**Abstract No.265**

## The Physicochemical and Biochemical Survey of the Honey Types from Iran: Protein and Enzyme Profiling

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Honey has been used since ancient time as both a sweet feed and a traditional medical treatment of many diseases. The greatest biochemical fraction of the honey weight is pertains to the sugars and their sugar profile are applied to characterize the honey quality the origin of honey. Moreover, the acidity of honey is one of the most important factors in quality control of honey. Over time, simple sugars and acids present in honey provides condition to produce the hydroxyl methyl furfural (HMF), therefore its quantity is an important but small percentage of the honey weight. factor in controlling of honey freshness. Proteins are the most important constitute however, at 0.1-0.5% of the honey weight. This amount differs according to the plant origin, and also diverse species of bees. Determination of protein content of honey is considered a new method for evaluating honey quality. In this study, the physicochemical properties and protein contents and enzyme profiling of 20 honey samples collected from different region of Iran were investigated. In this survey physicochemical parameters such as pH, acidity, ash, HMF and sugar content of samples were determined and compared. Upon extraction and concentration by dialysis, centrifuge and ammonium sulfate precipitation methods, the protein content were detected by Bradford assay. The result of this study showed that the physicochemical parameters and protein contents of honey types were different. These data can be used in determination of honey quality and its therapeutic features and provide beneficial information for future studies in this field.

**Keywords:** Honey, Quality Control, Physicochemical Properties, Protein Content.

**Abstract No.266**

## 40 Years of Protein Biochemistry with Escapades

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Biochemistry covers a vast area, and I was fortunate to visit a number of spots in it. Membranes caught my attention already when I was a student. In my first encounter during my diploma thesis I calculated the vibrational modes of different triglyceride esterbackbone conformations. The purpose was to characterize the "real" backbone conformation from the infrared spectra of lipid multilayers. I then switched the topic for my PhD, where I helped to establish an in vitro system of mammalian protein synthesis with purified components. At that time such a system was deemed necessary to elucidate the possible role of protein synthesis in the generation of antibody diversity. As a postdoctoral fellow - back on the main track - I synthesized a photoaffinity label for the analysis of apolar membrane lipid-protein interactions. Unfortunately, the label in the apolar environment reacted by intramolecular rearrangement instead of crosslinking and thus was useless. Benefitting from my experience in protein purification I then started to purify the glucose transporter of the Escherichia coli phosphotransferase system (PTS), the protein that became the golden thread of all subsequent research. One reason to choose this protein and no other was the fact, that it also has phosphotransferase (kinase) activity that is much easier to assay in vitro than transport. Along the way of cloning the permease gene a second PTS transporter was picked up, which appeared even more interesting because it also serves as membrane gate for the penetration of bacteriophage DNA and of pore-forming bacteriocins (toxic peptides). Gene sequencing took almost two years, an effort that payed off because it allowed us to overexpress the proteins, to purify them by metal chelate chromatography and to study their function by site-directed as well as random mutagenesis. Because PTS occur and play an important role only in bacteria, it appeared that it could be a target of antibacterial agents. This perspective motivated us to develop in vivo and in vitro assays for high throughput inhibitor screening, to characterize the attenuated virulence of a PTS knock-out strain, and to solve the X-ray structures of several soluble PTS protein subunits. Some of the projects were done in collaboration with a start-up company. Proteome analysis of the knock-out strain then draw our attention to a new family of dihydroxyacetone kinases. They are homologous to the eucaryotic Dha kinases but are supplied with high energy phosphate by the PTS rather than by ATP. The elucidation of their structure, catalytic mechanism and gene expression were the

conclusion of 40 exciting years spent in the biochemistry laboratories of different countries with various cultures.

**Keywords:** Protein Biochemistry, Backbone Conformation, Phosphotransferase System, Phosphotransferase, Proteome Analysis.

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**Abstract No.267**

### Effect of Butachlor Herbicide on Hemoglobin Structure and Species

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Butachlor (2-chloro-2', 6"-diethyl-N-(butoxymethyl) acetanilide) is a member of chloroacetanilide class of chemistry and is the herbicidal active ingredient in MACHETER EC. This herbicide is used as a pre-emergence control for the undesirable grasses and broadleaf weeds .The consumption of butachlor in Iran is among the most used pesticides which is mostly applied to rice fields. Extensive use of this herbicide beside other types of pesticides is now a concern for human health, as these chemicals can enters the body through our foods. Although most of these pesticides and their metabolites are excreted from the body, high daily intake cause permanent existence of these pesticides in the body. As a consequence, entrance of this herbicide into the blood stream, brings one of most abundant blood protein, hemoglobin, into contact with butachlor which may manipulate hemoglobin function in the body. In this report, the interaction of hemoglobin with butachlor under physiological condition was assessed and found the changes in protein structure and function which was analyzed by various methods such as UV-Vis, fluorescence as well as biophysical and biochemical investigations. The hemoglobin species upon interaction of butachlor were studied in this report.

**Keywords:** Herbicide, Butachlor, Hemoglobin, Spectroscopy.

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**Abstract No.268**

### Interaction of Cationic Peptide Drugs with Bacteria and Lipid Bilayers: Short R-, W-Rich Hexapeptides as a Case Study

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The global emergence of resistance to antimicrobial agents is increasingly limiting the effectiveness of current drugs. The treatment of multidrug-resistant Gram-negative germs represents a particular challenge. Antimicrobial peptides are effective molecules in the innate immune system and might provide a promising alternative towards classical antibiotics. Understanding the structural basis of activity and bacterial selectivity provides one basis for the development of effective peptide-based antimicrobial drugs. Peptides rich in arginine (R) and tryptophan (W) residues are of particular interest as they are found as small antimicrobial motifs in much larger natural compounds. Our recent strategy to induce constraints in RW-rich hexapeptides by cyclization did result in pronounced peptide activity against Gram negative Escherichia coli, for instance, cyclo-RRRWFW (c-WFW). The activity of c-WFW against E. coli is modulated by lipopolysaccharides (LPS) in the outer bacterial membrane.To elucidate the role of the two tryptophan residues in interactions with E. coli membranes, we replaced them by analogues having altered hydrophobicity, dipole and quadrupole moments, hydrogen-bonding ability, amphipathicity, or ring size. The biological activitiy against Bacillus subtilis and erythrocytes increased with increasing peptide hydrophobicity, whereas the effect on E. coli revealed a more complex pattern. Isothermal titration calorimetry demonstrated that peptide partitioning into model lipid membranes is driven by both electrostatic and hydrophobic interactions and follows the order: POPC/smooth-LPS >> POPC/rough-LPS > POPC/lipid A = POPC/POPG > POPC. The hydrophobic contributions to binding to POPC and mixed POPC/POPG were comparable. Low hydrophobicity and peptide conformational flexibility reduced binding, showing that peptide–membrane interactions correlate with the biological effect. The highly differentiated activity pattern against E. coli was poorly reflected in peptide binding to POPC/lipid A and disappeared in studies with LPS-containing membranes. Stronger partitioning into POPC/smooth-LPS as compared with POPC/r-LPS uncovered a significant role of O-antigen and outer-core oligosaccharides of LPS in anti-E. coli activity.

**Keywords:** Cationic Antimicrobial Peptides, Lipid Bilayers, Lipopolysaccharide, Peptide Partitioning, Isothermal Titration Calorimetry.

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