J Huazhong Univ Sci Technol [Med Sci]
DOI 10.1007/s11596-008-0228-0 28 (2): 222-226, 2008

Identification and Characterization of Peptide Mimics of Blood Group A Antigen

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Summary: In order to investigate peptide mimics of carbohydrate blood group A antigen, a phage display 12-mer peptide library was screened with a monoclonal antibody against blood group A antigen, NaM87-1F6. The antibody-binding properties of the selected phage peptides were evaluated by phage ELISA and phage capture assay. The peptides were co-expressed as glutathione S-transferase (GST) fusion proteins. RBC agglutination inhibition assay was performed to assess the natural blood group A antigen-mimicking ability of the fusion proteins. The results showed that seven phage clones selected bound to NaM87-1F6 specifically, among which, 6 clones bore the same peptide sequence, EYWYCGMNRTGC and another harbored a different one QIWYERTLPFTF. The two peptides were successfully expressed at the N terminal of GST protein. Both of the fusion proteins inhibited the RBC agglutination mediated by anti-A serum in a concentration-dependent manner. These results suggested that the fusion proteins based on the selected peptides could mimic the blood group A antigen and might be used as anti-A antibody-adsorbing materials when immunoabsorption was applied in ABO incompatible transplantation.
Key words: amino acid sequence; blood group A antigen; hemagglutination test; molecular mimicry; peptide library

To overcome the ABO-incompatible rejection, removal of the anti-A/B natural antibody is very important. Rituximab, an anti-CD20 antibody, was used before operation to inhibit the appearance of antibody against B cells[1]. As to the existent anti-A/B antibodies, plasmapheresis is an efficient way to reduce it, but other beneficial antibodies could also be diminished at the same time. Recently, specific immunoabsorption was applied instead of non-specific plasmapjeresis[2-5]. This method can remove the circulating A/B antigen-specific antibodies and let the other antibodies perform their natural functions. Synthetic carbohydrate A/B antigens were used to specifically absorb the corresponding antibodies in these studies. However, the large-scale syntheses of carbohydrate antigens are difficult and expensive. Attachment of these carbohydrate antigens to various matrices is also complex. A possible alternative to the use of carbohydrate would be the development of protein or peptide mimics that could bind to the specific antibodies[6,7]. In this study, we attempted to select peptide mimics of the blood group A antigen from a phage display random peptide library. These selected peptides may have potential to be used as anti-A antibody adsorbing materials when immunoabsorption was applied in ABO incompatible transplantation.

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1 MATERIALS AND METHODS

1.1 Biopanning

NaM87-1F6 (BD Co., USA), an anti-A monoclonal antibody (mAb), was coated on the immunosorbent plates (Greiner Bio One, Shanghai, China) and used as a target to screen the Ph.D.-12 phage library (New England Biolabs, USA) according to the instruction. The panning processes were carried out three times. The stringency of selection was gradually increased each round by decreasing the coated antibody from 10 μg to 7.5 μg and then to 5 μg, raising the concentration of Tween-20 in washing buffer from 0.1% to 0.5%, shortening the incubation time from 1 h to 30 min. The blockade buffer was converted from BSA to gelatin and then to BSA in the three rounds of panning. Phage titering was carried out according to the instruction manual.

1.2 Assay of the Positive Phage Clones

Phage ELISA was performed. Briefly, each phage clone or eluted phage pool (1×10^{10} TU/well) in TBS was coated on wells of a 96-well immunosorbent plate and blocked with 5% BSA in TBS. The anti-A antibody was added to each coated well (100 ng/well), and the plates were incubated for 2 h at room temperature. Unbound antibody was removed by washing with TBST (0.5% [v/v] Tween-20), and bound antibody was detected with biotin-labeled anti-mouse IgG antibody, avidin-labeled horseradish peroxidase and tetramethylbenzidine (TMB) substrate (Zhongshan Golden Bridge, Beijing, China). The absorbance (*A* value) at 450 mn was measured by a microplate reader. The wells coated with

the phage from unselected phage library were used as control. The assay was performed in triplicate.

Phage capture assay was carried out as described previously[8], except that each microplate well was coated with 10 ng of anti-A antibody. Individual input phage (1×10^4 TU/well) was used to bind anti-A antibody and TBST containing 0.5% (v/v) Tween-20 was used as washing buffer. The eluted phage was titered. The BSA-coated wells were used as control.

1.3 Sequencing of Phage DNA

The single strand DNA of selected phage clones was extracted according to the phage library kit manual. DNA sequences encoding the displayed peptides of selected phage clones were determined. The primer 5'-CCCTCATAGTTAGCGTAACG-3' was used to sequence the DNA sequences encoding the displayed peptides.

1.4 Phage ELISA in the Presence of Peptide-GST Fusion Protein

The peptide-GST fusion proteins containing the selected peptides were constructed as described previously[9]. The GST cDNA was amplified by PCR with the vector, pGEX-5X-1 (GE Healthcare, USA), as a template and inserted into vector pET28b (Novagen, USA). The resulting plasmid pET28b-GST was used to express the GST fusion protein attached with a hexahistidine tag at the C terminus.

About 1×10^{10} TU particles of each phage clone were coated on the microplate wells. BSA (5%) in TBS was used to block the wells. The anti-A antibody was pre-incubated with increasing concentrations of peptide-GST fusion protein for 1 h at 37°C, and then added to the microplate wells. In the control, GST protein instead of peptide-GST fusion protein was used. The plate was developed as the "phage ELISA" method described above.

1.5 RBC Agglutination Inhibition Assay

Human blood for *in vitro* studies was obtained from healthy donors from Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The fresh human group A RBCs were washed three times and suspended in PBS at a concentration of 1%. An agglutination reaction system including 4 μL of heat-inactivated anti-A sera, 20 μL 1% RBCs, and dilutions of each peptide-GST fusion protein with PBS was added to a 40 μL volume. The reaction lasted for 1 h at 37°C. Agglutination was assessed by microscopic examination. The Eppendorf tubes were flicked several times gently, and then about 10 μL of the mixture was pipetted onto a glass slide and examined at ×200. In the GST control groups, GST protein instead of peptide-GST fusion protein was use while in the other control system, the group B RBCs instead of that of group A RBCs and human anti-B sera instead of human anti-A sera were used.

2 RESULTS

2.1 Selection of Positive Phage Clones

The ELISA showed anti-A antibody-binding property of the elution from the three rounds of panning increased gradually (fig.1). Thirty-two phage clones were randomly chosen from the elution of the third round panning. The phage clones were amplified and immobilized to determine the anti-A antibody-binding ability by phage ELISA. Most of the chosen phage clones could bind to anti-A antibody well (fig.2). To further confirm the specific binding between the phage clones and the anti-A antibody, phage capture assay was carried out. Among the 11 phage clones having strong signal in ELISA, seven phage clones (C5, C17, C22, C23, C26, C27, and C31) bound to anti-A antibody and the other four clones did not (data not shown).

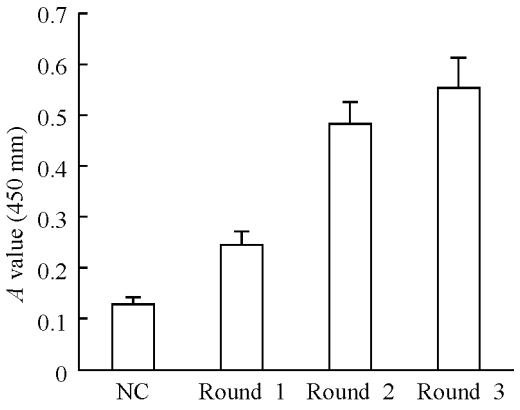

Fig. 1 Binding of anti-A antibody to the eluted phage pools

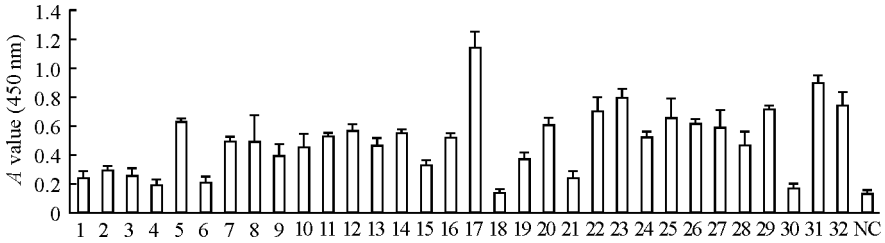

Fig. 2 Binding of anti-A antibody to each selected phage clone

2.2 The Peptides Displayed on The Positive Phage Clones

The ssDNA of the seven phage clones was extracted and the DNA encoding the peptides was sequenced. Among the 7 phage clones, 6 displayed the same amino acid sequence (EYWYCGMNRTGC), and

the other phage clone displayed a different one (QIWYERTLPFTF).

2.3 Mimicking Ability of Peptide-GST Fusion Proteins

In order to facilitate the construction of the natural conformation, the selected peptides were expressed at the N terminus of the GST protein. The continual amino acids, GGGS, were introduced into the site between the expressed peptide and GST protein as a linker.

The competitive phage ELISA was carried out to test the ability of the peptide-GST fusion proteins to mimic the peptides displayed on the phages. When anti-A antibody was allowed to bind to the C5 phage-coated wells in the presence of increasing concentrations of each peptide-GST fusion protein, a dose-dependent decrease in binding was observed (fig. 3A). The two fusion proteins could also inhibit the binding between anti-A antibody and C17 phage (fig. 3B). The GST control protein could not inhibit the binding.

2.4 Inhibition of Agglutination with Peptide-GST Fusion Proteins

To further assess the natural blood group A antigen-mimicking ability of the fusion proteins, RBC agglutination assay was performed. Both of the two fusion proteins could inhibit the agglutination of group A RBCs induced by serum from group B donor (fig. 4). The inhibition was not observed when serum from group A donor and blood group B RBCs were used to perform agglutination assay (Data not shown). In another control, GST protein could not inhibit the agglutinations of group A RBCs either.

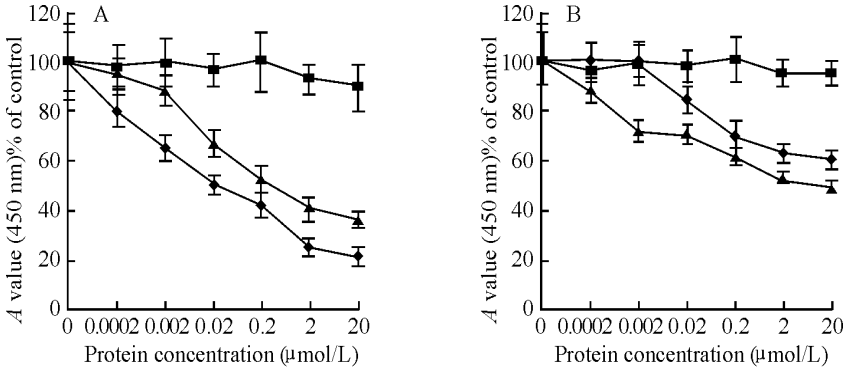

Fig. 3 Inhibition of phage binding by peptide-GST fusion protein

Anti-A antibody was incubated in phage-coated wells in the presence of different concentration of either EYWYCGMNRTGC-GST fusion protein (♦) or QIWYERTLPFTF-GST fusion protein (▲). GST protein (■) was used as negative control. The assay was performed in triplicate. Percent inhibition was calculated as the decrease in the absorbance (*A*) at 450 nm compared with controls (no fusion protein). A: Wells coated with C5 phage clone; B: Wells coated with C17 phage clone

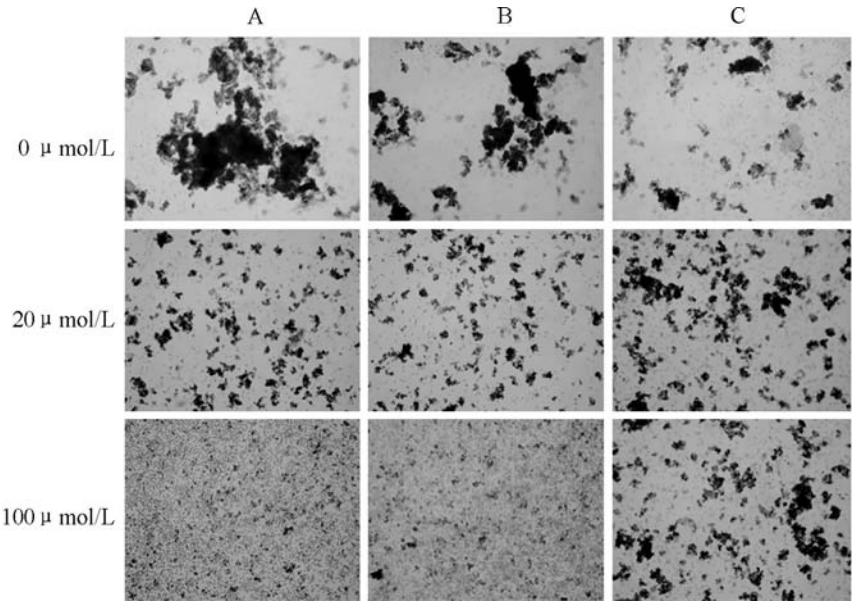

Fig. 4 Inhibition of agglutination by peptide-GST fusion protein

Sera from group B donors were incubated with group A RBCs in the presence of indicated concentration of EYWYCGMNRTGC-GST fusion protein (A), QIWYERTLPFTF-GST fusion protein (B) and GST protein (as control, C). Results were representative of three independent experiments

3 DISCUSSION

In this study, we described the identification of two peptides that mimic the blood group A antigenic epitope using a phage random dodecapeptide library. The similar library has been used to identify the peptides mimicking peptide or non-peptide ligands[10-13]. As carbohydrate antigens are complex and not convenient to manipulate, many studies focused on the peptide mimics instead of carbohydrate antigens[14-16].

The selected peptides in this study showed several antigenic characteristics of carbohydrates. In the selected EYWYCGMNRTGC peptide, CG and GC presented at the middle and the C terminal respectively. As double cysteines may form a bridge[17] and the little residue, glycine, can reduce the stereospecific blockade, the combination of CG and GC may make positive contributions to constraining a motif mimicking the conformational blood group A antigenic epitope. Several studies on peptide mimotope of carbohydrate antigen suggested aromatic amino acids were critical in modulating binding [8,18,19]. In our selected peptides, the aromatic amino acids (YWY) were also intensively presented beside the middle cysteine in EYWYCGMNRTGC peptide. As to QIWYERTLPFTF peptide, the three continual amino acids, FTF, may also facilitate the binding between the peptide and the anti-A antibody, possibly because of hydrogen bonding with the hydroxyl group of threonine residues (which mimic sugar hydroxyl) and hydrophobic interaction with carbons on the aromatic side chain of phenylalanine residues (which mimic sugar carbons)[8]. The proline may be also important for the formation of the antigenic mimic, since its cyclically bonded structure fixes its conformational degree of freedom.

As expected, the peptide-GST proteins could inhibit the binding between the corresponding phage clones and the antibody. The peptide-GST proteins could also inhibit their mutual binding and the GST control can not do this, indicating that the two peptides mimic the same (or overlapped) epitope (fig. 3). Both of the two peptide-GST proteins blocked the agglutination in a concentration-dependent manner. Compared with millimole concentratins of peptide mimics needed to inhibit the RBC agglutination in a similar study [20], only micromole concentrations of fusion proteins in this study could inhibit the agglutination. It is probably that the short peptides are too unstable to reveal natural mimic function, while peptide-GST fusion protein can overcome this to some extent. As blood group A antigen is similar to B antigen, the agglutination of group B RBCs induced by anti-B antibody was performed as control to demonstrate that the peptide-GST proteins were specifically binding to the anti-A antibody. Both of the peptide-GST proteins did not inhibit the agglutination. These data suggested that the two peptide-GST proteins could mimic the blood group A antigen and bind to the anti-A antibody specifically.

In conclusion, the fusion proteins based on the two peptides identified in this study can mimic the epitope of blood group A antigen. The efforts to determine the anti-A antibody-absorbing activity of these peptide fusion protein attached to a matrix are in progress. The peptides and the peptide-GST fusion form may serve as a clue to develop additional materials used in blocking or removing anti-A antibody during ABO-incompatible transplantation.

Acknowledgement

We thank Dr. Yuan TIAN for his technical assistance.

REFERENCES

1 Sawada T, Fuchinoue S, Teraoka S. Successful A1-to-O ABO-incompatible kidney transplantation after a preconditioning regimen consisting of anti-CD20 monoclonal antibody infusions, splenectomy, and double-filtration plasmapheresis. Transplantation, 2002,74:1207-1210

2 Norden G, Briggs D, Cockwell P *et al*. ABO-incompatible live donor renal transplantation using blood group A/B carbohydrate antigen immunoadsorption and anti-CD20 antibody treatment. Xenotransplantation, 2006,13:148-153

3 Kumlien G, Ullström L, Losvall A *et al*. Clinical experience with a new apheresis filter that specifically depletes ABO blood group antibodies. Transfusion, 2006,46: 1568-1575

4 Tyden G, Kumlien G, Genberg H *et al*. ABO incompatible kidney transplantations without splenectomy, using antigen-specific immunoadsorption and rituximab. Am J Transplant, 2005,5:145-148

5 Tydén G, Donauer J, Wadström J *et al*. Implementation of a protocol for ABO-incompatible kidney transplantation--a three-center experience with 60 consecutive transplantations. Transplantation, 2007,83:1153-1155

6 Barenholz A, Hovav A H, Fishman Y *et al*. A peptide mimetic of the mycobacterial mannosylated lipoarabinomannan: characterization and potential applications. J Med Microbiol, 2007,56:579-586

7 Tang S S, Tan W S, Devi S *et al*. Mimotopes of the Vi antigen of salmonella enterica serovar Typhi identified from phage display peptide library. Clin Diagn Lab Immunol, 2003,10:1078-1084

8 Scott J K, Loganathan D, Easley R B *et al*. A family of concanavalin A-binding peptides from a hexapeptide epitope library. Proc Natl Acad Sci USA, 1992,89: 5398-5402

9 Sugimura Y, Hosono M, Wada F *et al*. Screening for the preferred substrate sequence of transglutaminase using a phage-displayed peptide library: identification of peptide substrates for tgase 2 and factor xiiia. J Biol Chem, 2006, 281:17 699-17 706

10 Sharma A, Saha A, Bhattacharjee S *et al*. Specific and randomly derived immunoactive peptide mimotopes of mycobacterial antigens. Clin Vaccine Immunol, 2006,13: 1143-1154

11 Carter D M, Gagnon J N, Damlaj M *et al*. Phage display reveals multiple contact sites between FhuA, an outer membrane receptor of Escherichia coli, and TonB. J Mol Biol, 2006,357:236-251

12 Hou Y, Gu X X. Development of peptide mimotopes of lipooligosaccharide from nontypeable Haemophilus influenzae as vaccine candidates. J Immunol, 2003,170: 4373-4379

13 Li M, Yan Z, Han W *et al*. Mimotope vaccination for epitope-specific induction of anti-CD20 antibodies. Cell Immunol, 2006,239:136-143

14 Clément M J, Fortuné A, Phalipon A *et al*. Toward a better understanding of the basis of the molecular mimicry of polysaccharide antigens by peptides: the example of shig-

ella flexneri 5a. J Biol Chem, 2006,281:2317-2332
15 Gevorkian G, Segura E, Acero G *et al*. Peptide mimotopes of Mycobacterium tuberculosis carbohydrate immunodeterminants. Biochem J, 2005,387:411-417
16 Beninati C, Arseni S, Mancuso G *et al*. Protective immunization against group B meningococci using anti-idiotypic mimics of the capsular polysaccharide. J Immunol, 2004,172:2461-2468
17 Zhong G, Smith G P, Berry J *et al*. Conformational mimicry of a chlamydial neutralization epitope on filamentous phage. J Biol Chem, 1994,269:24183-24188
18 Oldenburg K R, Loganathan D, Goldstein I J *et al*. Peptide ligands for a sugar-binding protein isolated from a random peptide library. Proc Natl Acad Sci USA, 1992,89: 5393-5397
19 Westerink M A, Giardina P C, Apicella M A *et al*. Peptide mimicry of the meningococcal group C capsular polysaccharide. Proc Natl Acad Sci USA, 1995, 92:4021-4025
20 Lang J, Zhan J, Xu L *et al*. Identification of peptide mimetics of xenoreactive alpha-Gal antigenic epitope by phage display. Biochem Biophys Res Commun, 2006, 344:214-220

(Received Nov. 4, 2007)