mortality. Based on the safety, confirmed in experimental studies, peptide preparations can be recommended both for premature aging prevention and for primary or adjunctive therapy in case of various diseases.

20.7 Prospective Cellular and Molecular Mechanism of Action of Short Peptides

It is essential for understanding specific effects of the peptide behavior in the cell and intracellular structures to reveal binding of short peptides with specific DNA sites. In our research, fluorescently labeled short peptides penetrated into cells and intracellular structures.[74] In the HeLa cells, the most intensive fluorescence of the labeled peptides was observed in the nucleus and nucleoli, while the least intensive was observed in the cytoplasm. Investigation of interaction of fluorescence-labeled deoxyribooligonucleotides with short peptides showed that peptides with different primary structures bind with one and the same deoxyribooligonucleotide differently. By using the specific oligonucleotides (FAM–deoxyribooligonucleotides), it has been revealed that Epitalon binds primarily with oligonucleotides that include more cytosine (C) than guanine (G) residues. The constant of binding of Epitalon with FAM–CGC CGC CAG GCG CCG CCG CGC (12 C residues) was almost 2-fold higher than that with FAM–GCG CGG CGG CGC CTG CGC CGC (10 C residues). Introduction of 5-methylcytosine residue into the nucleotide sequence independent of C or G content increased the binding of oligonucleotides with Epitalon. Thus, the binding of peptide Ala-Glu-Asp-Gly is sensitive to the cytosine methylation status of oligonucleotides. Epitalon was shown to preferably bind with single stranded oligonucleotide, containing methylated cytosine.[74] As is commonly known, cytosine DNA methylation is the most extensively studied epigenetic genome modification playing a significant role in stable changes of gene activity upon cell differentiation and aging in mammals.[75–77]

Consequently, there are specific sites for binding of a peptide with a particular amino acid sequence and oligonucleotide with a particular nucleotide sequence. The short peptide may bind to the DNA in various ways depending on its methylation nature; obviously it will cause different effects on the gene functions in various tissues/cells—young and old, normal and cancerous *etc.*[67] Our study shows that unlike the temperature of melting of the DNA double helix (+69.5 °C), in the DNA–tetrapeptide (Ala-Glu-Asp-Gly) system, the melting point occurs at a significantly lower temperature (+28 °C) and is characterized by smaller changes in free energy and an approximately 2-fold decrease in the enthalpy and entropy values.[78] This fact demonstrates that the thermodynamically simplified way of the DNA–peptide complex separation at lower temperature settings is typical of the biochemical processes occurring in living organisms. It also suggests that the mechanism of DNA–Epitalon interaction is based upon the natural mechanism of functioning of a living organism.