The Technology of Incorporating Unnatural Amino Acids into Proteins, Expanding the Genetic Code, and Applications

An investigator interested in modifying a protein’s characteristics or in designing a protein de novo has a variety of methods at his or her disposal. These techniques include conventional site-directed mutagenesis, where one can designate the replacement of specific amino acid residues in a target polypeptide chain with any of the other 19 naturally occurring amino acids (Hendrickson et al. 2004), 21 if we consider Pyl and Sec (Sackmann-Sala, 2009). Another technique for protein engineering is that of solid-phase peptide synthesis, which allows for many modifications to protein structure but is currently limited to the production of smaller size proteins (Wang et al. 2006). Techniques allowing the incorporation of novel or “unnatural” amino acids into proteins hold great promise for expanding molecular knowledge.

Hypotheses about the structure-function relationship of proteins might best be tested using a molecular tool that could site-specifically insert any chemical structure into a protein. Insertion of amino acid residues beyond the 20 “natural” amino acids can allow the inclusion of groups having side chains with finely tuned chemical characteristics (for example see Magliery et al. 2005). Some characteristics now demonstrated to be tunable include acidity, steric size, and redox potential (Hendrickson et al. 2004) among others. Other work has shown the introduction of residues useful as spectroscopic probes (Celitti et al. 2008). The incorporation of residues bearing synthetically functional groups capable of accommodating numerous chemical modifications is a highly promising technique using unnatural amino acids (UAAs) (Brustad et al. 2008). Modifications include photocleavable (Wu et al. 2004) and photoisomerizable groups (England et al. 2004; Gorostiza et al. 2008), as well as the addition of fluorescent moieties at defined locations in a protein (Kajihara et al. 2006; Joo et al. 2008).

A number of techniques have been developed to direct the site-specific incorporation of UAAs (reviewed in Magliery, 2005). A majority of the work done with directed UAA incorporation has been carried out in cell free translation systems wherein all the necessary components for protein synthesis are supplied by cell lysates (for example see England, 2004). The in vivo incorporation of UAAs has also been recently demonstrated (Anderson et al. 2004), where some investigators implement UAA incorporation using an expanded genetic code (for review see Wang et al. 2006). The features and requirements of both cell-free and in vivo systems of UAA incorporation will be discussed.

The potential applications for specific UAA incorporation into proteins are limited only by the investigator’s imagination. The current presentation will examine recent work on the incorporation of UAAs with side chains having photoactive groups, fluorescent moieties, and reactive functional groups. Therapeutic applications of UAA mutagenesis that will be discussed include the directed PEGylation of protein-based drugs (see Hendrickson et al. 2004). Some examples for using UAAs to control ion channels will be presented as well, such as the use of photoswitchable receptor agonists (for review see Gorostiza et al. 2007).
References


