426e Incorporation and Labeling of an Aliphatic Ketone in Recombinant Proteins

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Recent work in our laboratory and others has demonstrated that engineering aminoacyl-tRNA synthetases can lead to efficient incorporation of nonnatural amino acids into proteins. In this work we examine the incorporation of oxonorvaline, an aliphatic ketone analogue of leucine, into a recombinant protein in *E. coli*. While oxonorvaline is nearly isosteric with leucine, protein expression in the presence of the analogue is not supported in an *E. coli* leucine auxotroph that over-expresses the wild-type *E. coli* leucyl-tRNA synthetase (LeuRS). However, significant protein yields are obtained in the presence of oxonorvaline when the leucine auxotrophs over-express LeuRS variants with a mutation at amino acid position 252. The threonine residue that appears in the wild-type LeuRS at position 252 is known to be a critical residue in the editing domain of the synthetase. Protein expression experiments with LeuRS bearing different residues at position 252 suggest that the editing function of the synthetase is dependent upon both the size and hydrophilic/hydrophobic character of the residue. In addition to studying the editing properties of LeuRS, we have also investigated chemoselective labeling strategies for the ketone functionality in recombinant proteins. We have demonstrated reactions with biotin hydrazide and hydroxylamine, and we are investigating the use of these labeling reactions to directly measure the amount of tRNA charged with oxonorvaline by wild-type and mutant LeuRS. These labeling strategies should be applicable to a wide range of biochemical and bioengineering problems.