Synthesis and Method Development for the Purification of Peptide Components Utilized in Multi-Epitope and Neoantigen Vaccine Constructs

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Introduction

Peptides have recently found applications in multi-epitope-based vaccine constructs as the conventional method of vaccine designing using larger proteins leads to undesired antigenic load in addition to increasing the chances of allergenic responses. Vaccine constructs that use short immunogenic peptide fragments can produce specific immune responses whilst reducing the probability of allergic responses. Synthesis and purification of such short immunogenic peptides is of growing interest. As a part of our ongoing projects, we have a continued interest in peptides that have applications in two therapeutical categories

(i) MHC class I and MHC class II restricted peptides which find potential application in multi epitope vaccines for infectious diseases.

(ii) Peptides with an N-terminal acetyl protection with improved half lives and potential application in neoantigen personalized vaccines for cancer treatment.

The above category of peptides have recently attracted significant interest from the research community, therefore a practical method to accomplish their synthesis and purification which can be employed for a range of peptides with a structural diversity is highly desired. Moreover, synthesis and purification of therapeutic peptides must be robust and should be adaptable to the different structural features of Amino Acids (AAs) used in the sequence. Keeping in view of these facts we describe herein the solid phase peptide synthesis (SPSS) and a general method of purification of a few examples of MHC Class I & II restricted peptides and Neoantigen peptides from our ongoing projects.

Workflow

Peptide Synthesis

Crude Peptide Isolation & Analysis

- Deprotection & Cleavage
- Crude peptdide isolation
- LCMS analysis
- HPLC analysis and method development for purification

Purification

Purification by Prep-HPLC

Pure Peptide

HPLC (purity)
LCMS/MALDI-TOF (identity)

- SPPS strategy using Fmoc method was followed
- Crude peptides isolated by precipitation after deprotection & cleavage

Peptides Synthesized using SPSS Fmoc Method

Peptides for Multi-epitope Vaccine	No. of AA's	AA's with charged side chains	AA's with polar uncharged side chains	Special AA's	Crude purity[1] (%)	Final purity[1] (%)
ALNTLVKQL (MHC Class I)	9	Lys	Asn, Gln	---	72.4	97.9
VLNDILSRL (MHC Class I)	9	Arg, Asp	Asn, Ser	---	76.9	97.3
RLNEVAKNL (MHC Class I)	9	Glu, Arg, Lys	Asn	---	89.0	95.1
NLNESLIDL (MHC Class I)	9	Glu, Asp	As, Ser	---	59.6	97.1
KGYHLMSFPQSAPHG (MHC Class II)	15	Lys, His	Ser, Gln	Pro	59.2	95.7

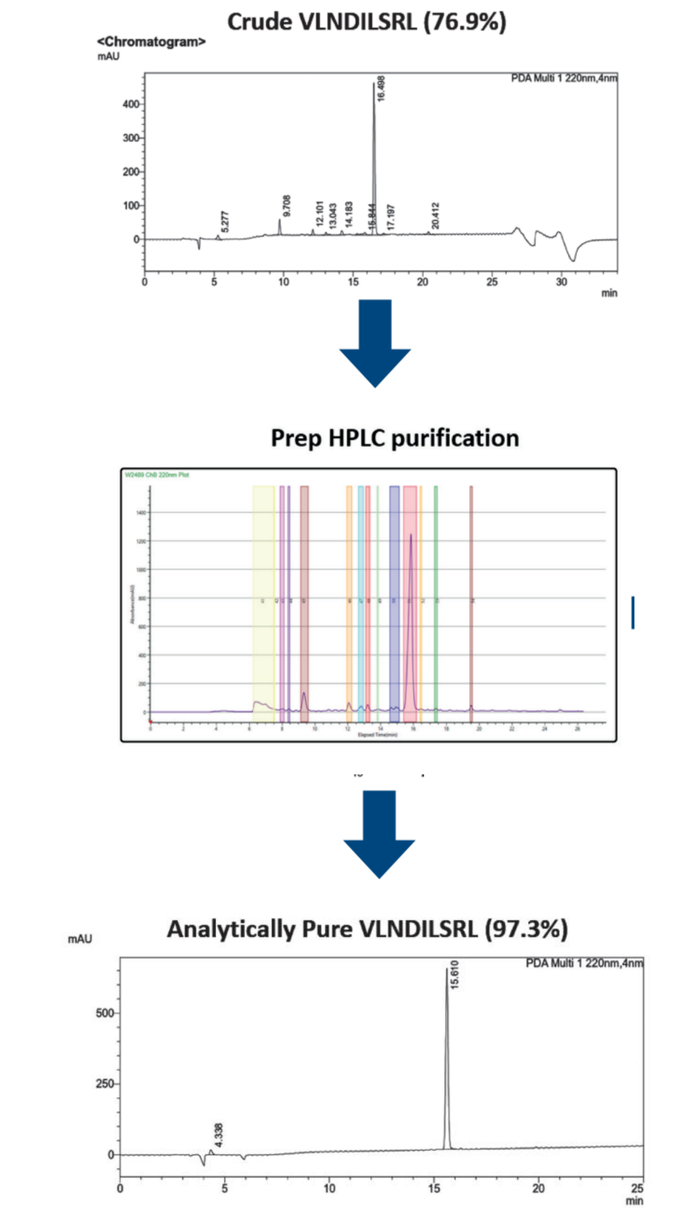
- Equipment: CEM Liberty Blue
- Scale: 0.1 mmol
- Resin: Rink Amide resin (0.59 meq/g)
- Activator: DIC (1.0 M in DMF)
- Activator base : Oxyma-pure (1.0 M in DMF)
- Cleavage cocktail: TFA/H_2O/TIS/DODT

Neoantigen Peptides	No. of AA's	AA's with charged side chains	AA's with polar uncharged side chains	Special AA's	Crude purity[1] (%)	Final purity[1] (%)
MNLRPILTI	9	Arg	Asn, Thr	Pro	57.2	95.7
SSCMGGMNRPILTIIT	17	Arg	Asn, ser, Thr	Cys, Pro, Gly	24.5	95.3
RCAFCKHLVATIKCCEE	17	Arg, Lys, His, Glu	Thr	Cys	28.6	93.7

1. As synthesized purity
2. Purity analysis after purification using Prep HPLC
3. Overall isolated yield 40-60%

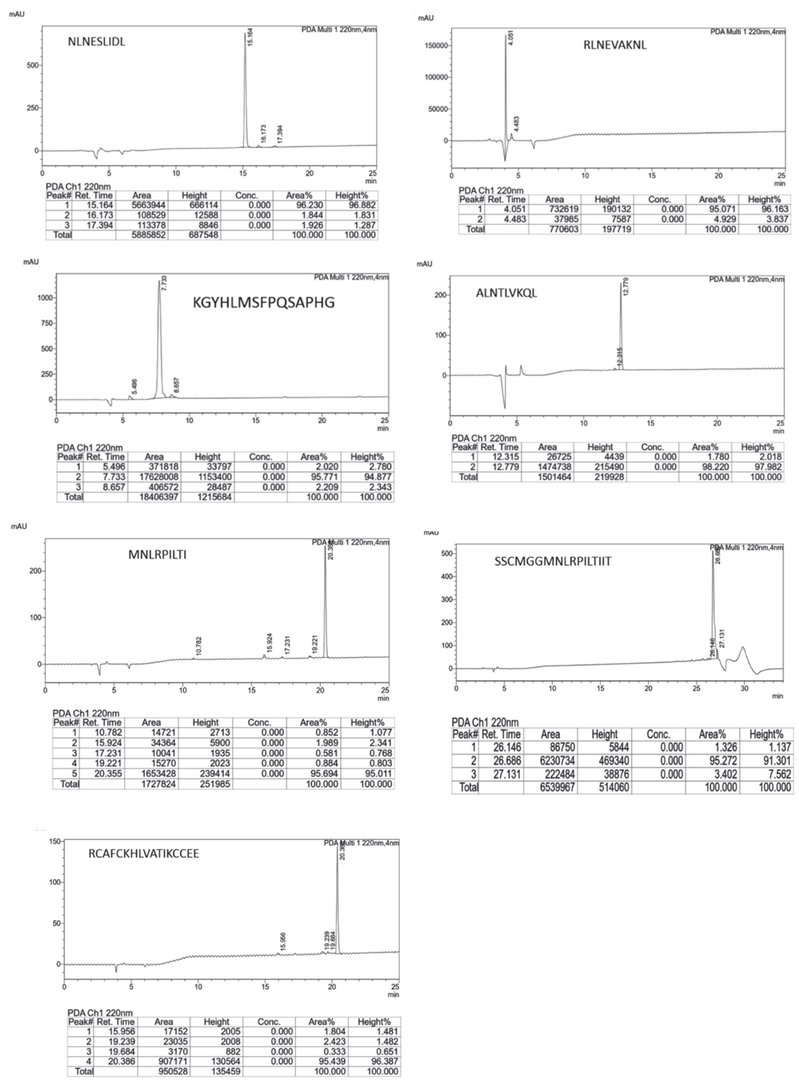
Method Development, Purity Analysis and Identification by Mass Spectral Analysis

Method Development for Purification and Analysis



- Mobile Phase A: 0.1 % TFA in H_2O
- Mobile Phase B: 0.1% TFA in CH_3CN
- Screening different analytical HPLC conditions using a gradient from 18% to 95% of Mobile phase B using different run times and flow rates led to an optimal method.

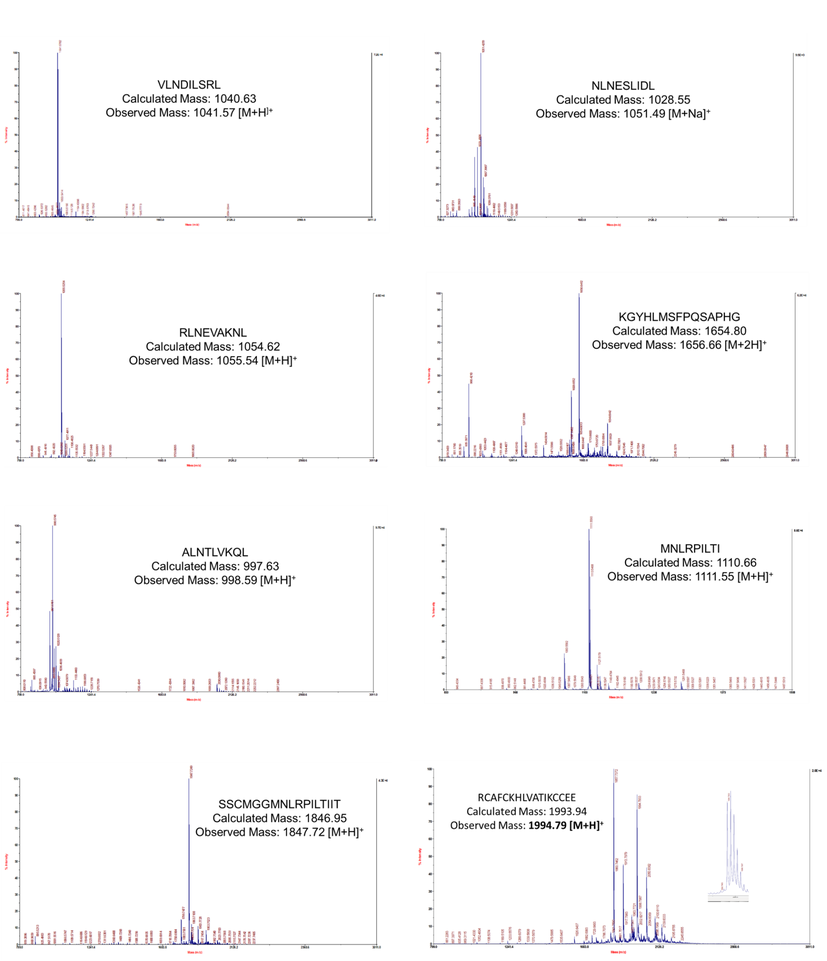
Purity Analysis (HPLC)



- **All peptides were obtained in a purity >95% after purification except RCAFCKHLVATIKCCEE (93.7%)**

- Equipment: **Shimadzu LC-2030 *i*-series HPLC**
- Column: **Waters X SELECT CSH C18 column 5 uM, 4.6 X 250 mm**
- Mobile Phase A: **0.1 % TFA in H_2O**
- Mobile Phase B: **0.1% TFA in CH_3CN**

MALDI TOF Analysis



- Equipment: **ABI 4800 (Applied Biosystems)**
- Matrix : **α-Cyano-4-hydroxycinnamic acid (CHCA)**

- All the peptides show the desired mass mostly as a proton ion adduct except NLNSLIDL (observed as a sodium ion adduct)

Overall, the synthesis and purification method developed by us can be extended to accomplish the synthesis and purification of a wide variety of peptides that have therapeutical applications with minimal optimization.