International Journal of Natural and Engineering Sciences 3 (2): 35-38, 2009
ISSN: 1307-1149 www.nobel.gen.tr

Investigation of Protein and Polymer as a Carrier on Immune System

Banu MANSUROGLU* Serap DERMAN Kadriye KIZILBEY Zeynep MUSTAFAEVA AKDESTE
Department of Bioengineering, Faculty of Chemical and Metallurgical Engineering, Yildiz Technical University, Davutpasa Campus, 34210 Esenler-Istanbul, Turkey

*Corresponding Author
e-mail: bmansur@yildiz.edu.tr

Received: December 08, 2008
Accepted: January 19, 2009

Abstract:
The immunogenic properties of water soluble BSA-Peptide and VP/AA-Peptide conjugates were investigated, and specificity of antibodies produced was analyzed. This epitope of Foot-and Mouth Disease virus VP1 protein (135-161 amino acid sequences) were used as a polypeptide antigen. Immunogenic activity of BSA-Peptide and VP/AA-Peptide conjugates were investigated depending on the peptide amount in conjugates per mouse and carrier (synthetic polyelectrolyte or protein molecule) structure. VP/AA-Peptide and BSA-Peptide conjugates revealed very high immunogenicity which are different in regards to the specificity of the antibody produced. The BSA-Peptide conjugates were able to generate specific antibodies both for 135-161 peptide epitope and BSA. In contrast to VP/AA-Peptide conjugates system was able to generate only 135-161 peptide epitope antibodies.

Key words: Polyelectrolyte, biopolymer systems, immunogen, polymer-peptide conjugates, synthetic vaccine, foot-and-mouth disease virus.

INTRODUCTION

Synthetic polyelectrolytes (PE) have been widely used to modify proteins via covalent attachment, increasing (or reducing) the immunoreactivity and/or immunogenicity of originally antigenic proteins, and improving their in vivo stability with prolonged clearance times. Besides, the conjugates of PE with individual microbe antigens develop strong protective properties [1] and they can be considered as a new generation of vaccinating compounds [2-3].

Its is well known that in order to elicit a high immune response to such antigens after coupling small hapten molecules to carrier molecules, this prepared bioconjugates are administered into the body with classical adjuvants-$Al(OH)_3$ and Freund's mineral oil adjuvant [4].

One of the promising alternatives to classical adjuvants is using nonimmunogenic synthetic polyelectrolytes (PE) (negatively or positively charged polymers) and proteins (e.g., keyhole limpet hemocyanin, ovalbumin, bovine gamma-globulin, or bovine serum albumin) as carriers for antigens. Peptides can be conjugated to these carrier reagents through either its amino- or carboxyl-terminal ends [5]. The attachment of weak microbial and viral protein antigens to various charged organic polymers allows the manipulation of the immunogenicity as well as the protective activity [6].

Foot-and-mouth disease virus (FMDV) is the causative agent of the economically most important animal viral disease world-wide. Although mortality associated with FMD is usually low, the disease decreases livestock productivity, and affected countries cannot participate in international trade of animals and animal products [7]. Particles are about 30 nm in diameter and are composed of 60 copies of each of four capsid proteins termed VP1, VP2, VP3 and VP4. Cleavage studies of VP1 identified two immunogenic fragments composed of residues 138 to 154 and 200 to 213 [8], and synthetic peptides corresponding to these regions are capable of inducing a neutralizing antibody response [9-10].

The advent of technological advances in antigen delivery has led to new strategies for the design and development of vaccines. This is especially highlighted by the progress in the design of synthetic peptide-based vaccines which have the potential to overcome a number of obstacles associated with current vaccine delivery technologies, especially pertaining to the issues of MHC and antigen diversity, immunogenicity and adjuvanticity [11].

The purpose of the present study is to examine the covalent binding mechanism of 1-Vinyl-2-pyrrolidone/acrylic acid (VP/AA) copolymer and Bovine Serum Albumin (BSA) molecules as carrier with 135-161 peptide sequences of VP1 capsid protein of Foot-and-Mouth Disease Virus. The immunogenic properties of BSA-Peptide and VP/AA-Peptide conjugates were investigated depending on the peptide amount in conjugates per mouse and carrier (synthetic polyelectrolyte or protein molecule) structure.

MATERIAL AND METHODS

Synthetic peptides

Peptides containing the VP1 (135-161) (Ser-Lys-Tyr-Ser-Thr-Thr-Gly-Glu-Arg-Thr-Arg-Thr-Arg-Gly-Asp-Leu-Gly-Ala-Leu-Ala-Ala-Arg-Val-Ala-Thr-Gln-Leu-Pro-Ala) epitope were synthesized by using the continuous solid-phase method by Sigma Gynosys system. Peptides were purified by semi-preparative HPLC and the achieved purity was greater than 95%. To enhance the immunogenicity of the VP1 epitope, this sequence was administered in several forms: combined with Freund's adjuvant (incomplete), conjugated with polyelectrolyte (PE) and BSA molecule at different ratio of components.

Synthesis of 1-Vinyl-2-pyrrolidone/acrylic acid (VP/AA) copolymer

Poly(N-vinylpyrrolidone-2-co-acrylic acid) (VP/AA = 2:1 ratio) was synthesized and fractioned as explained in the

literature [12]. Glacial acrylic acid and N-vinylpyrrolidone-2 were polymerized in THF with benzoyl peroxide and cobalt naphthenate at 70°C under nitrogen for 3 hr. The white solid was filtered and dried at 40°C in vacuum. Acrylic acid (Aldrich, Germany), vinylpyrrolidone-2, THF benzoyl peroxide and cobalt naphthenate analytical grade reagents. Fractionally distilled solvents were used in the reaction**.** The molecular weight of VP/AA was around 120kDa.

Preparation of VP/AA-Peptide Covalent Conjugates

VP/AA-peptide conjugates have been synthesized by the well-known carbodiimide method [13] VP/AA was dissolved in water, stirred at 4 °C and pH values of mixture adjusted to pH 5 with NaOH. Peptide (Mw=2556) was dissolved in water, stirred at 4 °C. Peptide solution was added to VP/AA solution which pH 5 values of mixture stirred at 4 °C. After 1 h, carboxyl groups of VP/AA were activated water-soluble 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (Sigma E7750). Dry EDC was added to mixture and stirred at 4 °C. Then, pH values of mixtures were adjusted to pH 7 by NaOH. After removal of O-acylisourea intermediate by dialyzes, the sample was lyophilized. The purified conjugates were redissolved in 0.9% NaCl at 4 °C for the immunization.

Preparation of BSA-Peptide Covalent Conjugates

BSA protein dissolved in phosphate buffer saline (PBS) (pH 7.2) and peptide (Mw=2556) dissolved in PBS (pH 7.2). Peptide solution added to BSA protein solution and stirred for 30 minutes at 4 °C. pH values of mixture adjusted to pH 5 with HCl. After 30 minutes, carboxyl groups of protein and peptides were activated water-soluble EDC. After the addition of EDC to the mixture were stirred at 4°C overnight. pH values of BSA-Peptide bioconjugates were adjusted to pH 7 by NaOH. After removal of O-acylisourea intermediate by dialyzes, the sample was lyophilized. The purified conjugates were redissolved in 0.9% NaCl at 4 °C for the immunization.

Immunization

40 male BALB/c mice, 6-8 weeks old were used for the study. Male BALB/c mice weighing 20-24 g were used for immunization. The immunizations were carried out with 5 BALB/c mice for each group. BSA-Peptide and VP/AA-Peptide covalent conjugates were used as the immunogens. NaCl isotonic solution (0.9%) was injected as control. VP/AA-Peptide covalent conjugates including 1.5 mg and 3 mg peptide/mouse were injected. BSA-Peptide conjugates including 1 mg/mouse and 0.5 mg/mouse were injected. Serum was collected after immunization weekly for 5 weeks. For antibody level determinations, the mice were bled through the tail vein. The blood was collected in a microfuge tube in sodium citrate, centrifuged at 2500 rpm to remove red blood cells. Serial dilutions of the serum (1:50 and 1:100) were prepared in PBS. The serum samples were tested with ELISA.

ELISA for the Antibody Responses

In order to confirm the synthesized for 135-161 peptide sequences of VP1 capsid protein of FMDV antibodies, 96-well polystyrene plates (GREINER immunoplates) were coated with 200 ng BSA-Peptide conjugate in parallels, with BSA in 100 µl PBS [6, 14-15]. Coating of plates was carried out at 4°C overnight. The plates were washed three times with washing buffer (0.005 % Tween-20 in PBS). Then, 0.2% casein in PBS was added to the wells, and the plates were incubated for 1 h at 37°C followed by washing as above. The mouse serum in dilution buffer (PBS) was added to each well and the plate was incubated at 37°C for 1 h. The plate was washed three times with washing buffer. Alkaline-phosphates conjugate of antimouse polyvalent immunoglobulin at 1:1000 dilution buffer (PBS) was added to each well and incubated for 1 h at 37°C. After repeating the washing step five times with washing buffer as above, the substrate buffer (1 mM ZnCl, 1 mM MgCl, 0.1 M Glycine, pH 10.4) and 1 mg/ml para-nitrophenyl phosphate (PNPP) were added. After 45 min, the absorbance at 405 nm was determined.

RESULTS

135-161 peptide sequences of VP1 capsid protein of FMD antibody produced by using conjugates of synthetic polyelectrolyte and BSA molecule as carrier (adjuvant activity and structure formed) compared to the classical Freund's incomplete adjuvant.

Immunogenicity of BSA-Peptide Conjugates

Using the regular coupling method with EDC, conjugates of BSA-135-161 peptide sequences of VP1 capsid protein of FMD were synthesized. This conjugates were varied depending on the peptide amount of conjugates per mouse (1 mg/mice, 0.5 mg/mice). For the immunization of mice, different concentrations of BSA-Peptide conjugate solutions were used.

The dynamics of antibody formation (OD_{405}) induced by BSA-Peptide conjugates are presented in Figure 1. A single immunization of mice with dilute solution of BSA-Peptide conjugates which elicited the production of very high number of antibodies [practically compared with peptide combined with Freund's adjuvant (incomplete) and control]. Immune response for all conjugates could be detected in the blood sera (serum dilution: 1/50) on the seventh day after-immunization and the highest level of immunogenic activity lasted more than 35 days.

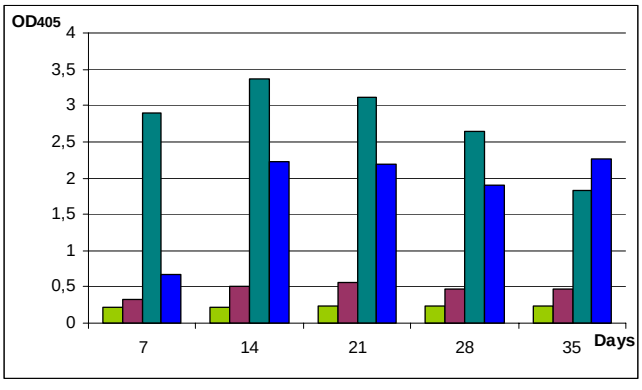

Figure 1: The dynamics of Peptide-Specific antibody formation [as assayed by ELISA (OD_{405})], induced by BSA-Peptide conjugates at different peptide doses; ( ) BSA-Peptide conjugates including 1 mg/mouse peptide, ( ) BSA-Peptide conjugates including 0.5 mg/mouse peptide, ( ) 1 mg free peptide combined with Freund's adjuvant (incomplete), ( )%0.9 NaCl isotonic solution as control**.**

Immunogenicity of VP/AA-Peptide Conjugates

VP/AA-135-161 peptide sequences of VP1 capsid protein of FMD conjugates were varied depending on the peptide amount in conjugates per mouse (1.5 mg/mice, 3 mg/mice). The dynamics of antibody formation induced by VP/AA-Peptide conjugates at different doses are shown in Figure 3. A single immunization of mice with dilute solution of VP/AA-Peptide conjugates which elicited the production of very high number of antibodies [practically compared with peptide combined with Freund's incomplete adjuvant and control].

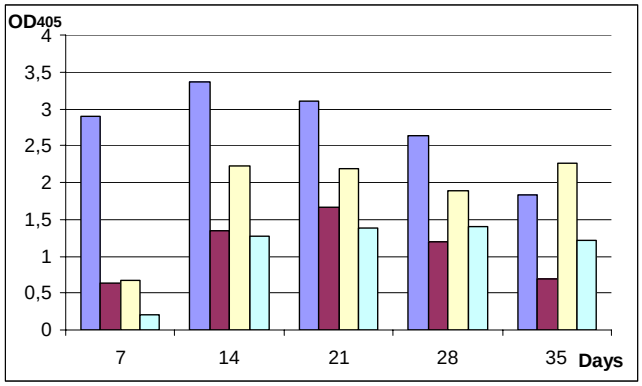

Figure 2: The dynamics of Peptide-Specific and BSA-Specific antibody formation [as assayed by ELISA (OD_{405})], induced by BSA-Peptide conjugates at different peptide doses; ( ) BSA-Peptide conjugates (including 1 mg/mouse peptide) Peptide-Specific antibodies, ( )BSA-Peptide conjugates (including 1 mg/mouse peptide) BSA-Specific antibodies, ( ) BSA-Peptide conjugates including (0.5 mg/mouse peptide) Peptide-Specific antibodies, ( ) BSA-Peptide conjugates including (0.5 mg/mouse peptide) BSA-Specific antibodies.

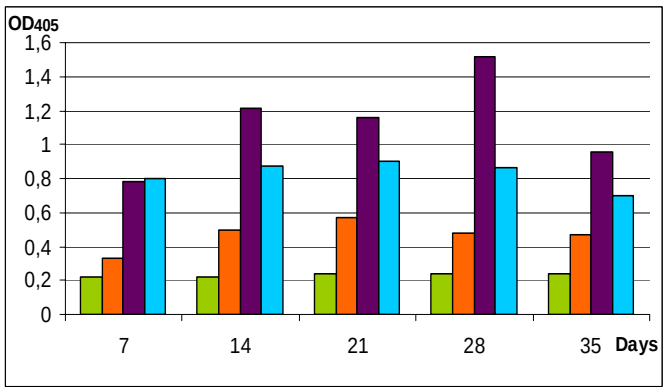

Figure 3: The dynamics of Peptide-Specific antibody formation [as assayed by ELISA (OD_{405})], induced by VP/AA-Peptide conjugates at different peptide doses; ( ) VP/AA-Peptide conjugates including 1.5 mg/mouse peptide, ( ) VP/AA-Peptide conjugates including 3 mg/mouse peptide, ( ) 1 mg free peptide combined with Freund's incomplete adjuvant, ( ) %0.9 NaCl isotonic solution as control.

DISCUSSION

It was shown that the covalent coupling of peptides using water-soluble carbodiimides can be very efficient by the linkage of (synthetic) peptides with polyelectrolyte (VP/AA) and carrier protein (BSA) in order to produce conjugates able to elicit peptide-specific immune responses. A comparative study of immunogenic properties of VP/AA-Peptide and BSA-Peptide conjugates revealed very-high immunogenicity that they are different in regards to the specificity of the antibody produced. The BSA-Peptides conjugates were able to generate both 135-161 peptide epitops specific and BSA specific antibodies (Figure 2). When PE is compared with BSA and the other carrier proteins it develops more specific immune response. PE is more advantageous as a carrier than BSA with this property.

FMD VP1 capsid proteins' 135-161 peptide sequences about 29 amino acids and 2950 kDa molecular weight does not compose immune response by itself. When immunization was carried out by peptide with classical Freund's adjuvant the immune response is lower than it was carried out by BSA-peptide or VP/AA-peptide conjugates. This molecular weight is generally not sufficient to elicit an antibody response. For this reason, the synthetic peptides must be converted to high-molecular weight products. Conjugation of a carrier protein is important because peptides are small molecules, which do not tend to be immunogenic alone, thus possibly eliciting a weak immune response. The carrier protein contains many epitops that stimulate T-helper cells, which help to induce the B-cell response. Many different carrier proteins can be used for coupling to synthetic peptides [16, 17].

CONCLUSION

In conclusion, a method is described for increasing the immune response to 135-161 peptide sequences of VP1 capsid protein of FMD antibody of immunological and practical interest. Selective use of BSA molecule and polyelectrolyte (VP/AA) as well as coupling methods may lead to more efficient use of weak antigens like 135-161 peptide sequences of VP1 capsid protein of FMD on immunogenicity in conjugates.

Finally as demonstrated in this study, the use of VP/AA as a carrier in single immunizations enhanced the immune response against polypeptide antigens and produced high level peptide-specific antibodies in the sera.

ACKNOWLEDGEMENT

Many thanks for Prof. Dr. Mamed Mustafaev AKDESTE who was the Constitutive Head of Bioengineering Department of Yildiz Technical University.

This research was supported by grant from T.R. Prime Ministry State Planning Organization (Project No. 25-DPT-07-04-01).

REFERENCES

[1] Petrov RV, Mustafaev MI, Norimov AS. 1992. Physico-chemical criteria for the construction of articial immunomodulators and immunogens on the bases of polyelectrolyte complexes. Sov. Med Rev D Immunol 2;4:1113.

[2] Salman Dilgimen A., Mustafaeva Z., Demchenko M., Kaneko T., Osada Y., Mustafaev M. 2001. Water-soluble covalent conjugates of bovine serum albumin with anionic poly(*N*-isopropyl-acrylamide) and their immunogenicity. Biomaterials 22:2383-2392.

[3] Mustafaev M., 2004. Functionally Biopolymer Systems. Sigma, Journal of Engineering and Natural Sciences ISSN: 1304–7191, Printed in Yildiz Technical University, 34349, İstanbul.

[4] Mustafaev M.I, Mustafaeva Z. 2002. Novel polypeptide-comprising biopolymer system. Technology and Healthcare 10:217-226.

[5] http://www.lerner.ccf.org/services/molecbiotech/peptide.php

[6] Mustafaev M., Mustafaeva Z., Ergen E., Uraki Y. and Sano Y. 2002. Novel Betulin-Containing Polyelectrolyte Conjugates Journal of Bioactive and Compatible Polymers 2:17; 251.

[7] Domingo, E., Escarmís, C., Martínez, M.A., Martínez-Salas, E. ve Mateu, M.G.1992. Foot-and Mouth Disease Virus Populations are Quasispecies. Curr. Topics Microbiol. Immunol. 176, 33-47.11.

[8] Sobrino F, Sa´iz M, Jime´nez-Clavero MA, Nu´n˜ez JI, Rosas MF, Baranowski E, Ley V. 2001. Foot-and- mouth disease virus: a long known virus, but a current threat. Vet Res 32:1–30.

[9] Bittle, J. L., Houghten, R. A., Alexander, H., Shın'nıck, T. M., Sutclıffe, J. G., Lerner, R. A., Rowlands, D. J. & Brown, F. 1982. Protection against foot-and-mouth disease by immunisation with a chemically synthesised peptide predicted from the viral nucleotide sequence. Nature, London 298, 30-33.

[10] Pfaff, E., Mussgay, M., Bohm, H. O., Shulz, G. E. & Schaller, H. 1982. Antibodies against a preselected peptide recognise and neutralise foot-and-mouth disease virus. *EMBO Journal* 1, 869-874.

[11] Strategies C. Olive, I. Toth and D. Jackson 2001. Technological Advances in Antigen Delivery and Synthetic PeptidenVaccine Developmental Mini Reviews in Medicinal Chemistry*, 1,* 429-438.

[12] Uelzmann, H. Copolymers of acrylic acid and N-vinylpyrrolidone-2. 1958 Journal of Polymer Science, 33 (126), 377-379.

[13] Dilgimen, A.S., Mustafaeva, Z., Demchenko, M., Kaneko, T., Osada, Y., Mustafaev, M., 2001, Water-soluble Covalent Conjugates of Bovine Serum Albumin with Anionic Poly(N-isopropyl-Acrylamide) and Their Immunogenicity, Biomaterials, 22 (17): 2383-2392.

[14] Yücel F., Çırakoğlu, B., 1999, Production of Monoclonal Antibodies Specific for Progesterone, Tr. J. of Biology 23 393–399.

[15] Başalp, A., Mustafaeva, Z., Mustafaev, M., 2002, Immunogenic Cu^{2+}-Induced Biopolymer Systems Comprising A Steroid Hormone, Protein Antigen, and Synthetic Polyelectrolytes, Hybridoma and Hybridomics, 21 (1): 45-51.

[16] Kabanov, V. A., 2004, From Synthetic Polyelectrolytes to Polymersubunit Vaccines. Pure Appl. Chem., 76 (9): 1659–1677.

[17] Posnett D.N., McGrath H., Tam J.P. 1988. A novel method for Producing Anti-peptide Antibodies. The Journalof Biological Chemistry. 263(4):1719-1725.