Keywords: Aequorin, Site Directed Mutagenesis, Luminescence Properties, Double Mutants.

Abstract No.258

Protective Effects of 6 Genotypes of Walnuts Against free Radical-Mediated Protein Oxidation

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The role of oxidative protein damages in the pathophysiology of human diseases is currently a topic of considerable interest as oxidized proteins has been implicated in a wide spectrum of clinical disorders. In this study, the antioxidant activity of 6 genotypes of walnuts, were investigated employing various established in vitro systems including ferric reducing ability (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and inhibitory effect on protein oxidation as well as the inhibition of Fe2+/ascorbate induced lipid peroxidation in human plama samples. Total phenolic content (TPC) and total flavonoid content (TFC) of the samples were also determined by a colorimetric method. The addition of Fe2+/ascorbate to the plasma samples significantly increased the of protein oxidation by loss of protein-bound sulphydryl (P-SH) groups and increased lipid peroxidation (LPO) The plant extracts showed inhibitory effects against P-SH oxidation, and LPO to varying degrees. Based on this study, the protective effects of walnuts extract could be due to its TPC. In that respect, free radical induced protein oxidation was suppressed significantly by the addition of walnut over a range of concentration. These results clearly demonstrated that in the shells of walnut have higher antioxidant activities than the hulls of walnut. KH501, KH403, KH509 genotype with the highest phenolic content in its shells has more antioxidant activity against protein oxidation.

Keywords: Protein Oxidation, Juglans Regia, Antioxidant Capacity, Lipid Peroxidation.

Abstract No.259

The Fibrillation Study of β-Lactoglobulin Upon Incubation with Aflatoxin M1

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Aflatoxin M1(AFM1) appears in milk as a direct result of the ingestion of food contaminated with aflatoxin B1 by cattle. The role of milk in human nutrition is well-known. The formation of AFM1 occurs in liver and it is secreted into the milk, which is cytotoxic and genotoxic. AFM1 is bound to milk proteins. As a result of the binding affinity of AFM1 for milk proteins, the toxin is distributed unevenly between whey and curd. The purpose of this report is to study the effect of AFM1 on β-lactoglobulin (β-Lg) fibrillation. Regards to this proposal that AFM1 enters to whey proteins (especially, β-Lg), supposed it would interact with this protein and affects on β-Lg fibrillation. β-Lg solution with concentrations of 1(W/V%) at pH 2 was prepared and interacted with different concentration of AFM1. After heating of the solutions at 85 °C for 24 h, the fibrillation of them were investigated. Strange results showed that AFM1 reduces the intensity of fluorescence of fibrils. By increasing the concentration of AFM1, the intensity of fluorescence of fibrils was decreased. This means AFM1 as a toxin reduces the fibrillation of β-lactoglobulin.

Keywords: Aflatoxin M1, β-Lactoglobulin, Fibrillation, Milk proteins.

Abstract No.260

Fibrillar Protein Aggregation May be Detrimental Via Different Oxidative Routes: Relevance to the Etiology of Amyloid-Related Neurodegenerative Disorders Using the Experimental-Based Evidences

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The exact mechanism of cell death in neurodegenerative diseases remains obscure, but the aberrant assembly of proteins into fibrillar

aggregates is accused to be the primary cause of pathogenesis. Furthermore, the structural determinants of protein fibrils that are responsible for cell dysfunction are not yet clear. The main objective of the present study was to discuss the potential role of peroxidase activity of "heme-amyloid fibril" complex in neurodegenerative disorders onset/progression using the protein-based experimental models, in vitro. The results of the present study also suggest that oxidative stress may be involved in neurodegenerative cell toxicity via several independent (mechanistic) routes, highlighting a possible functional link among formation of excessive amounts of reactive (oxidant) species and protein/DNA metabolism. The present findings emphasize that data on the origin of oxidative stress-related damages may help us to postpone/attenuate the onset/extent of irreversible part of neurodegenerative pathogenesis.

Keywords: Amyloid Aggregation, Toxicity, Heme, Peroxidase, α-Crystallin, α-Chymotrypsin.

Abstract No.261

Prediction of Aggregation Propensity and Amyloidogenic Regions in Proteins, Based on Their Primary Sequences

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The misfolding and extracellular amyloid depositions of specific proteins are associated with a large family of human pathologies, often called protein conformational diseases (PCDs). Despite many efforts expended to characterize amyloid formation in vitro, there is no deep knowledge about the environment (in which aggregation occurs) as well as mechanism of this type of protein aggregation. Thus, the development of methods to anticipate the aggregation properties of polypeptides is receiving increasing attention. In the last decade, data have begun to accumulate suggesting that the composition and the primary structure of a polypeptide determine to a large extent its propensity to aggregate and that small changes may have a huge impact on solubility and stability. The ability of identification of individual amyloidogenic regions in disease-linked polypeptides would be of much value. In this study, "aggregation-prone" sequences in 2 native/modified nondisease related amyloidogenic proteins, calculated by three prediction servers (Tango, Aggrescan and Waltz) were analyzed. Then, the outputs of computer-designed algorithms were compared with experimentally derived available information. There is the possibility that these algorithms become useful tools for screening therapeutic approaches against amyloidoses as well as to improve the solubility of recombinant proteins. We will discuss the importance of our analyses.

Keywords: Aggregation propensity, Tango, Aggrescan, Waltz.

Abstract No.262

Purification of Lipid-Transfer Protein-1 (LTP-1) from Rice Grain and Study of Drug Binding to Normal and Modified LTPs

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Plant non-specific lipid transfer proteins (nsLTPs) are small basic proteins which transport phospholipids between membranes and are subdivided into two subfamilies, nsLTP-1 (9 kDa) and nsLTP-2 (7 kDa) according to the molecular weight. All of nsLTPs are highly stable proteins because they possess eight highly conserved cysteine residues forming four disulfide bonds. These proteins have received an increasing interest as potential drug carriers in drug delivery systems. These highly stable proteins have potential to protect drugs against oxidation or degradation. In the present study, citraconylation was employed to modify the accessible lysine residues of LTP-1. Then amphotericin B, which is widely used as an antifungal drug, has been used to compare the drug binding behaviors of the native and modified proteins. Fluorescence spectroscopy was used to compare the thermodynamics as well as PSH properties of native and modified LTP-1. Our results showed that Kbinding, the number of binding site and PSH have been increased in modified LTP. We may propose that new binding sites have been created upon LTP modification or binding ability of protein to drug has increased compared to the native one. We will discuss the importance of our observations.

Keywords: LTP, Drug, Modification.
