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Biosynthetic introduction of aryl bromide functionality into proteins

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Abstract

Incorporation of aryl bromide functionality into proteins was achieved via engineering the bacterial biosynthetic apparatus. A phenylalanine auxotrophic *E. coli* host was equipped with a phenylalanyl-tRNA synthetase (PheRS) variant that has a broadened substrate specificity. The mutant *pheS* gene (*pheS**), which codes for the α-subunit of the enzyme PheRS and confers relaxed substrate specificity, was encoded on a multiple-copy plasmid that also bears the target gene dihydrofolate reductase (DHFR). Constitutive over-expression of *pheS** and subsequent expression of the target gene in the presence of phenylatanine analog, *para*-bromophenylalanine (*p*-Br-phe), allowed over 85% replacements of phe residues by *p*-Br-phe in DHFR. The level of bromination can be controlled by varying the relative amounts of phe and *p*-Br-phe in the culture medium. Introduction of aryl bromide functionality into proteins offers great potential for selective chemical modification of proteins via transition metal-catalyzed reactions, which are orthogonal to existing protein chemistry. Moreover, bromination may be useful in X-ray studies of proteins via the multiwavelength anomalous diffraction (MAD) technique. ^ The utility of the aryl bromide as a unique functionality was investigated in collaboration with Isaac Carrico. Artificial extracellular matrix (ECM) proteins were synthesized using the principles of recombinant DNA technology. These proteins were designed for eventual application in vascular grafts. The engineered ECM proteins contained alternating blocks of cell-binding domains derived from CS1or CS5 regions of human

fibronectin for endothelial cell attachment, and elastin-like repeats for mechanical integrity. One phe residue per elastin block [$(VPGVG)_2VPGFG(VPGVG)_2$] was designed, which could be replaced with *p*-Br-phe and subsequently used for chemical cross-linking of the proteins. Protein expression yields of 75–90 mg/L were obtained with 50–60% substitution of phe by *p*-Br-phe. Preliminary exploration of Pd(0)-catalyzed Heck and Sonagashira couplings with *p*-Br-phe demonstrate feasibility of these reactions under mild conditions required for protein modification as well as compatibility with side chains of all natural amino acids (except cysteine). ^ Site-specific incorporation of *p*-Br-phe was tested in an *E. coli* strain equipped with a yeast PheRS/$tRNA^{Phe}$ amber supressor pair. While *p*-F-phe can be site-specifically incorporated using this system, attempts at *p*-Br-phe incorporation were unsuccessful, probably due to unfavorable interaction of *p*-Br-phe with the bulky and polar tyrosine residue in the binding pocket of yeast PheRS. ^

Subject Area

Biology, Molecular|Chemistry, Biochemistry

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