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Stabilized Helical Peptides: A Strategy to Target Protein−Protein Interactions

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ABSTRACT: Protein−protein interactions are critical for cell proliferation, differentiation, and function. Peptides hold great promise for clinical applications focused on targeting protein−protein interactions. Advantages of peptides include a large chemical space and potential diversity of sequences and structures. However, peptides do present well-known challenges for drug development. Progress has been made in the development of stabilizing alpha helices for potential therapeutic applications. Advantages and disadvantages of different methods of helical peptide stabilization are discussed.

KEYWORDS: *stabilized peptide, α helix, protein−protein interactions*

In the last two decades, there has been great progress in new therapies for several disease types. The highest number of new drugs have been for oncology. In oncology, there has been a new era in treatment. Many would consider the introduction of imatinib mesylate (Gleevec) for chronic myelogenous leukemia as the start of this new era.[1] That discovery led to numerous efforts in developing new small molecule kinase inhibitors with the goal of finding the next "Gleevec". Many of these small molecule inhibitors follow Lipinski's "Rule of 5s".[2] While there have been successes in the development of small molecule inhibition of enzymes important in cell function and growth, many small molecule inhibitors have not been as effective as desired when given as single agent therapy. On the basis of these results, other strategies are needed to achieve better disease control across the spectrum.

■ TARGETING PROTEIN−PROTEIN INTERACTIONS

Strategies to target protein−protein interactions are numerous. One has been to enlist combination therapy utilizing small molecule inhibitors in combination. The vast majority of small molecule inhibitors are enzyme inhibitors. One of the disadvantages in targeting enzyme sites is that the sites may be conserved among different enzymes of the same family. Much of the specificity in cellular pathways includes the myriad protein−protein interactions that occur in the cell. Interest in protein−protein interactions in the cell recently has led to coining of the term "interactome".[3] One estimate is that there may be about 650,000 significant protein−protein interactions in the cell.[3] When thinking about the complexity of intracellular protein interactions in addition to the dynamics within each protein that may affect those protein−protein interactions, the number of potential targets seems to increase exponentially. However, targeting protein−protein interactions has been thought by some to be "undruggable". In light of recent successes and the vast potential for the development of more effective, less toxic therapies, a significant research and development effort seems to be in order.

Previous efforts have included different strategies. The nature of protein−protein interactions have been thought to be difficult to target due to the nature of the binding sites. Namely, the sites tend to be hydrophobic and shallow.[4] However, the discovery of "hot spots" has led to the hypothesis that disrupting protein−protein interactions does not need to target the entire surface but rather instead only a few, key smaller sites. Preclinical work in Bcl-2 inhibition led to development of ABT-263 and related compounds.[5] The thrombopoietin agonist eltrombopag (utilized in immune thrombocytopenic purpura) is an example of a small molecule peptidomimetic that mimics the activity of a larger protein. Another intriguing approach has been efforts examining modification of peptides to target protein−protein interactions.

■ STABILIZING HELICES

Small peptides (often consisting of a domain or smaller in size) have been evaluated extensively. The drawbacks of peptides as drugs are well-known. Native peptides outside of a parent structure can be quite sensitive to protease degradation. In addition, oral absorption is quite difficult to achieve. One of the most utilized peptides in the clinic is octretotide, and it has been used in several conditions, including carcinoid syndrome.[6] This peptide is able to be delivered subcutaneously. The cyclic nature of this peptide makes it more "drug-like" than a native, nonmodified peptide would be. Research into other modifications of peptides to get past the drawbacks listed above has increased over the last 10 years. While there are numerous studies that have been done, three approaches will be discussed here.

Probably the best known is the use of "stapled peptides" originally described by Walensky and Verdine.[7] In 2000, Verdine and colleagues first reported the synthesis of stabilized peptides,[8] and in 2004, Walensky, Verdine, and Korsmeyer published their results on the effects of stabilizing pro-apoptotic BH3-mimetic peptides.[7] Taking an isolated peptide sequence out of its larger parent protein is thought to significantly decrease the propensity to fold into the same secondary structure that is found in the parent protein. However, such

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peptides usually are in an array of conformations; it is a matter of the population of structures in a conformation that is more biologically relevant. Using olefin tethers, peptides were stabilized via links at positions i,i + 4 or i,i + 7. The stabilized peptides had increased propensity toward helicity, were able to be taken up by cells, and were effective *in vivo* against leukemia cells. Other groups have started to utilize this approach. This approach is quite promising, but it has engendered some debate. There have been few pharmacokinetic studies published, so how broadly this approach will be applicable in broader drug discovery is unclear. Positioning of the staples and the stereochemistry of the nonnatural amino acids introduced into the sequence to act as the olefin base is being worked out. The obvious concern is that the olefin linker could interfere with the key residues important for target binding; this could negate the benefits of tethering. The Genentech group was unable to reproduce some of Walensky et al.'s results. As is common in scientific endeavors, it is unclear why there are discrepant results obtained by different laboratories. (Identification of the reasons for the discrepancy could be very useful to the scientific community. Walensky et al. have proposed reasonable possibilities.)

As there are multiple ways to potentially stabilize helical peptides, other approaches have been studied. Gellman's group has extensively studied the introduction of beta peptides into peptide backbones (containing an extra carbon in the backbone) in order to determine the effects of backbone modification.[9] An advantage to this approach is that the side chains are not blocked or removed by a tethering functional group. It also may remove a separate chemical modification step, which may not be trivial in thinking downstream about manufacturing. Several alpha/beta sequences have been studied ($\alpha\beta\alpha\beta$, $\alpha\alpha\beta\alpha\alpha\alpha\beta$, $\alpha\alpha\beta\alpha\alpha\beta$, $\alpha\alpha\alpha\beta\alpha\alpha\alpha\beta$, etc.). This approach has been utilized to identify analogues of the Bim BH3 domain that are able to bind Bcl-2 family proteins and induce apoptosis in mice embryonic fibroblast extracts.

A third approach has been to use a hydrogen bond surrogate approach.[10] This includes mimicking the hydrogen bonds that stabilize the helix backbone. An advantage to this approach includes not requiring side chain substitution. A potential disadvantage is that thus far, only the amino terminal is likely amenable to this technique. While that may be enough to overcome the entropic penalty to fold a peptide into a helix, one can envision that the C-terminus is potentially left vulnerable in comparison to the other techniques discussed above.

MOVING FORWARD

A daunting advantage of the utilization of peptides as drugs includes the immense number of possible different peptides that can be developed. As there are 20 amino acids that may be substituted in each position, a 10 amino acid has 1.024×10^{13} possible sequences. A 30 amino acid has about 1×10^{37} possible sequences. It is evident that with current technology screening every possible combination of peptides, or even a significant fraction of that, is not possible. Other strategies are needed. As computational power increases, it may be possible to better model peptides. Much of the work described above has occurred in the past 10 years or so. It is exciting to see what the next 10 years hold in this field.

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