Hormone Mimicry

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Two research articles in this issue (*1*, *2*) are enough to reinstate one's belief in Santa Claus. A three-way collaboration between researchers at Affymax, R. W. Johnson Research Laboratories, and the Scripps Research Institute has dispelled the naysayers by showing that small peptides can be found that mimic large polypeptide hormones. These groups have isolated a 20–amino acid peptide that binds to and activates the receptor for erythropoietin (EPO), a 165–amino acid cytokine (*1*). On top of that, they determined the structure of the peptide-receptor complex (Fig. 1), which reveals in atomic detail how two molecules of the peptide dimerize the extracellular domain of the EPO receptor (called EPO binding protein, or EBP) (*2*). EPO, used for stimulating production of red blood cells, is a huge product in biotechnology with worldwide sales of $2.6 billion in 1995. Thus, not only is this work a major advance in molecular mimicry, it could have huge commercial implications as well.

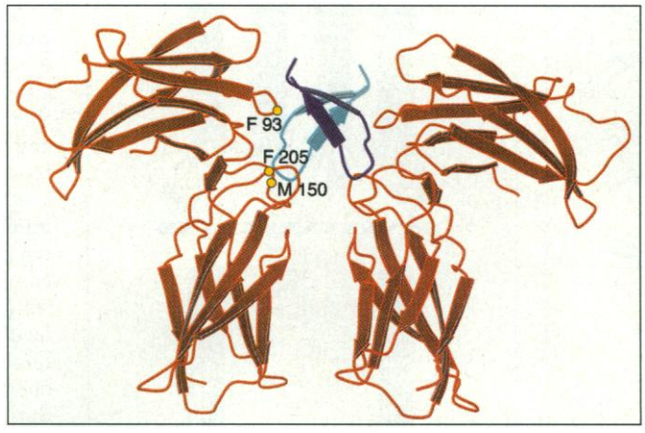

Fig. 1. Mimetic peptide. The dimeric peptide flanked by two molecules of the extracellular domain of the EPO receptor (EBP). [Reproduced from (*2*)]

Deciphering the "rules" for how proteins bind to small molecules or other proteins is largely an empirical science. There is no simple code for binding in proteins like the hybridization code for base pairing in nucleic acids, which was cracked by Watson and Crick over 40 years ago. Thus, most drug design efforts have relied on laborious, and generally unsuccessful, screening techniques to identify small-molecule "leads," compounds that are targeted for possible development into pharmaceuticals.

Six years ago several groups published (almost simultaneously) the concept of "peptides on phage," which promised to revolutionize the drug discovery process (*3*, *4*). In a logical extension of the pioneering work of Smith and co-workers on phage display (*5*), along with the concepts of molecular diversity developed by Geysen and colleagues on peptide libraries (*6*), small gene fragments coding for random peptides were fused to the coat proteins on filamentous phage [geneIII or geneVIII (*7*)]. A library of random peptide-phage was allowed to bind to and subsequently eluted from a target protein of interest. This binding selection and repropagation of phage was repeated many times to enrich for tighter binders. After three to six rounds, the identity of the selected peptide was simply determined from the packaged DNA sequence of the peptide-geneIII or -geneVIII fusion. Although promising, the only peptide mimics reported were for small molecules such as biotin, sugars, or other small peptides (*8*). Others have used phage display in a stepwise fashion to reduce the size of polypeptide binding domains while preserving the existing binding epitope (*9*). In the new studies, a small peptide was isolated from thoroughly naïve libraries; it shares no sequence or structural homology to the large cytokine it mimics.

How did they do it? There are several key aspects to the selection procedure (Fig. 2). First, try everything you can think of. The Affymax group sorted a myriad of phage and phagemid libraries containing either linear peptides (up to 20 amino acids long) or disulfide-constrained peptides (containing two to eight randomized residues between fixed cysteines). Second, gear the selection to ensure the capture of weak binders and then optimize. Multivalent display allows for better capture of weakly binding peptides (*4*, *7*) than does monovalent display (*10*), because of multivalent attachment of the phage to the target protein on the solid support (avidity effects). Thus, they first sorted for binders from peptide-geneVIII libraries that display on average 100 to 200 copies of fusion peptide per phage, then moved the selectant to a lower valency format on geneIII. Third, to avoid nonspecific "plate binders," a significant problem in sorting weakly binding peptide-phage (*11*), they used two different elution conditions. For the peptides on geneVIII, they cleverly introduced a thrombin cleavage site at the base of the EBP and cleaved the phage from the plate with thrombin, thus leaving behind a preponderance of unwanted phage. For eluting peptides on geneIII, they added increasing concentrations of EPO and increased the elution time and temperature as the sorting progressed.

Thus, from a Cys-X_8-Cys-geneVIII library (in which eight random residues were flanked by fixed cysteines that presumably form a disulfide bond), they harvested a consensus weak binder [dissociation constant (K_d) ~ 10 mM] with the sequence CRIGPITWVC (*12*). For selecting the higher affinity peptides on geneIII, they added three random residues plus diglycine linkers to either side of the disulfide constraint and allowed mutations to the interior as well. By this means, the group isolated a 20–amino acid peptide (GGTYSCHFGPLTWVCKPQGG) with a K_d of 200 nM, compared to 200 pM for EPO.

One of the most remarkable aspects of the work is that this peptide activates the EPO receptor in a variety of cell-based and animal assays (*1*). The EPO receptor requires dimerization for activation (*13*), much like the growth hormone receptor (*14*, *15*). Indeed, the structure of the complex (Fig. 1) reveals a peptide dimer flanked by two molecules of the EBP. How did they select for a peptide that can both bind and dimerize the EPO receptor? The answer is still a mystery; however, one possible clue lies in the manner in which the target was presented. They immobilized the EBP by coating plates with a bivalent antibody directed to an epitope tag at the carboxyl terminus of the EBP. This strategy would present the EBP in dimeric form and thus could have placed selective pressure for symmetrical peptide dimers on the phage (Fig. 2).

The structure of the complex is thrilling. Figure 1 shows the first structure of the EBP, which reveals striking structural homology to the growth hormone and prolactin receptors (*15*), as anticipated (*16*). However, in the dimeric complex the two molecules of the EBP have very little contact in the stem regions (~75 Å^2) as compared to the growth hormone receptor (~500 Å^2) (*15*). Mutational studies on growth hormone and the growth hormone binding protein show that a small region of the contact interface (a "hot spot") is crucial for binding (*17*). Per-

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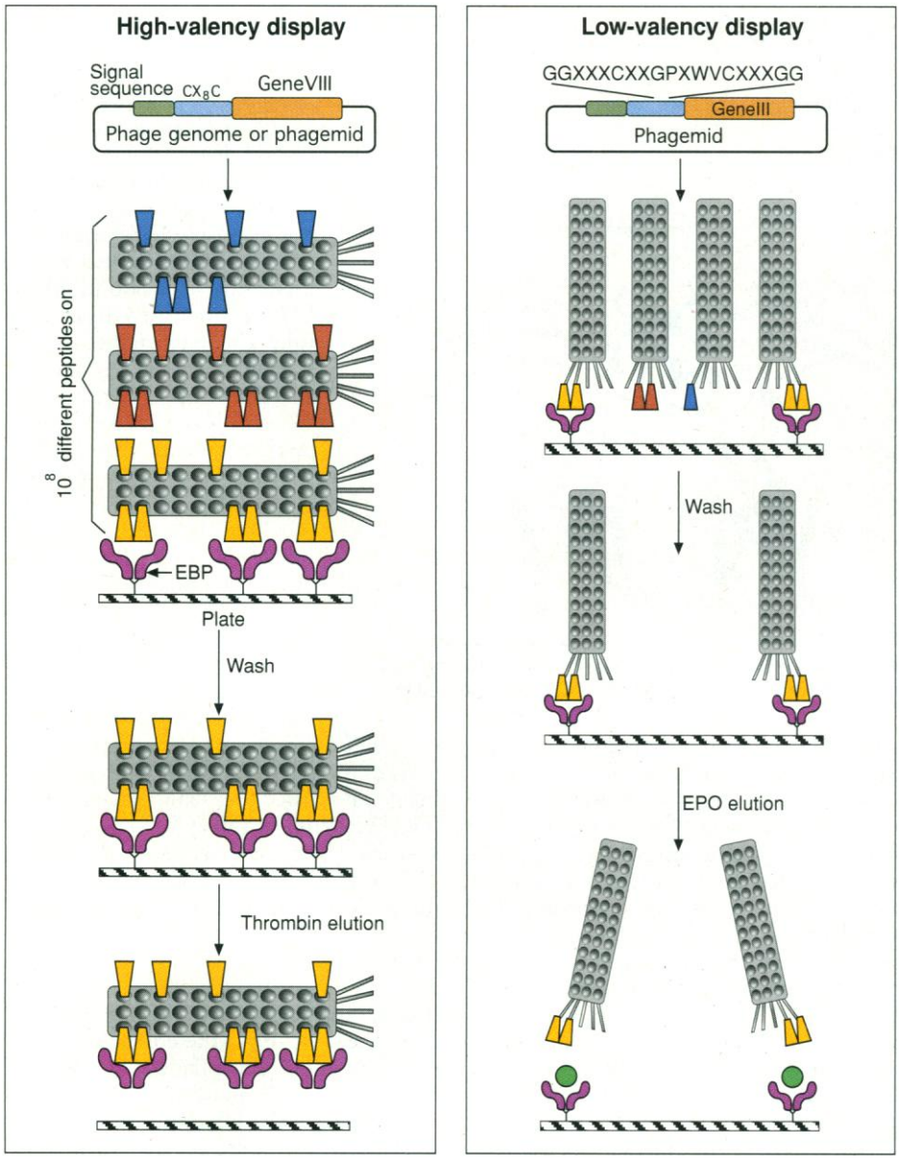

Fig. 2. Selection strategy for EPO mimics. Peptides were fused in a high-valency format to geneVIII on filamentous phage (left). Those sequences that bound, probably to a dimeric form of the EBP, were eluted by proteolysis of the EBP with thrombin. Consensus peptides (K_d ~ 10 μM) were further randomized and displayed in a low-valency format on geneIII (right). After several rounds of binding selection and elution with EPO, some peptides bind tighter (K_d ~ 200 nM) and dimerize the EBP. It is possible that dimeric peptides were present in the selection as "neighbors" on the phage.

haps the most amazing aspect of the peptide-EBP complex is that the dimeric peptide binds to the EBP at a site analogous to the energetic hot spot where growth hormone binds to its receptor. Like growth hormone, most of the important interactions of the EPO-mimetic peptide with the EBP are thought to involve the hydrophobic contact residues (Phe^{93}, Phe^{205}, and Met^{150}; shown in yellow in Fig. 1).

And let's not forget the peptide, whose compact structure and dense functionality are a wonder. The 20–amino acid peptide has a β-hairpin structure containing a highly selected Gly^9-Pro^{10} turn; it is stabilized by the Cys^6-Cys^{15} disulfide bond, a largely buried Phe^8, and hydrogen bonds in the β sheet. The two β hairpins in the dimer are arranged face-to-face at about right angles. The hairpins are apparently stabilized by hydrogen bonding where the two β sheets cross and a hydrophobic core consisting of the two disulfides, Tyr^4, Phe^8, and Trp^{13}. Amazingly, each peptide interacts not only with its peptide partner but with both EBPs as well. To do so, it uses most of the same hydrophobic residues that stabilize the dimer core as well as main-chain amides at the β turn. Most of the structurally important elements of this peptide were present in the initial 10–amino acid cyclic selectant; yet it wasn't until the researchers selected in later rounds for Tyr^4 (which adds additional stability to the dimer and contacts to the EBP) that the peptide was able to dimerize the EBP in vitro.

The skeptics will surely point out that what has been isolated here is a far cry from a drug: It is a peptide, with a molecular weight of ~2100 (multiplied by 2 because it is a dimer). It binds to the receptor with an affinity that is weaker (by a factor of 1000) than that of the native hormone in vitro and is even less potent in animal studies. However, a crosslinked form of the peptide binds much more tightly (*18*) and peptidemimetic strategies have succeeded in making small organic mimics from peptide lead compounds (*19*). Clearly, these new studies (*1*, *2*) demonstrate the power of molecular diversity methods coupled to structural analysis for identifying peptide mimics of large polypeptide hormones, something these same skeptics might say could never be accomplished. Sometimes even one's wildest dreams come true.

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