A novel screening method for combinatorial chemistry for low affinity interactions

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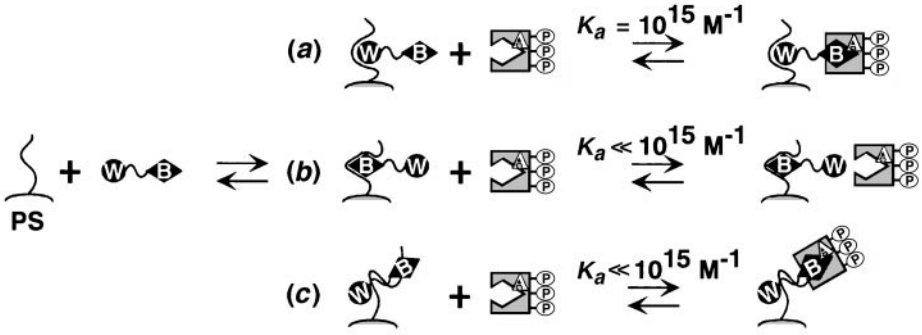
A novel screening method for combinatorial chemistry was developed by utilizing the high binding affinity of the avidin–biotin complex (K_a = 10^{15} M^{-1}), making possible the efficient detection of low affinity interactions between tryptophan and pentapeptides.

Combinatorial chemistry has attracted enormous attention because it can greatly facilitate the drug discovery process.[1,2] It is also an extremely powerful tool in the biotechnology of peptides[3,4] and nucleic acids. In nucleic acid research, this technology (*in vitro* selection) has been employed for finding small ribozymes and recently its reaction mechanism has been elucidated.[5,6] In the field of peptides, several screening methods have been employed using labeled target compounds such as colored dyes,[7] fluorescent dyes,[8] magnetic beads[9] and radioisotopes such as [γ-^{32}P].[10] However, many difficulties have been encountered in the selection of short peptides for a small target with low affinity. In particular, the above screening methods were not successful in extracting tryptophan (Trp) selective peptides from the pentapeptide library.

In this study, we developed a novel screening method utilizing the formation of the avidin–biotin complex, which has a large binding constant of 10^{15} M^{-1}. This screening method made possible the effective detection of low affinity interactions between tryptophan and pentapeptides. Trp was chosen as a target molecule since it is known to play a key role in biological processes, such as the functional peptide that recognizes porphyrin *via* a sandwiching interaction[11] and the *Trp* repressor in which binding Trp enhances DNA affinity as an allosteric effector.[12] Therefore, we have attempted the selection of short peptides that would recognize Trp from a combinatorial library to establish a general model system of low affinity interactions.

The random synthetic pentapeptide library was constructed by the 'split synthesis approach' as previously described.[1,2]† Nineteen natural L-amino acids were used, excluding cysteine to avoid intramolecular cyclization or intermolecular disulfide formation. The library theoretically contains about 2.5 million pentapeptide sequences. Biotin and a hydrophilic linker {biotinyl-ε-aminocaproic acid *N*-hydroxysuccinimide and [2-(2-*tert*-butoxycarbonylaminoethoxy)ethoxy]acetic acid (Boc-AEEA-OH)} were covalently attached to Trp. The introduction of a hydrophilic linker was performed to avoid aggregation of target molecules and such a linker is also effective in raising the activity of the avidin–biotin interaction because its affinity increases with linker length.‡ Moreover, the interactions of individual compounds on both sides of the linker can be treated independently by its introduction.

Our new approach is a novel screening method utilizing the avidin–biotin interaction in two steps. The first step of this screening method is mixing of the pentapeptide library and biotinylated Trp with the hydrophilic linker (BTHL) in an empty column, by gently shaking for 16 h and then washing the library beads.§ There may exist three binding modes between the pentapeptide library and BTHL during this step. These are between the pentapeptide library and the Trp site (W) [Scheme 1(*a*)], the biotin site (B) [Scheme 1(*b*)], and the linker site (L) [Scheme 1(*c*)] of BTHL, respectively. It is also possible that there may be no interaction between pentapeptide library and BTHL. The only desirable binding mode is between the pentapeptide library and W; the other cases must be eliminated. The second step is the addition of fluorescein labeled streptoavidin (FLS) to the library containing BTHL. The solution was gently shaken for 30 min and the library beads were washed with buffer solution to eliminate nonspecific interactions.¶ The concentration of added FLS was evaluated according to the amount of interacting target molecule, whose concentration was determined by UV absorbance of tryptophan in aqueous solution, using an extinction coefficient of 5500 cm^{-1} M^{-1}. Only the desirable interaction in the first step (formation of the W–library complex) will be able to form the avidin–biotin complex preferentially upon FLS addition. The other undesirable interactions, the B–library or L–library complexes, were eliminated since the binding constant of avidin–biotin complex formation (10^{15} M^{-1}) is much larger than those with other sites of BTHL, including W. Furthermore, the activity of the avidin–biotin interaction increases with the linker length of biotin. By regulating the concentration of added FLS, the case of no interaction in the first step can also be eliminated.



Scheme 1 Schematic drawing of the new selection method using the avidin–biotin interaction: Trp, biotin, avidin (streptoavidin), and the probe (fluorescein) are designated by W, B, A and P, respectively. Schemes (*a*), (*b*) and (*c*) show interactions of the pentapeptide library with W, B and L (linker), respectively. The pentapeptide library theoretically contains 2 480 000 pentapeptide sequences. PS designates PEG-PS resin which contains poly(ethylene glycol)-grafted polystyrene.

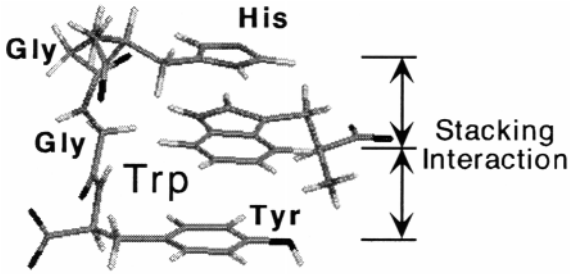


Fig. 1 Structure of the (His-Gly-Gly-Tyr) + Trp complex drawn *via* molecular modeling calculation using QUANTA 96/CHARMm 23.2. The energy of the complex was minimized by the Newton–Raphson method with an adopted basis set. Dynamic simulation of the energy-minimized structure was performed to obtain various conformations as initial coordinates for the calculation. The VERLET algorithm with a time step of 1 fs was used in the dynamic simulation. The system was heated from 0 to 1000 K for the conformational search in the MD simulation. All calculations were performed by running IRIX 5.3 on a Silicon Graphics Indigo2 workstation.

The library beads were subsequently poured carefully onto a plate. By exposing the pentapeptide library beads on the plate to UV light, only the beads which contain the avidin–biotin complex between FLS and TLB are able to generate fluorescence light. As a result, fluorescent beads (positive for the target molecule) were observed in the 2.5 million pentapeptide library. The fluorescent beads which contain the pentapeptide selective for Trp (target molecule) were physically removed from the library and subsequently microsequenced by protein sequencer. A total of ten pentapeptide sequences from the library were determined.

A consensus sequence of the selected peptides is Tyr/His-Gly-Gly-Tyr or His-Pro-Gly-His. The binding behavior of tryptophan with the peptide (Glu-His-Gly-Gly-Tyr) immobilized on the resin was analysed by a Langmuir isotherm. The binding constant was approximately 1.2×10^3 M^{-1} at room temperature. The consensus sequence has aromatic residues (His and Tyr) at the *i* and *i* + 3 positions, and a glycine residue at the *i* + 2 position in the motif can induce a β-turn structure.[13,14] When the motif forms the β-turn structure, two aromatic residues may orient in the same side to produce the sandwiching interaction with Trp between the two aromatic residues possible. To estimate the detailed interaction of the motif peptide (His-Gly-Gly-Tyr) with Trp, energy minimization calculations were performed using QUANTA 96/CHARMm 23.2. The result shows that the motif peptide may form a β-turn structure and the (His-Gly-Gly-Tyr) + Trp complex forms a stacking interaction between the two aromatic residues (His and Tyr) and Trp *via* the sandwiching interaction shown in Fig. 1. It was suggested that the motif peptide specifically recognizes Trp *via* the β-turn motif.

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Notes and references

† A split synthesis approach was carried out with standard peptide synthesis methods on solid phase with fluorenylmethoxycarbonyl (Fmoc) chemistry. Coupling was initiated by the addition of four-fold molar excess concentration of HATU and an eight-fold molar excess concentration of Pr^i_2NEt. The coupling reaction was driven to completion with a four-fold molar excess of Fmoc amino acids and monitored by the standard ninhydrin test. Occasionally, double coupling was needed. The resin was first divided into 19 aliquots of poly(ethylene glycol)-grafted polystyrene (PEG-PS) × HCl which has a hydrophilic linker (PEG) and was chosen as a solid phase support.

‡ Biotinyl-ε-aminocaproic acid *N*-hydroxysuccinimide and Boc-AEEA-OH were purchased from Calbiochem-Novabiochem Corporation and PerSeptive Biosystems, respectively. The coupling was carried out with *tert*-butoxycarbonyl (Boc) solid phase synthesis methods.

§ All experiments were conducted in buffer including 100 mM NaCl, 10 mM Na_2HPO_4 and 1 mM Na_2EDTA (pH 7.0) at room temperature in a same empty column.

¶ The composition of fluorescein labeled streptoavidin is F/S (molar) = 4.6 where F and S indicate fluorescein and streptoavidin, respectively. It was purchased from Vector Laboratories.

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