

## Modification of short peptides using $\epsilon$ -aminocaproic acid for improved coating efficiency in indirect enzyme-linked immunosorbent assays (ELISA)

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### Abstract

The hydrophobicity of short synthetic peptides of 5–10 residues was enhanced for high coating efficiency as antigens in indirect ELISA. To obtain enhanced hydrophobicity, coupling of  $\epsilon$ -aminocaproic acids to the synthetic peptides was carried out during solid phase peptide synthesis. As a short peptide model, three analogues of a streptavidin binding peptide consisting of 5 amino acid residues were prepared with four  $\epsilon$ -aminocaproic acid residues. HPLC analysis showed a dramatic increase in hydrophobicity after modification and the modified peptides showed a better adsorption ability than the unmodified peptides in indirect ELISA. The whole process from antigen coating to color development was carried out within 2.5 to 3 h by dissolving the peptide in methyl alcohol and evaporating the solvent in each well of the microplate. As an application of this method, a peptide assumed to function as one of the epitopes of the human 60 kDa Ro/SSA antigen was selected from hydrophilicity, acrophilicity and hydrophobicity plots. The peptide was synthesized having an  $\epsilon$ -aminocaproic acid modification at both N and C terminal ends and was tested with 30 sera from patients with systemic lupus erythematosus (SLE), 20 normal sera and a standard anti-Ro/SSA serum. The ELISA results revealed that the method gave a high signal-to-background ratio without altering the specificity of the assay. Moreover, our process was far simpler and more rapid than conventional methods used in indirect ELISA. Thus this method could be useful in the development of techniques for the diagnosis of SLE. © 1997 Elsevier Science B.V.

**Keywords:** ELISA;  $\epsilon$ -aminocaproic acid; SLE; Epitope; Peptide; Ro/SSA

Abbreviations: ELISA, enzyme-linked immunosorbent assay; SLE, systemic lupus erythematosus; RIA, radioimmunoassay; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, benzotriazolyl-oxymethyltetramethyl-uronium hexafluorophosphate; HOBT, hydroxybenzotriazole; TFA, trifluoroacetic acid; NMP, 1-methyl-2-pyrrolidinone; *p*NPP, *p*-nitrophenyl phosphate; DMF, dimethylformamide; DCM, dichloromethane; PBS, phosphate-buffered saline

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## 1. Introduction

A number of immunoassay techniques have been developed using either an antigen or an antibody bound to a solid support. Widely used immunoassay techniques such as enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) were designed to use microplates made of polystyrene or polypropylene from a variety of solid phase carriers. Proteins, either antigens or antibodies, are adsorbed to the plastic surface of the microplate as a result of interactions between hydrophobic regions of the protein and the nonpolar plastic surface. Even though only small amounts of protein can be adsorbed to each well of a microplate, the sensitivity of ELISA or RIA is such that only a small amount of antigen or antibody is needed. Each well of microplate has an actual binding capacity of 0.2–0.3  $\mu\text{g}$  protein (Winston et al., 1989).

However, there are some limitations in using short synthetic peptides of 5–10 residues as an antigen in ELISA procedures. First of all, peptides must, in general, be at least 15 residues long in order to be adsorbed on the plastic surface after overnight incubation in the microplate (Plaue and Briand, 1988). Moreover, very short peptides may be partially lost during normal ELISA washing stages leading to loss of precision. Some authors have recommended the pretreatment of polystyrene plates with direct coupling agents, such as glutaraldehyde (Corthier and Franz, 1981; Kasprzyk et al., 1988; Suter, 1982), cyanogen bromide (Lehtonen and Viljanen, 1980), poly-L-lysine or poly-L-aspartate (Brennand et al., 1986) to improve the attachment of peptide to the solid-phase. Modified microplates with chemical functional groups are now commercially available. Another problem of the short synthetic peptide is that the conformation of the peptide may be so altered by its interaction with the plastic as to diminish interactions with antibody. So, in spite of the numerous advantages of solid-phase assays, it should always be borne in mind that (a) the surface of any polypeptide immobilized on a solid phase will only be partially available for binding to the antibody, (b) adsorption of a polypeptide on a polymer surface will alter its conformation, (c) the kinetics and equilibrium characteristics observed in the liquid-phase

antigen–antibody interaction change when one of the reactants is immobilized (Stevens et al., 1986).

Using streptavidin binding peptide as a short peptide model, we have attempted to show that the problem of poor adsorption and conformational alteration of short peptides can be solved by modification with  $\epsilon$ -aminocaproic acids.  $\epsilon$ -Aminocaproic acid having an n-pentyl group as its side chain was chosen to increase the hydrophobicity of short peptides and function as an anchor for the peptide to be fixed on the surface of the microplate.

We designed a simple and rapid ELISA procedure to improve the coating of the modified peptides to the solid support and examined the immobilization of the short peptides modified in various ways. We also examined the sensitivity and the specificity of the assay compared to a conventional method. Finally, we evaluated the value of our ELISA procedure in the diagnosis of systemic lupus erythematosus (SLE).

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Chemicals

Fmoc-amino acids, HBTU, HOBT were purchased from AnaSpec (San Jose, CA). *N*- $\alpha$ -Fmoc- $\epsilon$ -aminocaproic acid was purchased from Nova Biochem (La Jolla, CA). *p*-Alkoxybenzyl alcohol resin, anti-human IgG conjugated with alkaline phosphatase and streptavidin conjugated with alkaline phosphatase were obtained from Sigma Chemical Co. (St. Louis, MO). Microplates (Ref. 3912) were purchased from Falcon Plastics (Oxnard, CA). TFA, NMP, piperidine and other solvents for peptide synthesis were purchased from Aldrich Chemical Co. (Milwaukee, WI). *p*-Nitrophenyl phosphate was purchased from Fluka Chemical Co. (Buchs, Switzerland). HPLC grade acetonitrile, methyl alcohol were purchased from Fisher Chemical Co. (Fair Lawn, NJ).

#### 2.1.2. Systemic lupus erythematosus (SLE) patient sera

Thirty sera from patients with SLE and twenty normal sera were obtained from the Clinic of

Rheumatism in Kang-nam St. Mary's Hospital (Seoul, Korea). All patients with SLE fulfilled the American Rheumatism Association classification criteria for the disease. For use as a positive control in the ELISA test, a reference human anti-Ro/SSA autoantibody was purchased from Immunovision (Springdale, AR).

## 2.2. Methods

### 2.2.1. Peptide preparation

Peptides were synthesized manually by standard methods on a solid support using Fmoc-chemistry (Gloor et al., 1994). *p*-Alkoxybenzyl alcohol resin (Wang resin, 60 mg) was used as a solid support. Coupling of the Fmoc amino acids including *N*- $\alpha$ -Fmoc aminocaproic acid was performed with 0.25 M HBTU/HOBT/NMP. For Fmoc deprotection, 30% piperidine in DMF was used. The coupling and deprotecting steps were then repeated as necessary until the desired sequence had been constructed. After synthesis, the resin was washed with DMF, DCM, methyl alcohol and dried under vacuum for at least 6 h. The cleavage of the peptides from the resin and side chain deprotection were carried out using 82.5% TFA, 5% thioanisole, 5% distilled water, 5% phenol and 2.5% 1,2-ethanedithiol. Each peptide was then isolated by several ether precipitations. After removal of the ether, each peptide was dissolved in distilled water and lyophilized. All crude peptide preparations were stored desiccated at 4°C until purification. Peptides that failed to produce a single homogeneous peak by analytical C-18 reverse phase column, 6.5  $\mu$ m (4.6  $\times$  250 mm, SynChrom, WI), were further purified with a preparative C-18 reverse phase column, 10  $\mu$ m (22  $\times$  250 mm, Vydac, CA). The programmed gradient elution was eluted at a flow rate of 0.7 ml/min for analysis and 8.0 ml/min for purification, and the gradient at 0 to 21 min was 10 to 45% solvent B, at 21 to 23 min was 45 to 100% solvent B, 27 to 29 min was 100 to 10% solvent B. Solvent A was 0.1% TFA in water, solvent B was 0.1% TFA in acetonitrile. About 10 mg of final peptide were obtained and purity was over 95%.

### 2.2.2. Modification of the streptavidin binding peptide

Among various streptavidin binding pentapeptides, GHPQG was chosen as the short peptide model and some modifications were performed. HPQ#1 had no modification at each terminal. HPQ#2 and HPQ#3 had four  $\epsilon$ -aminocaproic acid residues at amino and carboxyl terminals, respectively. HPQ#4 had two  $\epsilon$ -aminocaproic acid residues at both terminals. Because the four peptides had no lysine residue, peptide quantification was performed with ninhydrin tests (Sarin et al., 1981) and the peptide concentration for the indirect ELISA was adjusted to 30  $\mu$ M.

### 2.2.3. Selection of an epitope of 60 kDa Ro/SSA antigen

Amino acid sequences assumed to function as epitopes of the human 60 kDa Ro/SSA antigen were selected from the hydrophilicity, acrophilicity, flexibility plots (Hopp, 1985; Van Regenmortel, 1985). The plots were made by averaging the index values of each amino acid and the three flanking residues before and after it. The results from these plots were then compared with the ELISA results of sequential overlapping octapeptides from human 60 kDa Ro/SSA antigen with a reference human anti-Ro/SSA serum (Scofield and Harley, 1991). Among several candidates selected, one contained four amino acid residues (K<sup>133</sup>DLK<sup>136</sup>) which are part of a central antigenic octapeptide of the reference anti-Ro/SSA serum (Table 1). Two peptides for the selected residues 127–137, were prepared, one of which was designed to have two  $\epsilon$ -aminocaproic

Table 1  
Synthetic peptides assumed to function as epitopes of the human 60 kDa Ro/SSA antigen

Name	Amino acid positions	Sequence <sup>a</sup>
EP	127–137	TFIQFK <b>KDL</b> KEW
MEP	127–137	(Ahx) <sub>2</sub> TFIQFK <b>KDL</b> KEW(Ahx) <sub>2</sub>
Ro/SSA	133–136	<b>KDLK</b> <sup>b</sup>

<sup>a</sup>Amino acid residues are represented by the single-letter code except  $\epsilon$ -aminocaproic acid (Ahx) and in their order on the 60kDa Ro/SSA sequence.

<sup>b</sup>Amino acid residues in the Ro/SSA sequence which are part of a central antigenic octapeptide of the reference anti-Ro/SSA serum are indicated by bold letters (Scofield and Harley, 1991).

acid residues at each terminal. To facilitate peptide quantification, a tryptophan residue was inserted into each peptide (Grant, 1992). Peptide quantification was performed at 278 nm using a spectrophotometer (UVikon 930, Kontron Instruments, Switzerland).

The unmodified peptide (EP) and modified peptide (MEP) of the selected epitope have the peptide sequence of the 60 kDa Ro/SSA antigen, T<sup>127</sup>FIQFKKDLKE<sup>137</sup>W and (Ahx)<sub>2</sub>T<sup>127</sup>FIQFKKDLKE<sup>137</sup>W(Ahx)<sub>2</sub>, respectively.

#### 2.2.4. ELISA

The ELISA used to measure the binding of autoantibodies from human sera was performed as follows: for the modified peptide (MEP), each well of microplate was coated with 100  $\mu$ l of a 200  $\mu$ M peptide solution and the plate was gently shaken at 37°C. The solvent (methyl alcohol) was completely evaporated over 1 h. Thereafter the microplate was rinsed twice with the washing buffer (0.02 M PBS, 0.05% Tween 20, 0.01% NaN<sub>3</sub>). Thirty sera from SLE patients and twenty normal sera diluted 1/100 in 0.02 M PBS were added 100  $\mu$ l per well and the reference human anti-Ro/SSA antiserum was also added to the plate as a positive control. After 1 h incubation at 37°C, the plate was rinsed twice with the washing buffer. Anti-human IgG conjugated with alkaline phosphatase diluted 1/5000 in 0.02 M PBS was added to the microplate. After 30 min incubation at 37°C, the plate was washed four times with the washing buffer. 100  $\mu$ l of *p*-nitrophenyl phosphate solution (1 mg/ml) were added to each well of the microplate. The color development was quenched after 10 min with an equal volume of 3 M NaOH, and absorbance values were measured at 405 nm using a microplate reader (Emax, Molecular Devices, Germany). As a negative control, an ELISA procedure for an unmodified peptide (EP) was performed simultaneously. For the coating of EP, a 200  $\mu$ M peptide solution dissolved in 0.05 M carbonate buffer, pH 9.6, was added to the microplate, followed by overnight incubation at 4°C. Further steps of the assay were as described above. For the modified GHPQG, 30  $\mu$ M peptide solutions were used and 100  $\mu$ l of alkaline phosphate conjugate of streptavidin diluted at a ratio of 1/1000 were incubated at 37°C for 1 h.

### 3. Results and discussion

#### 3.1. Modification of ELISA methods

A more facile and rapid ELISA procedure was designed for the peptides modified with  $\epsilon$ -aminocaproic acids. Though simple adsorption of peptides to standard ELISA plates from carbonate buffer, pH 9.6, overnight at 4 or 37°C is a commonly used procedure (Barakat et al., 1990), a new peptide-coating method was used for the modified peptides. Among water, methyl alcohol, ethyl alcohol, acetonitrile and their mixtures, methyl alcohol had the optimum properties for the coating of modified peptides with  $\epsilon$ -aminocaproic acids, high solubility of peptides and fast evaporation at 37°C. In conventional ELISA procedures, blocking with BSA or non-fat dried milk has been regarded as an essential step in preventing nonspecific binding of antibodies. In this new procedure, however, on the basis of the duplicate and triplicate test results of the ELISA (data not shown), the blocking step only lowered the absorbance values without influencing the trends of the results, and there was little effect on the nonspecific binding of antibodies. Even in the case of short peptides of 5 residues, the blocking step seemed to hinder the appropriate exposure of the peptide needed for the interaction with antibodies.

When the new ELISA was performed as described above, the total assay time from antigen coating to color development was only about 2.5 to 3 h.

#### 3.2. The effect of $\epsilon$ -aminocaproic acid residues on the hydrophobicity of the peptide

It was suggested that  $\epsilon$ -aminocaproic acid residues inserted in short peptides could increase hydrophobicity because they have an *n*-pentyl group as a side chain. In order to prove that the short peptides coupled with  $\epsilon$ -aminocaproic acid residues have an enhanced coating efficiency for polystyrene microplates, we chose streptavidin binding peptides as a model system and coupled them with  $\epsilon$ -aminocaproic acids. Among the streptavidin binding peptides consisting of 5 residues, GHPQG was selected as a short peptide model (Lam et al., 1991). Four

peptides, a streptavidin binding peptide, GHPQG, and three peptides modified with  $\epsilon$ -aminocaproic acids, were analyzed with HPLC using a C-18 reverse phase column. As shown in Fig. 1., the elution times of GHPQG (HPQ#1) and the peptides modified with  $\epsilon$ -aminocaproic acid residues (HPQ#2, HPQ#3, HPQ#4) were about 5 and 13–15 min, respectively. As can be seen from the elution times, the three peptides with  $\epsilon$ -aminocaproic acid modifications have highly increased hydrophobicity compared with the unmodified peptide, HPQ#1. But, no significant difference in elution time was found between peptides having four  $\epsilon$ -aminocaproic acid residues at the N- or C-terminal end of GHPQG and a peptide having two  $\epsilon$ -aminocaproic acid residues at each terminal. From the hydrophobicity change results, one could assume that similar amounts of the three modified peptides (HPQ#2,3,4) would attach to the well of microplate. Nevertheless, the ELISA

results of the peptides modified with  $\epsilon$ -aminocaproic acid residues (HPQ#2, HPQ#3, HPQ#4) were very interesting. As shown in Fig. 2, the reactivity of the peptide with  $\epsilon$ -aminocaproic acid modification at the C-terminal end (HPQ#3) was even higher than the peptides with  $\epsilon$ -aminocaproic acid modification at both terminals (HPQ#4) or only at the N-terminal end (HPQ#2). Surprisingly, HPQ#2 had hardly any affinity for streptavidin conjugated with alkaline phosphatase. We conclude that these results reflect the screening condition of the streptavidin binding peptides with a pentapeptide library (Lam et al., 1991). The library was prepared using resins binding the C-terminal of each peptide and only the N-terminus of each peptide was freely exposed to streptavidin as the HPQ#3. Thus, among the modified peptides, HPQ#3 had a conformation most similar to the peptide used in the pentapeptide library. Though the N-terminus of HPQ#4 was fixed,

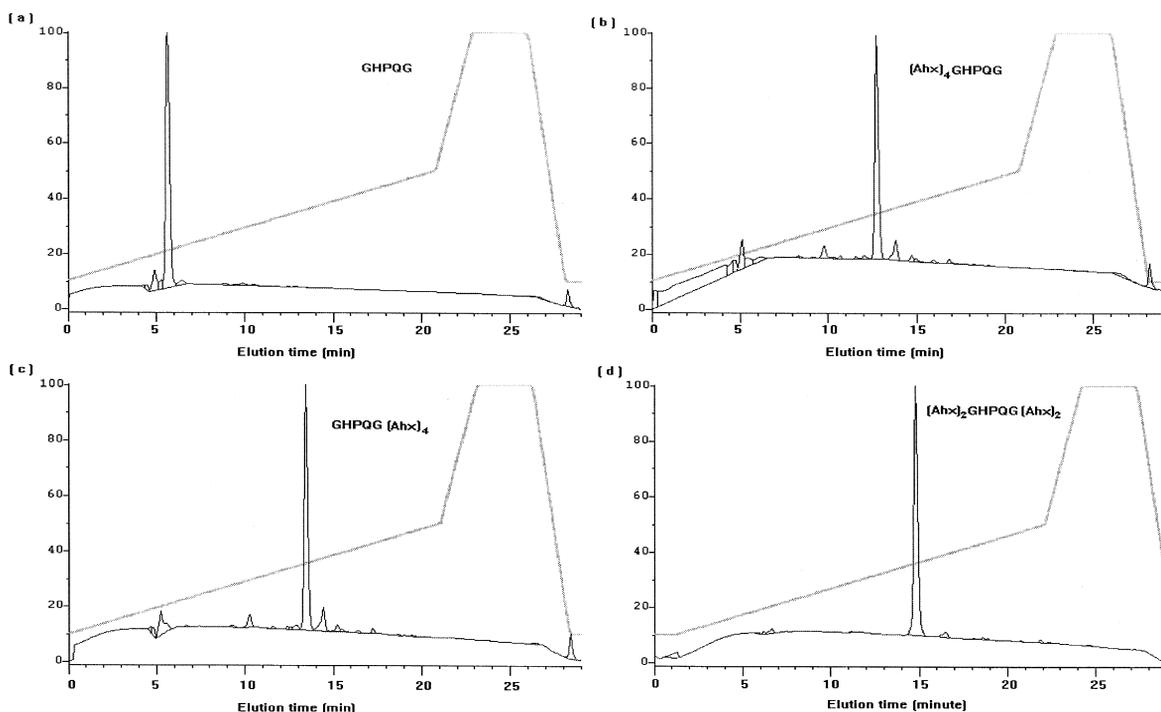


Fig. 1. HPLC analysis of the modified streptavidin binding peptides. 'Ahx' represents the  $\epsilon$ -aminocaproic acid residue. HPQ#1, HPQ#2, HPQ#3, HPQ#4 represent GHPQG, (Ahx)<sub>4</sub>-GHPQG, GHPQG-(Ahx)<sub>4</sub>, (Ahx)<sub>2</sub>-GHPQG-(Ahx)<sub>2</sub>, respectively. The HPLC profiles were obtained at 215 nm using a reverse phase C18 column as explained in the text.

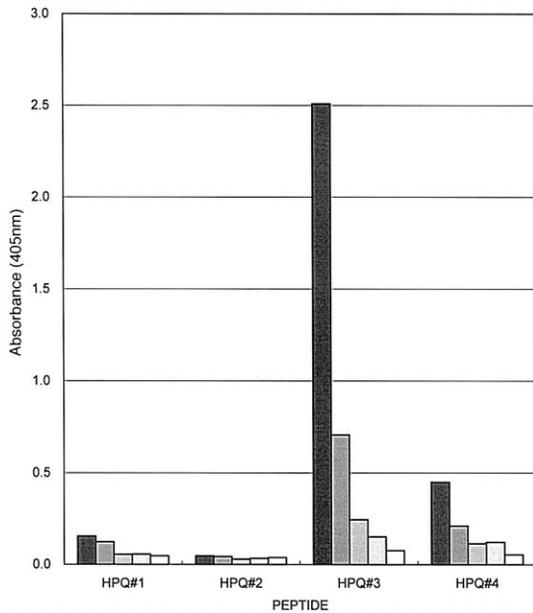


Fig. 2. Tests using the modified streptavidin binding peptides. HPQ#1, HPQ#2, HPQ#3, HPQ#4 represent GHPQG, (Ahx)<sub>4</sub>-GHPQG, GHPQG-(Ahx)<sub>4</sub>, (Ahx)<sub>2</sub>-GHPQG-(Ahx)<sub>2</sub>, respectively. Five 3-fold serial dilutions were carried out for each peptide as noted with the bar graphs. Original solutions for each peptide were prepared as 30 μM solutions.

HPQ#4 had a greater affinity for streptavidin than HPQ#2 following fixation of the C-terminal to the wall of microplate together with the N-terminal. Accordingly, the HPQ#2 of which only the N-terminal was fixed had a conformation which resembled HPQ#3 less than it resembled HPQ#4. On the basis of this, we conclude that the ε-aminocaproic acid residues inserted in short peptides not only contribute to the enhanced hydrophobicity of the modified peptides, but also act as a conformational anchor in adsorption to the solid phase. In addition, from these results, we were able to confirm that our method worked well even with the short peptides consisting of only 5 residues.

### 3.2.1. ELISA results using the peptides selected to function as epitopes against 60 kDa Ro/SSA antigen

We have described a simple and rapid assay using the short peptides modified with ε-aminocaproic acids. Short synthetic peptides, in conjunction with ELISA procedures, have become a major tool to define and characterize B-cell epitopes owing to their usefulness for epitope mapping. To examine the

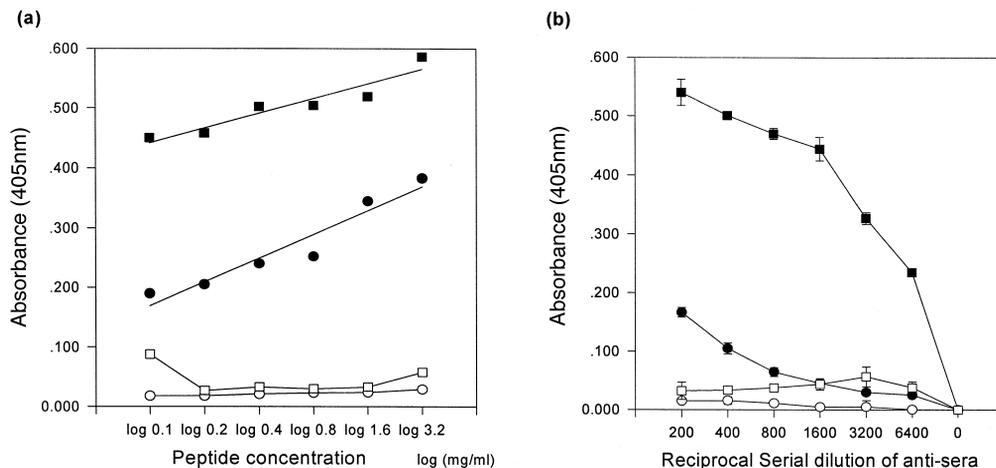


Fig. 3. (a) Test for peptide coating concentration for indirect ELISA. Microplates were coated with various concentrations of EP and MEP. EP and MEP represent the peptide sequence of 60 kDa Ro/SSA antigen, T<sup>127</sup>FIQFKKDLKE<sup>137</sup>W, (Ahx)<sub>2</sub>T<sup>127</sup>FIQFKKDLKE<sup>137</sup>W(Ahx)<sub>2</sub>, respectively. Optimal coating concentrations for EP and MEP were determined with the anti-sera diluted 1/100 in PBS. (b) Reciprocal serial dilutions of anti-sera. The peptides, both EP and MEP, were coated at a concentration of 0.2 mg/ml. The standard anti-Ro-SSA serum was tested for binding to its target peptides. A control normal human serum was also tested as a negative control. Six 2-fold serial dilutions of these anti-sera were evaluated for each peptide. In (a) and (b), ■, MEP was tested with the standard anti-Ro/SSA serum; ●, EP was tested with the standard anti-Ro/SSA serum; □, MEP was tested with a control normal serum; ○, EP was tested with a control normal serum.

value of our ELISA procedure, we applied the method to the diagnosis of systemic lupus erythematosus (SLE). In this study, we attempted to show that  $\epsilon$ -aminocaproic acids in peptides consisting of about 10 residues could function as a hydrophobic anchor without augmenting non-specific signals or altering the specificity of the assay.

On the grounds that anti-Ro/SSA antibodies of SLE patients react with multiple epitopes on 60 kDa Ro/SSA protein (Daniel et al., 1994), we prepared a peptide assumed to function as one of the epitopes of the human 60 kDa Ro/SSA antigen. In a study using synthetic peptides, the sequence of 21–41 was already known to be the 1st epitope site of 60 kDa Ro/SSA antigen (Barakat et al., 1992). Likewise, we selected another amino acid sequence assumed to function as an epitope of the 60 kDa Ro/SSA antigen on the basis of hydrophilicity, acrophilicity and flexibility plots (Hopp, 1985; Van Regenmortel, 1985). The results from these plots were then compared with the ELISA results of sequential overlapping octapeptides from human 60 kDa Ro/SSA antigen with a reference human anti-Ro/SSA serum (Scofield and Harley, 1991). Among several candidates, one representing residues 127–137, was so selected as to contain the sequence, KDLK, which is part of a central antigenic octapeptide of the reference anti-Ro/SSA serum as shown in Table 1. Though the streptavidin binding peptide was screened with its C-terminus fixed, epitopes of the 60 kDa

Ro/SSA antigen were assumed to have fixed N- and C-termini in the 60 kDa Ro/SSA antigen molecule. Accordingly, this peptide was prepared with  $\epsilon$ -aminocaproic acid modifications at both ends.

The ELISA result for this peptide (MEP) was compared with the result of the conventional ELISA method using the unmodified peptide (EP). For the coating of EP, 0.05 M carbonate buffer, pH 9.6, was added to the microplate, followed by overnight incubation at 4°C. First, an optimal peptide-coating concentration was determined in the range characterized by (a) linearity between the log scale of peptide concentration and the linear scale of absorbance and (b) low levels of negative background readings. As shown in Fig. 3(a), non-specific binding was minimized when both peptides were coated at a concentration of 0.2 mg/ml. Then, serial dilutions of a standard anti-Ro/SSA serum and a control normal serum were carried out with a peptide-coating concentration of 0.2 mg/ml. As shown in Fig. 3(b), both peptides appeared to work as epitopes for anti-human Ro/SSA antibody when the ELISA results of the positive control serum were compared with the results of a control normal serum. It was especially noticeable that the ELISA using MEP gave a much higher signal-to-background ratio than the conventional method used.

As an application of our method, these peptides were tested with 30 sera from SLE patients and 20 normal sera. In order to determine the threshold

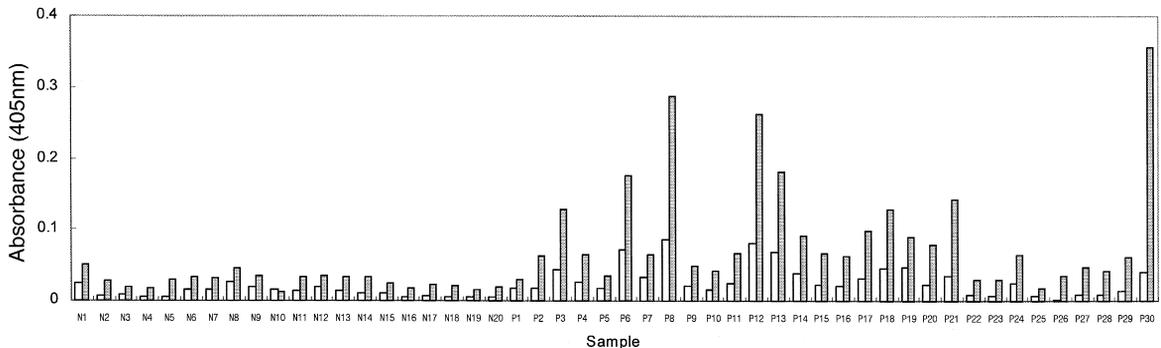


Fig. 4. ELISA using the unmodified and modified peptides assumed to function as epitopes of the 60 kDa Ro/SSA antigen. Sample sera from N1 to N20 represent the normal sera, and those from P1 to P30 represent the sera of SLE patients. Sera were considered positive when the absorbance values were higher than the threshold values. The threshold values for the diagnosis of SLE were 0.028 and 0.048 for EP and MEP, respectively. The results are averages of duplicate determinations with standard deviations being less than 5% for each bar. EP and MEP represent the peptide sequence of 60 kDa Ro/SSA antigen, T<sup>127</sup>FIQFKDLKE<sup>137</sup>W, (Ahx)<sub>2</sub>T<sup>127</sup>FIQFKDLKE<sup>137</sup>W(Ahx)<sub>2</sub>, respectively.

values for the diagnosis of SLE, a series of 20 sera from healthy individuals were tested for each peptide. Sera were considered positive when the absorbance values were higher than the average absorbance value plus two standard deviations of the 20 normal sera. The threshold values for EP and MEP were 0.028 and 0.048, respectively. Depending on the techniques used, anti-Ro/SSA antibodies are known to be found in 15–70% sera of patients with SLE (Slobbe et al., 1991). In this test, about 60% of sera from 30 patients with SLE were found to react with both EP and MEP. However, the signal difference between negative and positive sera was far more evident for MEP than for EP as can be seen from the bar graphs in Fig. 4. This efficient ELISA result could be achieved by the improved coating efficiency and conserved conformation of the modified peptide (MEP) as mentioned previously for the streptavidin binding peptides.

### 3.2.2. Application of new ELISA procedure

To improve the attachment of peptide to the solid-phase, some authors have recommended the pretreatment of polystyrene plates with glutaraldehyde (Corthier and Franz, 1981; Kasprzyk et al., 1988; Suter, 1982), cyanogen bromide (Lehtonen and Viljanen, 1980), poly-L-lysine or poly-L-aspartate (Brennand et al., 1986). Such methods have certain limitations in that (a) the conformation of the peptide may be so altered by its interaction with polystyrene plastic as to diminish the interaction with antibody when it is simply adsorbed to the plastic, (b) the covalent attachment of the peptide is restricted to certain specific orientations depending on the coupling agents used and (c) the introduction of chemical functional groups in polystyrene usually involves severe chemical treatment (Chin and Lanks, 1977; Rubin et al., 1980; Neurath and Stick, 1981). Furthermore, no method can be applied in every case and workers should be prepared to evaluate several methods for their particular application.

Here, we have described a simple and rapid assay using short peptides modified with  $\epsilon$ -aminocaproic acids. In comparison with the conventional methods described above, the main advantages of our method are (a) peptide-coating is very simple and rapid, (b) it gives a higher signal-to-background ratio without any significant increase of non-specific binding and

(c) it is applicable to a variety of synthetic peptides, especially, short peptides. Most of all, when compared with the conventional ELISA method, it is noticeable that the conformational problem of the short peptides can be solved by using an  $\epsilon$ -aminocaproic acid as a hydrophobic anchor. N-, C-, or both terminals, if necessary, can be anchored simply by coupling  $\epsilon$ -aminocaproic acids into the peptides. Moreover, the microplates coated with the modified peptide in this technique were stable for several weeks at 4°C.

If diagnostic kits are prepared adopting this technique, a reduction in both test time and cost can be achieved. Although diagnostic kits in which microplates are coated with the Ro/SSA antigen molecule are already commercially available, using our method would be a low cost alternative solution. In addition to SLE, our method should constitute an alternative to the common ELISA procedure in detecting antibodies of autoimmune sera.

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