Abstract No.118

Cloning, Sequencing and Characterization of the α-Amylase Gene from a Native Strain of Basillus cereus

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Among various extracellular enzymes, α-amylase is a commercially valuable enzyme which randomly cleaves the 1,4-α -D-glucosidic linkages between adjacent glucose units in the linear amylose chain. An α-amylase producing Basillus strain was isolated from hot springs of Sabalan mountain. The 16S rDNA was amplified and sequenced using universal primers and the isolate was characterized as B. cereus. Two specific primers for upstream and downstream sequences of the gene were designed using genome sequence of the bacteria (NCBI Reference Sequence: NC_012472.1). The polymerase chain reaction was performed according to standard three-step reaction. The DNA band with approximately 2500 bp in size was obtained and cloned into pBluescript II SK+ vector and sequenced. The results were analyzed and confirmed that the isolated Basillus cereus produces a thermostable alkaline α-amylase.

Keywords: α-Amylase Gene, DNA Isolation, PCR Amplification, Cloning.

Abstract No.119

The effects of Radiofrequency Electromagnetic Fields (RF-EMFs) on the Structure of DNA

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Currently, the biological effects of two kinds of nonionizing electromagnetic fields (EMFs) including extremely low and high frequency EMFs including radiofrequency (RF) radiation have been the subject of various experimental and theoretical studies. RF energy has been employed in majority of the areas for instance cell phones and pagers. The potential effect of RF-EMF on DNA is very important because damage to the DNA of somatic cells can lead to the development of cancer or cell death. In this study, the effects of mobile phone RF (940 MHz) on the structure of DNA were investigated by UV-Vis, circular dichroism and fluorescence spectroscopic techniques. The results indicated that mobile phone EMFs can altere the structure and conformation of DNA significantly. Accordingly, our results indicate that we cannot confidently exclude any possibility of an increased risk of DNA damage, with important implications for the possible health effects of exposure to 940 MHz EMFs.

Keywords: DNA, Radiofrequency Electromagnetic Fields, UV-Vis Spectroscopy, Circular dichroism Spectroscopy, Fluorescence Spectroscopy.

Abstract No.120

A Novel Eukaryotic Cell Based Bioluminescence Assay for Detection of Oxidative-Stress Inducing Compounds

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The modern life of human is accompanied by encounter with various environmental stresses which lead to the accumulation of free radicals in the body. These free radicals give rise to damages to cellular biopolymers including nucleic acids, proteins, carbohydrates and fatty acids. Hence, the goal of our work was to develop a biosensor with high sensitivity and specificity able to detect a wide range of compounds with oxidative capacity in biological samples.Two single-stranded oligonucleotides containing the ARE core promoter, after annealing, were cloned into PGL4.26 vector which comprise the luc2 gene encoding luciferase as reporter. Following amplification of the vector harboring the ARE sequence in E.coli, it was transfected into HUH7 cells (human hepatoma cells) grown in DMEM medium. After 24 hours, the cells were treated with two chemicals (hydroquinon and benzoquinone each with 10 μM concentration). Next, lussiferase assey was performed.The data obtained here exhibited that the ARE sequence is active in the presence of oxidative stress-inducing compounds hydroquinone and benzoquinone. Also, it was revealed that he HUH7 cells treated with hydroquinone and benzoquinone possess a higher level of luciferase expression, up to 10 and 6 times more respectively, in comparison with the control cells which were not exposed to oxidative-stress inducers.According to the data obtained in the first phase of our study presented here we were able to construct a

transient eukaryotic-cell based biosensor which has the capability to detect hydroquinone and benzoquinone compounds in the medium. The next phase of our study has been focused on the construction of a permanent biosensor with the ability of detecting a wide variety of oxidative-stress inducing chemicals. The results will be presented.

Keywords: Bioluminescence, Oxidative-Stress, Free radical, Biosensor.

Abstract No.121

Preparation of anti-CD4 Monoclonal Antibody-Conjugated Magnetic Nanoparticles and their Application on T lymphocyte Separation

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Detection and separation of specific classes of cell through their surface markers by Monoclonal antibodies are very valuable for research and clinical diagnosis. Nanoparticles can effectively bind to monoclonal antibodies to identify their targets in different populations of cells. In the present study, anti-human CD4 antibody was covalently immobilized on the surface of core-shell Fe3O4/SiO2nanoparticles to analyze CD4 T lymphocyte in human blood.In this study, first Fe3o4 nanoparticles were synthesized using a chemical precipitation method then these particles coated with Tetraethylorthosilicate. Silica coated nanoparticles where chemically modified in order to react with amino (−NH2) groups of antibody. Attachment of antibody to nanoparticles is confirmed by various spectroscopic and biological methods. The amount of immobilized antibody was estimated by Bradford method. After conjugation, the binding ability of antibody to its receptor was investigated. An agglutination test was performed to prove the presence of fixed antibodies. The presence of nanoparticles on the lymphocytes is confirmed by iron staining and fluorescence methods.Microscopic images were taken from the culture lymphocytes clearly showed the presence of nanoparticles at the cellular level. Moreover, after each step of synthesis and antibody conjugation with nanoparticles, spectrum of the nanoparticles confirmed the successful conjugation of functional groups. The results show that functional antibodies conjugated to nanoparticles successfully.Anti human CD4 monoclonal antibody could be conjugated to chemically modified nanoparticles efficiently. The conjugated antibody has biological activity and could be used in different application such as detection and separation of CD4 T lymphocytes. Compared with the conventional fluorescent antibody, the conjugated antibody was simple, rapid and stable method for detection and isolation of human CD4 T cells.

Keywords: Magnetic Nanoparticles, T Lymphocyte, Antibody, CD4 Molecule.

Abstract No.122

Copper/zinc-superoxide Dismutase 1 Mutants and the Propensity to Oligomerization

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Copper/zinc-superoxide dismutase 1 (SOD1), as an important antioxidant enzyme, scavenges intracellular superoxide anion radical and catalyszes the dismutation of this radical into molecular oxygen and hydrogen peroxide. More than 125 different mutations have been identified in the SOD1 gene that is related to the familial form (fALS) of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease, in which motor neurons of spinal cord and brain cortex are selectively destroyed. The presence of SOD1 aggregates in the motor neurons of fALS patients is well documented. The mechanism(s) by which these aggregates result in disease phenotype are still unknown, but, it is revealed that mutations can induce structural instability of SOD1 protein, which in turn, make the protein prone to aggregation. In this study, we selected two SOD1 mutants (Asp124His and Glu100Lys), both of them reported as pathological, and characterized them investigating their propensity to aggregation using different techniques, from ThT-binding fluorescence to Circular Dichroism spectra, light scattering and Transition Electron Microscopy (TEM). We show here that these two SOD1 mutants, only when they are under destabilizing conditions, undergo the same general mechanism of oligomerization as found for the Wilde type protein. The rates of