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A synthetic complement C1q-like peptide selectively interacts with immune complexes

Err-Cheng Chan*, Mai-Zon Pan, C. Allen Chang[#], Tzong-Zeng Wu[&]
and Yong-Bing Kuo

[#] Institute of Biological Science and Technology, National Chiao Tung University
[&] Institute of Biotechnology, National Dong-Hwa University
* School of Medical Technology, Chang Gung University, Taoyuan, Taiwan, ROC
Fax:886–3–3288741, E-mail:chanec@mail.cgu.edu.tw

A water-soluble peptide possessing an immune complex selective affinity was synthesized and its primary structure established as: Leu-Glu-Gln-Gly-Glu-Asn-Val-Phe-Leu-Gln-Ala-Thr-Ser-Asp-Asp-Cys. This peptide, designated as C1q-like peptide (CLP), represents a possible immune complex binding epitope of complement C1q. CLP has a hydrophilicity value of 0.21. At 0.5 μM, it inhibited by 50% natural human C1q from binding to horseradish peroxidase-rabbit anti-peroxidase immune complex. CLP failed to inhibit *Staphylococcus aureus* protein A from binding monomeric IgG. When coated to a microplate, CLP showed selective binding to the immune complex, and could be used for application in immunochemical detection of immune complex.

Keywords: human complement C1q-like peptide, immune complex, hydrophilicity

Introduction

Human complement subcomponent C1 is a glycoprotein involved in the recognition of activators by the classical pathway of complement, and is composed of three subunits, C1q, C1r and C1s (Muller-Eberhard, 1975). Among them, C1q interacts with immune complex selectively (Knobel *et al.*, 1974). The C1q molecule is composed of six globular heads linked via six collagen-like stalks to a fibril-like central region (Reid and Porter, 1976; Porter and Reid, 1978). The detailed structure shows that C1q contains 18 polypeptides chains (six A-, six B- and six C-chains), and contains a *N*-terminal collagen-like region of approximate 81 amino acid residues and a *C*-terminal globular region of approximate 136 residues (Reid, 1983). Baumann and Anderson (1990) have developed a synthetic peptide corresponding to residues 189–200 on the B-chain of human C1q globular head region that binds immune complex (IC) selectively. This peptide <LEQGENVFLQATLLC designated complementary binding peptide 2 (CBP2) was shown to be able to inhibit *Staphylococcus aureus* protein A and C1q from binding IC. We calculated the peptide hydrophobicity by using the Hopp & Woods index table, and the result showed that <LEQGENVFLQAT and CBP2 had the hydrophobicity values of -0.43 (Hopp and Woods, 1981). The aqueous insoluble characteristics of these peptide may limit them to apply in immunoassay.

In order to increase hydrophilicity of the immune complex-binding peptide, we have designed and tested a peptide designated C1q-like peptide (CLP), and which was chemically modified thepeptide <LEQGENVFLQAT by addition of four-residue <SDDC to the C-terminus. CLP is very soluble in aqueous phase and has a calculated hydrophobicity value of 0.21. In this study, we studied the biological activity of CLP and compared solid-phase CLP and CBP2 for a selective affinity with immune complex.

Materials and methods

Materials

The peptide based on the C1q sequence (residues 189–200), C1q-like peptide (CLP), and the peptide control *Helicobacter pylori* antigen fragment (HPAF) <AKNDKNESAKNDKQES, were synthesized by Chiron Mimotopes Pty. Ltd. (Australia, Clayton). Protein A and C1q, horseradish peroxidase-rabbit-anti-peroxidase immune complex (PAP), human IgG, and Tween 20 were purchased from Sigma. o-Phenylenediamine dihydrochloride and horseradish peroxidase conjugated to goat anti-human IgG (anti-IgG/HRP) were obtained from Pierce. The ELISA microplates were obtained from Costar. Trifluoroacetic acid (TFA) and acetonitrile for HPLC were from Merck.

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Purification of CLP

CLP obtained from Chiron Mimotopes Ltd. was 78% pure by a HPLC analysis and 73% pure by a mass spectrometry analysis, respectively. Further purification was performed using semipreparative reverse-phase HPLC on a Econosil C18 column (250 × 10 mm i.d., 10 μm particle size) with 0.1% TFA/acetonitrile gradient in 0.1% TFA/water as eluent. Peptide purity wasapproximately 99%, as analyzed on a Bondapal C18 analytical column (300 × 3.9 mm i.d., 5 μm particle size, Waters) with 0.1% TFA/acetonitrile gradient in 0.1% TFA/water as eluent. The lyophilizedly CLP was readily soluble in 0.2 M (pH 8) $NaHCO_3$ buffer.

Titration of PAP and anti-IgG/HRP bound by solid-phase CLP

The ELISA microwells were coated with 100 μl of an aqueous CLP (diluted to 8.6 μg/ml in a coating buffer, 0.1 M $NaHCO_3$, pH 9.0) and dried for 2 h at 37° C. A 300 μl blocking buffer containing 3% skim milk in PBS (9 mM Na_2HPO_4, 1 mM NaH_2PO_4, p7.2, 154 mM NaCl) was then added onto each microwell for 1 h at 37° C to block the remaining surface area. After the plate was washed three times with washing buffer (PBS containing 0.1% Tweeen 20), dilutions of PAP or anti-IgG/HRP were then incubated in 100-μl aliquots in the wells for 90 min at 37° C. Then the unbound material was discarded by washing the microplate three times with washing buffer. Next, a 200 μl substrate buffer containing 0.8 mg/ml OPD and 0.01% H_2O_2 in 0.1 M Na_2HPO_4 plus 0.05 M sodium citrate (pH 5.5) was added to each microwell. The color produced was measured after 30 min in a microtiter plate reader (MR5000, Dynatech Laboratories, Torrance, CA) at 405 nm.

Enzyme immunoassay of CLP inhibition

The ELISA microwells were coated with 100 μl of 3 μg/ml solution of C1q diluted in coating buffer for 2 h at 37° C, then blocked with 300 μl/well blocking buffer for 1 h at 37° C. PAP was coincubated with increasing concentrations of CLP diluted in PBSfor 90 min at 37° C. The samples in 100-μl aliquots were then added to the wells and incubated for 1 h at 37° C. The plate was then washed three time with washing buffer, OPD and H_2O_2 substrates were added, and absorbances were measured.

Enzyme immunoassay of Protein A inhibition

The ELISA microwells were coated with 100 μl of an aqueous protein A solution (2 μg/ml) and dried for 2 h at 37° C. Remaining surface area was blocked by adding 300 μl/well of blocking agent. Goat anti-IgG was coincubated with increasing concentrations of protein A, CLP, or HPAF in PBS for 90 min at 37° C, then 100 μl aliquot of each sample was added to the microwell and incubated for 1 h at 37° C. The microplate was washed three time with washing buffer, OPD/H_2O_2 substrate was added, and absorbances were measured.

Results

Application of the method of Hopp and Woods (1981) for calculating hydrophilicity revealed that CLP has a value of 0.21. The positive value of hydrophilicity makes CLP readily soluble in aqueous buffers, i.e. sodium bicarbonate buffer.

In this study, PAP was used to test the immune complex affinity of CLP. Using an enzyme-linked immunosorbent assay (ELISA), titration analysis for each dilution of purified CLP immobilized on the ELISA microwells against PAP diluted serially from 1:1 to 1:64 was performed. As seen in Fig. 1, PAP was able to recognize CLP, and the maximum slope of the linear regressionbetween absorbance and PAP dilution was observed as a concentration of

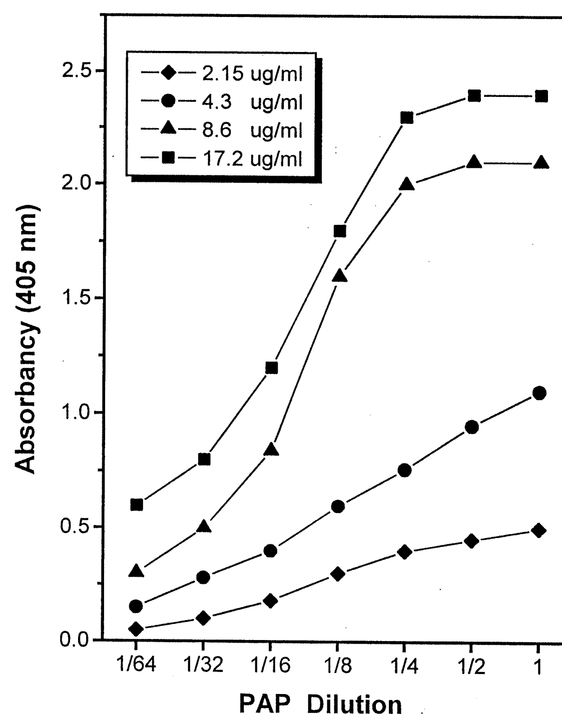

Figure 1 Box titration analysis of purified C1q-like peptide (CLP). CLP was serially diluted with a coating buffer at pH 9, and incubated at 37° C for 2 hr for adsorption onto a microplate. PAP solution was diluted serially from 1 to 1/64 for each CLP dilution and assayed as described in the "Materials and Methods".

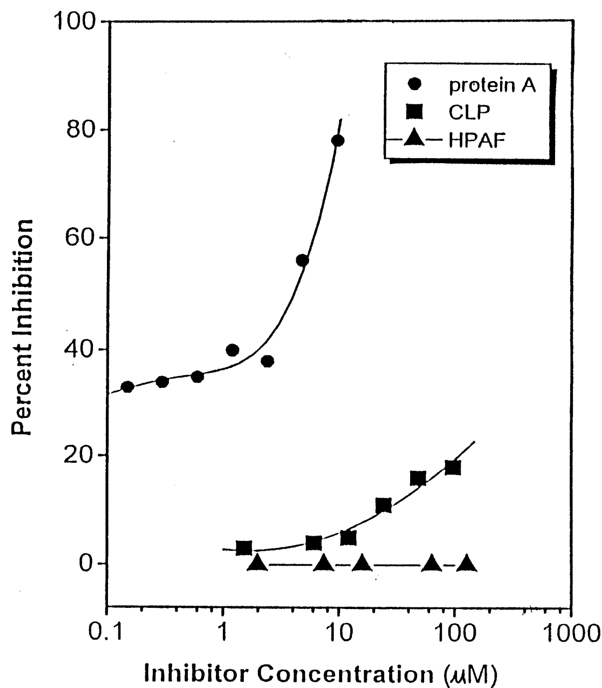

Figure 2 Inhibition of protein A by CLP. The solid-phase protein A binding human anti-IgG/HRP was inhibited by liquid-phase CLP, protein A and HPAF. Specific conditions are given under "Materials and Methods". Percent inhibition was calculated by subtracting the inhibited sample absorbance from the no inhibitor control absorbance. The net absorbance was then divided by the no inhibitor control absorbance and multiplied by 100.

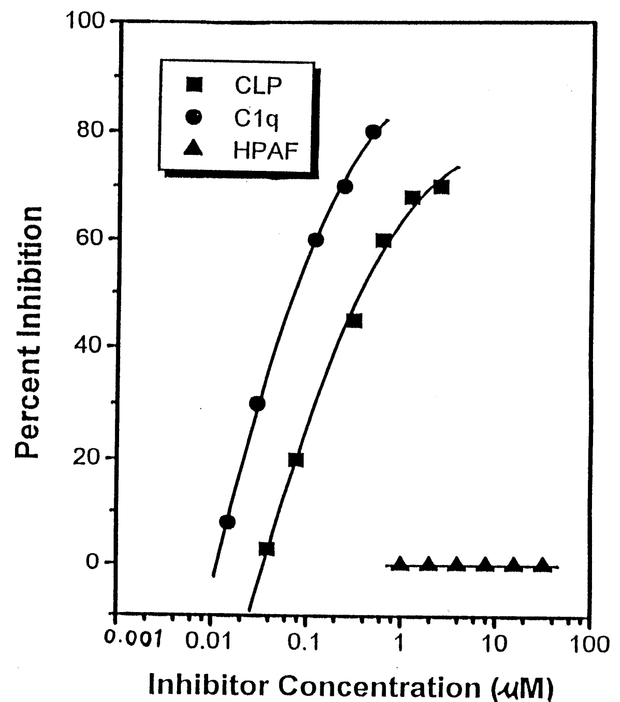

Figure 3 Inhibition of C1q by CLP. The solid-phase C1q binding PAP by liquid-phase C1q, CLP and HPAF. Specific conditions are given under "Materials and Methods". Percent inhibition was calculated as Fig. 2.

8.6 μg/ml of CLP used. For further experiments the dilution of the antigen prepared was fixed at a concentration of 8.6 μg/ml of CLP for coating plates.

To determine whether CLP interacted with free IgG, inhibition assay was performed according to the conditions described under "Materials and methods". In the assay, liquid-phase CLP competed with solid-phase protein A for the binding of liquid-phase of monomeric anti-IgG/HRP. Figure 2 shows a curve for CLP inhibition and the concentration of CLP at 20 % inhibition was approximately 100 μM. Since peptide HPAF had no sequence similarity with CLP, it was used as a negative control. The HPAF showed no inhibition of protein A at all. The data also showed that the concentration of protein A at 50 % inhibition was approximately 5 μM when liquid-phase protein A was used a positive control for a solid-phase protein A inhibition. CLP inhibition assay exhibited that only high concentrations of CLP interacted marginally with monomeric immunoglobulin.

Since CLP was based on the sequence of natural complement subcomponent C1q, it was examined for inhibitory activity in the C1q/immune complex binding assay. The assay condition was described under "Materials and methods". The inhibition curve in Fig. 3 shows that CLP competed with C1q for PAP binding, and the concentration of CLP at 50 % inhibition was approximately 0.5 μM. The negative control HPAF showed no inhibition.

To determine whether CLP possessed an immune complex selective affinity, direct binding experiments were developed especially for this purpose. A concentration of 8.6 μg/l of CLP immobilized on the ELISA microwells against monomeric anti-IgG/HRP diluted serially from 1:1 to 1:64 was performed. Peptide CBP2 described by Baumann and Anderson (1990) was also used for parallel comparison. In the titration analysis, a diluted solution of anti-IgG/HRP in the presence of an excess of IgG was immobilized on the microplates as an immune complex solution. Figure 4 shows the representative curves for CLP interacting with anti-IgG/IgG immune complex selectively. Insignificant binding of Peptide CBP2 to immune complex might be due to its hydrophobic characteristics.

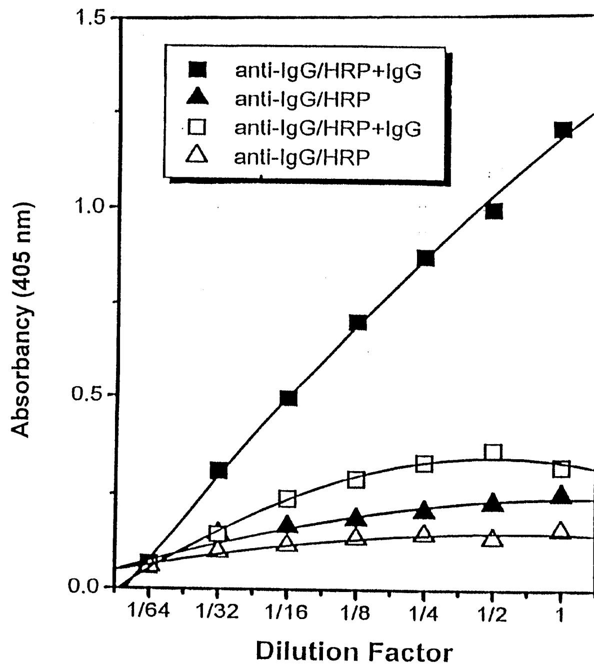

Figure 4 Selective binding of immune complex by CLP. CLP was directly immobilized on a microplate, and the serially diluted anti-IgG/HRP or anti-IgG/HRP plus IgG (as an immune complex) was added and incubated in the microplate (indicated by close symbol). CBP2 immobilized on a microplate was used for parallel comparison (indicated by open symbol).

Discussion

Appropriate antibodies complexed with multivalent antigens bind C1q efficiently (Brown and Koshland, 1975). It is believed that C1q possesses a low affinity for the Fc region of monomeric IgG. If several immunoglobulins bind epitopes which are close spatially, the multiple globular heads of C1q can bind adjacent IgG molecules resulting in a relatively high interaction between C1q and immune complex. In other words, C1q exhibits a selective binding affinity for immune complex in the presence of monomeric IgG. Therefore, C1q can be used for the detection, quantitation or removal of circulating immune complexes. Circulating immune complexes appear in elevated levels in a variety of diseases, such as rheumatoid arthritis, systemic lupus erythematosus, glomerulonephritis, and schistosomiasis (Williams, 1980; Theofilopoulos and Dixon, 1979; Peeter, 1979). The method was based on ELISA principle with peroxidase-labelled C1q as a tracer, and it was claimed to be a rapid and quantitative test for antibodies or antigens.

Baumann and Anderson (1990) demonstrated that a 12-residue sequence of C1q B chain (residues 189–200) would selectively bind immune complex in an inhibition experiment (Baumann and Anderson, 1990). They further added two Leu residues to the carboxyl-terminus as spacing residues to separated the potentially active residues from a future solid phase matrix. A Cys residue was also added to the carboxyl-terminus to allow for coupling to solid phases. The final amino acid sequence was designated CBP2. However, the hydrophilicity value of CBP2 was −0.43, and which was difficult to dissolve in aqueous solution. In addition, CBP2 exhibited less efficiency for binding immune complex when directly immobilized on a microplate.

In this study, we designed a synthetic peptide designated complement-like peptide (CLP) which was based on the sequence of residue 189–200 of C1q B chain with a modification by adding Ser-Asp-Asp-Cys residues at the peptide C-terminus. CLP has a hydrophilicity value of 0.21, which is readily dissolved in aqueous solution. This property make CLP be easily immobilized on a microplate and efficiently interact with binding immune complex. CLP is also nearly unable to inhibit *Staphylococcus aureus* protein A from binding monomeric IgG. An experiment of CLP competing with protein A for binding free monomeric IgG showed that theconcentration of CLP at 20 % inhibition was approximately 100 μM (Fig. 2). On the contrary, the concentration of CBP2 at 50% inhibition of protein A from binding free monomeric IgG was 1 μM (Baumann and Anderson, 1990). This result implicates that CLP is more selectively binding immune complex than CBP2 is. In the future the CLP-ELISA method will be used for quantitation of immune complexes in serum.

Acknowledgements

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