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Visions & Reflections

On the evolution of ligands: did peptides functionally precede metals and small organic molecules?

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While receptors are almost always proteins, their ligands may be proteinaceous or nonproteinaceous substances, including but not limited to metals, ions, steroids, sugars and immune haptens. The topic of this commentary is whether there might be an evolutionary and structural relationship between the two ligand groups and whether this might be employed to rationally design peptide mimetics of nonpeptide ligands and vice versa. An understanding of how this might be possible first requires a discussion of the concept of 'binary coding' of hydropathy and its role in molecular recognition between proteins and peptides [1]. Specifically, accumulating evidence suggests that a simple binary code of polar and nonpolar amino acids arranged in the appropriate order is an important determinant of gross shape and rudimentary function. That is, only the sequence location, not the identity of the polar and nonpolar R groups, must be explicitly specified for the formation of a stable structure or biologically active peptide. Such binary coding of hydropathy has been successfully used to synthesize biologically active analogs of peptide hormones [2, 3]; design proteins which fold into compact α-helical structures [4]; and to develop computer programs which simulate or predict certain aspects of protein folding [5]. Because there are multiple amino acid members within the polar and nonpolar groups, one would expect a marked degree of sequence degeneracy for a given shape, since any member of a group could occupy a given position in the sequence. Experimental evidence has borne out the idea that gross shape or structure is degenerate with regard to primary sequence in that any number of different primary amino acid sequences with the same binary code can fold into compact α-helical structures [4] or form biologically active peptides [2, 3].

If the gross shape of a peptide or protein is determined by its binary code or pattern of hydropathy, then exactly inverting a particular binary code should result in inverted or complementary shape because the same driving force is involved but in apparent reversed orientation (fig. 1) [1]. Inversion of the pattern of hydropathy of one sequence with respect to another can be achieved by computer programs designed for this task [6–8] or by simple reliance on an interesting aspect of the genetic code [9]. Specifically, amino acids of opposite hydropathy are specified by the coding and noncoding strands of complementary DNAs. Such peptides encoded by complementary nucleotide sequences [10] or designed by simply inverting the hydropathic pattern [7] are termed complementary peptides. They have the following characteristics expected of complementary structures: (i) Approximately 40 different complementary peptide pairs have been shown to bind one another with great specificity and moderate affinity (for review see [1, 11]; for a complete bibliography contact the author by e-mail at Blalock@uab.edu); (ii) complementary sequences delineate or denote the interactive sites of ligands and their receptors [12–15]; (iii) immunization with complementary peptide pairs generates the expected pairs of interacting idiotypic and antiidiotypic

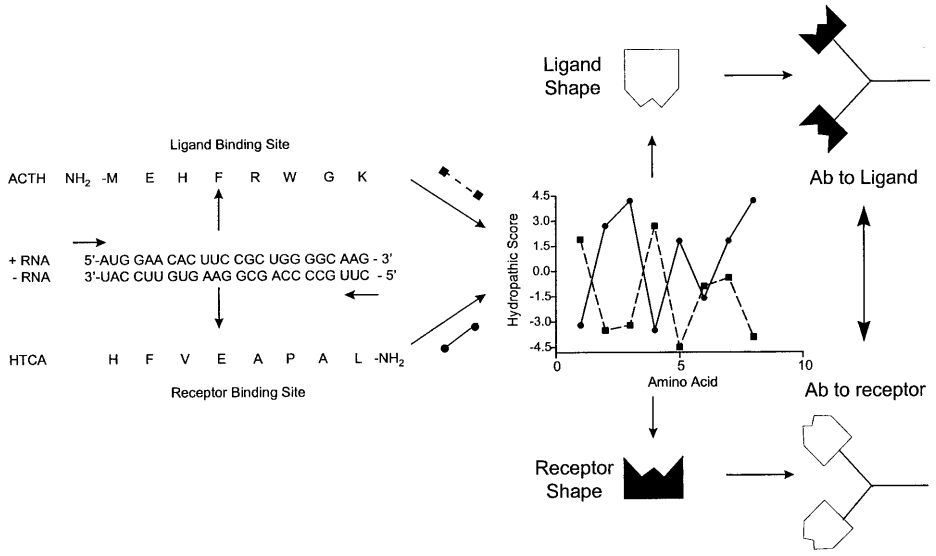

Figure 1. The MRT. Complementary nucleotide sequences encode peptides which have inverted or mirror-image hydropathic profiles. As a result, binding sites for a receptor and a ligand assume complementary shapes which allow for their interaction. Antibodies (Ab) made against the ligand and receptor binding sites will themselves consequently have complementary combining sites. By way only of example, a portion of adrenocorticotropic hormone (ACTH) is shown.

antibodies whose combining sites are complementary [16–25]; (iv) antibodies against a ligand's complementary peptide recognize the binding site on the ligand's receptor [10, 16, 25–34].

We have demonstrated that the pattern in the genetic code which results in complementary peptides was not a chance occurrence [9, 35] and have developed a molecular recognition theory (MRT) as a hypothesis for its existence (fig. 1; for review see [1]). It seems readily apparent that a peptide or protein without an interacting proteinaceous partner would be of little value and rarely, if ever, is seen. In the MRT, we propose that there developed in nature a fail-safe method to assure that a peptide or protein will always have an interactive site on a partner. Most simply, the genetic code evolved such that complementary strands of nucleic acid encode potential pairs of peptides that specifically interact via complementary shape or contours resulting from the 'mirror-image' hydropathy patterns of one amino acid sequence relative to the other. As long as the two nucleotide sequences are juxtaposed, the system is self-correcting in that a changed base on one strand would lead to compensatory change on the other and appropriate amino acids would be found in the resulting complementary peptide pairs. We originally envisioned [10] that at some point in time, the 5′ and 3′ ends of the primordial complementary nucleotide sequences would be ligated, complemented and each segment eventually translocated to be surrounded by new genetic information that would serve as scaffolding to hold the binding site on the newly evolved receptor or ligand in a preferred conformation so that the affinity of the interaction would increase. The most compelling molecular biologic evidence for this appeared in 1995 with the cloning of a novel dual receptor for arginine vasopressin (AVP) and angiotensin II (AII) by essentially screening a kidney complementary DNA (cDNA) library with AVP and AII cDNA [14]. The receptor was found to be the first member of a new type of seven-transmembrane-spanning, Gs-coupled receptor which has the unusual characteristic of binding two different ligands (i.e. AVP and AII) at two different extracellular loops. More important, the sites of AVP and AII cDNA hybridization demarcated the receptor's AVP and AII binding sites, respectively. Since the AVP and AII oligonucleotides hybridized in frame with the receptor, the amino acid sequence of the receptor contained a homolog of the AVP and AII complementary peptides. Interestingly, these homologs were also present in all of the more prototypical AVP and AII receptors, respectively [14]. This strongly suggests that through evolution, the primordial complementary peptide binding sites were retained and perhaps shuffled as miniexons between multiple sites that became different types of receptors. Most recently, virtually identical results were found for a novel dual receptor for endothelin 1 and angiotensin II [15].

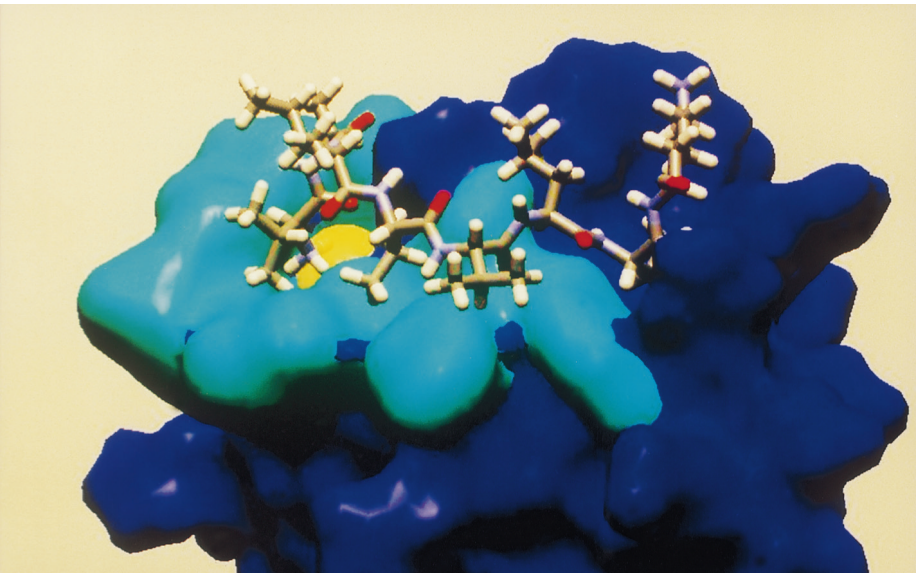

Figure 2. Theoretical model of the interaction of CALP1 with the C-terminal domain of CAM. CAM is in blue with EF hand 4 as light blue. The van der Waals radius for Ca^{2+} is shown in yellow. CALP1 is shown as a stick model. As can be seen, the ' amine of the amino terminal valine of CALP1 protrudes into the Ca^{2+} binding pocket. The model was obtain using the docking program Gold [49] (Cambridge Crystallographic Data Center, Cambridge, UK). The protein input file was the C-terminal domain of CAM (PDB entry 1CMG). The ligand file was an NMR structure of CALP1 in solution which was arbitrarily chosen from the ones available. The center of the docking site was O369 (Tyr 99) and 15 Å was the docking radius, which cover all the amino acids present in the EF hand loop 3 and 4. No constraints were given for the ligand. The solution presented was ranked first in the 20 different docking runs, with a fitness of 17.64.

Assuming that the above is correct, genes which encode receptors for peptide or nonpeptide ligands will retain an imprint of the original ligand in the form of the sequence which is complementary to that of the receptor's ligand binding site [36]. In terms of nonproteinaceous ligands, this offers a design opportunity to potentially make peptide agonists or antagonists of small organic molecules and metals by simply decoding the complementary information or making a peptide with a 'mirror-image' hydropathy code relative to the receptor's binding site. There are presently a number of examples which clearly demonstrate the feasibility of this concept. Perhaps the best characterized are a series of 8- and 12-residue peptides designed on this principle and targeted to the EF hand motif of the Ca^{2+}-binding protein, calmodulin (CAM) [36; and unpublished observations]. These peptides, but not unrelated peptides or those with the same amino acid composition but a scrambled sequence, interacted with the two carboxy-terminal Ca^{2+} binding sites or EF hands of calmodulin. The interactions resulted in a conformational change whereby the 8-mer peptide/calmodulin complex could activate CAM/dependent phosphodiesterase in the absence of Ca^{2+}. In contrast, the 12-mer peptide/calmodulin complex did not activate phosphodiesterase but rather inhibited activation by Ca^{2+}. Although these peptides were termed $\underline{Ca}^{2+}$-like peptides, or CALP, to indicate Ca-like effects or antagonism, nuclear magnetic resonance (NMR) determination of a CALP structure coupled with computer-assisted docking to a known CAM structure suggest that the interaction with CAM is unlike that of Ca^{2+}. Specifically, CALP has a complementary contour to a large part of certain EF hands and only the positively charged amino group on the N-terminal amino acid residue protrudes into the Ca^{2+} binding pocket (fig. 2). Despite the different binding modalities and obviously different shapes, Ca^{2+} and CALP interactions both lead to the expected biochemical and biological effects. Pharmaceutically, complementary peptides, such as CALP, might be viewed as lead compounds on which more potent constrained analogs might be designed once the peptide's structure is determined. In any event, the above would seem to support the notion that peptides can functionally substitute for certain actions of metals or small organic molecules. In the latter instance, and although not understood at the time, it is worth noting that endorphins and enkephalins can mimic the activity of and compete for binding of morphine to opiate receptors [37].

Likewise, aspartame (H-asparagine-phenylalanine-O methyl) interacts with taste receptors which ordinarily

detect sugars. Interestingly, four different complementary peptides for aspartame were all sapid compounds [38]. One was bitter, one was salty and two acted as taste enhancers. These results suggest that peptides can be designed to mimic the taste effects of acetic acid, sodium chloride and monosodium glutamate, respectively. Although unexpected, the findings also imply that since the ligands are complementary, there may be complementarity in terms of ligand binding sites between different types of taste receptors. That is not to say, nor is it derivative for the ligands, that, for example, sodium chloride is complementary in shape to glucose since, as was discussed for CALP, complementary peptides do not interact in the same way as a small organic molecule or metal with the active site of their target proteins. Rather, the results suggest that each partner in a primordial complementary peptide pair could evolve into a receptor's binding site as well as a ligand. In this situation, the ligands themselves and the receptors themselves would show complementarity of sequence and structure as is implied for the aforementioned aspartame system.

Although the discoveries of endogenous opiates and aspartame were through an entirely empiric process, there are now a number of instances, summarized below, where complementary peptide mimetics or antagonists of small organic molecules have been designed or deciphered using the MRT. The apparent first published account was the finding that a peptide representing a self-complementary sequence within the second hypervariable region of the heavy chain of a self binding antibody (Ab) could mimic the Ab's epitope, phosphorylcholine, in terms of blocking the self association of the Ab [39]. Thus, the complementary peptide was recognized as 'phosphorylcholine-like' by the immunoglobulin. Subsequently, a complementary peptide for a dopamine binding site on the D_2 dopamine receptor was reported to act as a dopamine agonist or antagonist depending on the dose [40]. Similarly, an antagonist of the nicotinic acetylcholine receptor was made by synthesizing a complementary peptide to a region of 13 amino acids representing the acetylcholine, as well as snake neurotoxin, binding site on the α-subunit of the acetylcholine receptor. This peptide inhibited in vivo neural transmission mediated by nicotinic cholinergic receptors [41].

In this issue of *Cellular and Molecular Life Sciences*, J. D. Neill and colleagues [42] have used the MRT to produce a complementary peptide with pure agonist activity which mimics the cardiotonic, natriuretic glycosteroid of the digitalis family, ouabain. Specifically, a hexapeptide (termed ouabain-like peptide, OLP) complementary to the ouabain binding site on its target protein, the sodium/potassium-dependent adenosinetriphosphatase (Na^+/K^+ ATPase), competed with radiolabeled ouabain for binding to the enzyme. The hexapeptide, like ouabain, also inhibited the enzymatic activity of the Na^+/K^+ ATPase. Ouabain stimulates renal sodium excretion (natriuresis) in vivo by inhibiting Na^+/K^+ ATPase [43]. Interestingly, polyclonal Ab to OLP was able to inhibit natriuresis and water excretion as urine in salt-loaded rats, supporting the notion of an endogenous OLP [44, 45]. A candidate endogenous OLP was identified by a computerized search of a protein database when an identical sequence to four amino acids of OLP was found in the glycopeptide moiety of the vasopressin-neurophysin precursor [42]. The glycopeptide or a derivative was recognized by Ab to OLP and blocked the activity of, as well as ouabain binding to, the Na^+/K^+ ATPase. Thus the glycopeptide would seem to fulfill the expected criteria of an endogenous ouabain-like substance, especially considering that the vasopressin-neurophysin precursor resides at an anatomical site regulating natriuresis, the neurohypophysis.

Collectively, the findings discussed in this commentary would seem to marshal considerable support for the MRT and its central hypothesis that because of binary patterning within the genetic code, sites of interactions between and perhaps within [11, 46] proteins and peptides arise on complementary strands of nucleic acid. While the numerous theoretical implications as well as practical consequences of this idea are beyond the scope of this treatise, a few are worth noting. First, there has developed in nature a fail-safe method, which likely facilitates evolution, by assuring that proteins or peptides will always have interactive partners. Second, although polypeptides do not physically predate metals, salts or certain small organic compounds, they were likely used functionally as ligands in advance of nonpeptides. It is our contention that complementary peptides were the original ligands for receptors which evolved to ultimately detect nonpeptides. During evolution, metals and small organic molecules began to subserve this role as a result of different stability, kinetics, pharmacology and so on. Today, of course, there remain examples of peptide ligands which mimic or inhibit the action of nonpeptides, and these include endogenous opiates [37], snake and snail neurotoxins, and the 'sweet-tasting' proteins thaumatin and monellin [47]. Although not usually genetically expressed, it is interesting to consider that our genomes may contain an imprint of an endogenous ligand for every receptor. This information resides in the nucleic acid sequence complementary to that of the receptor's binding site. As previously discussed, this information can be practically used to design peptide mimetics of nonpeptides, clone receptors, define interactive sites on proteins and identify novel protein interactions by searching protein databases. With the completion of sequencing of the

human genome, we anticipate that algorithms based on the MRT [48] may well have utility in the field of genomics by elucidating relationships between novel gene products [12, 14, 15, 42]. To end on a purely philosophical note, it is also interesting to think that if shape is encoded on one DNA strand and complementary shape on the other, then our genomes contain not only the information defining ourselves but also the potential for a complementary self.

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