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Review

Interactions and uses of antisense peptides in affinity technology

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ABSTRACT

Antisense peptides, amino acid sequences encoded in the antisense strand of DNA, can interact with significant affinity and selectivity with their corresponding sensepeptides. Experimentally, sense–antisense peptide recognition has been observed repeatedly. However, skepticism about the biological relevance of this phenomenon has persisted. This is due in part to the unexpected and somewhat couterintuitive nature of the interaction as well as to its non-universality as an empirical observation. Nonetheless, antisense peptides in several cases investigated so far have been used as immobilized ligands for the succesful affinity chromatographic separation of native (sense) peptides and proteins. For example, immobilized antisense peptides corresponding to Arg^8-vasopressin (AVP) have been used to separate vasopressin from oxytocin chromatographically as well as to affinity capture AVP–receptor complex. These results, together with improved understanding of the general features of amino acid sequence which drive antisense–sense peptide interactions as well as new ideas for making antisense peptides chimeras, are beginning to suggest improved ways to make antisense-related peptides as affinity agents for separation as well as for other biotechnology applications.

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1. INTRODUCTION

Antisense peptides are sequences of amino acids encoded in the antisense strand of DNA (Fig. 1). Such peptides are not believed to be synthesized cellularly except in some simple organisms such as phage and viruses. However, they can be synthesized chemically. Synthetic antisense peptides have been found to have unexpected interaction properties. As predicted by Mekler [1] and first shown experimentally by Bost *et al.* [2], an antisense peptide can interact with some degree of selectivity with the corresponding sense peptide. In the first experimental studies [2], adrenocorticotropic hormone (ACTH) was found to interact with the antisense peptide encoded in the strand of DNA antisense to the sense strand encoding ACTH. Since this early observation, a number of other cases of sense–antisense peptide interaction have been identified [2–13] (see Table 1), and a growing body of quantitative

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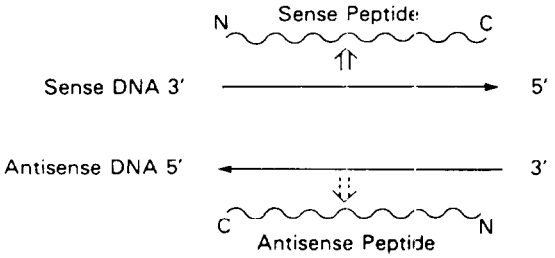

data has been obtained on antisense–sense peptide binding using analytical solid phase methods including analytical affinity chromatography [4,8,9,12].

The biological relevance of antisense peptide interaction with sense peptide has met with considerable skepticism. This is in part due to evidence that such peptide recognition is not universally observed. Skepticism has been fueled additionally by the lack of full mechanistic understanding for the antisense–sense interaction process (see below). On the issue of non-universality, no interaction was observed between angiotensin II and its antisense peptide by Guillemette *et al.* [16], De Gasparo *et al.* [17], and Eaton *et al.* [18]. And neither Najem *et al.* [15] nor Eberle and Huber [14] observed an interaction between ACTH and its antisense peptides. Angiotensin II and ACTH represent two cases for

TABLE 1

EXPERIMENTAL OBSERVATIONS OF ANTISENSE PEPTIDE RECOGNITION

Sense peptide or protein denoted in bold, AS peptide size given in parentheses.

System	Citation [ref. no.]
ACTH (1–24) (24 res. AS)[a]	Bost, Smith and Blalock, 1985 [2]
δ-Endorphin (17 res). (17 res. AS)	Bost, Smith and Blalock, 1985 [2]
IL-2 Related fragment (6 res). (6 res. AS)	Weigent, Hoeprich, Bost, Brunck, Reiher and Blalock, 1986 [3]
RNase S-peptide (20 res.) (10-20 res. AS)	Shai, Flashner and Chaiken, 1987 [4]
Fibronectin (2000 res.) (6 res. AS)	Brentani, Ribeiro, Potocnjak, Pasqualini, Lopes and Nakaie, 1988 [5]
Angiotensin II (8 res.) (8 res. AS)[b]	Elton, Dion, Bost, Oparil and Blalock, 1988 [6]
Insulin (51 res.) (6 res. AS)[c]	Knutson, 1988 [7]
Arg^8-vasopressin-GKR (12 res.) (12 and 20 res. AS)	Fassina, Zamai, Brigham Burke, Chaiken, 1989 [9]
Neurophysin II (95 res.) (8 and 20 res. AS)	Fassina, Zamai, Brigham-Burke and Chaiken, 1989, [9]
Substance P (11 res.) (11 res. AS)	Pascual, Blalock and Bost, 1989 [10]
Cystatin C (120 res.) and fragment 55-59 (4 res. AS)	Ghiso, Saball, Leoni, Rostagno and Frangione, 1990 [11]
c-raf protein and fragment 356-375 (20 res. AS)	Fassina, Roller, Olson, Thorgeirsson and Omichinski, 1989 [12]
α-MSH and β-MSH (13 and 18 res.) (13 and 18 res. AS)	Al-Obeidi, Hruby, Sharma, Hadley and Castrucci, 1990 [13]

[a] Failed to be observed by: Eberle and Huber, 1991, by inhibition of receptor action [14]; Najem, Corigliano-Murphy and Ferretti, 1989, by NMR [15].

[b] Failed to be observed by: Guillemette, Boulay, Gagnon, Bosse and Escher, 1989, by inhibition of receptor action [16]; De Gaspora, Whitebread, Einsle and Heusser, 1989, by inhibition of ligand-receptor interaction [17]; Eaton, Austin, Fesik and Martin, 1989, by NMR [18].

[c] K_d observed in nM in this case [7], compared to the mM–μM K_d values observed for most other cases.

which earlier data (refs. 2 and 6, respectively) had demonstrated and antisense peptide interaction. It is possible that some of the above-cited failures to observe sense–antisense peptide interactions could result from inappropriate analytical procedures which would not detect weak-affinity interactions (typical for most antisense–sense peptide recognition) and interactions which are conformationally degenerate (again, a property typical for this type of peptide interaction [12]). Nonetheless, there is no biological or mechanistic principle identified so far which would argue that all antisense peptides should interact with their sense peptides with experimentally observable affinity. Thus, at least for now, it appears likely that antisense–sense peptide recognition is not a universal phenomenon.

In spite of the caveats, the repeated observation of selective antisense peptide recognition of sense peptides has led to attempts to use this type of recognition process in molecular separation. Thus, antisense peptides have been immobilized on affinity chromatographic supports and tested for their ability to selectively separate native (sense) peptides, proteins and complexes containing these molecules. The results obtained so far suggest a generally useful new approach to peptide and protein separation science in which affinity chromatographic supports are designed with immobilized ligands (peptides and eventually their mimics) to target separation of a particular native sequence (or general class of native sequences). The current paper summarizes some initial observations of selective chromatographic separation of native peptides using immobilized antisense peptides, the range of peptides and proteins tried so far, and related use of the immobilized antisense peptides to affinity capture– receptor complexes containing sense peptide as part of a multimolecular complex. Some comments also are added on the emerging understanding of mechanism of antisense peptide recognition which may help guide the redesign of these peptides as separation agents.

2. SELECTIVITY IN THE ARG^8-VASOPRESSIN–BOVINE NEUROPHYSIN II SYSTEM

The chromatographic selectivity of immobilized antisense peptides for eluting sense peptides was first shown in the Arg^8-vasopressin system [9]. The root of this observation actually was an attempt to evaluate antisense peptide selectivity in the vasopressin–neurophysin system. An antisense (AS) peptide was synthesized based on the antisense DNA strand corresponding to the amino terminal 20 residues of proAVP/BNPII, the biosynthetic precursor of arginine vasopressin (AVP) and bovine neurophysin II (BNPII). The carboxyl terminal 9 residues of this peptide are antisense to AVP, whereas the amino terminal 8 residues of the peptide are antisense to the N-terminal 8 residues of BNPII. The 20-residue antisense peptide binds to both immobilized AVP and BNPII. More importantly in the effort to establish specificity, fragments of AS(proAVP–BNPII) that are antisense to AVP bind only to immobilized AVP but not to immobilized BNPII. In contrast, the fragment that is antisense to BNPII binds only to immobilized BNPII and not to immobilized AVP.

Based on these results, AVP antisense peptides were immobilized and tested for chromatographic effectiveness and selectivity [9]. When the full 20-residue AS(proAVP–BNPII) is immobilized on a solid support, the resulting affinity column binds both AVP and BNPII more efficiently than the two structurally-related polypeptides, oxytocin and BNPI (oxytocin-associated neurophysin). The fragment of AS(proAVP–BNPII) that is antisense to AVP also shows differential affinity for several peptides that are structurally related to AVP. With antisense peptide immobilized (defined as M) and the various sense peptides eluting as soluble peptides (defined as P), the heirarchy of affinities measured was AVP ($K_{M/P} = 5.3 \cdot 10^{-5}$ M) > arginine vasotocin ($K_{M/P} = 8.6 \cdot 10^{-5}$ M) > lysine vasopressin ($K_{M/P} = 1.1 \cdot 10^{-4}$ M) > oxytocin ($K_{M/P} = 5.3 \cdot 10^{-4}$ M). The differential affinity of the immobilized antisense peptide support for AVP *versus* oxytocin is sufficient to chromatographically resolve these two neuropeptides from each other with close to baseline separation (Fig. 2).

Vasopressin-oxytocin separation by immobilized antisense peptide has several similarities to other, more established types of chromatography. The interactions of analyte with solid phase are weak, similarly as found in such cases as ion-exchange chromatography. Chromatographic separations by weak interaction can work if there is sufficient capacity (number of theoretical plates of separation).

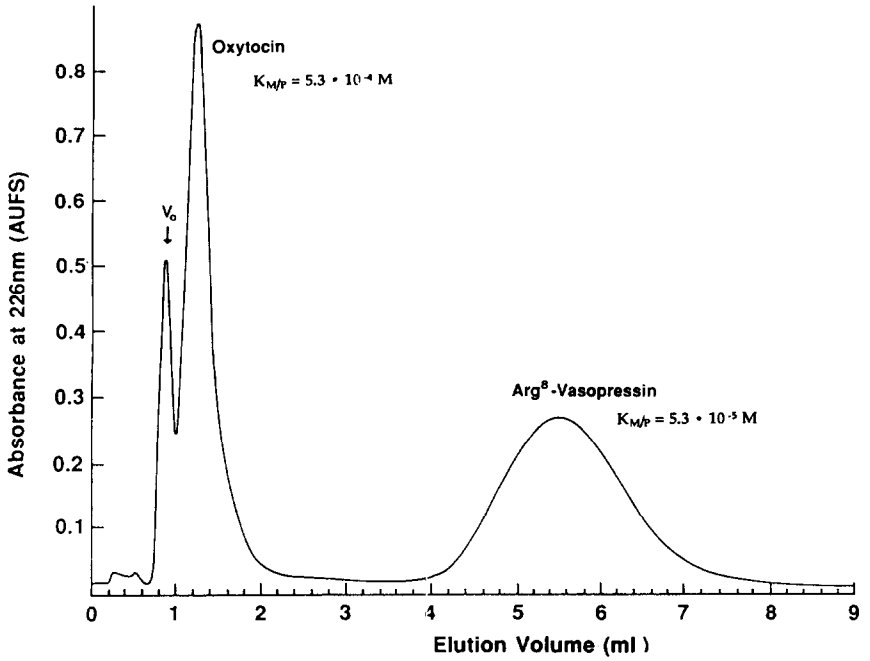

Fig. 2. Separation of arginine[8]-vasopressin and oxytocin (AVP and OT) on AS(proAVP–BNPII) 12 mer immobilized on ACCELL (Waters-Millipore). The sample containing 100 μg each of a mixture of AVP and OT, and dissolved in 0.1 *M* ammonium acetate, pH 5.5, was injected onto an immobilized 12-mer column of 15 cm × 3 mm (amount AS peptide attached = 0.223 μmoles), and elution (400 μl/min) was monitored at 226 nm, 1.0 AUFS. Peak identities were verified by comparison with authentic peptide elution positions and by amino acid analysis. The affinity chromatographic peptide separation was carried out on a Beckman System Gold liquid chromatograph. Figure adapted from ref. 9.

When sufficient capacity is attainable, weak interactions with a chromatographic support make it possible to achieve gentle elutions, often by isocratic elution with non-chaotropic solvents. This is the case in Fig. 2, for which a ten-fold difference in affinity is sufficient to achieve separation by using isocratic elution under binding conditions.

The immobilized antisense peptide separation of vasopressin also represents an example of general ligand affinity chromatography. While antisense peptides are selective, chromatographic overlap is likely given the nature of antisense peptide recognition as a composite (see below) of rather generic hydrophilic and hydrophobic interactions with limited conformational driving force [4,8,9,19]. At first blush, chromatographic overlap would seem anathema for high selectivity separation, and to some extent overlap can reduce resolution between closely related molecules. However, on the positive side, a particular antisense affinity support could be used to separate several different peptides from crude mixtures by relying on differential affinity to separate multiple interacting forms.

As one example of multimolecular affinity chromatography, vasopressin separation has been accomplished from posterior pituitary extracts [9]. Here, the affinity chromatographic fractionation of one such extract, from posterior pituirary, was carried out on immobilized AS(proAVP–BNPII) 20 mer. This immobilized AS peptide was expected to recognize both AVP and BNPII. Pituitary extracts contain two species of neurophysins, BNPII and BNPI. Gratifyingly, the order of neurophysin and peptide retardation on this antisense peptide column was BNPII > BNPI and AVP > oxytocin (OT).

Chromatographic separation on immobilized antisense peptide affinity supports also has been observed in the c-raf system [12]. The immobilized c-raf antisense peptide was synthesized with a se-

quence corresponding to a fragment of the c-raf protein. The antisense peptide affinity support could bind the synthetic protein fragment (residues 356–375). In addition, recombinant c-raf protein could be separated from crude cell extracts using the antisense peptide affinity support. For this antisense peptide system, the intriguing observation also was made that antisense peptide models containing a more perfected hydropathic oppositeness to the sense peptides interacted with sense peptide with greater affinity than the direct readout antisense peptide. These results not only confirmed the role of amphipathicity as a mechanistic element in sense–antisense peptide recognition but also suggested a general synthetic route to obtain higher affinity ligands, analogous to antisense peptides, for separation purposes (see below).

3. AVP–RECEPTOR COMPLEX AFFINITY CAPTURE

The bulk of quantitative data on antisense peptide interactions with sense peptides suggests that binding occurs by multipoint contact between relatively extended peptide chains (4,8,9, 19). This view led to a prediction, Fig. 3, that sense peptides, when bound to their receptors, might still have enough structural elements exposed to interact with antisense peptides. Hence, a particular immobilized antisense peptide might be useful to affinity capture a corresponding peptide–receptor complex.

This idea has been tested recently with the AVP system [20]. To do this, a solubilized [^{3}H]AVP–receptor complex was obtained from rat liver membranes as a partially purified fraction from ion-exchange chromatography. When, this fraction was eluted on immobilized AS(proAVP–BNPII) 20 mer, a radiolabelled fraction indeed was obtained as shown in Fig. 4. The late-eluting radiolabelled fraction subsequently was treated with a crosslinking agent to covalently attach [^{3}H]AVP to bound protein and the crosslinked forms were examined by gel electrophoresis followed by gel slice counting to determine the molecular weights of label-containing complexes. The major labelled forms so

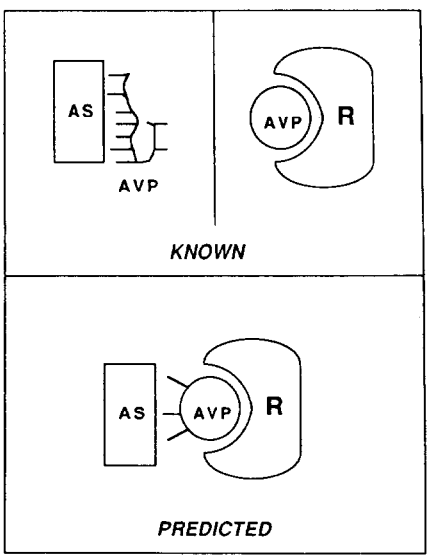

Fig. 3. Predicted binding of arginine^8-vasopressin-receptor (AVP–R) complex to immobilized antisense peptide affinity support. This prediction (bottom) was based on the observed selective binding of AVP to immobilized antisense peptide (upper left; see Fig. 2) and assumption (upper right) that AVP when bound to receptor would have some of its structural elements remaining exposed. Adapted from ref. 20.

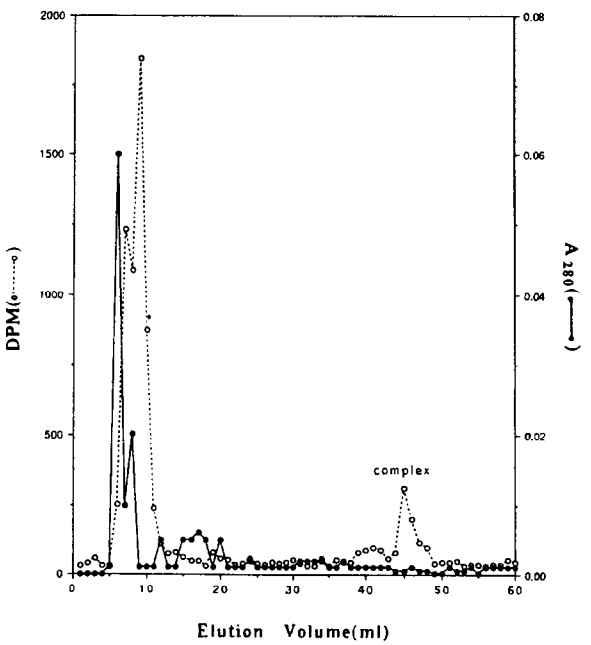

Fig. 4. Affinity capture of [3H]arginine^8-vasopressin–receptor complex on immobilized AS(proAVP–BNPII) 20-mer. Column bed was 2 ml. Elution was isocratic with 0.1 *M* sodium acetate containing 0.005% maltoside (pH 5.4). Flow-rate was 1 ml/min. DPM = desintegrations per minute. Adapted from ref. 20.

identified were of 31 000 and 38 000 dalton. Intriguingly, these molecular weights agree well with those identified before for the AVP receptor.

These data argue that AVP–receptor complex indeed can be affinity captured by immobilized AS peptide. This conclusion was supported by showing that binding of [^{3}H]AVP in the affinity-captured complex could be competed by both AVP and an AVP antagonist selective for rat liver (V1 subtype) receptor (but not by an AVP agonist selective for kidney (V2 subtype) AVP receptor [20]. In addition, elution of [^{3}H]AVP–receptor complex on immobilized AS peptide after crosslinking still yielded a retarded labelled fraction. This fraction could be competed from the matrix with soluble AVP.

Whether antisense peptide affinity capture of sense peptide–receptor complexes in the AVP case can be generalized to other peptide- and/or -protein complexes remains to be determined. Recent preliminary results in our laboratory have shown that the 21-residue AS peptide corresponding to endothelin 1, when immobilized, can bind selectively to eluting ET-1 [21]. Given the emerging availability of ET receptors, this system can provide another opportunity for peptide–receptor affinity capture by immobilized antisense peptide.

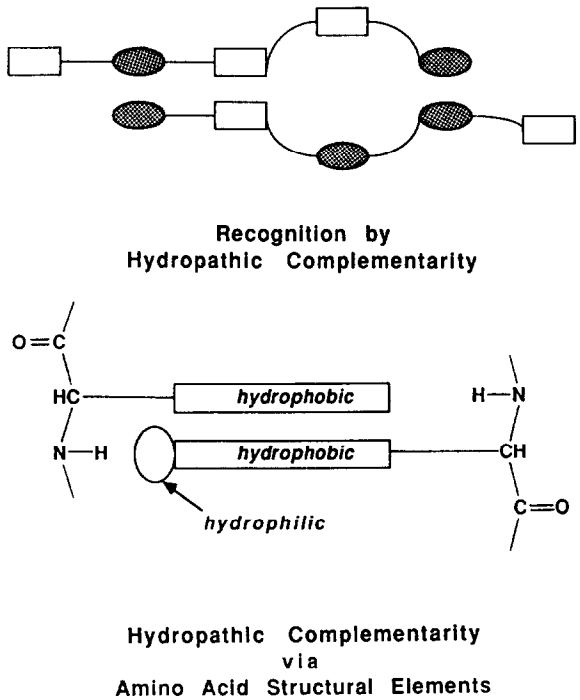

Fig. 5. Scheme depicting (top) hydropathic complementarity between sense and antisense peptides (shaded areas arbitrarily taken as hydrophilic, open areas as hydrophobic) and (bottom) the way such complementarity could occur between hydrophilic and hydrophobic amino acid residues encoded in opposite strands of DNA. Top scheme is adapted from ref. 19, bottom scheme from ref. 24.

4. MECHANISM AND DESIGN WITH ANTISENSE PEPTIDES

Mechanistic understanding of antisense peptide recognition remains incomplete. The nature of the genetic code [1,22] and mechanism-directed experimental data [4,8,9], along with the original ideas of Mekler [1], have led to several current hypotheses. These include models emphasizing a dominant role of hydrophobic interactions of side chain elements of residues [23] as well as models invoking amphipathic recognition through a combination of hydrophobic and hydrophilic forces provided by both main chain and side chain components of residues [24].

Most attempts to explain antisense–sense peptide interactions start with the pattern of hydrophilic and hydrophobic residues built into the genetic code. As pointed out by Mekler [1] and Blalock and Smith [22], when the sense strand of DNA encodes a greatly hydrophobic amino acid (*e.g.* Leu), the antisense strand of DNA encodes a greatly hydrophilic residue (Lys), and *vice versa*. Thus the genetic code dictates that antisense peptides have hydropathic patterns which are opposite those of sense peptides.

At the same time, a significant body of experimental data on antisense–sense peptide binding argues that both hydrophilic and hydrophobic interactions are important. This was shown, for example, by the disruptive effects of both increasing organic solvent and increasing ionic strength on the affinity of AVP with its corresponding antisense peptide [9]. In addition, mutation studies with AS peptides in the ribonuclease system [4,8] show the disruptive effect of replacement of both hydrophilic and hydrophobic groups of the peptide interactors.

One way to explain these results in the light of the pattern of residues encoded in DNA is a model of antisense–sense interaction which envisions amino acid residues as amphipathic, that is having both

hydrophilic and hydrophobic structural elements (Fig. 5). In this view, hydrophobic elements of hydrophilic–hydrophobic residue pairs can interact with each other. These hydrophobic interactions can then shield hydrophilic groups from water so that they can interact with each other. Hydrophilic elements of the residue side chain could interact either with main chain hydrophilic groups or with other side chain hydrophilic groups. In any case the pattern of hydrophilic and hydophobic elements built into the sense and antisense peptide sequences would drive interaction of the amphipathic peptides.

The above amphipathic (or hydropathic) complementarity model argues that antisense peptide sequences may be derived either by direct readout from the AS DNA sequence or by creating hydropathically opposite peptides based on readout of sense peptide sequences (see Fig. 6). Affinity supports built with immobilized antisense peptides could be designed with peptides derived by either route. As cited above, some evidence suggests that amphipathically-perfected "antisense" peptides may be of higher affinity than direct readout antisense peptides [12]. It is likely that both of these types of peptides, when immobilized, will be useful as affinity supports for peptide and protein separation. Affinity differences likely will dictate which would be useful for a particular separation goal.

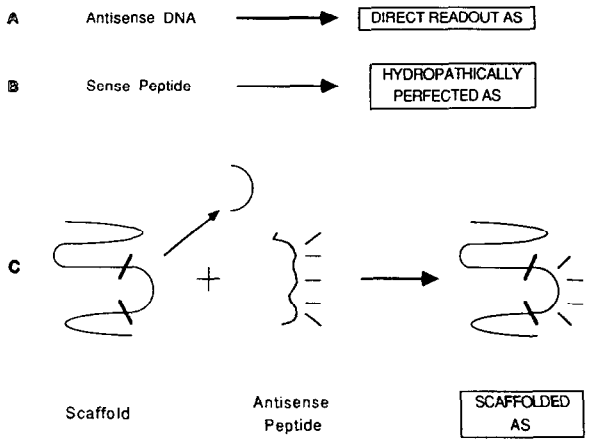

Fig. 6. Scheme depicting the design of antisense-related peptides by (top) direct sequence readout from the literal antisense DNA sequence; (middle) designing amphipathically-perfected "antisense" sequences guided by the sequence of amino acids in the sense peptide; and (bottom) antisense peptides inserted into conformationally constrained scaffolds. The choice of residues for the middle strategy has been attempted by maximizing the oppositeness of hydropathic moments *versus* sense peptide sequence but may also be based on conformational fit of side chains.

5. PERSPECTIVES

Since the initial observations of antisense peptide recognition of sense peptides, the rationale and therefore the biological relevance of these interactions has been debated. In particular, the mechanism of interaction has been elusive, and the interaction process has not been observed in all cases examined. Nonetheless, the repeated observation of this type of recognition process, and its selectivity, argue for its potential biotechnological utility. The usefulness of immobilized antisense peptides for separation has been demonstrated, at least in a few systems so far. Such usage is compatible with the weak affinity normally found for antisense–sense peptide binding. In contrast, whether antisense-related peptides can be made with high enough affinity and selectivity to warrant their use in therapeutics or diagnostics remains to be demonstrated. Perfecting the amphipathic pattern of residues may help.

In addition, it is likely that antisense peptide interaction affinity and selectivity would be increased if their productive conformations were limited by being inserted into some type of conformational scaffold (see Fig. 6). Peptides intrinsically are conformationally disordered. It is likely that this disorder is responsible, at least in part, for the low affinity and other complexities observed with antisense peptide recognition. Recently developed leucine zipper scaffolds [25] may provide one route to form constrained antisense peptide chimeras. Such chimeric constructs could be used in combination with molecular biological tools to derive higher affinity and more selective variants of antisense peptide sequences which could be used as improved tools in affinity separation and other biotechnology applications.

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