Abstract No.246

Positive Homotropic Effect in two-phasic Non-Michaelis Kinetics of Xanthan Lyase

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Xanthan gum, a bacterial anionic heteropolysaccharide, structurally consists of a main chain of D-glucose units which linked by β-1,4 bounds, as it is in cellulose, plus side chains attached to glucose residues alternately. Trisaccharide side chain contains of a D-glucuronic acid unit between two D-mannose residues. Xanthan, produced by xanthomonas campestris,has found commercial applications as a viscosity enhancing agent in aqueous solutions. Viscosity of xanthan solutions may be controlled via degrading its backbone or side chain by using hydrolyzing enzymes including xanthanase (for hydrolyzing the backbone) or xanthan lyase (for hydrolyzing the side chain).In this study, xanthan lyase activity was measured by monitoring ΔA235 of a solution containing appropriate concentration of xanthan in 50mM sodium phosphate buffer pH=7 and xanthan lyase enzyme secreted by a xanthan lyase producing bacterium at 30 °C. According to our results, the enzyme saturation curve showed non-Michaelis behavior (positive homotropic effect). More analysis, using Eadie-Hofstee and Hill plots, demonstrated the enzyme shows two phases during saturation by xanthan as substrate, resulting in enzyme activation. We concluded that xanthan is not only the enzyme substrate but also its activator. so that increasing concentrations of xanthan can induce a more active conformation in xanthan lyase.

Keywords: Xanthan lyase, Homotropic effect, Non-Michaelis-Menten, Xanthan.

Abstract No.247

Isolation of a β-mannanase Producing Bacterium, Secretion Optimization and Biochemical Properties of the Enzyme

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In the present work, a halo-alkali tolerant mannan-degrading bacterium (strain FH-1) was isolated from soil samples of Jahrom city, Fars, Iran. Phenotypic classification and 16S rDNA sequence analysis placed FH-1 in the genus Gracilibacillus. Strain FH-1 grew well at salinities and alkalinities of 0-20% NaCl and pH of 7-10. When grown on 1% locust bean gum (LBG) as the carbon source at 5 % NaCl and pH 9.0, maximal β-mannanase activity was produced in the culture supernatant after 3 days. Partial purification of the enzyme was done by a combination of ammonium sulfate precipitation and DEAE-Cellulose ion exchange chromatography. The endo-1,4-ß-D-mannanase activity of the enzyme was confirmed by using specific substrate, Azo-carob galactomannan. The optimum temperature and pH for β-mannanase activity on LBG as a substrate were 50 oC and 10.0 respectively. The enzyme exhibited its optimal activity at 0-1 M NaCl and 50 % of its initial activity remained when assayed in 2 M NaCl, indicating a high degree of halostability. These results suggest that the β-mannanase secreted by the newly isolated Gracilibacillus sp. strain FH-1 is a suitable candidate for using as a detergent ingredient due to its activity at a broad pH and NaCl concentrations.

Keywords: β-mannanase, Alkalophile, Gracilibacillus, Locust bean gum, Halostability.

Abstract No.248

Monitoring Surface Plasmon Resonance of Gold Nanorods in Biological Buffers

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Nobel metal nanoparticles with their shape and size dependent properties have the potential to be exploited in many research areas, such as nanoscale electronics, catalysis, optical sensing, imaging, gene/drug carrier, therapeutics etc. In the light of this, nanostructures of gold with rod morphology (GNRs) have attracted much more attention in novel medical and paramedical applications. It is important to investigate the stability of GNRs in different biological buffers, since presence of some ions might affect the structure and morphology of the nanostructures. Herein, we present the effect of three biological buffers on the longitudinal plasmon resonance of GNRs, to check the sensitivity of LSPR and maintenance of rod morphology. Short gold

nanorods were synthesized according to the conventional seed-mediated growth protocol and characterized by UV-Vis spectroscopy, transmission electron microscopy (TEM), and atomic absorption spectroscopy (AAS) for shape, size and yield determination. The typical surface plasmon absorption bands in the visible and near IR region confirmed the rod morphology of the nanostructures. Phosphate, Tris and HEPES buffer solutions were prepared and the pH was adjusted to be 7.4. Concentration of the buffer solutions was 0.05 M in the final working solutions. Prior to use, GNRs were purified by two rounds of centrifugation at 12000 rpm, for 6 minutes. Pellet of the second round with a fixed concentration of gold nanorods were diluted with each buffer solution. UV-Vis spectra of the interacted samples were recorded to monitor the plasmonic bands. Results showed that neither the transverse SPR nor the longitudinal one have changed notably. Although the intensity of LSPR peak has been somewhat affected in buffer medium, the rod morphology is still maintained. Sensitivity of gold nanorods to trace changes in the local environment/ refractive index encourages the possibility of utilizing these nanostructures in development of nanostructures for various biosensing applications.

Keywords: Gold Nanorods, Surface Plasmon Resonance, Nanobiosensor.

Abstract No.249

Investigation of Mechanistic Influence of Mutation D117G in Aequorin from Aequorea Victoria: a Molecular Dynamics Simulation Study

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The photoprotein aequorin, isolated from the jellyfish Aequorea victoria,is a bioluminescent complex formed with the protein apoaequorin and the prosthetic factor coelenterazine that emits light upon calcium binding. In order to understand the mechanism of the reaction, the study of structure-function relationships was undertaken with respect to modifying certain of its amino acid residues. One of these mutations is the D117G that is located out of substrate binding cavity and Ca2+-binding loops. In this study short time molecular dynamics simulations were performed to investigate the influence of this fine change on dynamic properties of substrate binding cavity and Ca2+ binding loops that direct emission light properties in this photoprotein. Previous studies showed that this mutation decreases affinity of loops to Ca2+ and increase half-life of emission light in the photoprotein. Our finding revealed that the replacement of ASP with GLY alters dynamics and energetic properties in functional regions of the structure and affects emission light and Ca2+- sensitivity properties in photoprotein.

Keywords: Aequorin, Molecular Dynamic Simulation, Ca2+-affinity, Life-time.

Abstract No.250

Surface Modification of Gold Nanorods with Polyethyleneglycol for Nano Biosensing Applications

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There has been great interest in the domain of nanobiotechnology to design and develop new generation of nanobiosensors. Amongst anisotropic nanoparticles, gold nanostructures of rod morphology (GNRs) have attracted significant attention, for having variety of applications in biomedicine and biosensing. Although many fruitful features of gold nanorods have been introduced so far, the nanostructure itself is believed to show strong cytotoxicity, since it has been synthesized in the presence of hexadecyltrimethylammonium bromide (CTAB). The cationic surfactant is also known to play important role in the stabilization of GNRs, and maintenance of rod morphology. Herein, polyethyleneglycol has been utilized for neutralization of the positively charged GNRs. Gold nanorods were synthesized according to the conventional seed mediated protocol. Formation of the rod morphology, size, monodispersity, concentration and yield of synthesis were characterized by UV-Vis spectroscopy, transmission electron microscopy (TEM) and atomic absorbance spectroscopy (AAS). Excess CTAB was removed by one round of centrifugation at 12000 rpm for 6 minutes. Sample of GNRs with 75 nM concentration was interacted with 400 µL PEG-4000 (600 mg. mL^{-1}). The mixture was incubated at ambient temperature for 1 hour. Excess PEG was removed by another round of centrifugation. Surface plasmon resonance of bare and pegylated GNRs was monitored via UV-Vis spectrophotometer. Results showed that transverse plasmon resonance (TSPR) and longitudinal plasmon resonance (LSPR) appeared at 528 nm and 708 nm, respectively. Intensity of SPR bands of the pegylated GNRs decreased upon treatment, without any shift in both regions. Although a considerable quantity of the cationic surfactant has been replaced by polyethyleneglycol, stability of GNRs