

Nonepitopic antibody binding sequence: implications in screening and development of peptide vaccines

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Abstract

We describe the interaction of a nonepitopic synthetic decapeptide sequence comprising, GQVLQGAIKG, derived from a random sequence with polyclonal IgGs from various animal sources. GQVLQGAIKG was screened for antibody binding activity using ELISA techniques. The peptide showed similar binding characteristics to the IgGs tested. The results were similar whether we used peptide acid or amide. MAP (multiple antigen peptide)-type construct of the peptide was synthesised and employed as an approach to enhance peptide-IgG interaction. The construct, (GQVLQGAIKG)₄-K₂-K, showed significant antibody binding activity relative to its monomeric form. These results show that nonepitopic sequences may contribute to binding activity observed in peptide library screening and development of peptide based vaccines. As a cautionary point the measure of antibody binding cannot alone be used to classify peptide as an antigen. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Polyclonal IgGs; Peptide vaccines; Nonepitopic sequences

1. Introduction

Research into peptide based vaccine development involves antibody epitope mapping to obtain information about specificity of antibody for particular antigen [1–3]. Several bacterial cell wall proteins with no homology have been shown to bind to IgG from various animal sources [4–7]. Protein A from *Staphylococcus aureus* and protein G from group C and G streptococci are well characterised examples of such proteins. Protein A and protein G are large multi-domain proteins containing repeats of 3–5 small IgG-binding domains, 55–58 residues in length. Both bind to the Fc portion of IgG. Studies by Diesenhofer [8], Derrick and Wigley [9], have shown that protein G also binds to the constant domains in the Fab fragment. Protein A and protein G derived sequences, 11–

33 residues in length, have been shown to mimic the binding of their respective intact parent protein [10,11]. Herein, we describe the antibody binding activity of one of several short nonepitopic sequences selected from random screening of several peptides in our laboratory. Such sequences may have implications in epitope screening methods, particularly in the screening of peptide libraries, and peptide vaccine development. Furthermore, these sequences are distinct from any known antibody-binding proteins.

2. Materials and methods

2.1. Reagents

4-Methylbenzhydrylamine (MBHA) and *t*-Boc-glycine-Merrifield resins, *t*-Boc and Fmoc protected amino acids were purchased from Calbiochem–Novabiochem Ltd (Beeston, Nottingham, UK), 5-amino salicylic acid, biotin, ExtrAvidin-peroxidase,

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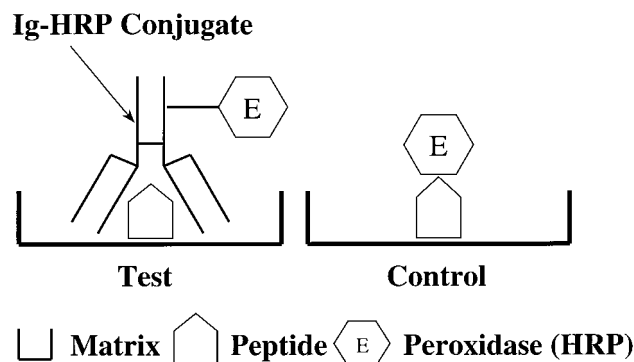


Fig. 1. Direct peptide-antibody binding assay.

horse radish peroxidase, goat anti-rabbit peroxidase conjugate (GARP) and polyclonal IgGs were purchased from Sigma Chem. Co. Ltd (Poole, Dorset, UK), Immulon M129AI and M129AIV plates were purchased from Dynatech Laboratories Ltd (Billingshurst, West Sussex, UK)

2.2. Synthesis, purification and characterisation

Peptide acids and peptide amides comprising the sequence, GQVLQGAIKG, were synthesised as described by Merrifield [12]. Resin free peptide were obtained by HF treatment. Peptide were purified by RP-HPLC (reverse phase hplc) on C-18 Vydac 218TP1022 preparative column (22 × 250 mm) using a 0.1% TFA (tri-fluoroacetic acid)/80% acetonitrile gradient. Characterisation was performed by electrospray mass spectrometry.

2.3. Enzyme-linked immunosorbent assays (ELISA)

2.3.1. Direct peptide-antibody binding assay (Fig. 1)

Dynatech M129AIV plates were coated overnight by incubation at 37°C with 100 µl/well of peptide solution at 20 µg/ml in carbonate buffer, pH 9.6 (15 mM Na₂CO₃; 35 mM NaHCO₃) with 0.2% DMSO content; the plates were then washed three times with phosphate-buffered saline (10 mM, pH 7.4) containing 0.1% Tween-20 (PBS-T); 100 µl/well of PBS-T was used to block the uncoated well surface [13], by incubation for 1 h at 37°C; plates were then washed as described previously; 100 µl of horse radish peroxidase conjugated goat anti-rabbit (GARP) in PBS-T was placed into each well followed by incubation at 37°C for 1 h. In control wells, 100 µl horse radish peroxidase (HRP) equivalent of GARP was placed and incubated appropriately; plates were washed as before to remove excess and unbound GARP or HRP; bound GARP or HRP was detected by incubation with 5-amino salicylic (0.1% w/v) containing H₂O₂ (0.006%

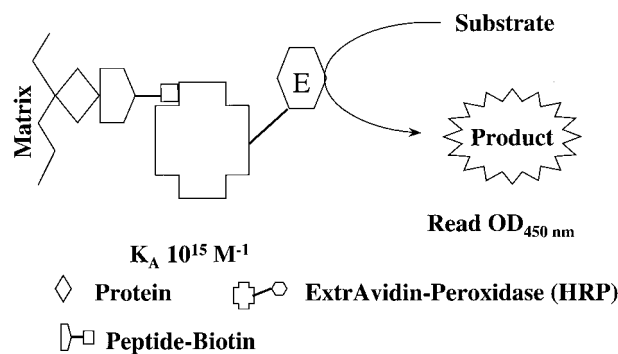


Fig. 2. Indirect peptide-antibody binding assay.

v/v) in 50 mM Na₂HPO₄/NaH₂PO₄, pH 6, for 30 min at 37°C; optical density reading was determined at 450 nm, using a Dynatech MRX microplate reader.

2.3.2. Indirect peptide-antibody binding assay (Fig. 2)

Dynatech M129AI plates were coated by incubation for 1 h at 37°C with 100 µl/well of IgG solution at 20 µg/ml in carbonate buffer, pH 9.6 (15 mM Na₂CO₃; 35 mM NaHCO₃); plates were washed three times with tris-buffered saline (25 mM, pH 7.4) containing 0.1% Tween-20 (TBS-T) on a Dynatech MRW washer; 100 µl/well of TBS-T was used to block the uncoated well surface by incubation for 3 h at 37°C; plates were washed as before; 100 µl of biotinylated peptide at 20 µg/ml in TBS-T containing 0.2% DMSO was placed into each well followed by incubation at 37°C for 1 h; plates were washed as before to remove excess and unbound biotinylated peptide; IgG-bound biotinylated-peptide in each well was detected by incubation with 100 µl of ExtrAvidin-peroxidase conjugate in TBS-T (1:1000 dilution as supplied and recommended by manufacturer) for 1 h at 37°C; plates were washed as before to remove excess and unbound conjugate; bound conjugate was then detected by incubation with 5-amino salicylic acid and optical readings at 450 nm were determined as described previously.

3. Results

3.1. Synthesis and characterisation

Peptides were successfully synthesised, purified and characterised by electrospray mass spectrometry. A typical mass spectrum, of the MAP-type construct; (GQVLQGAIKG)₄K₂K, is shown in Fig. 3.

3.1.1. Direct peptide-antibody binding assays

Monomeric GQVLQGAIKG showed highly significant binding to peroxidase conjugated polyclonal goat

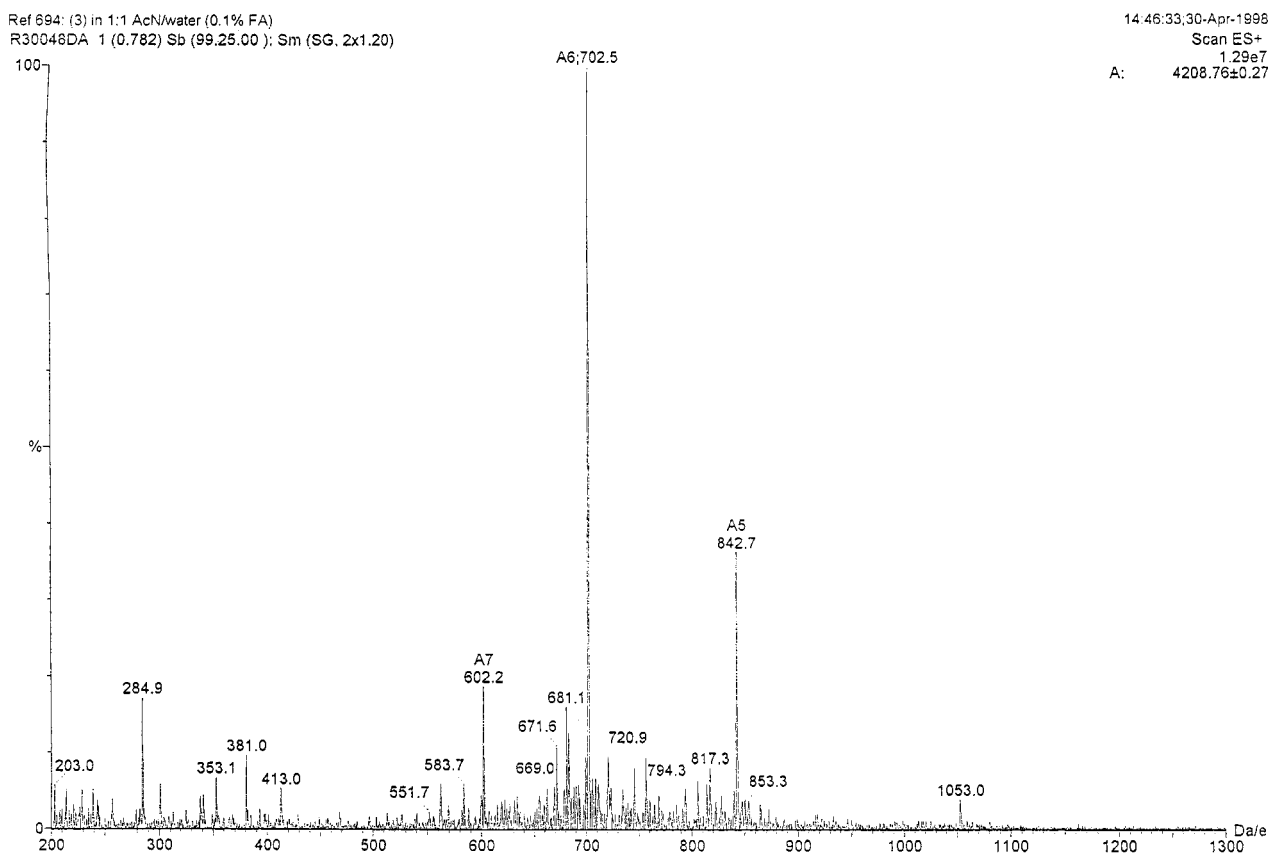


Fig. 3. Electrospray spectrum of MAP-type construct; (GQVLQGAIKG)₄K₂K. The annotation of the major peaks (A series) indicate that a single series is recognised, with the protonation states shown (A5, A6 and A7). Expected mass 4209; Observed mass: 4208.76 ± 0.27.

IgG relative to control tests. There was little or no interaction between the peptide and HRP. This data suggests that there was specific and significant interaction between the peptide and goat IgG (Fig. 4a). There was no significant difference between the binding characteristics of the free acid or amide, suggesting that the free C-terminal is not crucial for peptide-IgG recognition (Fig. 4b). Peptide multimerisation on a trimeric lysine tree was employed as a means to enhance peptide-protein interaction as well as to improve peptide adsorption to microwell. (GQVLQGAIKG)₄K₂K showed significantly higher antibody binding activity relative to its monomeric form, GQVLQGAIKG (Fig. 5).

3.1.2. Indirect peptide-antibody binding assay

Biotinylated GQVLQGAIKG showed significant binding to polyclonal IgG from various sources and to other proteins relative to the appropriate controls (Fig. 6). In this assay avidin exhibited some nonspecific binding to the proteins. Nevertheless, signal from peptide-IgG interaction was apparent. The level of peptide-IgG interaction, as judged by comparison of

optical densities, were similar across the range of polyclonal IgGs tested.

4. Discussion

Peptide, GQVLQGAIKG binds to IgGs from various sources and this binding can be improved by peptide multimerisation. The mechanism of binding is currently unknown. Several other random sequences (not reported here) have also been found to exhibit similar IgG binding characteristics which may have additional value in immunological methods. We conclude that screening methods can produce spurious positive results from non-epitopic sequences which have the ability to interact with a wide range of antibodies. The existence of such sequences can seriously lead to artefacts when screening for epitopes and may contribute to binding activity observed in peptide library screening and in the development of peptide based vaccines. As a cautionary point the measure of antibody interaction cannot alone be used to classify peptide as an antigen.

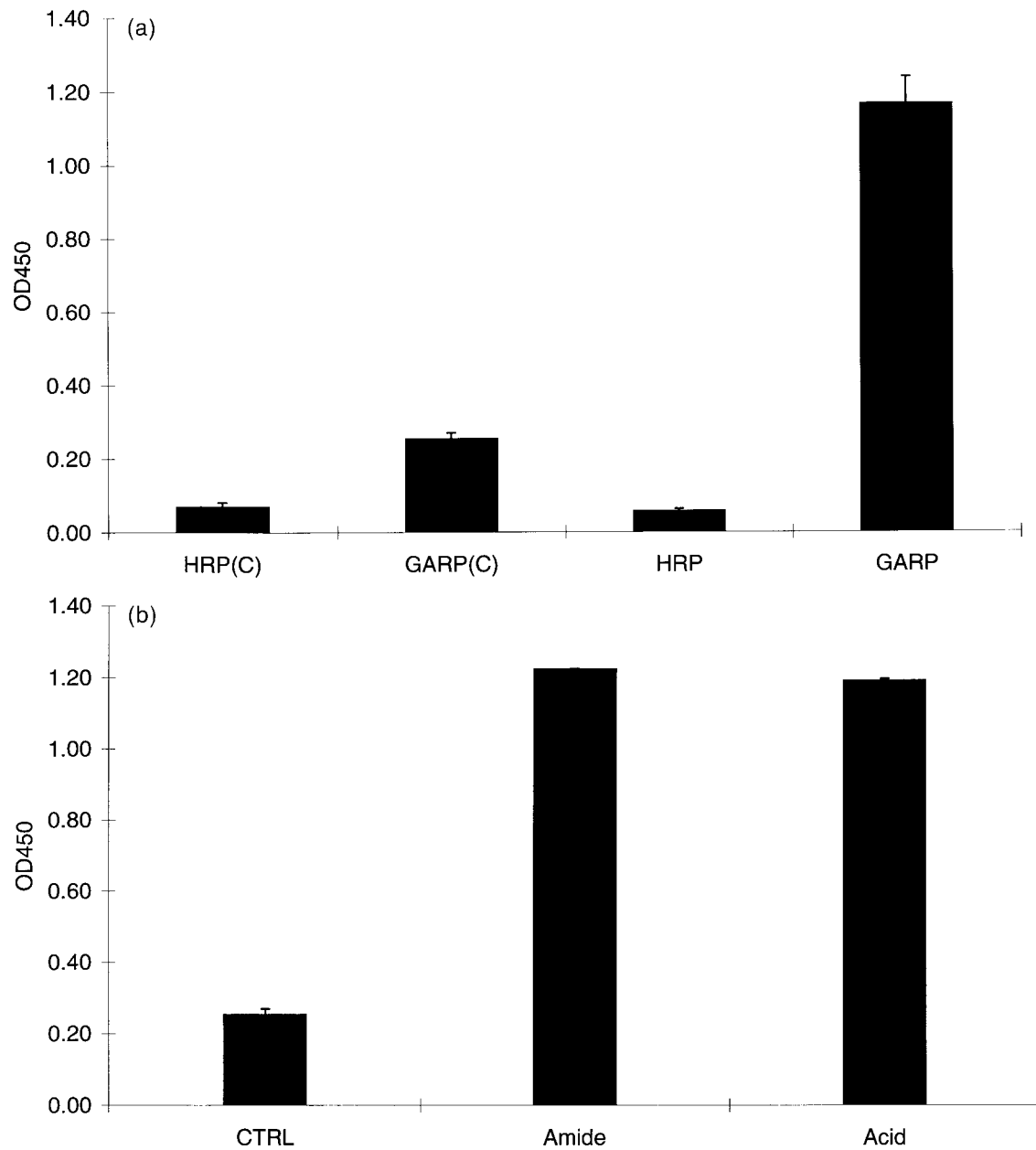


Fig. 4. Direct peptide-antibody binding assay showing (a) the interaction of goat IgG with monomeric GQVLQGAIKG and (b) comparison of the interaction of goat IgG with monomeric GQVLQGAIKG as an acid or amide. HRP(C) and GARP(C) are controls for nonspecific binding to plate wells. Data are mean \pm S.D. ($n = 8$) $P < 0.05$.

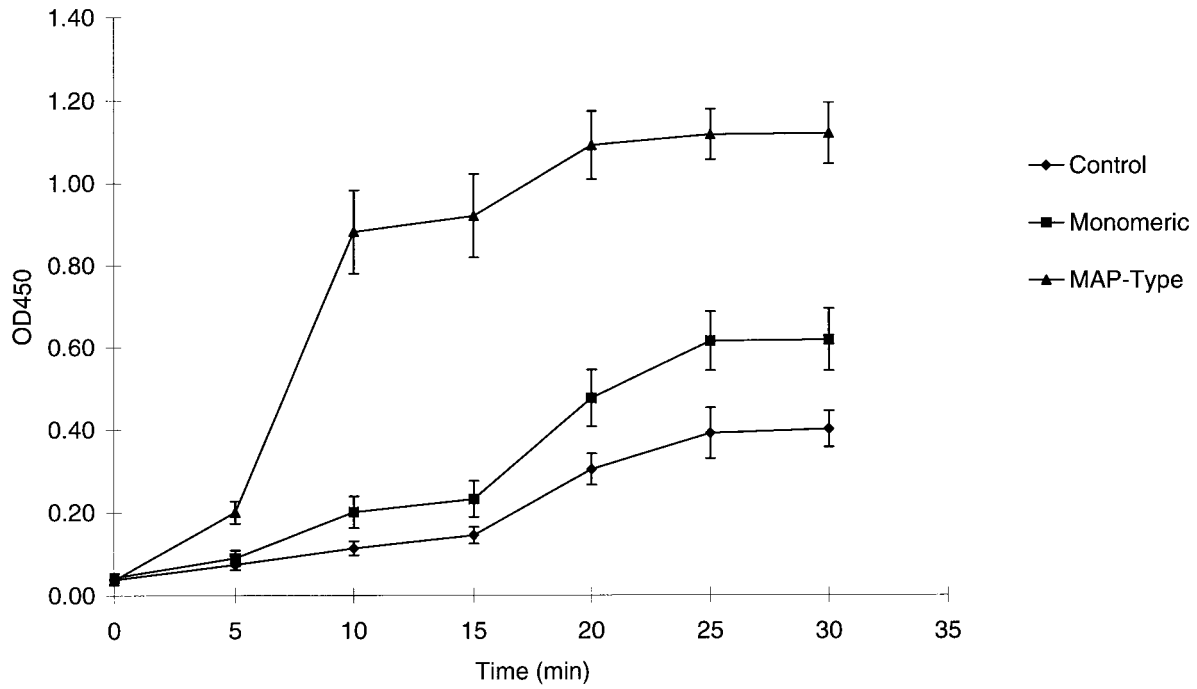


Fig. 5. Direct peptide-antibody binding assay. Comparison of the interaction of MAP-type construct; (GQVLQGAIKG)₄K₂K and monomeric GQVLQGAIKG with goat IgG.

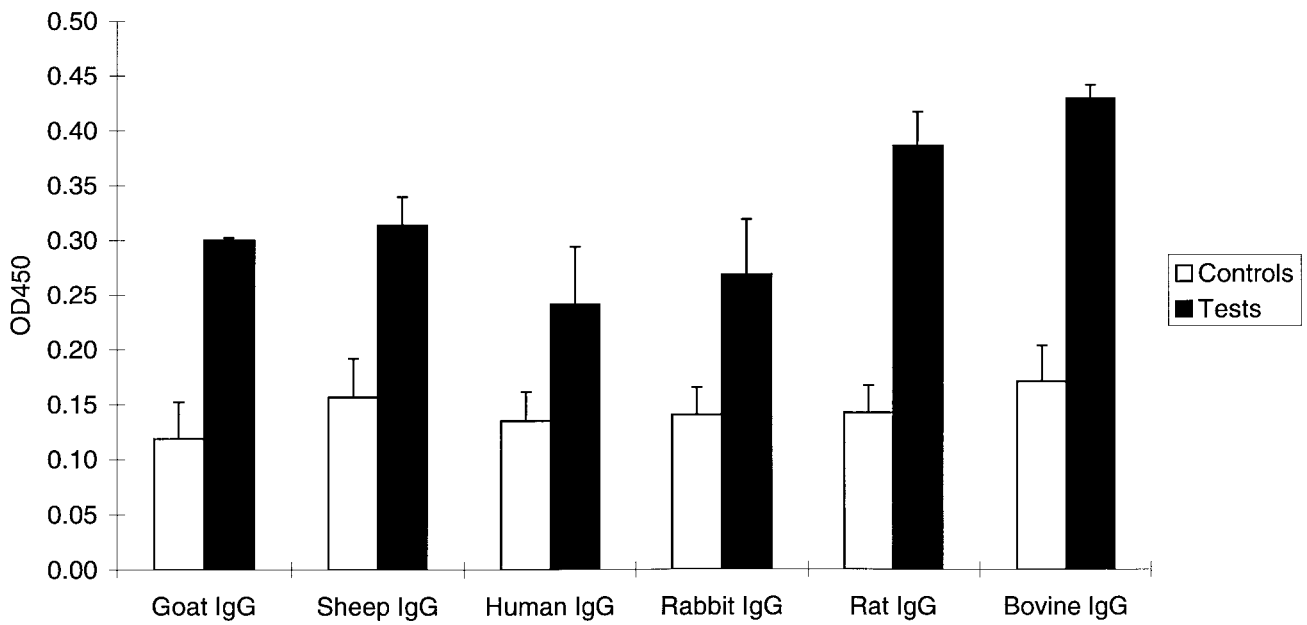


Fig. 6. Indirect peptide-antibody binding assay of biotinylated-GQVLQGAIKG with polyclonal IgGs from different animal sources. Data are mean \pm S.D. ($n = 8$) $P < 0.05$.

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