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Helical peptide arrays for lead identification and interaction site mapping

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ABSTRACT

Libraries composed of linear and cyclic peptides cannot fully represent the higher order structures of most antigenic sites. To map the binding site of ligands or antibodies, a larger part of the three-dimensional space should be sampled. Because parallel synthesis of large arrays of peptides on hydrogels is restricted to relatively small peptides, a simple and robust homodimeric helical system was chosen for antigen presentation. First, it was established in an heterodimeric system that the 26-mer peptide could be synthesized and that the helical coiled-coil peptides interact in the hydrogel in a predictable manner. Next, libraries of homodimeric coiled coils were synthesized into which the epitope was grafted. Using dedicated helical dimeric and trimeric coiled-coil libraries, the epitopes of two anti-HIV-1 gp41 monoclonal antibodies known to interact with helical structures were mapped at high resolution. These mappings precisely reflect existing X-ray data, and the arrays can be applied to lead identification, epitope mapping, and systematic analysis of amino acid contribution to coiled-coil systems.

Pepscan analysis has been a successful approach for the delineation of protein interaction sites by virtue of the systematic libraries of overlapping linear and cyclic peptides [1–3]. However, libraries of short peptides cannot fully represent the higher order structures of most antigenic sites; therefore, folded miniproteins are required to increase the chance of protein mimicry. The size of on-card synthesized peptides is limited to 25–30 residues, and because the smallest autonomously folding natural domains require at least 32–40 residues, they are too short for mimicry of a discontinuous epitope. One noteworthy exception is the α-helical coiled coil, amphipathic peptides that form stable multimeric super helical structures in aqueous solution by burying their hydrophobic patches in a so-called leucine zipper [4,5]. Coiled-coil peptides are characterized by a heptad repeat pattern, $(abcdefg)_n$, where n is the number of repeats and with apolar amino acids at the a and d positions, resulting in an amphipathic helix with a hydrophobic a, d face that can pack against that of another coiled-coil-forming peptide, exposing the hydrophilic stretches to the solvent [5–8]. Arrays containing helical peptides may increase successful epitope mapping, and the identified peptide can be easily translated into a soluble folded miniprotein. To prove association of coiled coils within the hydrogel of the solid support, we applied the optimized short heterodimeric (E/K)3 coil system, a 3-heptad heterodimeric parallel coiled coil with a K_d of 6×10^{-7} M, whose constituent peptides do not homo-oligomerize above pH 6.0 [9]. In this short coiled coil, most amino acid positions contribute to binding and orientation and mutations could cause instability, rendering it less suitable as a scaffold that can accept epitope grafts. Instead, based on the knowledge available for GCN4-like homodimeric parallel coiled coils, it is possible to make a homodimeric coiled coil that has 3.5 heptad repeats and in which all other positions (b, c, e, f, g) can be used as acceptors of functional or antigenic regions from other proteins, thereby presenting the epitope of interest [9–11].

We show here that K3 peptides synthesized on the hydrogel graft of the Pepscan minicards can efficiently form heterodimeric coiled coils with the E3 peptide. Moreover, homodimeric GCN4 coiled coils form spontaneously on the hydrogel surface and can be used as helical epitope scaffolds, as illustrated for the successful mapping of the broadly neutralizing anti-human immunodeficiency virus type 1 (HIV-1)[2] gp41 monoclonal antibodies (Mabs) 4E10 and HK20 [12,13].

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[2] *Abbreviations used:* HIV-1, human immunodeficiency virus type 1; Mab, monoclonal antibody; Fab, fragment of an antibody; PBS, phosphate-buffered saline; BSA, bovine serum albumin; RT, room temperature; ABTS, 2,2′-azino-di[3-ethyl-benzthiazolinesulfonate(6)]diammonium salt; CCD, charge-coupled device; ELISA, enzyme-linked immunosorbent assay; OVA, ovalbumin; HRP, horseradish peroxidase; HPLC, high-performance liquid chromatography; ACN, acetonitrile; TFA, trifluoroacetic acid; MPER, membrane proximal external region; HR1, heptad repeat 1.

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Materials and methods

Incubation of biotinylated soluble peptide with peptide on solid support

Peptide–peptide interactions were measured using Pepscan analysis [14]. Biotinylated peptides were incubated with peptides that were synthesized on a solid phase polypropylene surface of the Pepscan minicard (Fig. 1A). The credit-card-sized minicard that contains 455 wells with 455 different peptides was washed with phosphate-buffered saline (PBS)/Tween 80 (7.5 mM phosphate, 0.14 M NaCl, and 0.5% Tween 80, pH 7.4) for 1 h. Next, the cards were precoated in blocking buffer (5% bovine serum albumin [BSA], 5% horse serum, 1.5% Tween 80, 7.5 mM phosphate, and 0.14 M NaCl, pH 7.4), washed in PBS/Tween 80, and incubated with biotinylated peptide in PBS/Tween 80 containing 1% BSA for either 5, 60, or 120 min at room temperature (RT). Next, the cards were washed and incubated with streptavidin/peroxidase (1:1000) in blocking buffer for 30 min, washed, and incubated with the substrate (0.5 g/L of 2,2′-azino-di[3-ethyl-benzthiazolinesulfonate(6)]diammonium salt [ABTS]) with 0.006% H_2O_2 in 0.18 M Na_2HPO_4, and 0.22 M citric acid was added until pH 4.0. The signal was measured after 60 min with a charge-coupled device (CCD) camera.

Pepscan analysis

The binding of Mab to peptides was assessed in a Pepscan-based enzyme-linked immunosorbent assay (ELISA). Each Mab was titrated to ensure that optimal binding was achieved and non-specific binding was avoided. Each of the wells in the card contains covalently linked peptides that were incubated overnight at 4 °C with Mab, between 1 and 10 ng/ml in PBS containing 5% (v/v) horse serum, 5% (w/v) ovalbumin (OVA), and 1% (v/v) Tween 80 or in an alternative blocking buffer of PBS containing 4% (v/v) horse serum and 1% (v/v) Tween 80. After washing, the plates were incubated with a horseradish peroxidase (HRP)-linked rabbit anti-antibody (DakoCytomation, Glostrup, Denmark) for 1 h at 25 °C. After further washing, peroxidase activity was assessed using ABTS substrate and color development was quantified using a CCD camera and an image-processing system.

Peptide synthesis

Peptides were synthesized by Fmoc chemistry and purified by reversed phase high-performance liquid chromatography (HPLC). For the peptide–peptide interaction studies, some peptides were N-terminally biotinylated via an aminohexanoic acid (Ahx) spacer. The peptides were analyzed for identity by electrospray mass spectrometry. Idealized heterologous helix pairs are named IAAL-E3 for EIAALEKEIAALEKEIAALEK and IAAL-K3 for KIAALKEKIAALKEKIAALKE.

HPLC analyses

Samples were analyzed by ultra-performance liquid chromatography (UPLC, Alliance, Waters, Milford, MA, USA) with a C18 reversed phase column and were detected with a photodiode array detector and a mass sensitive detector. A gradient at 25%/min for 25–100% acetonitrile (ACN) with solvent A (H_2O + 0.05% trifluoroacetic acid [TFA]) and solvent B (ACN + 0.05% TFA) was used. All reagents were at least HPLC grade.

Results

Folding of helical coiled coils on the surface of Pepscan card hydrogel

To establish helical coiled coils on the Pepscan card hydrogel, a K3 peptide and variants thereof were synthesized on solid support and titrated with the complementary N-terminally biotinylated E3 peptide. For this purpose, conditions needed to be established to detect peptide–peptide interactions in the Pepscan format. Specific and sensitive binding between the complementary helices could be detected showing that complex coiled-coil assemblies can be constructed on the solid support. No binding was observed when the Ile and Leu residues in the zipper motif are replaced by Gly residues (Fig. 1). Binding was negatively affected by single Leu to Ala substitutions at the *a* or *d* position (Fig. 2) or more than one substitution of a Lys at the *e* or *g* position (Fig. 3A and B). Especially the C-terminal heptad has low tolerance to substitutions. As expected, mutations of the Glu at the *f* position, which is far from the interface site, had a minor influence on the binding (Fig. 3C).

This thorough mutation analysis shows the importance of each residue in this idealized coiled-coil system, marks the high specificity and sensitivity of the peptide–peptide interaction in this format, and shows that complex coiled-coil assemblies can be constructed on the card.

Epitope mapping using helical template

In this study, we demonstrate the possibility to exploit the synthesis of coiled-coil peptides for the mapping of two broadly neutralizing human Mabs directed against HIV-1 gp41. These two Mabs, 4E10 [12] and HK20 [13,15], target epitopes in the membrane proximal external region (MPER) and the N-terminal heptad repeat 1 (HR1) of gp41, respectively, which are accessible for antibody binding only during the HIV-1 fusion process.

A detailed mapping of the 4E10 epitope was done by measuring the binding of 4E10 with a set of overlapping 9-, 10-, and 12-mer peptides covering the gp41 MPER (Fig. 4). The best binding

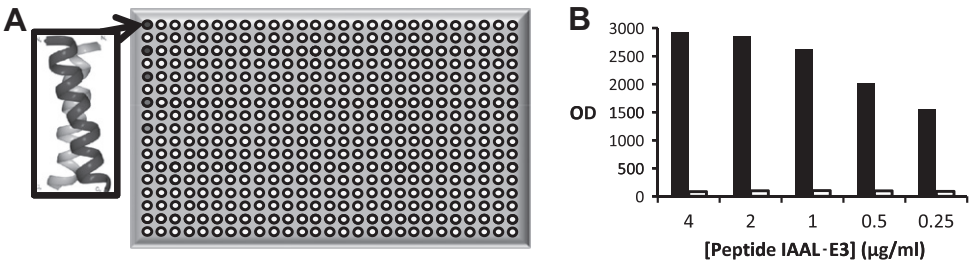

Fig.1. (A) Formation of heterodimeric coiled coils by titration of soluble 21-mer helical monomer biotin-IAAL-E3 (biotin-EIAALEKEIAALEKEIAALEK) with complementary helical monomer IAAL-K3 (KIAALKEKIAALKEKIAALKE) on the solid support at the bottom of the microwell of a credit-card-sized plate. (B) These titration results are also shown as histograms for the binding of biotin-IAAL-E3 to the IAAL-K3 peptide (filled bars) and the negative control GAAG-K3 (KGAAGKEKGAAGKEKGAAGKE) (open bars) synthesized on card surface.