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

transoidal-type mechanism. The assignment of the proton NMR of A-2-C has been deduced by applying NOE difference experiments, Gd(III) line-broadening experiments and 2D-NOESY experiments of regio-and stereospecificially deuterated A-2-C's.

Glutamine Synthetase of some Fermentation Bacteria: Function and Application

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Metabolic activity of inorganic nitrogenous compounds affects not only microbial growth but also metabolite production in fermentation technology. We have worked on the enzymes participating in ammonia assimulation of some fermentation bacteria. This paper summarizes the results on glutamine synthetase and its application in practical field.

Glutamine synthetase (L-glutamate:ammonia ligase, EC. 6.3.1.2) catalyzes the formation of glutamine from glutamate and ammonia at the expense of cleavage of ATP and inorganic phosphate. The enzyme plays a dual role in nitrogen metabolism in bacteria; it is a key enzyme not only in the biosynthesis of various compounds through glutamine but also in the regulation of synthesis of some enzymes involved in the metabolism of nitrogenous compounds. The detailed works with the *Eschericia coli* and other enterobacterial enzymes revealed that glutamine synthetase is controlled by the following complex of mechanisms: (a) feedback inhibition by end products, (b) repression and derepression of enzyme synthesis, (c) modulation of enzyme activity in response to divalent cation and (d) covalent modification of enzyme protein by adenylylation and its cascade control. Comparative studies have also been made on the enzymes from other organisms.

1) Glutamine synthetase of *Gluconobacter suboxydans*

G. suboxydans, one of the type species of acetic acid bacteria, has a remarkable oxidizing-ability toward sugars and polyols, and has been utilized in fermentative production of various substances. The organism is also characterized by the lack of conventional TCA cycle. Investigators have therefore attempted to elucidate the biosynthesis of certain amino acids generally formed from intermediates of the cycle. For glutamic acid, was suggested a route involving some of the TCA cycle enzymes in conjunction with transaminations. However, the enzymatic mechanism of incorporation of ammonia into amino acid has not been elucidated because direct amination of α-ketoglutarate by glutamate dehydrogenase was not demonstrable in the organism. We confirmed that the organism assimilates ammonia as a nitrogen source and indicated glutamine synthetase/glutamate synthase pathway operates as the ammonia-assimilating system. Glutamine synthetase was crystallized and characterized together with glutamate synthase.

2) Glutamine synthetase of bifidobacteria.

Bifidobacteria, gram positive and strictly anaerobic, are one of the predominant organisms of mammalian intestinal flora. The organisms are supposed to take some part in reduction of putrefactive bacteria and toxic metabolites of the host. We investigated the enzymes involved in their ammonia assimilation, which might related with their detoxification activity in host intestine. Glutamine synthetase occurred in the organisms besides glutamate dehydrogenase, but glutamate synthase was not found in any strain. Glutamine synthetase was isolated from *Bifidobacterium bifidum* (human origin), *B. breve* (human origin) or *B. pesudolongum* (pig orgin). In addition,we found that bifidobacterial glutamine synthetase was inactivated by an enzymatic reaction, and characterized the inactivating enzyme. The results suggested that glutamine synthetase activity in the organism is controlled through adenylylation which appears similar to that found in the gram