CHEMICAL REVIEWS

Volume 101, Number 10 October 2001

Introduction: Protein Design

This is a particularly exciting time to be working in the area of protein science. Recent advances in genomics and structural proteinomics are providing a wealth of protein sequences and three-dimensional structures at an astonishing pace. One potential outgrowth of this explosion of information is a deeper understanding of the relationship between protein sequence, structure, and function. As this understanding deepens, it should become increasingly possible to design proteins and unnatural oligomers that are tailor made to carry out a variety of functions unprecedented in nature. While this is a relatively recent endeavor, much progress has already been made, as documented in this issue of *Chemical Reviews*. This collection of reviews strives to cover most chemical aspects of this area, with particular emphasis being placed on the elucidation of the principles of folding and structure, as a fundamental first step in the ultimate goal of constructing entirely novel proteins and biomimetic molecular assemblies.

One approach to introducing novel functionality in proteins is to modify the sequence and chemical composition of existing natural proteins. Penning and Jez discuss a variety of methods to redesign enzymes to alter their substrate specificity, stereochemical preferences, and reaction mechanism, as well as the conversion of substrate-binding sites into catalytic sites. In a similar vein, Lu, Berry, and Pfister provide a comprehensive review of the design and alteration of metal-binding sites within natural enzymes and proteins. A variety of methods now exist for introducing new metal-binding sites into proteins of known three-dimensional structure. Additionally, one can readily alter the residues surrounding known metallo-sites in natural proteins. A primary question often asked in these studies is how the redox properties and chemical reactivity of a given metal site is shaped by the nature and geometry of the primary ligands as well as second-shell effects that include the site's rigidity, electrostatic potential, polarity, and solvent accessibility.

Qi, Tann, Haring, and Distefano review the field of semisynthetic enzymes, which provides another promising approach to the design of novel catalysts and sensors. Chemical methods provide an attractive method for covalently attaching a diverse array of functionality that either does not exist in natural proteins or cannot be easily introduced using genetic methods. In this way, one should be able to expand the repertoire of reactions catalyzed by proteins.

A rapidly progressing area of protein engineering involves the design of sequences de novo rather than through the modification of existing natural proteins. While this approach is significantly more challenging, it nevertheless is an important endeavor because it critically addresses our understanding of protein folding and function. A given design will succeed or fail, depending on the depth of our understanding of the underlying physical principles. In de novo protein design, it is essential to compute a sequence that will adopt a given structure and often also a function. This remains a challenging job because even a short protein chain can have an astronomical number of potential sequences and also because a given sequence can adopt an astronomical number of conformations. Saven discusses computational methods with varying degrees of molecular detail and their application to understanding the requirements for folding into a given three-dimensional structure. Venkatraman, Shankaramma, and Balaram as well as Baltzer, Nilsson, and Nilsson discuss the current status of de novo protein design. In early work, designed proteins often failed to adopt the desired conformation—not because the designed structure was unreasonable or not stable, but rather because alternatively folded states or ensembles of related states were more stable than the desired structure. With time, improvements in computational methods as well as our fundamental understanding have allowed one to design a variety of proteins that fold into well-defined three-dimensional structures. Further, chemical methods allow incorporation of conformational restraints, which predispose the protein toward the desired structure. This area of research is now sufficiently advanced that it is possible to design a variety of proteins that incorporate multiple redox-active cofactors. Lombardi, Nastri, and Pavone describe the design of heme-containing model proteins and their use as catalysts and molecular sensors.

A particularly large challenge in protein design is determining precisely which sequence to make. Computational methods and potential functions have not evolved to a state that one can necessarily choose the

10.1021/cr000663z CCC: $36.00
Published on Web 10/10/2001

single best candidate for experimental evaluation, but they frequently suggest families of possible solutions. Thus, combinatorial methods that allow one to prepare libraries of sequences and accompanying strategies for selecting the most favorable sequences have become an important facet in protein design. Moffet and Hecht discuss methods for designing libraries of sequences that are likely to adopt a given fold and/or function as well as progress in screening through such libraries for folded and functional molecules. Hoess provides an overview of phage display in which peptides or small proteins are displayed on the surface of bacteriophages. The advantage of this method is that the DNA encoding the sequence of the protein is encapsulated together with the protein, providing a rapid method to read out and amplify the sequence of interest.

If our understanding of protein folding is truly fundamental and molecular in nature, it should be relatively straightforward to translate this understanding into the design of oligomers that are not based on the standard backbone observed in proteins. Cheng, Gellman, and DeGrado discuss research in one specific area of unnatural oligomer design, the design of β-peptides. This class of polyamides is built from β-amino acids rather than α-amino acids. Although work in this area is relatively recent, much progress has already been made in the rational design of biologically active β-peptides that adopt well-defined secondary structures. Thus, they represent an excellent testing ground for determining whether the rules of de novo protein design can be extended into the design of unnatural biopolymers.

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