Methods in ENZYMOLOGY

Volume 462

Non-Natural Amino Acids

Edited by

Tom W. Muir
John N. Abelson

VOLUME FOUR HUNDRED AND SIXTY-TWO

METHODS IN ENZYMOLOGY

Non-Natural Amino Acids

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Preface

Proteins are the most versatile of nature's macromolecules. There seems to be no limit to what proteins can do, whether it is to interact with other biomolecules, in a sense acting as molecular Velcro, or to catalyze biochemical reactions involved in everything from secondary metabolism to the remodeling of chromatin. Understanding how proteins work at the molecular level is at the heart of biochemistry. Indeed, the pursuit of this fundamental question has helped spawn entire areas of study, including structural biology, protein engineering, and of course enzymology. The more we learn about protein function using the tools provided by these venerable fields, the more we appreciate the extraordinary complexity of their inner workings. Despite the enormous progress that has been made, it is clear that additional approaches are required if we are to further penetrate these seemingly Byzantine structure-activity relationships—just as a watchmaker requires extremely fine tools to put together (or fix) an analog timepiece, so too the protein biochemist needs precise methods by which to tweak the chemical structure of his or her favorite protein to figure out what the various cogs and gears (i.e. amino acids) are doing. Often, site-directed mutagenesis is too blunt an instrument for the question at hand; it is easy to perform, but it represents a compromise over what one might really want to do, namely alter the physiochemical properties of an amino acid in a subtle manner that minimizes collateral damage (i.e. unwanted secondary effects) to the protein. Clearly, the ability to incorporate nonnatural amino acids into proteins would go a long way toward correcting this deficiency. The past several years have seen an explosion of research directed at this problem. This volume offers a snapshot of the major developments in this area and highlights both the chemistry-driven techniques that have been devised, as well as the type of problems in protein biochemistry to which they are now applied.

Broadly speaking, two approaches have emerged in recent years that enable the generation of proteins containing unnatural amino acids; protein semisynthesis and nonsense suppression mutagenesis. The former approach involves building the target protein from premade polypeptide fragments, one of which is generated by recombinant DNA expression methods and others by chemical synthesis. The recombinant building block can be extremely large, while the synthetic piece can contain any number or type of noncoded element(s). Thus, by linking the two together we have the best of both worlds, size, and chemical diversity. Several of the chapters

in this volume review the various approaches available to link the protein fragments together and offer detailed practical guides to their use. Also covered in this volume is the nonsense suppression methodology. This approach allows for an unnatural amino acid to be incorporated site specifically into a fully recombinant protein via ribosomal synthesis. In the past few years, methods have been developed that allow for incorporation of a wide range of unnatural amino acids into proteins in cells. This *in vivo* methodology relies on the generation (by directed evolution methods) of mutant tRNA synthetases that aminoacylate suppressor tRNAs with the unnatural amino acid directly in living cells. Importantly, the suppressor tRNA/aminoacyl-tRNA synthesase pair is orthogonal to the host cell aminoacylation apparatus, thereby ensuring the fidelity of the unnatural amino acid mutagenesis.

Do we really need two entirely different approaches to the same problem? The answer is a resounding yes. This is because the two strategies are complementary in terms of what they can and cannot do. The suppressor mutagenesis approach has the big advantage of being easy to perform (at least once you have the orthogonal tRNA/aminoacyl-tRNA synthesase pair). Indeed, it is no more difficult than standard mutagenesis and can, in principle, yield large amounts of mutant protein using bacterial overexpression technologies. Also, one can mutate any residue in the protein simply by replacing the corresponding codon in the gene with an amber codon. In contrast, semisynthesis involves *in vitro* manipulations, which can be technically cumbersome by comparison. Moreover, these same technical issues mean that it is much easier to incorporate unnatural amino acids near (within $\approx$50 residues) the N- or C-terminus of a large protein than in the middle. Semisynthesis allows for a much wider range of unnatural amino acids to be introduced into the protein than suppressor mutagenesis; it is possible to incorporate almost any building block into a synthetic peptide, whereas there are significant restrictions on what amino acids the ribosome will accept. Furthermore, only a single unnatural unit can be introduced efficiently using the suppressor mutagenesis approach, whereas any number of unnatural units can be incorporated at once by semisynthesis; indeed, the synthetic building block can be nonpeptidic (e.g. DNA or a glass slide). Another often-overlooked strength of semisynthesis is that it allows NMR-active isotopes to be introduced into a single amino acid, or stretch of amino acids, in a protein, thereby allowing targeted spectroscopic studies to be performed. Thus, semisynthesis and suppressor mutagenesis have different strengths and weaknesses. It is also worth noting that there is no technical reason why they cannot be used in combination; that is to generate a protein containing nonnatural elements introduced by both strategies. Many otherwise intractable problems could yield to this integrated approach.

Many of the chapters in the book describe applications of these protein engineering technologies to specific biochemical problems that exploit their

respective strengths, some of which I have alluded to here. Indeed, one of the messages that I hope readers take from reading this volume is that the approaches are robust enough to tackle even the most challenging of problems, whether those be studying conductance of an ion channel or teasing apart proton-coupled electron transfer reactions in a multi-subunit enzyme. Thus, I think the future of this field will be increasingly application driven rather than focused on technology development per se. Hopefully, the reader will gain some inspiration from one or more of these articles.

TOM W. MUIR

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