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DESIGN OF PEPTIDES DERIVED FROM ANTI-IgE ANTIBODY FOR ALLERGIC TREATMENT

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Abstract: We have designed and synthesized peptides derived from an anti-IgE antibody which has a potential for the treatment of allergy. It was indicated that conformational restriction of peptide *via* an intramolecular disulfide bond improved the binding affinity for IgE and that the peptide might have an ability to inhibit the IgE-receptor interaction.

Introduction

The interaction between immunoglobulin E (IgE) and its cellular high-affinity receptor, FcεRI, is a central event of the allergic response.[1] Allergen-bound IgE mediates the allergic response by binding through its Fc region to FcεRI on mast cells and basophils, causing the release of inflammatory mediators such as histamine. One strategy to stop this reaction is the use of synthetic compounds which inhibit the interaction between IgE and FcεRI.[2] Over the years, considerable efforts have been devoted to design of peptides derived from IgE or FcεRI as inhibitors of the IgE/FcεRI interaction.[3] Even though the crystal structure of FcεRI α subunit was solved recently,[4] there is still no agreement on the actual binding region. Thus, such peptides based on a part of IgE or FcεRI have met with limited success and have not afforded the consensus knowledge.[2] Another potential approach to block the binding of IgE to FcεRI has been generated by anti-IgE antibodies.[5,6] Anti-IgE antibodies directed against the FcεRI binding region on IgE inhibited the IgE/FcεRI interaction without inducing mediator release from IgE sensitized cells. Intact antibody molecules, however, may cause an immunoresponse, thus it would not be acceptable as a therapeutic molecule.

One of promising approaches to overcoming these problems is to use peptides derived from the antigen binding site of the anti-IgE antibody. The antigen-specificity of antibodies is mainly determined by the limited number of amino acids in complementarity determining regions (CDRs).[7] Therefore, peptide analogues based on CDR might be able to retain the antibody function of antigen-binding. This strategy has been widely acceptable to develop small peptides that are capable of inhibiting intermolecular interaction or specific antigen binding.[8] Several studies have shown that synthetic peptides derived from CDR sequences have binding properties similar to the intact antibody.[9-12] Along with this aspect, we attempted to design peptides based on an anti-IgE antibody which has a potential for the treatment of allergy.

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Results and Discussion

The anti-IgE antibody, MaE11, was reported as a candidate for the treatment of allergic diseases.[6] It can block IgE-binding to its receptor and inhibit histamine release from IgE sensitized cells (Figure 1a). Furthermore, mutations at several residues in the CDRs suggested that three Asp residues in light chain CDR1 (CDRL-1) were involved in binding to the IgE.[6] On the other hand, it has been known that there are only small repertories of main-chain conformations of CDRs, referred to as 'canonical structures'.[13,14] According to the number of residues, the structure of CDRL1 in MaE11 was modeled to be the canonical structure 5 of L1 (Figure 1b).[14] As shown in Figure 1b, the canonical structure for L1 was characterized by a hydrophobic residue (Val, Leu, or Ile) at 29, and the conformation shows that residues between 29 and 32 form a hairpin loop. Three Asp residues are positioned in the loop region consisting of 8 residues (29Val to 32Tyr). Consequently, a part of CDRL-1 sequence of MaE11 was targeted for the design of synthetic peptides which might be inhibitors of the IgE/FcεRI interaction (Figure 1c). Two peptides, Ohg-C and Ohg-L, containing the sequence 29-32 of MaE11 were designed and synthesized to examine the binding ability to IgE. The former peptide was cyclized by the disulfide bond for examining the effect of the conformational restriction. These peptides were modified with a pyrene (Py) moiety as a probe for fluorescence measurements. In addition, designed peptides should be tested for not only IgE binding ability but also their ability to inhibit the IgE/FcεRI interaction. For this purpose, we also synthesized a peptide L262-C derived from FcεRI sequence. It was reported that the peptides which have the L262 sequence could be an effective inhibitor of IgE binding to its receptor and of histamine release.[3] As a reference compound, PyG, which has no peptide chain except glycine was also prepared.

The peptides were synthesized by the solid-phase method using the Fmoc-strategy.[15] After coupling all amino acids, 1-pyreneacetic acid was introduced to the N-terminus of Ohg-C and Ohg-L. Cyclic peptides were obtained by oxidizing cysteine residues to an intramolecular disulfide. Synthetic peptides were purified with RP-HPLC and identified by matrix assisted laser desorption ionization time-of-flight mass spectrometry.[16]

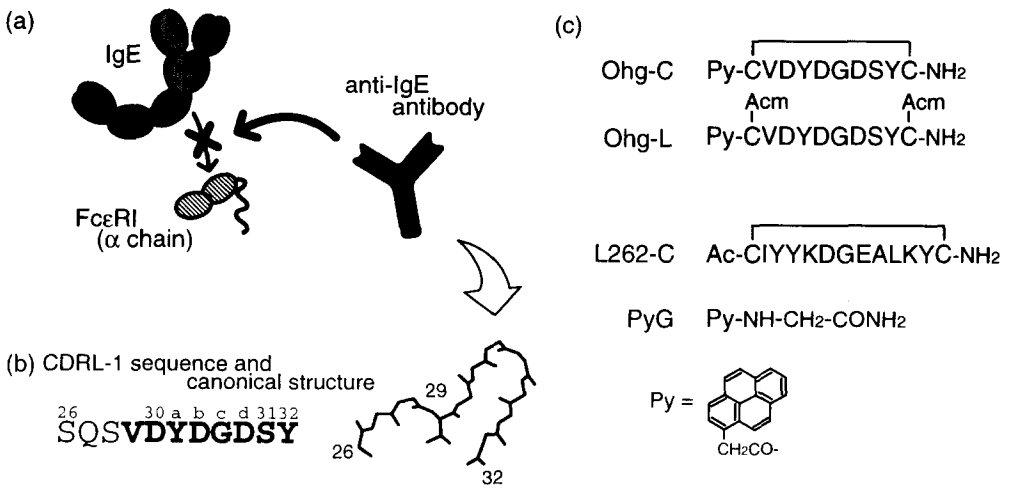

Figure 1 (a) A role of anti-IgE antibody for the inhibition of the IgE/FcεRI interaction. (b) The amino acid sequence and predicted structure of CDRL-1 of MaE11. The residue nunbers are according to Chothia *et al.*[14] (c) Structures of synthetic peptides.

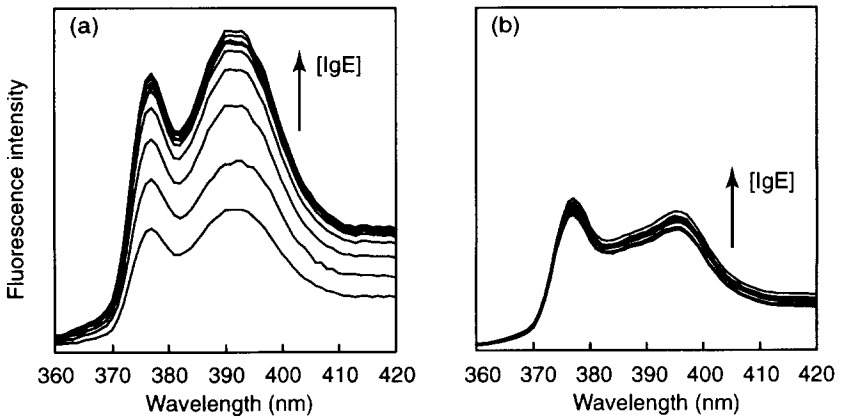

Figure 2 Change of fluorescence spectra of Ohg-C (a) and Ohg-L (b) by the addition of IgE. [peptide] = 0.1 μM, in PBS (pH 7.4), λex = 343 nm, at 25 °C.

Binding properties of the synthetic peptides for human IgE (Chemicon International, Inc.) were examined by the measurements of fluorescence spectroscopy. All measurements were carried out in PBS (pH 7.4) at 25 °C. As the increase of IgE concentration, fluorescence intensities of pyrene in the peptides were increased (Figure 2). In contrast, when IgE was added to PyG solution, no spectral change was detected. These results indicated that the peptide chain, but not pyrene moiety alone, interacted with IgE. The binding constants of Ohg-C and Ohg-L with IgE were calculated from the change of fluorescence intensity at 377 nm. On the assumption that there are two binding sites to peptides in the IgE molecule containing two heavy chains and that these sites are independent each other, binding constants of $6x10^5$ M^{-1} and $7x10^4$ M^{-1} were obtained for Ohg-C and Ohg-L, respectively (Figure 3). Ohg-C with the intramolecular disulfide bond had much higher affinity to IgE than Ohg-L, suggesting that the conformational restriction of peptide improved binding affinity

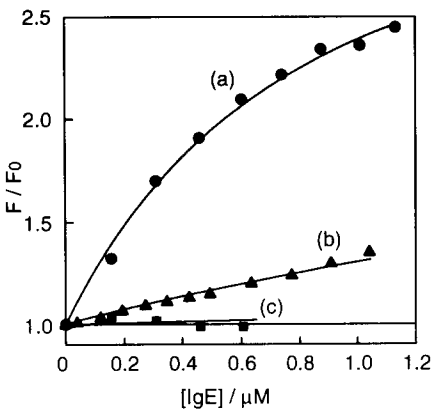

Figure 3 Plots of fluorescence change (F / Fo) of Ohg-C (a), Ohg-L (b), PyG (c) vs. concentration of IgE. [peptide] = 0.1 μM, in PBS (pH 7.4), λex = 343 nm, λem = 377 nm, at 25 °C.

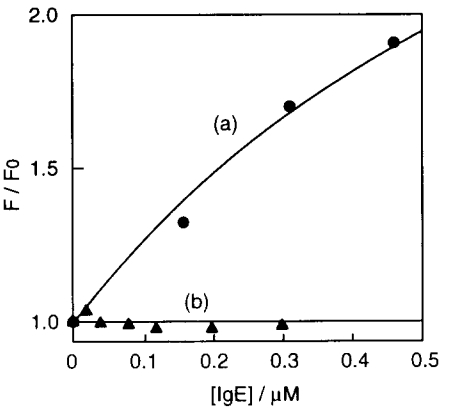

Figure 4 Plots of fluorescence change (F / Fo) of Ohg-C vs. concentration of IgE with and without L262-C. [Ohg-C] = 0.1 μM, [L262-C] = 0 (a) or 10 μM (b), in PBS (pH 7.4), λex = 343 nm, λem = 377 nm, at 25 °C.

for IgE. The interaction between Ohg-C and IgE was also analyzed by surface plasmon resonance experiments using BIACORE biosensor (Biacore AB). When PBS solution of Ohg-C (5-30 μM) was injected over a surface of immobilized IgE, the response signals were observed in a dose-dependent and a reversible manner. These results also supported that Ohg-C had an ability to bind to IgE.

Furthermore, Ohg-C was used for the competition assay using the FcεRI-derived peptide, L262-C. When the IgE concentration was increased, the fluorescence intensity of the mixed solution of Ohg-C and L262-C was not changed in contrast to that without L262-C (Figure 4), indicating that L262-C inhibited the Ohg-C binding to IgE. Since the peptides which have the L262 sequence have been demonstrated to block the IgE binding to its receptor,[3] these results suggest a possibility of Ohg-C to be a potent inhibitor to the IgE/FcεRI interaction.

In conclusion, it was elucidated that the synthetic peptides derived from the CDR of the anti-IgE monoclonal antibody bound IgE effectively, and the binding ability to IgE was improved by the restriction of the conformation according to the model structure of CDR. Furthermore, the interaction between Ohg-C and IgE was inhibited by the FcεRI-derived peptide, L262-C. These findings indicate that the designing approach using a CDR region of an antibody is effective to develop peptides that bind proteins as well as small functional molecules.[12] We are now constructing a new system sensing a variety of biologically important molecules.

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