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# Mimotope selection of blood group A antigen from a phage display 15-mer peptide library

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ABSTRACT

We select the peptide mimics of blood group A antigen by a monoclonal anti-A from a phage display 15-mer peptide library. Monoclonal anti-A was used in biopanning a phage display 15-mer peptide library. After four rounds of panning, ELISA was carried out to confirm the positive phage clones. The exogenous DNAs of the positive phages were sequenced and the corresponding amino acid sequences were deduced. Both the synthesized peptide and the phage clones were used to bind to anti-A in competitive ELISA. Erythrocyte agglutination inhibition tests were carried out to determine the mimic ability of the free synthesized peptide to the natural blood group A antigen. Computer softwares were used to simulate the interaction between the peptide and anti-A. After four rounds of biopanning, the eluted phage reached an enrichment of approximately 1600 times. Thirty-seven phage clones were chosen randomly and amplified. There were eleven clones that interacted specifically with anti-A in ELISA. DNA sequencing of the inserted oligonucleotide revealed that nine clones present a same peptide — TRWLVYFSRPYLVAT (named TRW) and each of the other two clones presented a different peptide. The synthesized free peptide TRW could inhibit the interaction of both phage displayed peptide and group A red blood cell with anti-A in competitive ELISA and hemagglutination inhibition test. Both the peptide TRW and the natural group A antigen were docked into a same cavity of anti-A in a computer simulation assay. The results indicate that peptide TRW can mimic blood group A antigen. It may be used as a proxy of natural blood group A antigen in clinical application.



## 1. Introduction

Carbohydrates and proteins are the most popular materials for antigen construction. Research on carbohydrate is more difficult than that on protein. An important reason is that a carbohydrate cannot be expressed directly by modern molecular biology technology. As a peptide can be expressed or synthesized easily, research on peptide mimics of carbohydrate antigens attracts more and more scientists [1]. Peptide is an important research field of life science. The organ or tissue specific peptides can be used to aid in constructing novel nanoparticles. Side effects can be decreased deeply when drugs are based on these kinds of nanoparticles [2–4]. Peptide based biosensors can increase the sensitivity and specificity [5–7].

Currently, scientists have achieved great success in ABO incompatible kidney transplantation. It is profit from using natural blood group A/B antigens to filter the anti-A/B antibodies before transplantation [8]. Natural blood group antigens are important to ensure success, but natural blood group A/B antigens are constructed by oligosaccharides which are difficult to synthesize and are very expensive. Actually, the shortage of natural blood group A/B antigen is a main barrier for researching on and extending ABO incompatibility organ transplantation [9]. To find substitutions for natural blood group A antigen, in our previous work, we have investigated the 12-mer peptide mimics of blood group A antigen [10]. In this study, we have identified 15-mer peptide mimics of group A antigen using the phage display technology platform. These peptides help us to enrich the database of proxy for blood group A antigen.

## 2. Objective

We hypothesized that some short peptides could mimic blood group A carbohydrate antigen and may be used as a substitute in clinical practice. The aim of this study is to get this kind of peptides from biopanning a phage display 15-mer random peptide library and a series of validation experiments.

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**Table 1**
Enrichment of the ratios of output to input for four rounds of biopanning.

| Cycle | Input (pfu) | Output (pfu) | Output/input |
|---|---|---|---|
| I | $1.0 \times 10^{12}$ | $6.0 \times 10^{2}$ | $6.0 \times 10^{-10}$ |
| II | $2.0 \times 10^{12}$ | $2.0 \times 10^{3}$ | $1.0 \times 10^{-7}$ |
| III | $1.6 \times 10^{12}$ | $6.0 \times 10^{5}$ | $3.8 \times 10^{-7}$ |
| IV | $1.2 \times 10^{12}$ | $1.2 \times 10^{6}$ | $1.0 \times 10^{-6}$ |

## 3. Materials and methods

### 3.1. Materials

Monoclonal anti-blood group A (Clone Number: 9A) was purchased from Abcam Inc. (Cambridge, USA). The horse radish peroxidase (HRP) labeled antibody against phage was obtained from Sigma Inc. The phage display 15-mer peptide library and the host strain *E. coli* K91 were a gift from Professor George P. Smith (Division of Biological Science, University of Missouri). The microcolumn gel cards were products of DiaMed AG (Cressier, Switzerland). Anti-A and Anti-B used in the hemagglutination tests were plasma from blood group B and blood group A volunteers of our laboratory. The other reagents were bought from Sangon Biotech (Shanghai, China). The peptide was synthesized by ShineGene Company (Shanghai, China).

### 3.2. Biopanning of the phage display 15-mer peptide library

The panning procedure was referred to Adda et al. [11] with some modifications. Anti-A (1 μg/well in 150 μL 0.1 M $NaHCO_3$, pH 8.6) was coated in one well of a microplate (Greiner bio-one, German). The well was incubated at 4 °C under moist condition. Blocking buffer (0.5 mg/ml BSA or 0.3% gelatin in 0.1 M $NaCO_3$ buffer) was used to block the well for 2 h at 4 °C. After washing six times with TBST, 10 μL phage library suspended in 100 μL TBST was added into the well and incubated for 1 h at 37 °C. Unbound phages were discarded and wells were washed 10 times by TBST (the concentration of Tween-20 was 0.1% in the first round and 0.5% in other rounds of panning). The bound phages were eluted by 0.1 M Glycine–HCl buffer (pH 2.2). Tris–HCl (pH 9.1) was used to neutralize the Glycine–HCl buffer. The procedures for phage amplification and tittering were the same as previously described [11]. Four rounds of panning were carried out. One uncoated well was used as negative control.

### 3.3. Phage ELISA

Thirty-seven phage clones were randomly chosen from the tittering plate after the fourth round of panning. The clones were amplified in 1 mL volume. The supernatants were retained for subsequent use. Anti-A (200 ng/mL in 0.1 M $NaHCO_3$) were coated in 96-wells plates at 4 °C overnight. The wells were blocked by BSA (0.5 mg/mL in 0.1 M $NaHCO_3$) for 2 h at 4 °C. Phages were added to the wells and incubated for 1 h at 37 °C. The primary phage library was used as negative control. The wells were washed six times with TBST (0.1% v/v Tween-20). The bound phages were detected by anti-phage-HRP antibody and enzyme substrate (TMB and $H_2O_2$). Each experiment was repeated three times.

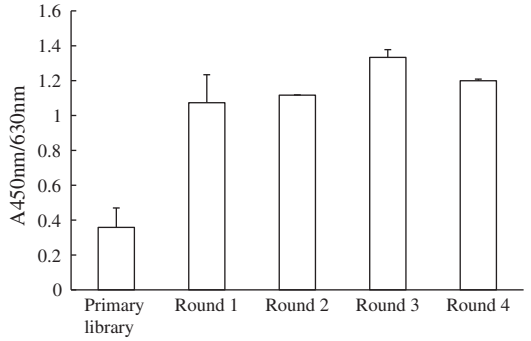


**Fig. 1.** ELISA result showing the phage's affinity for anti-A. The primary library given on the x-axis means the unpanninged phage display 15-mer peptide library.

### 3.4. DNA sequencing

Single strand DNAs of phages were purified by NaI method. The exogenous DNAs were sequenced in Sangon Biotech (Shanghai, China). The primer sequence was as follows: TGAATTTTCTGTATGAGG.

### 3.5. Competitive ELISA

Competitive ELISA was performed as ELISA described above except that $10^{10}$ pfu phage particles were added to the anti-A coated wells with the presence of decreasing amounts of synthetic peptide. Each experiment was repeated again.

### 3.6. Hemagglutination inhibition test

Anti-A and the synthetic peptide were incubated for 30 min at 37 °C. Twenty microliters of the mixture and ten microliters of washed group A erythrocytes (2%) were added to the microcolumn gel cards. After incubation for 30 min at 37 °C, the gel cards were centrifuged at 85 *g* for 10 min and then the results were recorded by camera. The hemagglutination inhibition tests with anti-B, group B erythrocytes and the synthetic peptide were performed as specific control.

### 3.7. Molecular docking between the peptide and anti-A

The web server, 3D-JIGSAW (http://bmm.cancerresearchuk.org/~3djigsaw/), was used to construct the 3D structure of the peptide. A 3D structure data of anti-A was retrieved from PDB database (No. 1JV5). Autodock software [12] was used to simulate the interaction between the peptide and anti-A. Lamarckian genetic algorithm, semi-flexible docking and semi-empirical free energy algorithm were used to estimate the minimum total energy of the complex structure. The natural blood group A antigen drawn from a complex structure (PDB No. 2vng) was also docked into anti-A.

## 4. Results

### 4.1. Biopanning and the specific phages enrichment

Four rounds of biopanning were carried out, anti-A monoclonal antibody was used as target molecule to biopanning the phage display 15-mer peptide library. BSA and gelatin were used alternately as blocking reagent. This operation may reduce the enrichment of nonspecific phage clones. The output phages of every round of elution were tittered. The ratios of output to input were calculated. As the biopanning rounds continue, the ratios increased gradually. The enrichment achieved about 1600 times (Table 1). There was no obvious increase of the output/input ratio in the negative control. The ELISA result also showed that the amplified elutes' affinity for anti-A were greater than that of the primary library phages (Fig. 1).

### 4.2. Phage clones' affinity for anti-A in ELISA experiment

Thirty-seven phage clones were randomly chosen from the tittering plate after four rounds of biopanning. Supernatant of the amplified phage clones were used to determine the affinity for anti-A by ELISA method. The primary library was diluted 100-fold and used as negative control. Those clones having absorbance values over three times higher than that of the negative control were considered as positive clones. There were eleven phage clones that satisfied this condition and the positive rate was 27.5% (Fig. 2).

### 4.3. DNA sequencing and the derivation of corresponding peptides

Eleven positive phage clones' exogenous DNAs were sequenced and the corresponding peptides were deduced. Among these eleven phage clones, nine clones

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