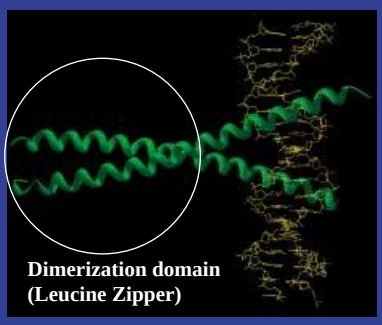
Design of new DNA binding peptides

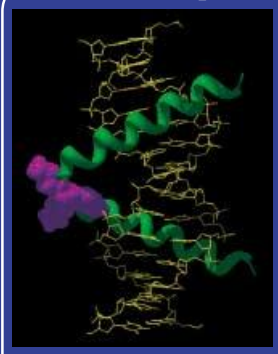
Structure-based design of a protein-DNA complex affords short peptides with an artificial dimerization domain. The strategy enables a specific formation of noncovalent peptide dimers with preorganized orientation on sepcific DNA sites.

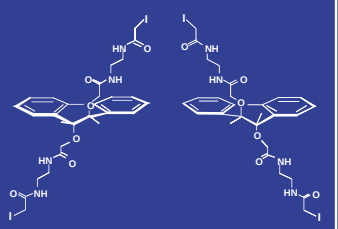


An X-ray crystal structure for the complex between GCN4 basic leucine zipper motif (bZIP) and DNA.

Structure-Based Design

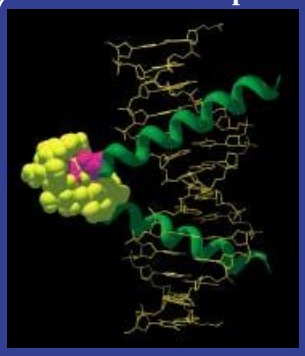
Covalent Peptide Dimers

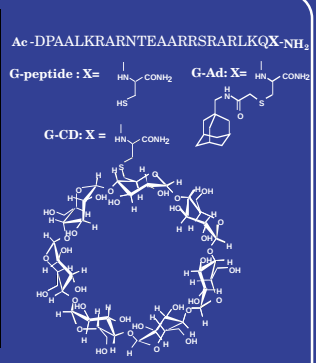




Sequence-specific DNA binding with sub nanomolar affinity.

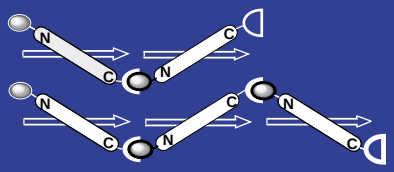
Noncovalent Peptide Dimers



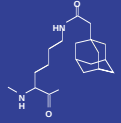


Cooperative DNA binding of homo- and hetero peptide dimers.

Cooperative Oligomerization of Short Peptides on DNA

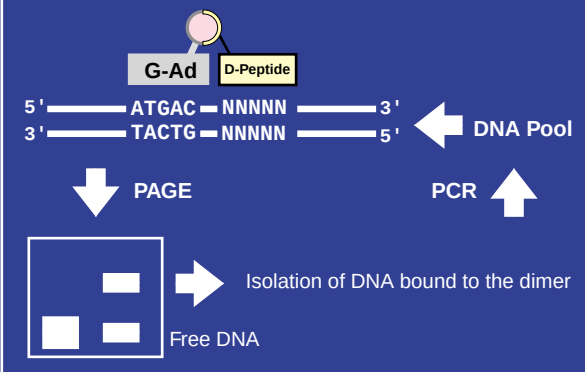


G-Ad-CD: Ac-DXAALKRARNTEAARRSRARKLQZ-NH2
X = Lys(Ad), Z = Cys(CD)



Efficient sequence discrimination mechanism by reducing the nonspecific DNA binding of peptide.

Strategy to Determine a Target DNA Sequence of a Short Peptide



Determination of preferential DNA-binding sequences of a D-peptide.

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Chemical Approaches Untangling Sequence-Specific DNA Binding by Proteins

Shin-ichi Sato,[a] Masaki Hagihara,[a] Kenji Sugimoto,[a] and Takashi Morii*[a, b]

Abstract: Structure-based design of novel DNA-binding proteins provides an ultimate test of our understanding of protein–DNA interactions. A combination of synthetic, organic, biochemical and molecular biological approaches has been developed to study the principle of molecular recognition associated with the protein–DNA interactions. The strategies enabled a specific formation of noncovalent peptide dimers and determination of the preferential DNA-binding sequence of short peptides.

Keywords: DNA recognition · host–guest systems · molecular recognition · protein design · peptide design

Introduction

High-resolution structures of sequence specific protein–DNA complexes provide a far greater understanding of how proteins and DNA assemble and function within these complexes to perform essential cellular processes.[1–4] While a universal code for the recognition between proteins and nucleic acids has yet to be generalized,[5] several features of how the proteins recognize specific DNA sequences have emerged: the proteins use relatively small regions to contact directly several base pairs of DNA, and most of the sequence-specific DNA binding proteins are active only in a dimeric form or in a multi-protein complex. The small regions often form α-helices, termed "recognition helices" in direct contact with the DNA major groove. Because most of the direct contacts between the amino acids and nucleic acid bases are made within such recognition helices, the rest of proteins can be regarded as architecture to position the recognition helices in a proper geometry with respect to the specific DNA sequences.[1, 2] The shape and size of the dimerization module are critical for final positioning of the recognition helices. The sequence-specific DNA binding of homo- or heterodimers by transcription factors and bacterial repressors is modulated by a combination of protein–protein and protein–DNA contacts and offers the simplest example of the recognition event including both the protein–DNA and the protein–protein interactions.

DNA-binding proteins generally consist of more than two DNA contacting regions to ensure recognition selectivity.[1, 2] One class of proteins binds DNA with multiple DNA binding modules connected through covalent linkages and the other with noncovalent formation of homo- and heterodimers. In the former class of proteins, the greatest progress to date has been achieved by studying the sequence specific DNA binding of the C_2H_2 zinc finger proteins.[6, 7] In the later class of proteins, the protein–protein interactions controlling the dimerization are considered to play significant roles in enhancing specificity of DNA binding and increasing the sensitivity of equilibrium binding to the change in protein concentrations.[1, 2] Because structural elements of natural proteins are harder to separated, many model systems have been developed in the structure-based fashion to understand the functional roles of the dimerization on the sequence-specific DNA binding properties of dimeric proteins to study the role of dimer formation in modulating the affinity and cooperativity of protein–DNA interactions. The model systems described here address the issues of protein–protein and protein–DNA recognition in far greater detail than is possible with native protein systems.

Covalently Linked Peptide Dimers

One of the best structurally characterized bZIP family of proteins is the yeast transcription activator GCN4.[8, 9] GCN4 is known to bind DNA as a homodimer with each basic region directly contacting the major groove of DNA. The native GCN4 dimer specifically binds 5′-ATGACTCAT-3′ (AP1) and 5′-ATGACGTCAT-3′ (CRE) sequences with similar affinity. The dimerization is mediated through a coiled-coil structure in the leucine-zipper domain that is located at the C-terminus of the basic region. The basic region of GCN4 has

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disordered structure when complexed with a nonspecific DNA, but is structured to an α-helix upon binding to a specific DNA sequence. The basic-region of the basic leucine zipper (bZIP) protein serves as the simplest short peptide that targets DNA sequences four to five base pairs in size. However, DNA binding affinity of the short basic region peptide is inherently low, and it is difficult to analyze sequence specific DNA binding without forming dimers of the peptide to achieve DNA binding of convincible sequence selectivity.

Kim and co-workers found that disulfide-bonded dimers of GCN4 basic region peptides specifically bound the AP1 sequence (5′-ATGACTCAT-3′) as observed for the native GCN4; this encouraged the use of the basic region as a DNA binding unit for the design of novel DNA binding peptides.[10] A peptide corresponding to the basic region of GCN4 was synthesized with a Gly-Gly-Cys linker added at the carboxyl terminus. The Gly-Gly-Cys was included to provide a flexible linker in the disulfide-bonded dimer. The 34-residue basic region peptide dimer binds an oligonucleotide containing the GCN4 recognition element (AP1 site) in a helical structure.

It is now possible to design peptide dimers that possess DNA binding specificities different from the native bZIP by appropriately arranging two basic region peptides with an artificial dimerization domain, such as a bulky metal complex[11, 12] or an enantiomeric bridged biphenyl derivative.[13–17] Size, shape, and chirality of the dimerization domain affect significantly DNA binding by peptide dimers. A bis(terpyridyl)iron(II) complex, $[G29TS]_2Fe$, was used in place of the GCN4 coiled coil to assemble two GCN4 basic region peptides (29 residues).[11] With a bulky metal complex as a dimerization domain for the basic region peptide of GCN4, the dimeric peptide $[G29TS]_2Fe$ preferentially bound the CRE sequence over the nonpalindromic AP1 sequence by a factor of 150. Because $[G29TS]_2Fe$ and the disulfide-linked G29 peptide dimer binds CRE sequence with comparable affinity; the shape, size and possibly the positive charge of the terpyridyl metal complex reduced the affinity of $[G29TS]_2Fe$ to the AP1 sequence. Also, one surprising effect of the chiral bridged biphenyl templates is a marked reduction in the affinity of the peptide dimer to nonspecific sequences.[16] An N-terminal basic region peptide dimer with chiral bridged biphenyl template targets specific 5′-GTCATATGAC-3′ sequence with a dissociation constant (K_d) of 0.1 nM, while it binds 5′-ATGACGTCAT-3′ sequence with K_d of 363 nM. When a template with opposite chirality is used, the resulting N-terminal basic region peptide dimer binds the specific and nonspecific sequences with K_d of 0.15 and 14 nM, respectively. Such destabilization of nonspecific binding complexes have also been observed for MyoD-derived peptide dimers.[14] The chiral template reduces the affinity of the peptide dimer for the nontargeted sequence, rather than increasing the affinity for specific sequences as has been suggested for the DNA binding of metallo-peptides.[11] The strategy of reducing nonspecific binding would help in the design of second-generation homo- and heterodimers that recognize a variety of DNA sequences with high selectivity.

Noncovalent Peptide Dimers: Dimerization Promoted by a Host–Guest Inclusion Complex

The noncovalent interaction between two monomers is another important feature in the sequence-specific DNA binding by protein dimers. The equilibrium governing the formation of dimers would be important in enhancing the selectivity of DNA binding and in increasing the sensitivity of DNA binding to change in protein concentrations. In order to understand the role of noncovalent dimer formation in modulating the affinity and cooperativity of protein–DNA interactions, DNA binding oligopeptides capable of forming a functional quaternary structure by noncovalent interactions were designed.

A host–guest inclusion complex of β-cyclodextrin (CD) and adamantane (Ad) provides a new method to associate oligopeptides in aqueous solution.[18] This module in essence mimics the specific protein–protein interactions that govern formation of homo- and heterodimers. A peptide modified with β-cyclodextrin and another peptide modified with an adamantyl group formed a noncovalent dimer that was mediated by formation of a 1:1 β-CD/Ad inclusion complex (Figure 1). The stability of the β-CD/guest dimerization

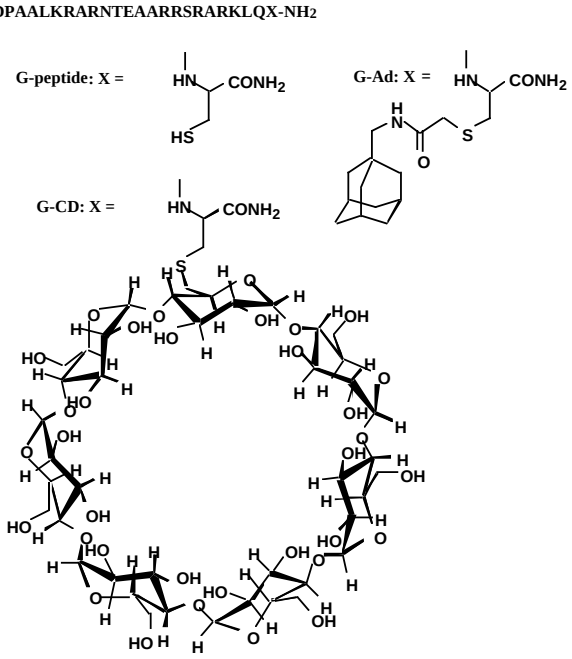

Figure 1. Amino acid sequence for the G-peptide and structures of the modified C-terminal Cys residue (X). The cysteine residues were modified with 6-deoxy-6-iodo-β-cyclodextrin for G-CD and with *N*-bromoacetyl-1-adamantanemethylamine for G-Ad.

domain can be independently controlled by changing the guest molecules without greatly affecting the DNA binding ability of the peptide itself.[19]

The GCN4 basic region peptide (G-peptide) modified at the C-terminus with an adamantyl group indeed dimerized with another peptide possessing the β-cyclodextrin attached to the C-terminus; the peptide dimer further showed specific DNA binding to the native GCN4 site (Figure 2). Binding

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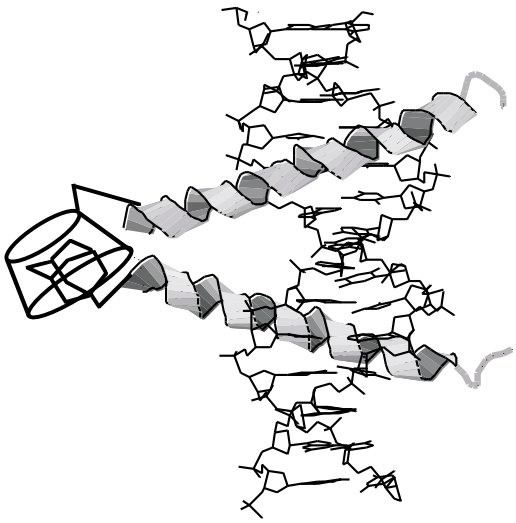

Figure 2. A schematic representation showing the G-Ad/G-CD dimer bound at the AP1 sequence. Gray ribbons represent the basic region peptides in the helical conformation. The β-cyclodextrin and the adamantyl group are shown at the C-terminus of the helices. Coordinates for the basic region peptides and DNA are adopted from the GCN4–AP1 complex.[6]

mixtures containing a 1:1 mixture of G-Ad and G-CD showed gradual appearance of a complex with lower electrophoretic mobility than CRE; this indicates that G-Ad and G-CD bind to DNA as a dimer. Half-maximal DNA binding occurs with $\approx$20 nM peptide for the CRE site, while G-Ad alone did not show any detectable binding to CRE in a dimeric form. The cyclodextrin–adamantane system effectively modulate a cooperative formation of peptide dimer–DNA complex.

Sequence-Specific DNA Binding by Peptide Heterodimers

Transcription factors often form heterodimers that recognize nonpalindromic DNA sequences with each monomer of the heterodimer binding to each half-site. The β-CD/guest dimerization domain effectively regulates specific formation of heterodimer.[20] Modification of a peptide by β-CD and another peptide by a guest molecule allows specific formation of a heterodimer of peptides that recognizes a nonpalindromic DNA sequence; this sequence in turn consists of two distinct half-sites corresponding to native protein binding sequences. The DNA-binding regions of two different basic leucine zipper proteins, the yeast transcriptional activator GCN4 (G-peptide) and an enhancer binding protein C/EBP (C-peptide), have been successfully used to design heterodimers that recognize nonpalindromic DNA sequences. Modification of the C-terminal cysteine of the peptides with mono-6-deoxy-6-iodo-β-cyclodextrin (CD) or *N*-bromoacetyl-1-adamantanemethylamine (Ad) afforded four different peptides (G-Ad, G-CD, C-AD, and C-CD) that are capable of forming specific homo- (G-Ad/G-CD and C-Ad/C-CD) and heterodimers (C-Ad/G-CD and C-CD/G-Ad). GCN4 and C/EBP are known to recognize palindromic sequences with a half-site of 5′-ATGAC-3′ (CRE) and 5′-ATTGC-3′ (CE), respectively. Combination of these half-sites gives a nonpalindromic sequence (CE/CR), 5′-ATGACGCAAT-3′, for the target of the peptide heterodimer. The heterodimer C-CD/G-Ad preferentially bind to the CE/CR sequence over the palindromic CRE and CE sequences, while homodimers G-Ad/G-CD and C-Ad/C-CD bind to the palindromic CRE and CE sequences, respectively.

Cooperative DNA Binding by Peptide Oligomers

Both homo- and heterodimers consisting of an adamantyl peptide and a β-cyclodextrin–peptide can target the palindromic and/or nonpalindromic DNA sequences. This strategy has been extended to cooperative DNA binding by peptide homo-oligomers of an oligopeptide derived from the basic region of GCN4.[21, 22]

A modified lysine residue bearing an adamantyl group at the ε-amino group was incorporated at the N-terminal position, and β-cyclodextrin was attached at the C-terminal cysteine residue of the parent basic region peptide (Figure 3).

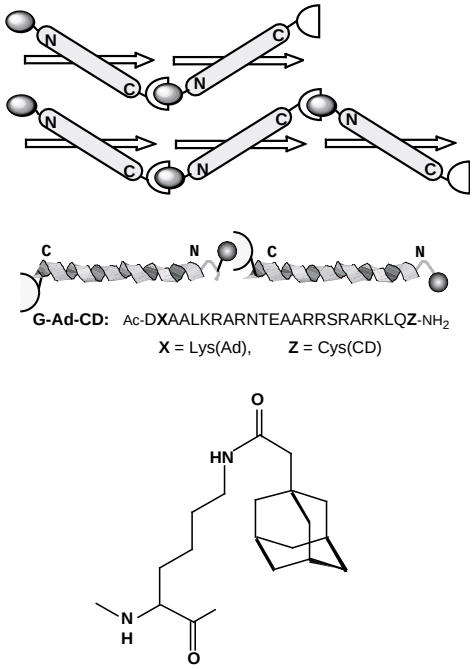

Figure 3. Schematic representations showing the G-Ad-CD homo-oligomer bound on the direct repeat of CRE half-site. Hatched bars represent the basic region peptide. N and C indicate the N-and C-terminus of the peptide, respectively. Half-circles at the C-terminus and filled circles at the N-terminus represent β-cyclodextrin and the adamantyl group, respectively. White arrows denote the CRE half-site. Also shown is a structure of Lys(Ad).

The resulting G-Ad-CD peptide possesses both host and guest moieties in the same peptide chain. The peptide without β-CD (G-Ad) binds direct-repeat sequences of a half-site of the native GCN4 binding site without any cooperativity. DNA binding of the G-Ad-CD peptide to single-, double- and triple-direct-repeats of the CRE half-site was compared using the Electrophoretic Mobility Shift Assay (EMSA). Interestingly, the G-Ad-CD peptide did not bind an isolated CRE

half-site (5′-ATGAC-3′), while a monomeric peptide lacking the β-cyclodextrin group formed a specific monomer-half site complex. G-Ad-CD bound the double-direct-repeat sequence (T2: 5′-ATGAC-ATGAC-3′) as a dimer in a cooperative manner. Moreover, cooperative formation of a 3:1 G-Ad-CD – DNA complex was observed for a triple-direct-repeat sequence with no monomer-DNA complex of G-Ad-CD being observed for the double- or triple-direct-repeat sequence.

G-Ad-CD binds to the target DNA sequence with a head-to-tail dimer configuration. The same head-to-tail configuration of a noncovalent heterodimer was achieved by using two basic region peptides: one with the N-terminal adamantyl group (AdG) and the other with the C-terminal CD (G-CD). G-Ad-CD showed much higher sequence selectivity to discriminate a single base-pair mutation within the target T2 sequence (Figure 4A).

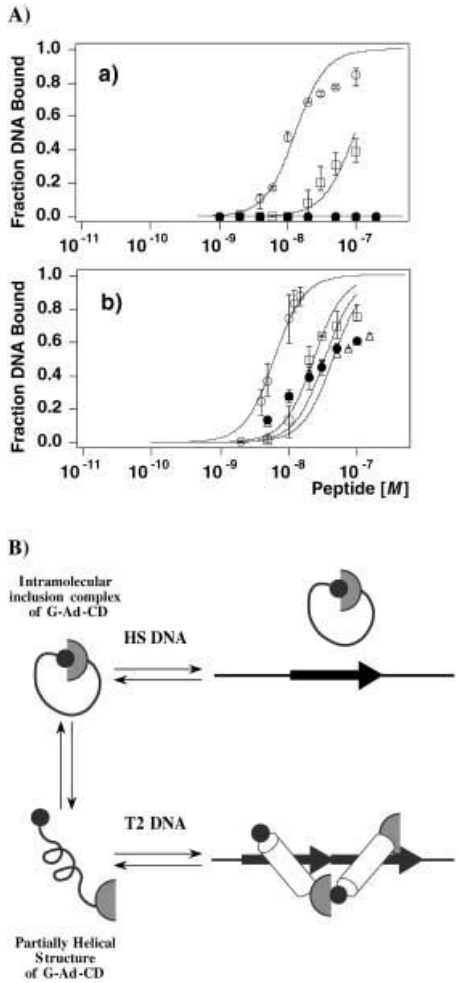

Figure 4. A) Semilogarithmic plots showing the fractions of ^{32}P-labeled T2 (○: 5′-ATGAC-ATGAC-3′), G3A (●: 5′-ATGACAT*A*AC-3′), HS (△: 5′-ATGACA*CTG*C-3′), and T2S (□: 5′-ATGAC*C*ATGAC-3′) bound to the G-Ad-CD (a) and G-Ad/G-CD (b) dimers as a function of peptide concentration. The solid curves represent the best fit for the titration data. B) G-Ad-CD forms the intramolecular inclusion complex in the absence of specific DNA sequence and forms an α-helical trimer – DNA complex with T2 DNA. The intramolecular inclusion complex remains stable in the presence of HS DNA. Half-circles and filled ovals represent δ-cyclodextrin and the adamantyl group, respectively. Filled arrows denote the 5′-ATGAC-3′ half-site. The cylinders represent the helical form of G-Ad-CD.

In the absence of DNA, G-Ad-CD forms an intramolecular host – guest complex (Figure 4B). Formation of this cyclic peptide appears to reduce the affinity of monomeric G-Ad-CD to the CRE half-site as compared to that of G-Ad. The observed highly selective binding of G-Ad-CD was accomplished by i) its cooperative nature of DNA binding and by ii) destabilization of its nonspecific DNA binding complex.

Design of DNA Binding Peptide Units

Native sequence-specific DNA binding proteins mostly bind in the major groove by using a simple secondary structure, usually an α-helix, which is complementary to the structure of the B-DNA major groove. Structural studies and sequence comparisons revealed that many DNA-binding proteins could be grouped into classes that use related structural motifs for recognition. It seems reasonable to assume that they may provide the most convenient scaffolds for design of new DNA-binding peptides and/or proteins.[1, 2] The X-ray crystallographic and NMR studies provide detailed information about the stereospecific interactions between amino acid residues in the protein and the functional groups on DNA. With this knowledge, design and synthesis of a novel DNA binding motif composed either of native or non-natural amino acids is possible. Such a study is a crucial test of our understanding of molecular recognition, and the results will aid in expanding our knowledge of sequence-specific DNA recognition mechanisms.

A major drawback of such studies is that the designed peptide usually possesses low DNA binding affinity and specificity. Determining sequence preference of weak DNA binders has been problematic because their low affinities complicate experimental results and usually produce unclear outcomes. This problem has been tackled by combining the selected and amplified binding site (SAAB) imprinting technique and cyclodextrin – adamantane dimerization module.[23]

A DNA pool containing a randomized five base-pair site (N5), which provides enough binding site size for a short peptide adjacent to 5′-ATGAC-3′ is chemically synthesized to cover all possible binding sequences for the heterodimer. The basic region peptide of GCN4 modified by the adamantyl group at its C-terminal cysteine (G-Ad) anchors at the 5′-ATGAC-3′ sequence in a heterodimer, thereby facilitating to locate the other peptide in the major groove of the randomized N5 sequence. A mixture of the 5′-^{32}P-end-labeled DNA pool and peptide dimer is separated into the peptide dimer-bound and the unbound DNA by polyacrylamide gel electrophoresis (PAGE). The peptide dimer-bound DNA is recovered from the gel and amplified by the polymerase chain reaction (PCR). The resultant DNA pool should contain a higher population of specifically bound by heterodimer, implying that the fraction of dimer-bound DNA increases with SAAB cycles. The SAAB cycle is repeated until no further increase in binding efficiency is observed. The resulting DNA is cloned and sequenced (Figure 5). The G-Ad peptide tethers a low-affinity DNA-binding peptide adjacent to a GCN4 binding sequence (5′-ATGAC-3′)

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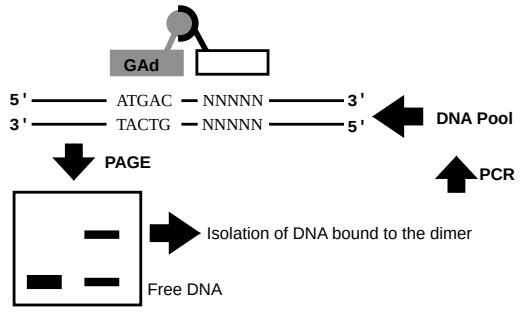

Figure 5. A SAAB cycle to determine a target sequence of short peptide (open box) by using its heterodimer with G-Ad (hatched box).

through the cyclodextrin–adamantane association, thereby increasing local concentration of the low-affinity peptide to degenerate neighboring DNA sequences. In principle, an increase of local concentration makes a "pseudo high-affinity peptide" of the low-affinity peptide, which allows the SAAB imprinting technique to work.

This combined technique succeeded in finding the C/EBP half site, 5′-GCAA(T/C)-3′, when ten rounds of SAAB cycles were applied for a short basic region peptide of the transcription factor C/EBP. Moreover, sequence-preference of a D-enantiomer of the GCN4 basic region peptide, which is expected to be a very weak DNA binder, has been determined after twelfth round of SAAB. The D-enantiomer of GCN4 peptide discriminated the obtained 5′-ACACA-3′ sequence from other sequences, such as 5′-ATGAC-3′ or 5′-ACTGC-3′. The result opens the possibility for design of DNA-binding D-peptides.

Once the sequence preferences of weak binders are determined, high-affinity ligands for a particular DNA sequence may be designed by simply tethering them together. The technique can also be extended to analyses of sequence preferences of organic molecules, especially basic oligomeric compounds, such as oligo-aminosaccharides, which have an intrinsic tendency to bind DNA. Application of this technique to organic molecules may permit the design of small molecules that bind a particular DNA sequence, but not a desired sequence. The heterodimer system can be extended to a library approach to obtain D-peptides that bind desired sequences. This would be conceptionally similar to the phage display technique reported in 1997, in which zinc finger proteins that recognize desired DNA sequences were discovered by extending a zinc finger module across the desired 9- or 10-base pair target site.[24] However, the advantage of the present technique is synthetic accessibility, as it is potentially applicable to any chemical entity.

Conclusion

The atomic view of DNA–protein interactions has changed how DNA binding peptides or protein mimetics are designed. The covalent dimerization module would be suitable for making high-affinity DNA binding ligands. The noncovalent dimerization strategy would achieve a narrower range of peptide concentrations required for saturating the specific DNA sequence. The mechanism involving an equilibrium that leads to a formation of less active DNA binding species, such as a conformationally altered monomer, would be quite important for increasing the selectivity and for exerting the cooperative nature of the DNA–protein complex formation. While structure-based design of new sequence-specific DNA binding proteins will occupy center stage, still much work remains to be done. The strategies mentioned here have successfully been used for design of DNA binding peptides derived from basic region of leucine zipper proteins, but it is not yet clear whether the same strategies could be applicable for other DNA binding motifs. In addition, short disordered regions such as the basic region of leucine zipper proteins are not suitable for DNA binding at physiological temperatures.[25, 26] Is it possible to design α-helices that can fit in the major groove to recognize any given three to four base pairs? It is not yet clear whether the α-helical motif has characteristic DNA sequences that are suitable for the binding site. Attempts to design novel DNA-binding proteins and/or peptides will provide an ultimate test of our understanding of protein–DNA interactions.

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