drug resistance. In this study a peptide corresponding to the N-terminal fragment of endostatin and some variants of this fragment including the insertion of a disulfide bond were designed and synthesized. Function of peptide variants were tested in vitro and in vivo. Results from endothelial cell proliferation assay showed that, in spite of some variations, all peptides can inhibit the proliferation of endothelial cells in vitro. Treatment of tumor-bearing mice with these peptides also showed that they can inhibit the growth of breast tumor in mice. Analyses of secondary structure of peptide fragments by far-UV CD showed that amino acid substitutions notably alter the secondary structure of peptides.

Keywords: Endostatin, Angiogenesis, Anti-Tumor, Disulfide Bond.

Abstract No.75

The Interaction of Polyphenol Flavonoids with β-lactoglobulin: Molecular Docking and Molecular Dynamics Simulation Studies

Mehdi Sahihi[1], Zobeideh Heidari-Koholi[1], Abdol-Khalegh Bordbar*[2]

1. Department of Chemistry, Shahreza Branch-Islamic Azad University, IR
2. Department of Chemistry, University of Isfahan, IR
(E-mail: msahihi@ch.iut.ac.ir)

The interaction of quercetin, quercitrin and rutin as, natural polyphenolic compounds, with β-lactoglobulin (BLG) using molecular docking and molecular dynamics simulation methods was examined. Molecular docking studies showed that the quercetin and quercitrin were bound to the internal cavity of the protein while rutin was bound to the entrance of the cavity because of its large structural volume. It was found that there were one, three and four hydrogen bond interactions between BLG and quercetin, quercitrin and rutin, respectively. This showed that, with an increase in the number of OH groups in the flavonoid structure, there was an increase in the number of hydrogen bond interactions. The binding constants for the binding of quercetin, quercitrin and rutin to BLG were 1.2×10^6 M^{-1}, 1.9×10^6 M^{-1} and 7.4×10^4 M^{-1}, respectively. The results of molecular dynamics simulation showed that the root mean square deviation (RMSD) of non-liganded BLG and BLG-ligand complexes reached the equilibration after 3500 ps. The study of the radius of gyration revealed that BLG and BLG-ligand complexes were stabilized around 2500 ps and unlike the two other complexes it did not exhibit any conformational change for the BLG-quercetin. Finally, analyzing the RMS fluctuations suggested that the structure of the ligand binding site remained approximately rigid during the simulation.

Keywords: Molecular Dynamics Simulation, Molecular Docking, β-lactoglobulin.

Abstract No.76

Expression of Recombinant Apaf-1 in Pichia Pastoris

Masoud Torkzadeh-Mahani[1], *Somayeh Karimzadeh*[1], *Farangis Ataei*[1], *Mojtaba Mortezavi*[1], *Maryam Nikkhah*[2], *Saman Hosseinkhani*[*1]

1. Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, IR
2. Department of Nanobiotechnology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, IR
(E-mail: Ataei_fa@yahoo.com)

Apoptosis, or programmed cell death, is an active regulatory response in both physiological and pathological conditions. One of the most important pathways of cell death activation is referred to as the mitochondria-associated apoptosome formation. In this pathway, It has been proved that the activation of procaspase-9 molecules is mediated by the oligomerization of the apoptotic protease-activating factor 1 (Apaf-1) molecules. The apoptosome formation is triggered by release of cytochrome c from mitochondria and oligomerization of Apaf-1 molecules which in turn promotes the activation of caspase -9. Apaf-1 comprises of an amino-terminal caspase recruitment domain, a central nucleotide-binding oligomerization domain, and carboxy-terminal multiple WD40 repeats. The activation of caspase-9 is thought to be due to the induced proximity of procaspase-9 molecules. In order to study the functional and structural details of Apaf-1, we have designed a construct for expression of Apaf-1 in Pichia pastoris.Total RNA was extracted from the fresh human spleen and the reverse transcription reaction was performed then the entire coding region of Apaf-1 gene was amplified using a hot start polymerase chain reaction. The amplified products were then digested by XhoI/ NotI and then cloned into the XhoI/ NotI digested/dephosphorylated pPICZa plasmids. Verified vectors were linearized with Sac1 endonuclease to target the integration of the expression cassette into the AOX1 locus of P. pastorisstrains X-33. Transformants of P. pastoris containing the Apaf1gene were selected on the basis of Zeocin resistance using YPD agar plates containing 100 µg ml-1Zeocin. The transformed yeast cells were grown in BMGY medium at 30◦C. The Apaf-1integration and expression was verified at mRNA level.

Keywords: Apoptosis, Apaf1, Pichia Pastoris, Protein Expression.
