Abstract

It is well-known that the large diversity of protein functions and structures derives from the broad spectrum of physicochemical properties of the 20 canonical amino acids that constitute modern proteins. According to the generally accepted coevolution theory of the genetic code, evolution of protein structures and functions was continuously associated with enrichment of the genetic code, with aromatic amino acids being considered the latest addition to the genetic code to increase structural stability of proteins and diversification of their catalytic functions.

The main objective of this master thesis was to test whether enzymatic catalysis could precede the appearance of aromatic amino acids in the standard genetic code. For that purpose, the effect of amino acid alphabet reduction on structure and function of dephosphocoenzyme A kinase (DPCK) was studied. Dephosphocoenzyme A kinase catalyses the final step in the biosynthesis of coenzyme A, a very conserved cofactor.

Two aromatic amino acid-lacking mutants of DPCK from a thermophilic bacterium, *Aquifex aeolicus*, were designed by substituting aromatic amino acid residues by (i) leucines and (ii) various non-aromatic amino acids to best preserve the structural stability of the protein. Wild type protein and the two mutants were cloned and successfully expressed in *Escherichia coli*, and the recombinantly produced proteins were characterized regarding the preservation of the secondary/tertiary structure and enzymatic activity. Structural characterization suggests that substitution of aromatic amino acids by non-aromatic residues can support rich secondary structure but leads to drastic loss of a firm globular arrangement, that resulted in a significant decrease or loss of enzymatic activities. Enzyme assays demonstrated that one of the mutants did not exhibit any catalytic activity. The other (where all aromatics were substituted by leucine residues) is capable of efficient ATP hydrolysis unlike the wild type and also less efficient phosphotransferase activity when compared with the wild type protein.

Using this exemplary study, the results presented in this thesis suggest that formation of protein structure and some catalysis could at some cost precede incorporation of aromatics into the genetic code.

Key words: protein reverse evolution, coenzyme A, dephosphocoenzyme A kinase, CD spectroscopy, limited proteolysis.