

Abstract No.205

Spectrofluorimetric Study of the Interaction of Daunomycin Antibiotic with Histone H1

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Histone H1 is a very lysine rich histone fraction of chromatin which appears to play an important role in the compaction and stability of the structure of chromatin. Daunomycin is an antitumor anthracycline antibiotic widely used as a chemotherapeutic agent for the treatment of various cancers. In the present study, we have investigated the interaction of daunomycin with the purified calf thymus histone H1 in solution using fluorescence spectroscopy technique in 20 mM phosphate buffer, pH=7.0 and 1 mM EDTA at room temperature. The results show that daunomycin decreases the fluorescence emission of histone H1 at 325 nm and induces hypochromicity at the emission spectrum of this protein. Also the fluorescence emission of daunomycin is increased at 595 nm and hyperchromicity is induced at the emission spectrum of this drug after incubating with histone H1. Increasing ionic strength elevates this effect. The results suggest that H1 can be considered as a target for daunomycin action at the chromatin level.

Keywords: Chromatin, Histone H1, Daunomycin, Fluorescence Spectroscopy.

Abstract No.206

Role of Hydrogen Bond Network Around the p-hydroxybenzyl ring of Coelenterazine in Mnemiopsis bioluminescence Activity by Site-directed Mutagenesis

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Photoproteins are Ca²⁺-regulated proteins that emit light. So far, the most investigations of photoproteins have been done on coelenterate

photoproteins such as aequorin and obelin and there is no information about the mechanism of coelenterate photoproteins and architecture of its coelenterazine binding site. In native aequorin and obelin photoproteins, exists a water molecule (W1) in the binding pocket that makes hydrogen bonds with the phenolic OH of the 2-p-hydroxybenzyl, the O_γ-atom of Thr166, and the carbonyl oxygen of Ile105. The main role of W1 is considered to be the stabilization of coelenterazine moiety. In the mnemiopsis, W1 that exists in the binding pocket of native aequorin and obelin is missing and the hydroxyl group of Ser128 establishes a hydrogen bond with 2-p-hydroxybenzyl one hand and the other to with Ile124 and thus possibly creating a network of hydrogen bonds in this position. In the well-known photoproteins, Ile105 and Thr166 are essential in the formation of this network and their corresponding residues are Ile124 and Val183 in mnemiopsis. Therefore, V183T and S128G substitutions based on hydrogen bond network around the ring of coelenterazine were performed. On the contrary, the V183T and S128G mutants showed 75% and 19% activity respectively when compared to wild type variant. V183T mutant was active in higher concentrations of calcium and its maximum wavelength of bioluminescence spectrum had about 20 nm blue shift relative to the wild type (470 nm vs. 490 nm). Other characteristics of the mutant did not show any changes. CD and fluorescence spectroscopy and molecular modeling studies showed some structural changes in the mutants. Based on homology modeling studies, this hydrogen bond network has been removed in S128G mutant, while in V183T mutant, an additional hydrogen bond was observed. The results indicated that the hydrogen bond network around the p-hydroxybenzyl ring in position C2 of coelenterazine is important in the bioluminescence activity of photoproteins.

Keywords: Site-directed mutagenesis, Photoprotein, Mnemiopsis, Coelenterazine.

Abstract No.207

Stabilization of Glucose Oxidase Upon Immobilization on Albumin Amyloid Nano-fibers

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Glucose oxidase (GOD, EC 1.1.3.4) is an oxidoreductase with numerous biomedical and industrial applications. It catalyzes oxidation of glucose to gluconic acid. Molecular oxygen accepts electrons to