PROTEIN ENGINEERING

Site-specific incorporation of amino acid analogues

(A general introduction)

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Why introduce unnatural amino acids into proteins ?

I. Synthesis of proteins with novel properties (protein engineering)

- Changes in structural properties
 - · Changes in physico-chemical properties
 - e.g. improvement in thermostability
 - e.g. improvement in stability in organic solvents
 - Changes in conformation (e.g. α-helix)
- Changes in catalytic properties
 - Increase in V_{max}
 - Decrease in K_m
 - Changes in pH & temperature optimum
 - Modification of specificity of reaction

Why introduce unnatural amino acids into proteins ?

II. Analysis of protein structure and function

- Fluorescent analogues for studying intracellular protein localization, protein folding, structure, binding to ligands etc.
- Photoactivatable amino acids for protein-protein interactions
- Chemically reactive groups
- Phospho amino acids for studies of signal transduction
- Heavy-atom derivatives for x-ray crystallography
- Spectroscopic probes for NMR, EPR etc.

The Genetic Code

			Second	1 letter			
2	_	U	C	А	G		200
First letter	U	UUU UUC UUA UUA UUG Leucine	UCU UCC UCA UCG	UAU UAC UAA Stop codon Stop codon	UGU UGC Cysteine UGA Stop codon UGG Tryptophan	U C A G	U C A G
	с	CUU CUC CUA CUG	CCU CCC CCA CCG Proline	CAU CAC Histidine CAA CAG Glutamine	CGU CGC CGA CGG	U C A G	letter
	A	AUU AUC AUA AUG Methionine start codon	ACU ACC ACA ACG	AAU AACAsparagineAAA AAGLysine	AGU AGC Serine AGA AGG Arginine	U C A G	Third
	G	GUU GUC GUA GUG	GCU GCC GCA GCG	GAU GAC Aspartic acid GAA GAG GIutamic acid	GGU GGC GGA GGG	U C A G	

Three codons (UAG, UAA, UGA) specify termination of translation



Role of specialized tRNAs (suppressor tRNAs) in translation of premature termination codons





Nonsense Suppression

Strategy for site-specific incorporation of unnatural amino acids into proteins using nonsense suppression



Strategy for site-specific incorporation of unnatural amino acids into proteins using nonsense suppression



Key requirement:

suppressor tRNA carrying the unnatural amino acid **IS NOT** recognized by any of the endogenous synthetases ... 'orthogonal' suppressor tRNA

Use of 'orthogonal' suppressor tRNAs in unnatural amino acid mutagenesis

1. In vitro protein synthesis



> E. coli S30 cell extracts

➤Wheat-germ system

➢Rabbit reticulocyte systems

21st aminoacyl-tRNA synthetase/suppressor tRNA pairs



Incorporation of unnatural amino acids into proteins



•Use of minimal media supplemented with analogue

random

in vivo

Nonsense suppression

Expanding the genetic code

