

A Simple and Effective Approach to Preparation of Radioimmunoconjugate with DTPA derivative

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1. Introduction

Radioimmunotherapy (RIT), which uses a monoclonal antibody labeled with a radionuclide to deliver radiation to target sites of disease, has been extensively studied in this population. It is important that the radionuclide be stably coupled to the antibody when administered in vivo and not interfere with immune activity of the antibody (Ab). Labeling the Ab directly with a radionuclide is inadequate because of the possibility that the radionuclide could attach to an immune determinant region and interfere with its activity or specificity. As an alternative to direct labeling, bifunctional chelating agent (BFCA) with a strong chelating group capable of covalently bonding the Ab and the labeling radionuclide is introduced. Any suitable chelator combined with a variety of reactive groups may be used, but the most popular are diethylene triamine penta acetic acid (DTPA) and ethylenediamine tetra acetic acid (EDTA). Often the formation of conjugates between Ab and DTPA resulted to a less stable agent due to differential position in antibody and leads to radiolabeling inefficiency.

The main goal of this study was to optimize the most important step in immunoconjugation using a BFCA for the preparation of radioimmunoconjugate. We introduced a derivative of DTPA, DTPA-NCS, to conjugate Ab. Characterization of immunoconjugation was carried out.

2. Methods and Results

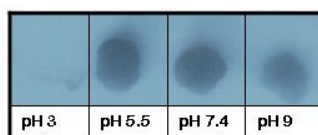
IgG, and the DTPA-NCS were supplied from Sigma-Aldrich (Milwaukee, USA) and Macrocyclics (Dallas, USA).

0.2mM DTPA-NCS was suspended in 1 ml buffer at various pH 3, 5.5, 7.4, and 9. Then 0.2mM IgG was added at room temperature for 10 min. Unbound DTPA-NCS was removed by ultrafiltration (Centricon filter 50kDa, Millipore, Netherlands), and the yields of conjugated DTPA-NCS with IgG was checked using a microplate spectrophotometer (Molecular Devices, California, USA) at 260nm.

The immunoconjugates of DTPA-NCS-IgG in various pH conditions were spotted 1-2 drops on nitrocellulose membrane. After incubation in 5% skim milk solution and washing with PBST (0.1% Tween 20 in PBS), the membrane was incubated with a HRP conjugated anti-human IgG diluted to 1: 8000 in PBS for 1 h. The proteins were exposed using enhanced

chemiluminescence ECL kit (Amersham). The IgG-DTPA-NCS remained immunoactive against anti-human IgG. (Fig.1)

a)



b)

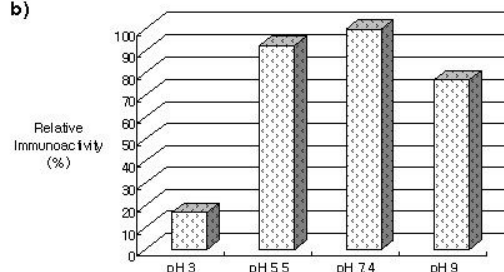


Fig.1. Immunoactivity of DTPA-NCS-IgG at various pH conditions (pH 3, 5.5, 7.4, and 9)

a) Dot blots of DTPA-NCS-IgGs against anti-human IgG

b) Relative immunoactivity of dots using densitometer

0.2, 0.4, 0.8mM DTPA-NCS solutions at pH 7.4 and 0.2mM IgG were gently mixed at room temperature in 10 min.

The yield of DTPA-NCS-IgG is rather low at high concentration of DTPA-NCS. The optimum molar ratio of IgG and DTPA-NCS was 1:1 for a successful bioconjugation. The yield is over 99% (Fig.2).

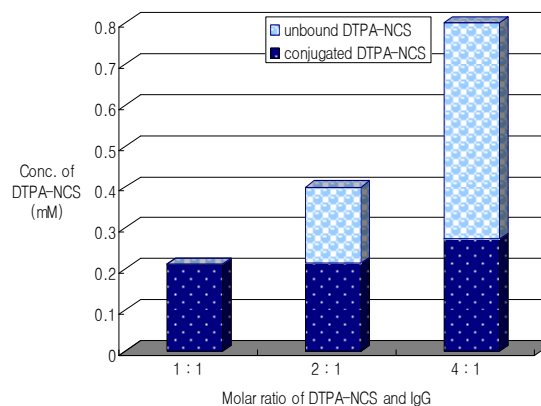
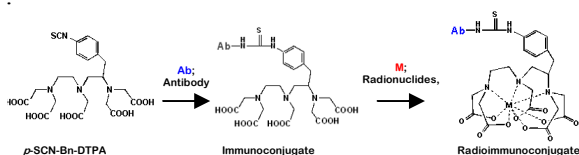


Fig.2. Concentration (mM) of conjugated DTPA-NCS with IgG under the various molar ratio conditions.

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The Synthetic route for Radioimmunoconjugate with DTPA-NCS-IgG is shown in Scheme 1. All the carboxylic acid groups of DTPA are available for chelation of radionuclide. The bioconjugate was stably formed by the reaction between the NCS of DTPA-NCS and an amine group of the antibody.



Scheme1. Synthetic route for Radioimmunoconjugate with DTPA-NCS-IgG

DTPA-NCS was stably bonded with radioisotope as well as Ab with functional NCS.

Among the many advantages of this study include the method of preparation for immunoconjugate that permits the introduction radionuclide to other biomaterials. The antibody, IgG, may be substituted with proteins, peptides, hormones, growth factors, enzymes, receptor proteins, any class of immunoglobulin including IgG family or antibody fragments. Any other biomaterials with free amine groups can be used for targeted radiotherapy.

3. Conclusion

For a successful bioconjugation, the optimum Ab and DTPA-NCS molar ratio was 1:1 carried out at pH 7.4 and in room temperature. Present study showed that DTPA-NCS is a simple and effective bifunctional chelating agent for developing a novel radioimmunoconjugates in targeting therapy.

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