Preparation of a Lysine based DTPA derivative and its Immunoconjugate for RIT

So-Young Lee, Mi-Sun Pyun, Young-Don Hong and Sun-Ju Choi
Radioisotope Research & Development center, Korea Atomic Energy Research Institute
Dukjin-dong 150, Yuseong-gu, Daejeon, Republic of Korea, 305-353,
solee31@kaeri.re.kr

1. Introduction

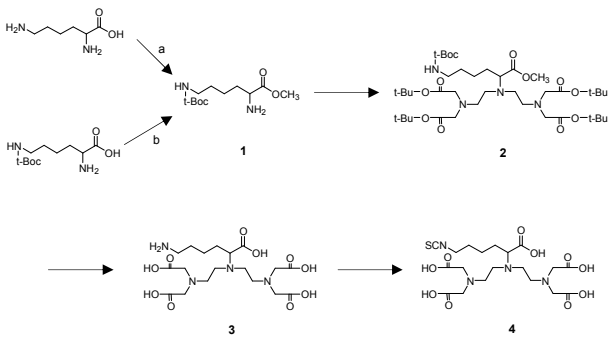
Radioimmunotherapy (RIT) has beneficiary effect of both immunotherapy and radiotherapy in cancer treatment. Those are the effect on predominant tumoricidal potency induced by radiation and intensified tumor cell targeting by antibody of radioimmunoconjugate.

For conjugation of radioisotope with antibody for RIT the introduction of proper BFCA (bifunctional chelating agent) is very important.

The most widely used BFCA is a diethylene triamine penta acetic acid (DTPA), However, it is known to form less stable conjugation due to competitive conjugation between radioisotope and antibody. In present study, to overcome the unstable chelation we synthesized the lysine based DTPA derivative. Furthermore, we prepared even more stable conjugate with human IgG using this DTPA derivative by its active isothiocyanate, demonstrated a stability of the immunoconjugate.

2. Methods and Results

The preparation scheme and chemical structures of all the intermediate compounds are indicated in scheme 1.



Scheme 1. Preparation of lysine-based DTPA.

2.1.1 N_ε -(tert-butoxycarbonyl)-L-lysine methylester**(1)** from **a**

To a suspension of L-lysine (1.0g, 5.5 mmol) in CH_3OH, chlorotrimethylsilane (2.43ml, 19.2 mmol) was dropwisely added for 10min. After a stirring for 24hrs, the reaction mixture was evaporated in vacuum to give the crude product (about 1.1 g) as a white powder. Without any further purification, the crude product was dissolved in 2 drops of water, CH_3OH(20 ml), and triethylamine (4 ml), and a solution of Di-tert-butyl-dicarbonate (1.31, 6.0 mmol) in CH_2Cl_2 (4 ml) was added for 10 min at 10℃ and stirred overnight. The reaction mixture was reduced to a 3 ml volume and purified by using a flash column chromatography with a gradient condition (CH_2Cl_2/CH_3OH 95:5) to yield 86% of product **1**.

2.1.2 1 N_ε -(tert-butoxycarbonyl)-L-lysine methylester **(2)** from **b**

A solution of diazomethane (35 mmol)[5] in diethyl ether (30 mmol) was added cautiously portionwise to a stirred solution of N_ε-(tert-butoxycarbonyl)-L-lysine (1g, 4.1 mmol) in CH_3OH (10 mL) at 0℃. The reaction mixture was maintained under a N_2 condition and stirred at room temperature for 4 hrs. Evaporation of the solvent under a reduced pressure gave the product **1** with a quantitative yield.

2.1.3 N_ε -(tert-butoxycarbonyl)-L-lys(tBu4-DTPA) methylester **(2)**

To **1** (0.8g, 3.1mmol) dissolved in CH_3CN (10 mL) and DMF (5 mL), a 2M phosphate buffer (pH=8, 10 mL) and N,N-bis[(tert-butoxycarbonyl) methyl] -2-bromoethyl-amine (3.78g, 10.7mmol) prepared a previous report was added. The resulting mixture was vigorously stirred for 48 hrs at room temperature. Organic layer was extracted with CH_2Cl_2 and repeated three times. The solvent was evaporated to afford a residue as a pale yellow oil form. Purification was performed using a flash column chromatography with a gradient condition (Hexane/Ethyl acetate, from 100:0 to 0:100 for 30 min) to give a product as pale oil form with 76 % yield of **2**.

2.1.4 Hydrolysis **(3)**

Hydrolysis was performed with 3N-HCl at 100℃ for 20 mins. After a evaporation, a recrystalization was performed by using CH_3OH and diethyl ether.

2.1.5 2-{Bis-[2-(bis-carboxymethyl-amino)-ethyl]-amino}-6-isothiocyanatohexanoic acid **(4)**

To the solution of 3 in water, 1.2 equivalent of thiophsgene($CSCl_2$) was added and stirred for 2 hrs. The water layer was collected in a vial, and then freeze dried to give the desired product **4**

2.2 Immunoconjugation with IgG

The differential concentrations of lysine based DTPA derivative were conjugated with 0.2mM IgG at room temperature, and pH 7.4. The molar ratios between DTPA derivative and IgG were 1: 1, 2: 1 and 4: 1. Unbound DTPA-NCS was removed by filteration system (Centricon). The purified immunoconjugates were analyzed by polyacrylamide gel electrophoresis, and the gel was visualized by Coomassie brilliant blue R-250 staining.

From the result, immunoconjugates showed no degradation products or other impurities. This demonstrates the stability of the IgG in DTPA derivative (Figure 1).

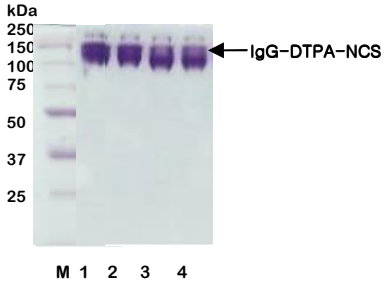


Figure 1. SDS-PAGE analysis of immunoconjugates
M; Protein size marker, 1; Concentrated IgG (control),
2~4 : Immunoconjugates using Lysine based DTPA derivative.
The molar ratios between DTPA and IgG were 1:1, 2:1 and 4:1.

3. Conclusion

We established the preparation method for a lysine based DTPA derivative as a bifunctional chelating agent (BFCA). The immunoconjugation with human IgG was successfully prepared at pH 7.4 with 10 mins of an incubation at room temperature. Present study showed that a lysine based DTPA derivative is a simple and effecitive BFCA for developing a novel radioimmunoconjugates for targeting therapy.

REFERENCES

[1] K. Kimpe, T.N. Parac-Vogt, S. Laurent, C. Pierart, L.V. Elst, R. N. Muller, and K. Binnemans, *Eur. J. Inorg. Chem.* p. 3021-3027, 2003.

[2] S. Liu, E. Cheung, M. Rajopadhye, N. E. Williams, K. L. Overoye, and D. S. Edwards, *Bioconjugate Chem.* vol. 12, p. 84-91, 2001.

[3] D. Rattat, C. Terwinghe, and A. Verbruggen, *Tetrahedrron*, vol. 61, p. 9563-9568, 2005.

[4] H. A. Tang, S. Yang, and R. D. Yang, *Synthetic Commun*, vol. 30, No. 16, p. 2811-2815, 2003

[5] A. Scozzafava, L. Menabuoni, F. Mincione, and C. T. Supuran, *J. Med. Chem.* vol. 45, p. 1466-1476, 2002

[6] J. M. Couchet, J. Azema, P. Tisnes, and C. Picard, *Inorg. Chem. Commun.* vol. 6, p. 978-981, 2003

[7] M. Ligeti, O. Gunduz, A. Magyar, E. Kato, A. Z. Ronai, C. Vita, I. Varga, F. Hudecz, G. Toth, A. Borsodi, and S. Benyhe, *PEPTIDES*, vol. 26, p. 1159-1166, 2005