF was added. After each coupling procedure, the PE foils were treated with phosphate-buffered saline containing 0.05% v/v Tween 20 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. 0.5% v/v Tween 20; 0.1% v/v Tween 20; 0.1 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 0.2 M DCC and 0.2 M HBT and 50 mg HMD dissolved in 300 µl methanol were added to the PE foil. The mixture was stirred for 30 min at 42 °C and then washed with THF and methanol (5 times each).