

Development of a Multivalent Heteroepitopic Group A Streptococcus Vaccine

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Introduction

Group A streptococcus (GAS) causes many diseases such as impetigo, necrotizing fasciitis, acute rheumatic fever, and rheumatic heart disease. More than 100 M-serotypes or *emm*-sequence types have been identified. Multivalent vaccine strategy would contribute to prevention against various GAS infections and provide better protective immunity.

We have developed the lipid-core peptide (LCP) system which has 4-copy of peptide epitope on Lys-based dendrimer and 3 lipoamino acids in single molecular [1]. Moreover we succeeded to assembly a homo-multivalent LCP vaccine with high purity and yield [2] using native chemical ligation (NCL) technique [3].

We designed the strategy of hetero-multiepitopic peptide vaccine by stepwise NCL using 3 building blocks; two peptide thioesters as epitopes and a LCP having 2 different Cys residues (Cys-LCP) (Figure). However we suffered from no or less production at the first NCL step. Hence we present the study on the effect of solvent system and various Cys residues on other branches on NCL reaction.

Results and Discussion

Peptides were synthesized by Boc-chemistry. Peptide thioesters; NS27-, Y504S- and Ac-J8-TAMPAL as B-cell epitopes were prepared according to Hackeng [3]; NS27: ADDHPGAVAARNVDVLSGFS, Y504S: TEVKAAGQ-SAPKGTNVSADL, and J8: QAEDKVKQSREAKK-QVEKALKQLEDKVQ. Compounds were purified and characterized by RP-HPLC and ESI-MS respectively.

NCL was performed using 1.5 – 2.0 equivalent of peptide thioester to Cys residue. Peptide thioesters and 2-mercaptoethanesulfonic acid sodium salt (MESNA) were dissolved in phosphate buffer (PB) and pH was adjusted to 7.6. The solution was left for 1hr at 37°C. The thioester-exchange was checked by HPLC and ES-MS. Cys-LCP and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were dissolved in PB solution and pH was adjusted at 7.5, and two solutions were combined and left at 37°C. Reaction was monitored by HPLC.

The effects on NCL reaction by using three different solvent systems: phosphate buffer (PB) alone, PB with 20% isopropyl alcohol (iPrOH) and PB with 1% sodium dodecyl sulphate (SDS) solution were studied. Cys-LCPs were hardly dissolved in PB alone due to its lipophilicity. Addition of iPrOH improved solubility; however NCL reaction did not occur. Using SDS as a detergent improved not only solubility and also reactivity due to reduction of inter- and/or intramolecular aggregation.

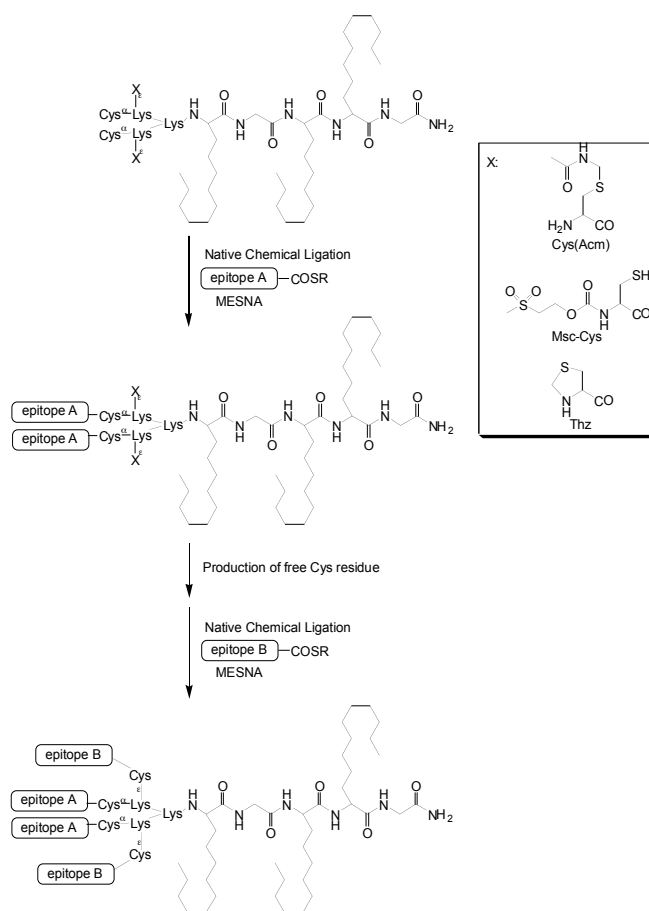


Figure. The overview of hetero-multiepitopic peptide synthesis by stepwise native chemical ligation.

As shown in Figure, in this work we used Cys(Acm), Msc-Cys (Msc: methylsulfonylethylloxycarbonyl) and Thz (thiazolidine-2-carboxylic acid) in which protecting group can be removed selectively for side chains. When using Cys(Acm) and Msc-Cys, the ligation yields in PB with 1% SDS solution were quite low (less than 5%) after 22 hour. As shown in Table, the ligation yield was improved (27%) by change to Thz, which is smaller compared with Cys(Acm) and Msc-Cys, suggesting the reaction is remarkably influenced by the size of Cys residue on other branches.

Table. The NCL yields after HPLC purification under various conditions.

	Solvent Systems		
	PB alone	PB with 20% iPrOH	PB with 1% SDS
Cys derivatives			
Cys(Acm)	No reaction	No reaction	4.49%
Msc-Cys	No reaction	No reaction	4.82%
Thz	Not tested	Not tested	27.6%

NCL between peptide thioester and LCP with 4 Cys residues on Lys-branches at 37 °C succeeded with high yield in previous work [2]; however the reaction at room temperature was not completed after 3 days.

Overall the stepwise NCL reaction to branching peptide such as LCP requires the detergent to reduce aggregation, the higher temperature to accelerate reaction, and the proper Cys residue on other branches.

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References

1. Toth, I., Danton, M., Flinn, N, and Gibbons, W. N. (1993) *Tetrahedron Lett.*, **34**, 3925-3929.
2. Moyle, P. M., Hari, Y., Huang, N., Olive, C., Good, M. F., and Toth, I. (2007) *Tetrahedron Lett.*, **48**, 4965-4967.
3. Hackeng, T.M., Griffin, J.H., and Dawson, P.E. (1999) *Proc. Natl. Acad. Sci.*, **96**, 10068-10073.