Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 344 (2006) 214–220
BBRC
www.elsevier.com/locate/ybbrc

Identification of peptide mimetics of xenoreactive α-Gal antigenic epitope by phage display

Jianshe Lang [a], Jinbiao Zhan [a,*], Linhai Xu [b], Zhikun Yan [b]

[a] Department of Biochemistry, Zhejiang University Medical School, Hangzhou 310006, China
[b] Department of Surgery, People's Hospital of Zhejiang Province, Hangzhou 310014, China

Received 9 March 2006
Available online 29 March 2006

Abstract

The prevention of hyperacute rejection (HAR) triggered by interaction between the human natural antibody and xenoreactive antigenic epitope (Gal-α1, 3Gal) present on pig cells is the key to success in pig-to-human xenotransplantation. The phage display technology offers an effective strategy for screening peptides which can interact with the anti-Gal antibody to block α-Gal antigen binding site. Two peptide libraries, linear 7 peptide library and C7C library, were panned on the anti-B monoclonal antibody which has the characteristic of binding to the α-Gal antigenic epitope. After four rounds of panning, 22 positive phage clones were selected. Highly homologous sequence PT and STL existed among these selected peptides. Stachyose competitive ELISAs revealed that these peptides specifically bound to α-Gal antigen binding site. Eight peptide mimics of α-Gal antigenic epitope could inhibit the agglutination of pig red blood cells mediated by human sera in a dose-dependent manner. These results demonstrated that the selected peptides can mimic the conformational structure of α-Gal antigenic epitope and have the therapeutic potential in xenotransplantation.

Keywords: Peptide mimics; Xenoreactive antigen; Xenotransplantation; Phage display

Transplantation has been a very useful strategy in the treatment of many diseases. However, the dilemma of medical service today is that many patients in need of heart, kidney, liver or other organs fail to receive the needed allograft, because of the shortage of human organs. To find an alternative source of organ from other mammals, scientists from all over the world made great efforts in this field. Because of similarities in physiology between human and pig organs, pigs have been selected as ideal source of organ supply. Subsequent studies have revealed that hyperacute rejection (HAR) induced by the interaction of human natural anti-Gal antibody with Gal-α1, 3-Gal antigenic epitope on the pig cells was a major immunological barrier in xenotransplantation [1,2]. The antibody binds to the carbohydrate structure Gal-α1-3Gal on glycolipids and glycoproteins abundantly expressed in the membrane of pig cells [3–5]. This obstacle can be overcome by blocking or removal of the natural antibody by affinitive ligands.

Phage display technology is a very powerful tool for epitope mapping [6], identification of critical amino acids responsible for protein–protein interaction [7], and discovery of new therapeutics [8,9]. A significant aspect of phage display lies in linking the phenotype of bacteriophage displayed peptide or protein with the genotype encoding that molecule, packaged with the same viron. This permits rapid selection and amplification of specific clones of phage representing desired binding sequences from the pools of billions of phage clones.

In our experiment, affinitive selection was carried out using the anti-B monoclonal antibody which has the characteristic of binding to the carbohydrate structure of α-Gal. Using two peptide libraries, linear 7 and C7C peptide libraries, we attempted to select peptides which can specifically bind to the α-Gal epitope binding site and may have

* Corresponding author. Fax: +86 571 87217044.
E-mail address: jzhan2k@cmm.zju.edu.cn (J. Zhan).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.03.112

the potential to prevent the hyperacute rejection in the pig-to-human xenotransplantation.

Materials and methods

Materials. The C7C peptide and linear 7 peptide libraries were purchased from New England Biolabs, USA. Stachyose was kindly provided by Keyi Wang (Shanghai Institute of Biochemistry, Chinese Academy of Sciences). Human sera for in vitro studies were obtained from Tongde Hospital of Zhejiang province, China. The anti-B monoclonal antibody was a product of the Changchun Brother Biotech Inc., China. HRP/anti-M13 monoclonal conjugate was purchased from Amersham Pharmacia Biotech Inc.

Biopanning process of two libraries. The two peptide libraries, linear 7 peptide library and C7C peptide library, were cycled through four rounds of affinity selection. In the first round, the immunosorbent plates were coated with 100 μl of Anti-B antibody (titer: 1:128) in sodium carbonate buffer (0.1 M $NaHCO_3$, pH 8.6) at 4 °C overnight, followed by blocking for 2 h at 37 °C with 300 μl of 5% bovine serum albumin (BSA) in Tris-buffered saline (TBS).

The phage libraries (approximately 10^{12} transducing units) were added to the coated wells. After incubation for 1 h at room temperature, the plates were washed 10 times with TBST (Tris-buffered saline containing 0.1% Tween 20). The phages bound to the antibody were eluted with 100 μl of 0.1 M glycine–HCl buffer (pH 2.2) and then were neutralized by 15 μl of 1 M Tris–HCl buffer (pH 9.1). The eluted phage was amplified by propagation in *Escherichia coli* ER2738 for 4.5 h and harvested by precipitation with PEG/NaCl. The selection procedure for rounds two, three, and four was identical to that for round one.

Detection of the positive phage clones by ELISA. The 96-well immunosorbent plates were coated with 100 μl of Anti-B antibody at 4 °C overnight and blocked with 300 μl of 5% BSA for 2 h at 37 °C. Approximate 10^{12} phage particles of the selected clones were added to each well and incubated for 1 h with shaking at room temperature. The plates were washed six times with TBST, then incubated with 100 μl of horseradish peroxidase anti-M13 antibody conjugate diluted 1:5000 in TBS for 1 h and washed six times with TBST. The plates were developed with the 100 μl of substrate tetramethylbenzidine (TMB), terminated by 50 μl of 2.0 M H_2SO_4, and read at 450 nm.

The phage DNA sequencing. The positive phage clones selected by ELISA were sequenced to determine the amino acid sequence of peptides. The single strand phage DNA was extracted as the protocol described. The primer 5′-CCCTCATAGTTAGCGTAACG-3′ was used for DNA sequencing.

Affinity measurement by stachyose competitive ELISA. The competitive ELISAs were carried out on the 96-well immunosorbent plates coated with Anti-B antibody. Fifty microliters of positive phage clones (approximately 10^{12} transducing units) and 50 μl of serially diluted stachyose solution were added into each well and incubated at room temperature for 1 h. Then the plates were washed and developed as the method described above. The inhibition rate was calculated by the formula:

$$\text{Inhibition rate} = [A_{450}(-\text{s}) - A_{450}(+\text{s})]/A_{450}(-\text{s}),$$

where $A_{450}(-\text{s})$ represents A_{450} without stachyose and $A_{450}(+\text{s})$ is A_{450} with stachyose.

DTT reduction assay. The 96-well immunosorbent plates were coated with 100 μl of Anti-B antibody at 4°C overnight and blocked with 300 μl of 5% BSA for 2 h at 37 °C. Approximately 10^{12} phage particles of each positive clone were incubated in TBS with 2 mM DTT for 30 min. Negative controls were phage supernatant without DTT. Each clone was added to plates and incubated for 1 h at room temperature. The plates were washed six times with TBST, incubated with 100 μl of horseradish peroxidase anti-M13 antibody conjugate diluted 1:5000 in TBS for 1 h, then washed and developed as above. The absorbance was read at 450 nm.

Peptide synthesis. Peptides were synthesized using standard solid phase method. Purity and mass of each peptide were verified by liquid chromatography mass spectrometry (HPLC/MS).

Inhibition assay of pig RBC agglutination. Freshly collected pig RBCs (3%) were washed and resuspended in PBS. RBC agglutination assay was performed in U-shaped agglutination plates [10]. Phage solutions were serially diluted in 40 μl PBS. Each phage dilution was mixed with 40 μl of 1% RBC which was washed three times and resuspended in PBS and 40 μl of 1% human serum diluted in PBS. The mixtures were shaken gently for 2 min and incubated for 1 h. The agglutination of RBC was visible when RBC homologically distributed on the bottom of the wells to form blood dumps. In contrast, RBC precipitated as a small dot when no agglutination happened.

Results

Identification of positive phage clones

The positive phage clones binding to the antibody were selected from two libraries, linear 7 peptide library and C7C peptide library, which expressing linear and disulfide constrained heptapeptide fused to the PIII coat proteins of the filamentous phage, respectively. In the panning process, the enrichment of phage was monitored by determining the yield of phage (number of phages eluted/number of phages applied). As shown in Tables 1 and 2, after four rounds of selection, approximate 17000-fold increase in the number of eluted phage for linear 7 peptide library and 20-fold for C7C peptide library were observed. Because the enrichment of phage correlates closely with the affinity of the phage for the target, we deduced that phage selected from the C7C peptide library have lower affinity for the antibody than that from linear 7 peptide library.

In order to identify the positive clones, we proceeded to screen the individual clones randomly picked from the fourth round of panning for specific interaction with antibodies by ELISA. Assessing the different interaction of individual phage clones with antibodies and BSA, we determined the phage clones which specifically bind to antibodies, but not BSA. In contrast, the wild type phage revealed

Table 1
Enrichment of linear 7 peptide library during four rounds of biopanning

Round of panning	Input	Output	Yield
1	2×10^{12}	3×10^{4}	1.5×10^{-8}
2	2×10^{12}	4×10^{4}	2×10^{-8}
3	2×10^{12}	3×10^{6}	1.5×10^{-6}
4	2×10^{12}	5×10^{8}	2.5×10^{-4}

The yield was determined as the ratio of the titer of applied phage (input) and the titer of eluted phage (output).

Table 2
Enrichment of C7C peptide library during four rounds of biopanning

Round of panning	Input	Output	Yield
1	2×10^{11}	1.3×10^{4}	0.75×10^{-7}
2	2×10^{11}	2×10^{4}	1×10^{-7}
3	2×10^{11}	2.2×10^{5}	1.1×10^{-6}
4	2×10^{11}	3.1×10^{5}	1.55×10^{-6}

The yield was determined as the ratio of the titer of applied phage (input) and the titer of eluted phage (output).

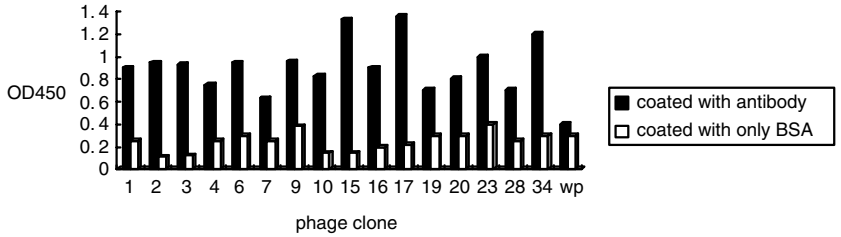

Fig. 1. Identification of positive clones of linear 7 peptide library by ELISA. Sixteen clones selected from the eluates of the fourth round were subjected to ELISAs. Absorbance at 450 nm of the positive clones binding to anti-B antibody was much higher than to BSA only. wp represents wild type phage clone and was used as negative control. For each clone, the absorbance at 450 nm was calculated as average of three duplicates.

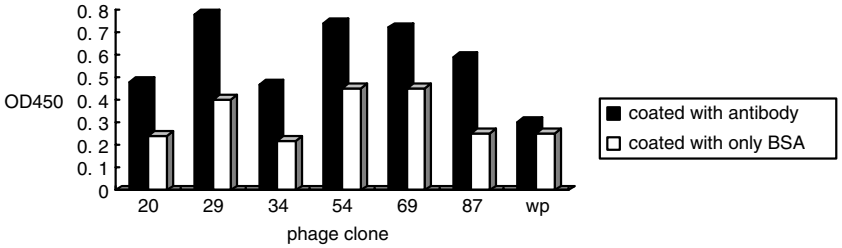

Fig. 2. Identification of positive clones of C7C peptide library by ELISA. Six clones selected from the eluates of fourth round were subjected to ELISAs. Absorbance at 450 nm of the positive clones binding to anti-B antibody was much higher than to BSA only. wp represents wild type phage clone and was used as negative control. For each clone, the absorbance at 450 nm was calculated as average of three duplicates.

only background binding to both targets. Using this method, we isolated 16 phage clones from linear 7 peptide library and 6 clones from C7C peptide library which displayed anti-Gal antibody-binding specificity (see Figs. 1 and 2).

Sequence analysis of the positive clones

Sequencing the single stranded DNA encoding the peptides displayed on the positive phages surface, we deduced the amino acid sequences of the selected polypeptides responsible for antibody binding. As shown in Table 3, alignment of the sequences revealed a number of conserved residues existed in the selected peptides.

Nine out of sixteen clones from linear 7 peptide library have the same sequence FHENWPS. Six clones also shared the same sequence FHEFWPT. There are only two amino acid residues different from each other: Asn and Phe at position 4, Ser and Thr at the C-terminus. However, Ser and Thr are both polar amino acids, having a very similar chemical characteristic. The amino acid sequence of clone 16 is SMLDTPT. Pro and Thr also appeared at the C-terminus. In the C7C library, the random heptapeptides are structurally constrained through a pair of cysteine residues. After four rounds of panning, six positive clones were selected which have different amino acid sequences. Further analysis indicated that these sequences exhibited some very conserved amino acid residues: His/Lys at position 1, Thr/Ser at position 3, 7, and Pro at position 2. In each peptide, there are three or four Ser/Thr forming a cluster of hydrophilic group mimicking the stereochemical feature of carbohydrate. There is a very clear consensus sequence PT among these peptides from two libraries. We used Clone 1 and Clone 15 in place of other clones with the same sequences in the subsequent experiments. For convenience, Clone 1, Clone 15, and Clone 16 from linear 7 peptide library, Clone 20, Clone 25, Clone 34, Clone 54, Clone 69, and Clone 87 from the C7C library were abbreviated to L1, L15, L16, C20, C25, C34, C54, C69, and C87.

Table 3
The amino acid sequences of positive clones deduced from DNA sequencing

	Phage clone	Amino acid sequence
Linear 7 peptide library	Clone 1, 2, 3, 4, 6, 7, 9, 10, 20	**Phe-His-Glu**-Asn-**Trp-Pro-Ser**
	Clone 15, 17, 19, 23, 28, 34	**Phe-His-Glu**-Phe-**Trp-Pro-Thr**
	Clone 16	Ser-Met-Leu-Asp-Thr-**Pro-Thr**
C7C peptide library	Clone 20	Leu-**Pro-Thr**-Ile-Thr-Asn-**Thr**
	Clone 25	**His**-Ile-Leu-Gly-Ser-Thr-Ala
	Clone 34	**His-Pro-Thr**-Trp-Ser-Ser-Leu
	Clone 54	**His**-Gln-**Thr**-Pro-Leu-Ser-**Thr**
	Clone 69	Ser-Ser-**Thr**-Ile-Ala-Asn-**Thr**
	Clone 87	**Lys-Pro-Thr**-Ser-Thr-Leu-**Thr**

The amino acid residues which existed in the sequences with high frequency are noted in bold.

Assessment of binding affinity by competitive ELISA

Stachyose can inhibit the interaction of α-Gal antigen with the antibody in that its structure is very similar to that of α-Gal antigen. If the selected peptides can mimic α-Gal antigenic epitope at conformational level, stachyose can block their binding to the antibody. The positive clones were examined by stachyose competitive ELISAs in which

phage-displayed peptide binding to the antibody was inhibited corresponding to the concentration of stachyose (see Figs. 4 and 5). This indicated that the positive clones bound to antibody at the α-Gal antigen binding site.

DTT reduction assay

To further analyze the function of disulfide bond in the peptides selected from the C7C library, we performed the DTT reduction assay to compare the results with or without DTT. DTT is a reductive reagent which can reduce the disulfide bond and change the disulfide strained peptide from ring to linear structure.

As shown in Fig. 3, when the disulfide bond was disrupted by DTT, the binding capacity of the peptides was decreased to different levels, indicating that the suitable conformation of binding to the antibody was changed. In contrast, no obvious variation of OD_{450nm} was observed with or without DTT for linear peptides.

Peptide synthesis

The peptides displayed on the positive clones L1, L15, and C34 were synthesized by solid phase method using standard fluorenylmethoxycarbonyl (Fmoc) chemistry, purified by reversed-phase HPLC, and characterized by Mass spectrometry. Their purity was all above 70%. For convenience, the peptides were named P1, P15, and P34, respectively.

Inhibition of RBC agglutination assay

The RBC agglutination assay was performed by pig red blood cells (RBCs) mixed with serially diluted stachyose, positive phage clones, and the synthetic peptides (Fig. 6). The result indicated that stachyose, positive phage clones, and the synthetic peptides could inhibit RBC agglutination. The α-Gal antigen expressed on the red blood cells has higher affinity for human natural antibody, so inhibition of RBC agglutination needed high concentrations of the peptides to competitively block the interaction of the α-Gal antigen with the antibody. C69 did not inhibit the agglutination, so it was very likely that the peptide have lower affinity for the antibody. From the data we detected

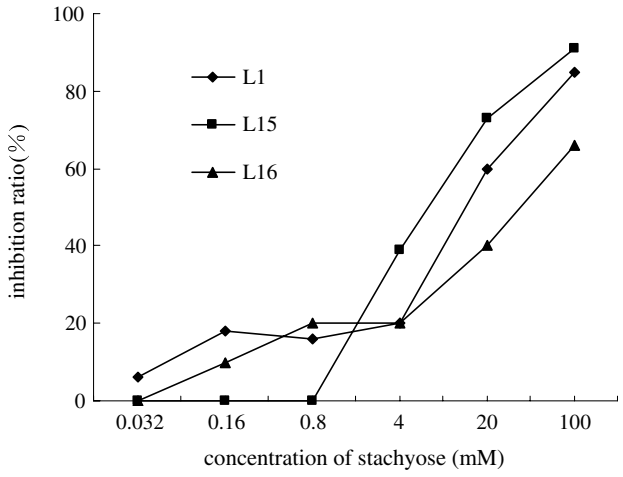

Fig. 4. Stachyose competitive inhibition of positive clones of linear 7 peptide library binding to anti-B antibody. For each clone, the absorbance at 450 nm was calculated as average of three duplicates. The inhibition ratio of each positive clone was determined by the formula: Inhibition rate = $[A_{450}(-s) - A_{450}(+s)]/A_{450}(-s)$.

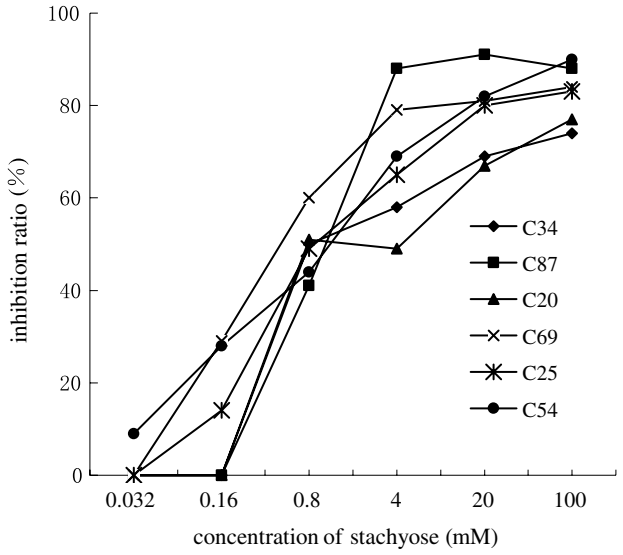

Fig. 5. Stachyose competitive inhibition of positive clones of C7C peptide library binding to anti-B antibody. For each clone, the absorbance at 450 nm was calculated as average of three duplicates. The inhibition ratio of each positive clone was determined by the formula: Inhibition rate = $[A_{450}(-s) - A_{450}(+s)]/A_{450}(-s)$.

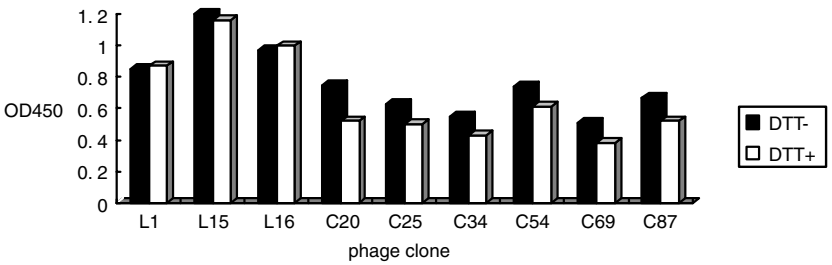

Fig. 3. Effect of DTT on the binding of the positive clones to the antibody. Each clone was incubated with and without 2 mM DTT, respectively. The absorbance at 450 nm was calculated as average of three duplicates.

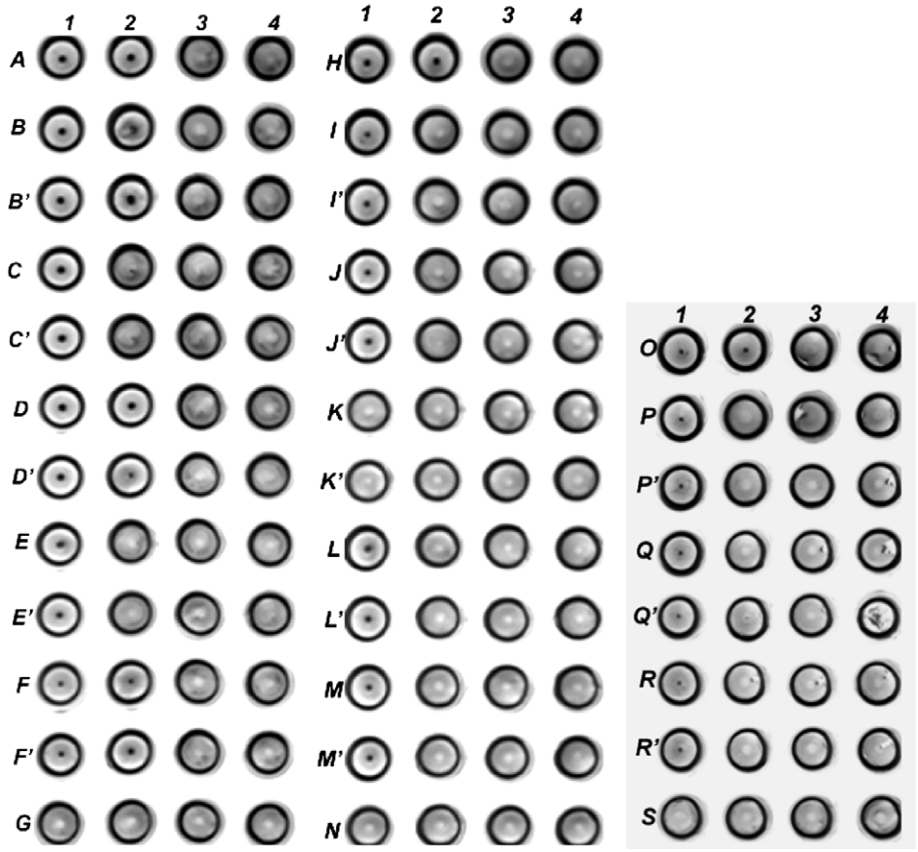

Fig. 6. The inhibition of agglutination of pig RBC induced by human sera with different concentrations of positive phage clones displaying the selected peptides, the synthetic peptides, and stachyose. A1–2, H1–2, O1–2 PBS control, no agglutination A3–4, H3–4, O3–4 positive control, agglutination. B1–4 and B′1–4, the concentration of stachyose was 200, 40, 8, 1.6 mM, respectively. The inhibition of the first two wells were observed. C1–4 and C′1–4, E1–4 and E′1–4, I1–4 and I′1–4, J1–4 and J′1–4, K1–4 and K′1–4, L1–4 and L′1–4, M1–4 and M′1–4, P1–4 and P′1–4, and Q1–4 and Q′1–4 was the positive clone L1, L15, C87, C25, C69, C20, C54, L16, and C34, respectively. From well 1 to well 4, the concentration of each clone was 0.1, 0.02, 0.004, and 0.0008 mM, respectively. The inhibition of the first well was observed. D1–4 and D′1–4, F1–4 and F′1–4, R1–4 and R′1–4 were the synthetic peptides P1, P15, and P34, respectively. From well 1 to well 4, the concentration of each peptide was 20, 4, 0.8, and 0.16 mM, respectively. P1 and P15 inhibited the agglutination of RBC with concentration of 4 mM. For P34, the concentration was 20 mM. G1–4, N1–4, The wild type phage clone was used as negative control, agglutination was observed. S1–4, the peptide CVQPSHSSC was used as negative control, agglutination was observed.

that the concentration of the synthetic peptides, either linear or cyclic, had to be much higher than that of the phage which displayed peptides on their surface. Probably the selected peptides were in a more stable conformation that was suitable for binding to the antibody than that of the free peptides. The removal of phage led to a dramatic decrease in binding affinity. The linear peptides have more flexible and unstable conformation. There is a dynamic equilibrium of conformation transition. The suitable conformation that can bind to antibody was only a small fraction. It has been argued that structurally constrained peptide might be better stable than the free-moving linear peptide. For this purpose, peptide libraries such as C7C library have been constructed that include cysteine residues flanking the randomized sequence with the hope of limiting the number of possible conformation. However, just as the case we encountered, Wrighton et al. [11] selected an eight amino acid cyclic peptide binding to a soluble recombinant erythropoietin receptor (EPOr) from a PVIII-displayed peptide library. The free soluble cyclic peptide had a rather low affinity for the EPOr. The mechanism underlying the phenomenon is not clear and needs further investigations.

Discussion

Phage display, first introduced by G. Smith in 1985, has evolved into a powerful technology providing opportunities to define protein–protein interactions [12,13] and to design polypeptides with novel functions [14]. Numerous phage-derived peptides that bind extracellular protein surfaces and mimic the receptor–ligand interaction have been proven remarkably successful [15]. Mapping protein functional epitope by phage display provided deep insight into protein–protein interaction underlying cellular processes [16].

In our experiment, eight peptide mimics of the α-Gal epitop were selected and identified from two peptide libraries. ELISAs suggested that these peptides bound specifically to anti-Gal antibody. It was further proven by stachyose competitive assay that the phage-displayed peptides and stachyose competitively bound to the anti-Gal

antibodies at the same binding site. The peptides displayed on the phage surface and the free synthetic peptides can inhibit the agglutination of pig RBC mediated by human natural anti-Gal antibody. These results revealed that the peptides can mimic the structure of Gal-α1-3Gal at the molecular level and are able to replace α-Gal antigen to interact with the human natural antibody.

The significant chemical characteristic of carbohydrates is that they have the capacity of forming hydrogen bonds and hydrophobic interactions with their ligands, which is attributed to the existence of many hydroxyl groups and hydrophobic ring in their structure [17]. Hydrogen bonds play a very important role in the affinity. So peptide which can mimic the carbohydrate should have a few polar residues with hydroxyl groups and hydrophobic residues in its sequence. This point was strongly supported by the occurrence of the amino acid residues: serine, threonine, leucine, isoleucine, phenylalanine, and tryptophan. The polar amino acids serine and threonine share a common chemical feature that they all have the hydroxyl group and are good hydrogen bond formers. In these selected peptides, serine and threonine are often present in a cluster of hydrophilic group, participating in hydrogen bonding interaction. Previous studies indicated that the nonpolar amino acids, particularly the aromatic amino acids, appeared with high frequency in the peptide mimics of polysaccharides [18]. Probably the nonpolar amino acid residues in the selected peptides substituted the role of hydrophobic ring and were involved in the hydrophobic interaction. The aromatic amino acids have strong potential to mimic the conformation of carbohydrates because of their molecular size, their hydrophobic aromatic ring, and the capability of forming hydrogen bond [18,19]. This was proved by our selecting result of linear 7 peptide library. Proline was also a very conserved residue in these selected peptides. Proline is more conformationally restricted than other amino acids. Since its cyclically bonded structure fixes its conformational degree of freedom, the proline-containing peptide therefore possesses the capacity to turn and reorient itself to produce the required compact structure in a relatively stable manner [20].

The α-galactosyl peptide mimic SSLRGF selected by Kooyman et al. [21] have very homologous sequence with our positive peptides. Comparing these peptide mimics of α-Gal antigenic epitope, we clearly found the conserved motif STL. As shown in Table 4 in some peptides, serine was substituted with threonine and leucine with isoleucine or alanine. But they have very similar chemical features. Further analysis indicated that motif PT always existed at C-terminal of the linear peptides. In the disulfide constrained peptides, motif PT was often present at N-terminus, whereas STL at C-terminus. It was very likely that the binding sites of the linear peptides and the cyclic ones on the antibody were not the same. But the two sites overlapped at the position where the motif PT interacted with the antibody. Moreover, the sites where motif PT and STL binding to the antibody were separated by just one amino acid residue, probably. Interestingly, both motifs were in a reverse orientation in C54. The variation in the primary structure can produce different conformation of the peptide. Perhaps, the peptide displayed by C54 still has the capability to mimic the structure of α-Gal antigenic epitope.

Table 4
Alignment of the sequences of amino acid of positive clones and the peptide selected by Kooyman group

Kooyman group	**Ser-Ser-Leu**-Arg-Gly-Phe	**SSL**RGF
Our group	His-*Pro-Thr*-Trp-**Ser-Ser-Leu**	H*PT*W**SSL**
	His-Ile-Leu-Gly-**Ser-Thr-Ala**	HILG**STA**
	Lys-*Pro-Thr*-**Ser-Thr-Leu**-Thr	K*PT***STL**T
	Ser-**Ser-Thr-Ile**-Ala-Asn-Thr	S**STI**ANT
	Leu-*Pro-Thr*-Ile-**Thr**-Asn-Thr	L*PT*I**T**NT

The conserved amino acid residues were noted in bold and the common motif PT was underlined.

In order to identify peptides with higher affinity, sublibraries shall be constructed on the basis of the conserved motifs PT and STL. The scaffolds used in designing the sublibraries for affinity maturation will include linear and disulfide constrained peptides. For linear peptide libraries, the scaffold $X_nPTXSTLX_m$ (X represents random amino acid, n and m may be 0, 1, 2, or 3) should be adopted. At the same time, random amino acid residues should be added at C- and N-termini in order to expand the epitope. For the purpose of selecting for the optimal ring size of cyclic peptides binding to the antibody, the scaffold $CX_nPTXSTLX_mC$ (X represents random amino acid, n and m may be 0, 1, 2, and 3) can be further investigated by site-directed mutagenesis at the random sites.

Our work is a first step towards further discovery of peptide ligands capable of removal or blocking the human natural antibodies. Once the ligands with desirable affinity were isolated, the peptides can be attached to an affinity column to remove the antibodies specifically or may be directly used in vivo as a blocking agent.

Acknowledgments

We thank Dr. Keyi Wang and Dr. Hongyu Jia for their technical assistance in phage display. This research was supported by National Natural Science Foundation of China (Grant #30470369).

References

[1] U. Galili, Significance of anti-Gal IgG in chronic xenograft rejection, Transplant. Proc. 31 (1) (1999) 940–941.
[2] U. Galili, The alpha-gal epitope in xenotransplantation, Biochimie 83 (7) (2001) 557–563.
[3] J.H. Lawson, J.L. Platt, Molecular barriers to xenotransplantation, Transplantation 62 (3) (1996) 303–310.
[4] J.L. Platt, R.J. Fischel, A.J. Matas, S.A. Reif, R.M. Bolman, F.H. Bach, Immunopathology of hyperacute xenograft rejection in a swine-to-primate model, Transplantation 52 (2) (1991) 214–220.
[5] U. Galili, Interaction of the natural anti-Gal antibody with alpha-galactosyl epitopes: a major obstacle for xenotransplantation in humans, Immunol. Today 14 (10) (1993) 480–482.

[6] R. Cortese, F. Felici, G. Galfre, A. Luzzago, P. Monaci, A. Nicosia, Epitope discovery using peptide libraries displayed on phage, Trends Biotechnol. 12 (7) (1994) 262–267.
[7] M.F. Renschler, R.R. Bhatt, W.J. Dower, R. Levy, Synthetic peptide ligands of the antigen binding receptor induce programmed cell death in a human B-cell lymphoma, Proc. Natl. Acad. Sci. USA 91 (9) (1994) 3623–3627.
[8] B. Pan, B. Li, S.J. Russell, J.Y. Tom, A.G. Cochran, W.J. Fairbrother, Solution structure of a phage-derived peptide antagonist in complex with vascular endothelial growth factor, J. Mol. Biol. 316 (3) (2002) 769–787.
[9] P.M. Keller, B.A. Arnold, A.R. Shaw, R.L. Tolman, F. Van Middlesworth, S. Bondy, V.K. Rusiecki, S. Koenig, S. Zolla-Pazner, P. Conard, et al., Identification of HIV vaccine candidate peptides by screening random phage epitope libraries, Virology 193 (2) (1993) 709–716.
[10] J.B. Zhan, Z. Xia, L.H. Xu, Z.K. Yan, K.Y. Wang, A peptide mimetic of Gal-alpha 1, 3-Gal is able to block human natural antibodies, Biochem. Biophys. Res. Commun. 308 (1) (2003) 19–22.
[11] N.C. Wrighton, F.X. Farrell, R. Chang, A.K. Kashyap, F.P. Barbone, L.S. Mulcahy, D.L. Johnson, R.W. Barrett, L.K. Jolliffe, W.J. Dower, Small peptides as potent mimetics of the protein hormone erythropoietin, Science 273 (5274) (1996) 458–464.
[12] G.P. Smith, Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface, Science 228 (4705) (1985) 1315–1317.
[13] M.A. Gallop, R.W. Barrett, W.J. Dower, S.P. Fodor, E.M. Gordon, Applications of combinatorial technologies to drug discovery. 1. Background and peptide combinatorial libraries, J. Med. Chem. 37 (9) (1994) 1233–1251.
[14] R. Schier, J. Bye, G. Apell, A. McCall, G.P. Adams, M. Malmqvist, L.M. Weiner, J.D. Marks, Isolation of high-affinity monomeric human anti-c-erbB-2 single chain Fv using affinity-driven selection, J. Mol. Biol. 255 (1) (1996) 28–43.
[15] J.M. Healy, O. Murayama, T. Maeda, K. Yoshino, K. Sekiguchi, M. Kikuchi, Peptide ligands for integrin alpha v beta 3 selected from random phage display libraries, Biochemistry 34 (12) (1995) 3948–3955.
[16] A. Folgori, R. Tafi, A. Meola, F. Felici, G. Galfre, R. Cortese, P. Monaci, A. Nicosia, A general strategy to identify mimotopes of pathological antigens using only random peptide libraries and human sera, EMBO J. 13 (9) (1994) 2236–2243.
[17] A. Varki, M. Chrispeels, et al., Essential of Glycobiology, Cold Spring Harbor Laboratory Press, New York, 2002, 30–56.
[18] K.R. Oldenburg, D. Loganathan, I.J. Goldstein, P.G. Schultz, M.A. Gallop, Peptide ligands for a sugar-binding protein isolated from a random peptide library, Proc. Natl. Acad. Sci. USA 89 (12) (1992) 5393–5397.
[19] J.K. Scott, D. Loganathan, R.B. Easley, X. Gong, I.J. Goldstein, A family of concanavalin A-binding peptides from a hexapeptide epitope library, Proc. Natl. Acad. Sci. USA 89 (12) (1992) 5398–5402.
[20] H.B. Lowman, Y.M. Chen, N.J. Skelton, D.L. Mortensen, E.E. Tomlinson, M.D. Sadick, I.C. Robinson, R.G. Clark, Molecular mimics of insulin-like growth factor 1 (IGF-1) for inhibiting IGF-1: IGF-binding protein interactions, Biochemistry 37 (25) (1998) 8870–8878.
[21] D.L. Kooyman, S.B. McClellan, W. Parker, P.L. Avissar, M.A. Velardo, J.L. Platt, J.S. Logan, Identification and characterization of a galactosyl peptide mimetic, Transplantation 61 (6) (1996) 851–855.