that BDMC and DABC bind to the surface of the protein by four and one hydrogen bond interactions, respectively. Finally, molecular dynamics simulation results show that the binding of BDMC to BLG causes the conformational change of BLG and the structure of binding site remains rigid during the simulation of two complexes.

Keywords: Bisdemethoxycurcumin, Diacetylbisdemethoxycurcumin, β-lactoglobulin.

Abstract No.72

A Comparison of Denaturation of Trypsin in the Presence of Urea and Guanidine Hydrochloride

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Trypsin (EC 3.4.21.4) is a serine-protease with a polypeptide chain of 223 amino acid residues and six disulfide bridges that hydrolyzes peptide bonds at the carboxylic end of the amino acid residues arginine (R) and lysine (K). It is a globular protein with predominance of antiparallel β-sheet secondry structure and it has two domains with similar structures. In this study, denaturation of trypsin in the presence 2 mM, 4 mM, 6 mM and 7.5 mM of urea and GdnHCl has been studies by spectrofluometry and UV-VIS spectrophotometry in different pH (3, 8 and 10) at 308 K. The intensity of the emission spectrum has a direct relationship with the increase of concentration of urea and GdnHCl. Adding of guanidine to 0.25 mg/ml trypsin solution, the intensity of the spectrum was increased more than adding urea and 13 nm red shift occurred with respect to native curve. Adding of 7.5 mM of urea to trypsin solution at pH 3.0, the intensity of the spectrum was reduced with respect to native curve. The value of absorbance was taken at 280 nm. The final results denote unfolding of trypsin occurred in the presence of GdnHCl and urea and the ionic nature of GdnHCl masks electrostatic interactions in trypsin, a phenomenon that was absent in the presence of urea.

Keywords: Trypsin, Urea, Guanidine Hydrochloride, Spectrophotometry, Spectrofluometry.

Abstract No.73

Synthesis, Characterization, Cytotoxicity and Interaction of a Newly Designed Anti-Cancer Palladium(II) Complex with Calf Thymus DNA

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Deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer platinum- and palladium-based drugs[1]. Several pd(II) and pt(II) complexes of dithiocarbamate derivatives have been prepared[2]. Here we report Synthesis, Characterization, Cytotoxicity and interaction of a palladium(II) complex of formula [Pd(en)(bpy)](NO3)2(where en is ethylendiamine and bpy is 2,2′-bipyridine) with calf thymus DNA(CT-DNA). The cytotoxicity assay of the complexe has been performed on chronic myelogenous leukemia cell line, K562, at micromolar concentration. This complexe showed cytotoxic activity far better than that of cisplatin under the same experimental conditions. The binding parameters of the complexe with CT-DNA was investigated using UV-visible and fluorescence techniques. It shows the ability of cooperatively intercalating in CT-DNA. Gel filtration studies demonstrated that palladium complex could not cleave the DNA. In the interaction studies between this Pd(II) complexe with CT-DNA, several binding and thermodynamic parameters have been determined, which may provide deeper insights into the mechanism of action of these types of complexes with nucleic acids.

Keywords: DNA Binding and Thermodynamic Parameters, Anti-Tumor Activity, Pd(II) Complexe.

Abstract No.74

Studies on the Anti-tumor Activity of an Endostatin Fragment

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Angiogenesis, a complex multistep process including the proliferation, migration and differentiation of endothelial cells, microtubule formation, and sprouting of new capillary branches, is a critical event in growth and metastasis of cancer and prevention of angiogenesis is one of the best strategies for treatment of cancer. Endostatin, the C-terminal fragment of collagen XVIII, is an endogenous inhibitor of angiogenesis that inhibits tumor growth without toxicity and acquired

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

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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

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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.
