Student's Name

Professor's Name

Course Number

Date

Biochemistry Reading Assignment

The primary objectives of this study were to examine the role of PyIRS(Y306A/Y384F) in recognition of a variety of amino acids, and to determine the crystal structures of 14 derivatives of non-natural lysine that are bound to a catalytic fragment of PyIRS(Y306A/Y384F). From the precise recognition of these substrates, structure-based design of novel amino acids which are non-natural, and are suspected to be restricted by the natural variety of L-amino acids, building blocks could be established. It was, therefore, necessary to expand the repertoire of the amino acids in translations so as to develop the novel protein structure and functions.

The study led to the establishment of precise structural accommodation bases of a variety of derivatives which are non-natural in the binding sites of M. mazel PyIRS(Y306A/Y384F) amino acid. Further, the study led to the demonstration of the usefulness of the synthesis method of cell-free protein, with its numerous advantages in the incorporation of non-natural amino acids in the system which is E. coli cee-based. For instance, excluding the non-positive factors of non-natural amino acids' cell membrane permeability, and achieving high productivity of proteins containing non-natural amino acids compared to the recombinant systems of the ordinary cell-based. Additionally, the consumption of non-natural amino acids during incorporation is smaller in quantity compared to that of the cell-based system. Another importance is the synthesis of

membrane proteins and toxic proteins that contain non-natural amino acids, which is impossible to produce in the cell-based order.

The relevance of the article to the material covered in this course

The expansion of genetic code is a useful crucial technological requirement in the incorporation of sites of non-natural amino acids precisely into the protein of focus during structural and functional analyses. The genetic coding expansion is mostly done using archaeal pyrrolysyl-tRNA synthetase (PyIRS) and $tRNA^{pyl}$. The most widely used enzymes in a variety of non-natural lysine derivatives, together with post-translationally modified lysines which are naturally occurring at UAG codons, are Methanosarcina mazei PyIRS mutant containing Y306A and Y384F mutations. The PyIRS(Y306A/Y384F) has a wide range of substrate specificity, and that makes it famous for these experiments.

The study analyses the underlying broad specificity mechanisms of the PyIRS(Y306A/Y384F). To begin with was the comparison of 17 derivatives of non-natural lysines, the chemistry of a variety of functional groups, photo cross-linking, among others, concerning the incorporation of in vivo protein. In vitro aminoacylation of $tRNA^{pyl}$ and ten derivatives of ZLys substituted at the ortho-, para-, or meta- position of benzene ring ligated to tRNA. Determination of the crystal structures of the catalytic fragments of PyIRS(Y306A/Y384F), complexed with 14 derivatives of non-natural lysines occurred, so as to clarify the structural basis for specificity of the broad substrate.

The para- and meta- substitution of the derivatives of ZLys snugly incorporate in the binding site of PyIRS(Y306A/Y384F), thus representing productive mode. On the contrary, the

derivatives of ZLys and the ortho-substituted ZLys exhibited non-productive binding mode, which is double-binding mode.

Accurate recognition of structural mechanisms of the derivatives of substrate lysines by use of PyIRS(Y306A/Y384F) is a step towards rational design based on the structure of novel non-natural amino acids, which is very useful in genetic-code expansion. Synthesis of cell-free protein is helpful for amino acid incorporation especially that which is not adequately incorporated in vivo.

This article is very relevant to the material discussed in this course as it gives a detailed view of the literally acquired knowledge during lectures. It provides sufficient insight into the role of PyIRS(Y306A/Y384F)in recognizing structures and functions of non-natural amino acids. The focus of this article on the role of a specific enzyme, which is PyIRS(Y306A/Y384F), has resulted in a better understanding of the role of similar proteins covered in this course.