Expanding the Genetic Code: Preparation of a Photo-Functional Protein by Site-Specific Incorporation of Unnatural Amino Acid

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Abstract

Site-specific mutagenesis of a certain codon to an amber codon and the suppression of this amber codon makes it possible to incorporate site-specifically an unnatural amino acid with novel functionality. We synthesized two caged unnatural amino acids, *L*-S-(2-nitrobenzyl)cysteine and *D,L*-(2-nitrophenyl)

Glycine which can be converted to cysteine and can be fragmented by UV irradiation, respectively when incorporated into the protein structure. Using *E. coli* cell-free protein synthesis system, we inserted the unnatural amino acids into the 38th position of human erythropoietin. Site-specific incorporation of unnatural amino acid was analyzed by liquid scintillation counting, SDS-PAGE/western blot and autoradiography. In these analyses, it was found that programmed suppression competes with not only the release factor but also background suppression. To maximize programmed suppression, we determined key factors out of several factors we tested. The site-specific incorporation of nonproteinogenic photocaged amino acids into protein provides an advanced tool for post-translational modification and functional analyses of protein.

References

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