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Difficulties In Synthesis and Characterization of Viral Capsid Peptides

Zafer Ömer Özdemir*, Erdem Karabulut, Murat Topuzoğulları, Zeynep Mustafaeva

Yildiz Technical University, Bioengineering Department, Istanbul, Turkey

Abstract

Peptides and proteins play a central role in numerous biological and physiological processes in living organisms.

Viral capsid pepdides are part of the viruses outer shell of genetic materials. Viruses are recognized by immune system via capsid peptides. Depending on this property of capsid peptides, totally synthetic vaccine prototypes can be developed. In this work we synthesised 3 different viral peptide sequences with microwave enhanced Solid Phase Peptide Synthesis (SPPS) method and characerized with LC-ESI-MS system. We briefly describe the difficulties in synthesis and characterization.

Key Words: Synthetic viral peptide, LC-ESI-MS analysis, Solid Phase Peptide Synthesis.

INTRODUCTION

Proteins and their smaller relatives are present in every living cell and possess a variety of biochemical activities. They appear as enzymes, hormones, antibiotics, and receptors. They compose a major portion of muscle, hair, and skin. Consequently, scientists have been very interested in synthesizing them in the laboratory. This interest has developed into a major synthetic field known as Peptide Synthesis.

The publication in 1901, by E. Fischer (with E. Fourneau) [1], of the preparation of the dipeptide glycylglycine by hydrolysis of the diketopiperazine of glycine, is considered to be the beginning of peptide chemistry. However, T. Curtius had synthesized and characterized a related peptide 20 years earlier, during his PhD studies with H. Kolbe [2], preparing the first N-protected dipeptide, benzoylglycylglycine, by treatment of the silver salt of glycine with benzoylchloride [3].

The general chemical requirements for peptide synthesis were to block the carboxyl group of one amino acid and the amino group of the second amino acid. Then, by activation of the free carboxyl group the peptide bond could be formed, and selective removal of the two protecting groups would lead to the free dipeptide [4].

After discovering of solid phase peptides synthesis (SPPS) by Merrifield [5], research on peptide

* Correspondence to: Zafer Ömer Özdemir

Yildiz Technical University, Bioengineering Department, Davutpasa Campus, Esenler, Istanbul 34210, Turkey

Tel: +90212 0212 383 4637; Faks: 0212 383 4625
E-mail: ozdemirz@gmail.com

synthesis rapidly increased. In Merrifield's SPPS method, peptide grows on a polymeric solid particle which called resin. This method is used t-Boc (tert-butyl oxycarbonyl group) [6, 7] as an amino protection group. t-Boc is acid labile and it is removed by TFA (trifluoro acetic acid) and HF (hydrogen fluoride) is used for cleavage. TFA and HF are dangereous and very corrosive materials, working with them is very hard. F-moc (9-fluorenylmethyloxycarbonyl group) [8] amino protection group is base labile and removed by Piperidine. Because of this reason removing the protection step easier in F-moc method than t-Boc.

Bruce Merrifield
Nobel Prize for
Organic Chemistry
1984

Linker — Resin

Anchoring

Repetitive cycle

Nα-deprotection

Coupling

(1) Nα-deprotection
(2) Cleavage from resin
(3) Side-chain deprotection

Figure 1 . Schematic presentation of the principles of solid-phase peptide synthesis [3].

Generally vaccines may consist of live or killed viruses [9]. Viral capside peptide is the viruse's outer shell protein part which recognized by element of immune system [10]. Depending on this property and preparation of their conjugation with biodegradable polymer, make a totally synthetic vaccine prototype is possible [11, 12]. In this work we synthesised 3 different viral capsid peptides for developing new vaccine prototype. These peptide sequences are below.

Viral capsid peptides of Foot and Mouth Disease (FMDV); VP1 200-213 WDRHKQRIIAPAKQLG, VP1 135-161 SKYSTTGERTRGDLGALAARVA-TQLPA [13], Viral capsid peptide of Influenza; NP 55-69 WRLIQNSLTIERMVLSA [14], Peptide synthesis and characterization are tedious work. Synthesis and characterization of peptides have lots of parameters. We classified these paramaters, a) choosing and swelling of resin, b) coupling reagents, c) deprotection reagent, d) cleavage conditions (cocktails and time), e) precipitation with cold ether, f) determination of gradient rate, etc.

Important steps in peptide synthesis and characterization;

Choosing and swelling of resin,

Resin is a polymeric particle which peptide grows on it. There are several types of resins. Wang resin is the mostly used resin in peptide synthesis, also we used preloaded Wang resin. Binding of the first amino acid to resin required special chemicals and synthetic methods. Because of this, using the preloaded resin is useful. Another parameter for the resin is loading parameter. It can be lower loading and higher loading. Resin has specific functional groups on it. These specific functional groups are used for binding of amino acids. If all these groups occupied with amino acid, these resins are called as higher loaded. If small part of these groups occupied with amino acid, these resins are called as lower loaded. Higher loaded resins is not preferred for long peptide sequences.

Resin must be swelled in properly before the synthesis. With swelling, binded amino acid for preloaded resin become homogenic on resin surface.

Coupling reagents,

Activation of the carboxylic group can always lead to racemization via the oxazolone intermediate; however, the introduction of additives such as 1-hydroxybenzotriazole (HOBt) to the reaction mixture was then shown to minimize this by the formation of a less-reactive HOBt ester. Over the years, numerous other coupling reagents have been developed, and a selection of the most widely used ones is shown in Figure 2.

If the coupling is not good enough, there will be a deletion in the sequence.

Deprotection reagent,

In F-moc protection method, mostly piperidine is used as a deprotection reagent, some synthesis piperazine can be used

Cleavage conditions (cocktails and time),

Cleavage is the cutting procedure from finished peptide from resin and removing of side chain protection groups. Cleavage is the vital part of the peptide synthesis. Because yield of the synthesis depend on this step. TFA is the main reagent for cleavage in F-moc strategy. Mostly EDT (ethanedithiol), Thioanisole, TIS (triisopropylsilane), Water and Phenol are used as a scavanger. Scavenger's rate change depends on the peptide sequence.

Cleavage time is also important and the time should be carefully decided. More time in the cocktail causes more oxidased peptide.

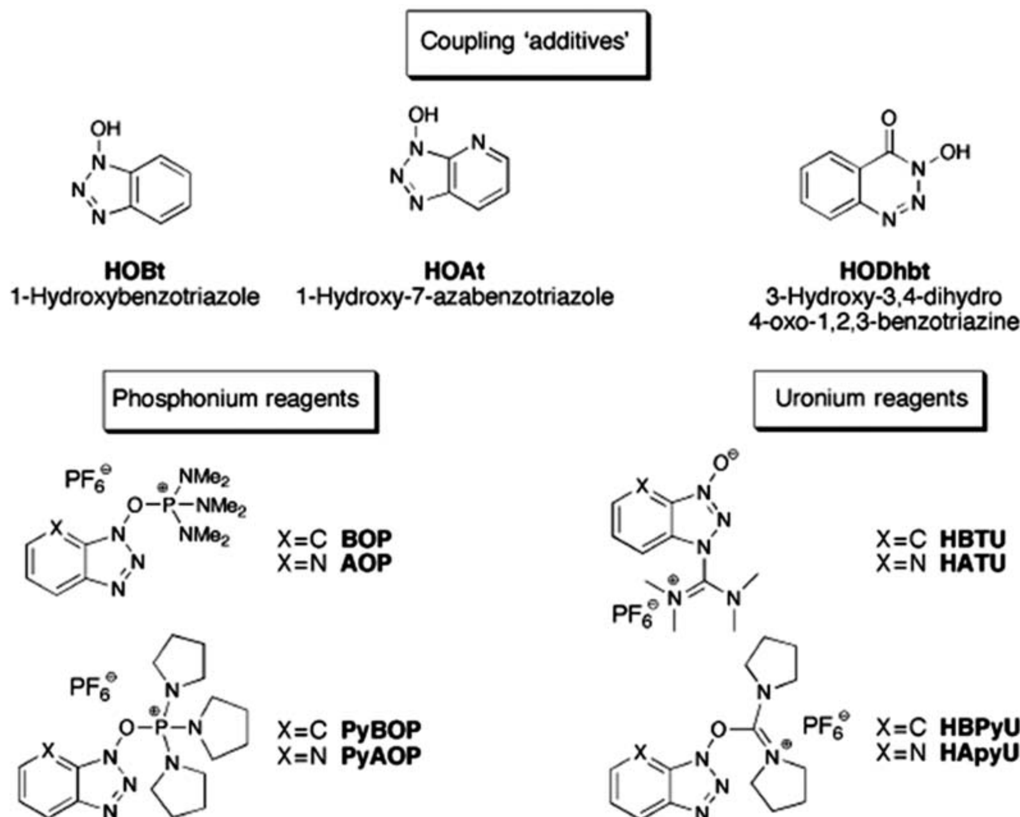


Figure 2. Molecular formula of some coupling reagents [3].

Precipitation with cold ether,

Cold (-20°C) ether (dietilether) is used for precipitation of peptide from cleavage cocktail. Ether must be cold enough because it is a solvent for peptide.

Determination of gradient rate,

Gradient solvents mostly Acetonitrile (AcN), and water. Sometimes Ethanol, Acetone can be used. In LC-MS analysis [15], TFA or Formic acid [16] is added to the gradient solvent as modifier for 0.1% rate. When ideal gradient solvent and gradient rate defined, HPLC (high pressure liquid chromatography) purification [17] is performed with these parameters.

In this work microwave energy is used for peptide synthesis. However microwave energy is not the main subject of this paper, we would like to mention briefly about microwave energy and effect mechanism of it.

Microwave irradiation is electromagnetic irradiation in the frequency range of 0.3 to 300 GHz. Microwave-enhanced chemistry is based on the efficient heating of materials by "microwave dielectric heating" effects. This phenomenon is dependent on the ability of a specific material (solvent or reagent) to absorb microwave energy and convert it into heat. The electric component of an electromagnetic field causes heating by two main mechanisms: dipolar polarization and ionic conduction [18,19].

MATERIALS AND METHODS

All chemicals used in this study were obtained from commercial sources. Wang resins, amino acids and coupling reagents purchased from NovaBiochem. The other chemicals purchased from Sigma-Aldrich.

These peptides was synthesized by solid phase peptide synthesis (SPPS) method with microwave

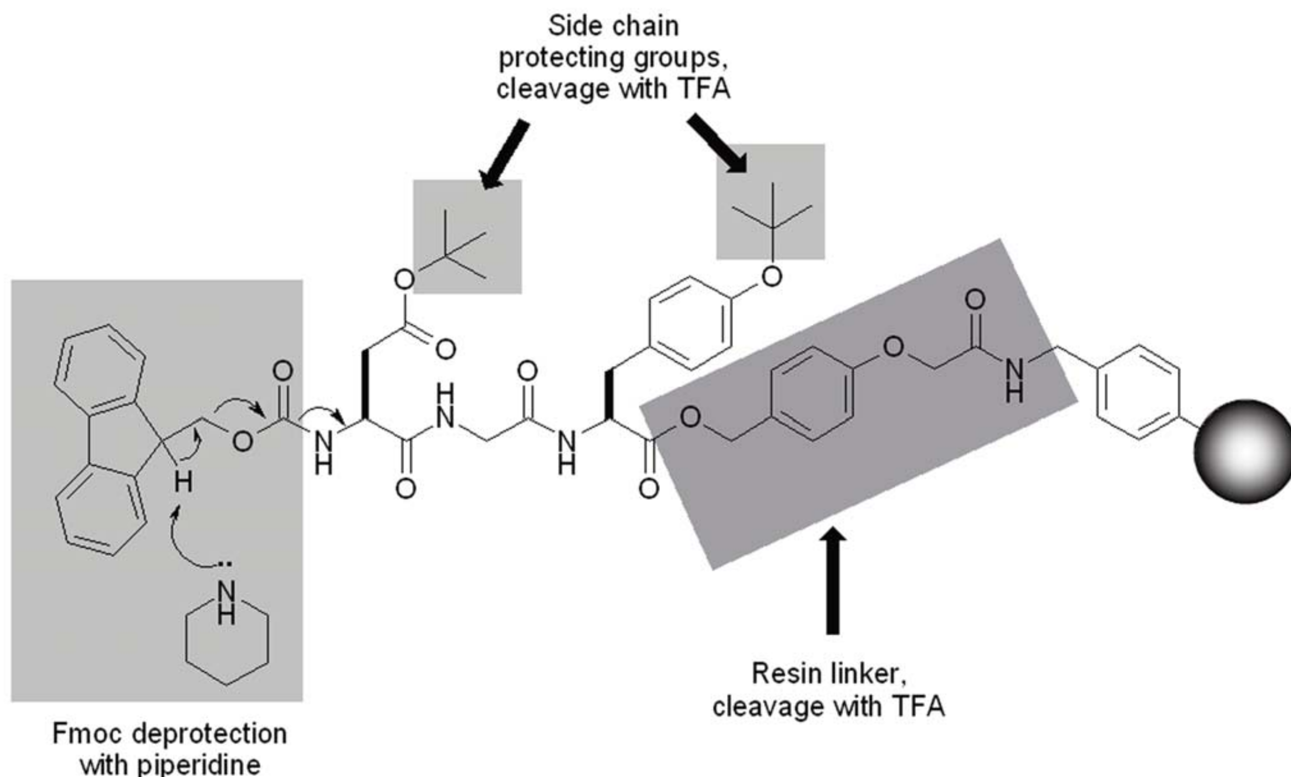


Figure 3. Fmoc strategy in solid-phase peptide synthesis. The Fmoc group is cleaved under basic conditions with piperidine, while the sidechain- protecting groups and the linker are cleaved under acidic conditions using TFA [3].

assisted (CEM, Liberty) in DMF media, using HBTU/HOBt as activator and DIEA/NMP as activator bases. Preloaded Wang resin is used in these peptides synthesis. Peptides were cleaved from resin using cleavage cocktail prepared with TFA/Thioanisole/EDT/Water 90/5/2.5/2.5 (v/v). Cold (-20°C) ether was used for precipitation. After the centrifugation precipitated peptides dried in vacuum. Synthesized viral peptides VP1 135-161, VP1 200-213 is defined from Foot and Mouth Disease Virus's (FMDV) capsid protein sequences and NP 55-69 is defined from influenza virus. Also tryptophan amino acid added the N-terminus of the sequences, because of to be able to fluorescence analysis for further research. Synthesized peptide sequences are;

VP1 135-161 SKYSTTGERTRGDLGALAARVA-TQLPA,
VP1 200-213 WDRHKQRIIAPAKQLG
NP 55-69 WRLIQNSLTIERMVLSA

Microwave energy is used for:
Deprotection; 55 W to max. 45°C for 2 min.
Coupling; 25 W to max. 75°C for 3 min.

LC-MS system (Shimadzu LC-MS 2010 EV) with ESI (Electro Spray Ionization) method is used for characterization of synthetic peptides. Elution was gradient (Acetonitrile-Water) and at a flow rate of 2.0 ml/min. Teknokroma Tracer Exel 120 ODS-A 5 - m 20x0.21 column was used for characterization of synthetic peptides. Crude peptides purified with preparative-HPLC system (Shimadzu). Elution is the same as LC-MS system. Shim-pack PRC-ODS HPLC column (20 mm x 25 cm was used for purification. Ultra pure water from Millipore MilliQ Gradient system is used.

RESULTS AND DISCUSSON

After the mass analysis we have got the following MS spectra results.

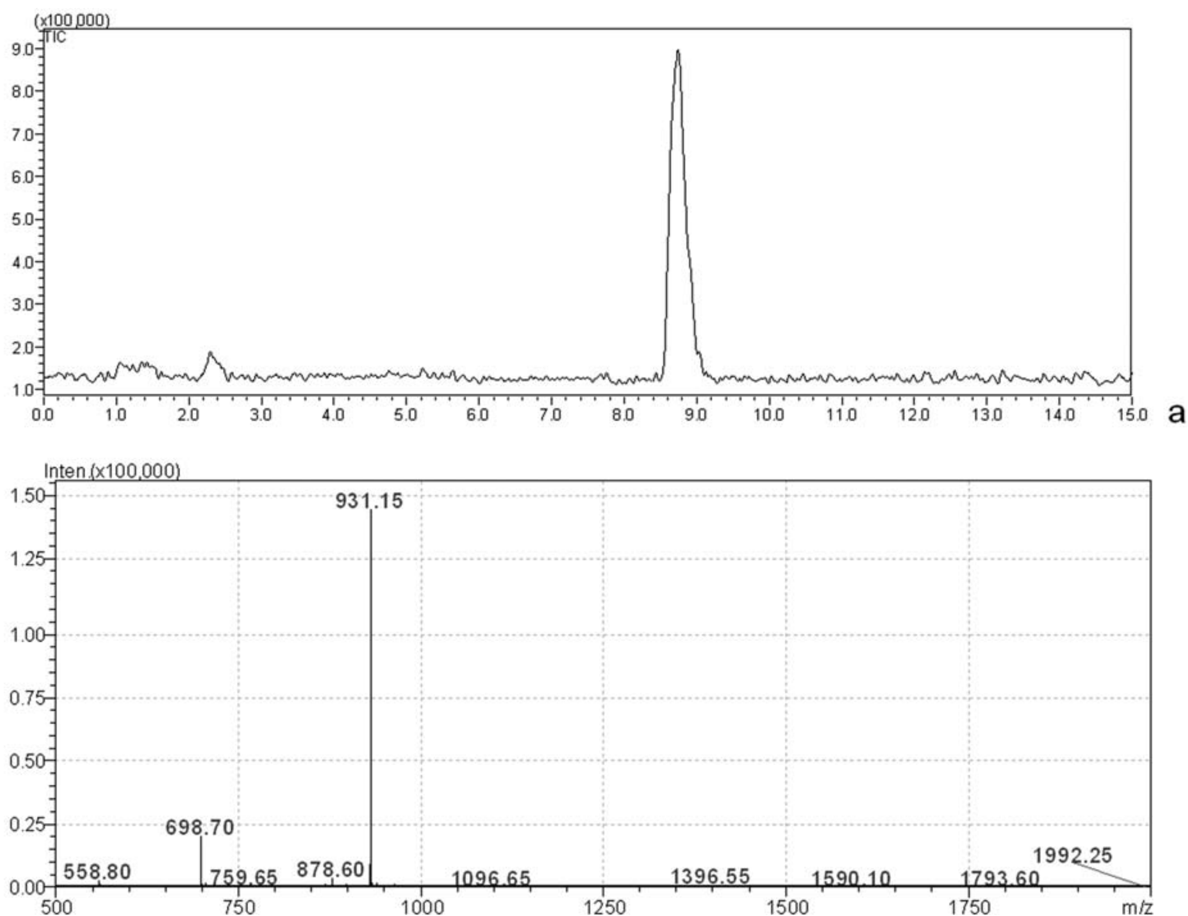


Figure 4. MS spectra for VP1 135-161 SKYSTTGERTRGDLGALAARVATQLPA.

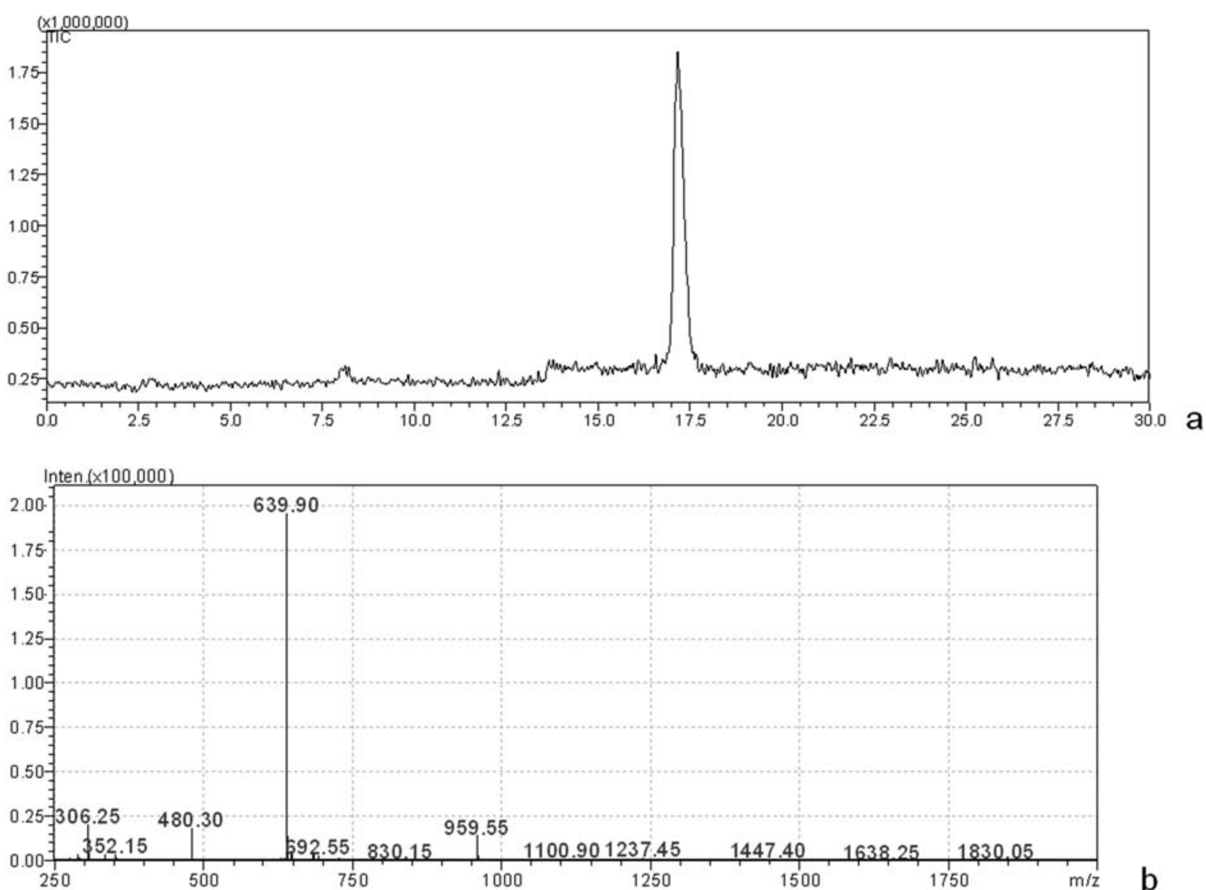
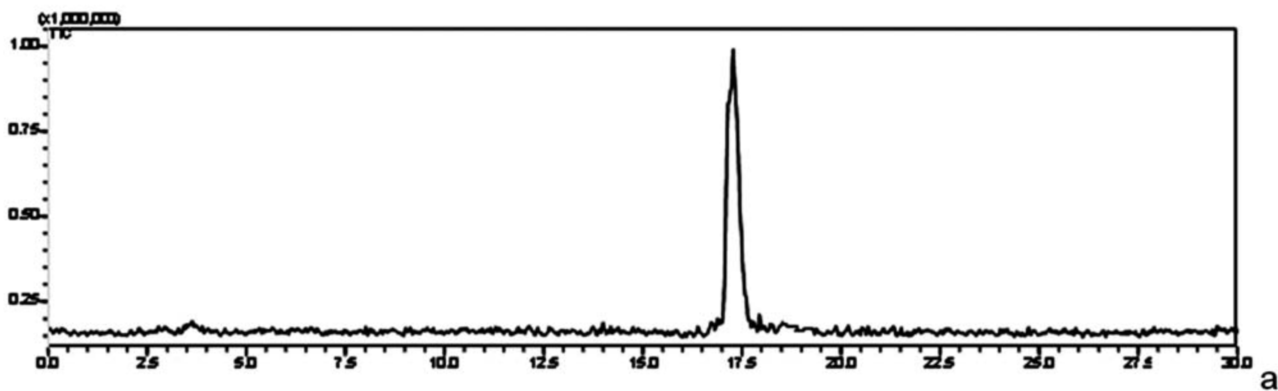


Figure 5. MS spectra for VP1 200-213 WDRHKQRIIAPAKQLG.



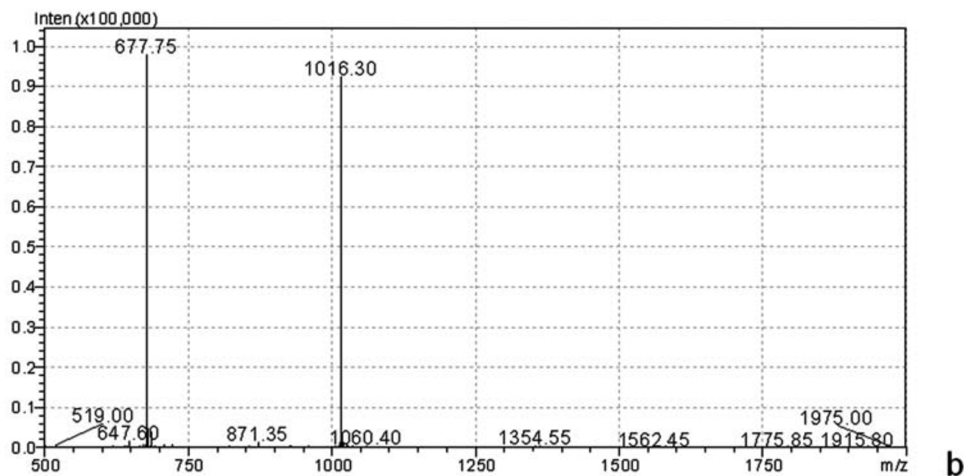
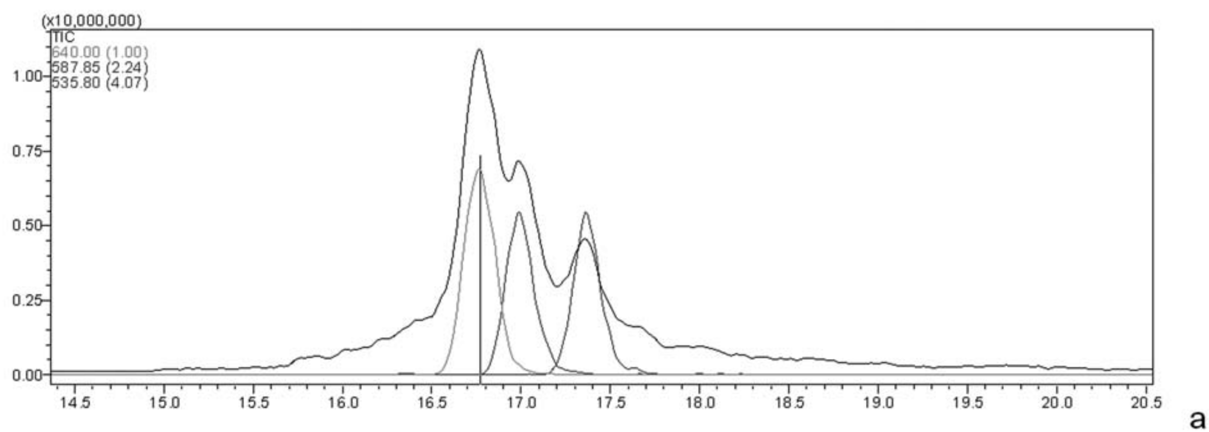


Figure 6. MS spectra for NP 55-69 WRLIQNSLTIERMVLSA.



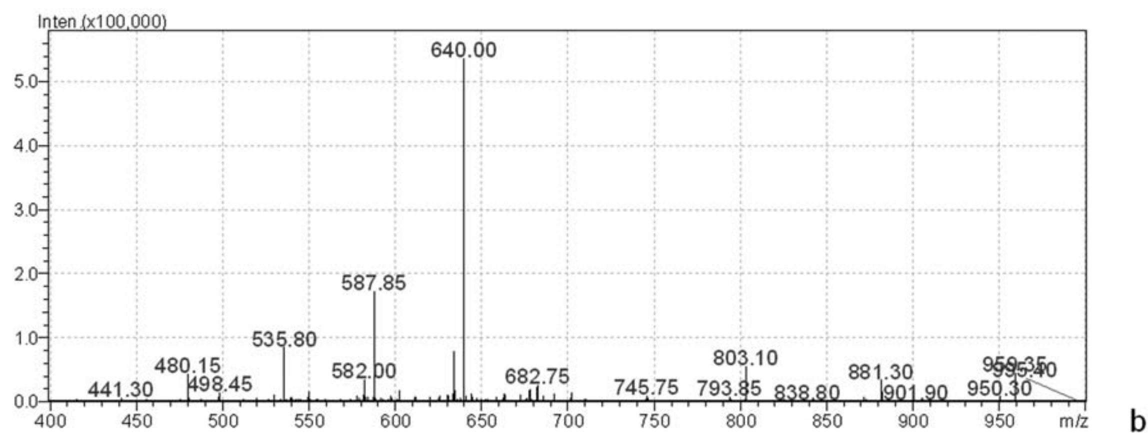


Figure 7. MS spectra for VP1 200-213 WDRHKQRIIAPAKQLG.

Figures 4, 5, 6 are ideal peptide spectra. There are no deletion, dehydration, etc.

In our first attempt to synthesised for VP1 200-213 WDRHKQRIIAPAKQLG we got spectra like the following.

In Figure 7a, there are 3 different peaks with different mass. These peaks mean, there are deletions in the desired peptide sequence. These deletions source are from Arginine. We understand this with basic calculation:

640,00 – 587,85 = 52,15
587,85 – 535,80 = 52,05

There are 2 arginine in the sequence. Arginine has hydrophilic character. Because of the arginines hydrophilic character the biggest peak (m/z=640.00) in the Fig. 7 is from real sequence (no deletion). The second peak (m/z=587.85) is from 1 deletion (one of the arginine is missing). The last one (m/z=535.80) is from 2 deletion (two of the arginine are missing).

We used double coupling against for deletion and we got synthesis result in Figure 5.

CONCLUSION

Microwave assisted peptide synthesis is briefly described. The important steps of peptide synthesis and characterisation are explained. LC-MS analysis of 3 different viral capsid peptide sequences are discussed. This work is the main part of the preparation of totally synthetic vaccine prototype which can be achieved by synthesis of viral capsid peptides and their bioconjugation with biodegradable polyelectrolytes.

ACKNOWLEDGEMENTS

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