Strategies for direct attachment of hapten to a polystyrene support for applications in enzyme-linked immunosorbent assay (ELISA)

Jasdeep Kaur, K.V. Singh, Manoj Raje, Grish C. Varshney, C. Raman Suri

Institute of Microbial Technology, Sector 39-A, Chandigarh 160 036, India

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Abstract

A new method describing direct attachment of carboxylated haptens on a polystyrene support, using 3-aminopropyltriethoxysilane (3-APTES) as a linker, is reported. The hapten coated polystyrene support showed excellent stability as a function of the buffer pH and reaction time, and was successfully used to demonstrate its application in enzyme-linked immunosorbent assay (ELISA).

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Keywords: Hapten; Polystyrene; Silanization; ELISA

Polystyrene microtiter plates are widely used for enzyme-linked immunosorbent assay (ELISA)-based applications. Biological macromolecules such as proteins are immobilized on a polystyrene surface by passive adsorption. The adsorption of these molecules to a polystyrene surface is due to intermolecular attraction forces (van der Waals forces etc.), which are based on intramolecular electrical polarities. It was demonstrated that protein molecules immobilized on a hydrophobic polystyrene surface by passive adsorption lose their activity and suffer considerable denaturation [1]. However, biological macromolecules are found to better retain their functional activity when immobilized through extended hydrophilic spacer arms, since sorption on the surface is substantially reduced [2].

A polystyrene surface can be modified to improve its hydrophilicity by incorporating various functional groups such as hydroxyl, amino, carbonyl, carboxyl etc. on its surface [3–6]. Haptens are usually bound to polystyrene microtiter wells indirectly by coating the wells with protein–hapten conjugates, since direct attachment of haptens to a polystyrene surface is not possible due to the lack of available functional groups on polystyrene. However, the formation of this conjugate is not always reproducible which makes it difficult to evaluate hapten–protein stoichiometries [7]. In this report, we describe a method for grafting secondary amines on polystyrene microtiter wells using simple one-step aqueous silanization chemistry. The generated amine groups on the polystyrene surface are further utilized for binding of a carboxylated pesticide, 2,4-dichlorophenoxyacetic acid (2,4-D) for ELISA applications.

For this purpose, the polystyrene (PS) plates were first acid treated with 47% (v/v) HNO₃ in concentrated H₂SO₄ (250 µl/well) for 30 min at room temperature (RT) with mild shaking to generate –NO₂ groups on the surface. The acid-modified wells were then treated with 5% (v/v) 3-aminopropyltriethoxysilane (APTES, Aldrich) solution in distilled water (250 µl/well) and kept at RT for 2 h with mild shaking. The pH of the APTES solutions (pH 4–7) was lowered with concentrated HCl. After incubation, the wells were washed with distilled water and cured at 62°C in an oven for 2 h to enhance the binding of APTES to the PS surface. The reaction to form the amino-PS surface with APTES treatment is shown below:

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The amino group density on the PS surface was determined by a fluorescence method as described in our earlier studies [8]. For this, polystyrene wells coated with 5% (v/v) APTES solutions (pH 4–7) were treated with freshly prepared fluoresceine isothiocyanate (FITC, Sigma, St. Louis) solution in borate buffer (100 mM, pH 8.5) for 30 min. After washing thoroughly with distilled water, the FITC-APTES complex was eluted by adding 100 mM trisodium phosphate buffer. Fluorescence of the eluant was measured with a Kontron spectrofluorimeter (SFM-25) at 512 nm with excitation at 491 nm. APTES coating at pH 6.9 on nitrated-PS showed the highest amino group density (1300 nmol) in comparison with that obtained for a plain PS surface treated at pH 6.9 (1080 nmol) and nitrated-PS treated at pH 4.0 (1485 nmol).

To facilitate the binding of hapten to the amino-PS surface, the carboxyl groups of the hapten were first activated by the carbodiimide activation method using dicyclohexylcarbodiimide (DCC) [9].

Table 1

<table>
<thead>
<tr>
<th>Amount of 2,4-D (nmol)</th>
<th>Absorbance of rabbit anti-2,4-D at 450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS (pH 6.9 and RT)</td>
<td>PS (pH 6.9 and 62 °C)</td>
</tr>
<tr>
<td>250</td>
<td>0.630 ± 0.065</td>
</tr>
<tr>
<td>500</td>
<td>0.707 ± 0.008</td>
</tr>
</tbody>
</table>

The hapten-coated PS wells without APTES treatment were used as a control for anti-2,4-D antibody binding.

PS surface after APTES treatment was cured at RT for 2 h.

PS surface after APTES treatment was cured at 62 °C for 2 h.

The amount of antibody, as measured by ELISA, on the modified PS surface is shown in Table 1. The results

![Diagram](image1)

Fig. 1. Reactivity of rabbit anti-2,4-D antibody on hapten-coated PS wells (A) 1st day (B) 21st days after coating of 2,4-D on APTES modified PS wells. Two antibody concentrations i.e., 0.4 and 1.6 µg/ml were selected for hapten-coated PS wells.
showed that the maximum binding of the antibody on the hapten-coated PS surface occurred when PS wells were treated first with 47% HNO₃ in concentrated H₂SO₄ (250 μl/well). An APTES-modified PS surface prepared at neutral pH (pH 6.9) and cured at RT showed slight binding of anti-2,4-D antibody to it. Almost negligible (absorbance <0.1) binding of selective antibodies on PS surface prepared without APTES treatment for hapten coating was observed, confirming no direct attachment of hapten to the PS surface. In order to determine the optimum concentration of hapten used for coating PS wells, two amounts of hapten i.e., 250 and 500 nmol were selected. Not much difference in absorbance was observed in both instances. The optimum amount of hapten thus selected was 250 nmol.

In order to determine the stability of the hapten-coated nitrated plates, the modified PS plates were treated with antibody solutions at different concentrations for a fixed time interval (weekly). Fig. 1 shows the stability of hapten-coated PS wells for anti-2,4-D antibody binding for a 21-day period. No significant loss in antibody binding capacity on plates was observed over 3 weeks when stored in a vacuum desiccator. The hapten-coated plates thus could be used without any significant loss of activity for at least 21 days.

To summarize, the method developed represents a well-controlled methodology, which can also be applied to bind different macromolecules like peptides, hormones and proteins to polystyrene supports (wells, beads, plates, etc.) without losing their activities.

Acknowledgements

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References