1. Symposium Abstracts

In Vitro Translation and Methylation of Iso-1-Cytochrome *C* from *Saccharomyces Cerevisiae*

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The gene for iso-1-cytochrome *c* for *Saccharomyces cerevisiae* was recloned into a pSP65 vector containing an active bacteriophage SP6 promoter. The iso-1-cytochrome c gene was cloned as an 856 bp Xho 1-Hind III fragment. When the resulting plasmid was digested at the Hind III site 279 bases downstream from the termination codon of the gene and transcribed *in vitro* using SP6 RNA polymerase, full length transcripts were produced. The SP6 iso-1-cytochrome c mRNA was translated using a rabbit reticulocyte lysate system and the protein products analyzed on SDS polyacrylamide gels. One major band was detected by autofluorography. This band was found to have a molecular weight of 12,000 Da and coincided with the Coomassie staining band of apocytochrome c from *S.* cerebisiae. The product was also shown to be identical with that of standard yeast apocytochrome c on an isoelectric focusing gel. The *in vitro* synthesized iso-a-cytochrome c was methylated by adding partially purified S-adenosyl-L-methionine: protein-lysine N-methyltransferase (Protein methylase III; EC 2.1.1.43) from *S. cerevisiae* along with S-adenosyl-L-methionine to the *in vitro* translation mixtures. The methylation was shown to be inhibited by the addition of the methylase inhibitor S-adenosyl-L-homocysteine or the protein synthesis inhibitor pu omycin. The methyl derivatives in the protein were identified as ε-N-mono, di and trimethyllysine by amino acid analysis. The molar ratio of methyl groups incorporated to that of cytochrome c molecules synthesized showed that 23% of the translated cytochrome c molecules were methylated by protein methylase III.

Biosynthesis of L-Azetidine-2-Carboxylic Acid In *Actinoplanes ferrugineus*

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L-Azetidine-2-carboxylic acid (A-2-C), a four-membered cyclic imino acid has been identified in certain plants, and the microorganism Actinoplanes ferrugineus. The imino acid A-2-C has a physiological significance as an antgaonist of proline during peptide synthesis. The biosynthetic mechanism for the formation of A-2-C has not been studied in any detail. By using various amino acids such as methionine and S-adenosyl-L-methionine labeled with deuterium or carbon-14, the details of the biosynthetic pathway and a possible mechanism for the formation of L-A-2-C in *A. ferrugineus* have been unravelled. Both *in vivo* and *in vitro* experimental results suggest the biosynthesis of L-A-2-C is mediated by a confactor containing a carbonyl group, probably pyridoxal phosphate. S-Adenosyl-L-methionine, which seems to be the direct biosynthetic substrate, has undergone a γ-displacement by an α-amino group of the amino acid portion of the substrate S-adenosyl-L-methionine potentially via a vinylglycine intermediate. The overall stereochemical events at the β-carbon of the substrate have been shown to inversion of configuration. The overall stereochemical events at the -position of the substrate have also been shown to occur with inversion of configuration. The β, γ-elimination reaction of the substrate seems to follow a cisoidal-type mechanism and the addition portion of the reaction a