

Abstract No.30

Study on chaperoning effect of the N-terminal propeptide precursor of a novel thermolysin-like metalloprotease

Hamid Reza Karbalaeei-Heidari

Department of Biology, Faculty of Sciences, Shiraz University, Shiraz
71454, Iran

The proper folding of some proteins requires the assistance of other proteins called molecular chaperones, while the folding of others needs an intramolecular chaperone (IMC). Extracellular proteases are generally synthesized as inactive precursors consisting of a signal peptide, an N-terminal propeptide, and/or a C-terminal propeptide, along with a mature region displaying catalytic activity. Typically, the N-terminal propeptides of proteases act as intramolecular chaperones and/or inhibitors of the cognate mature enzymes, and this is the case for subtilisin, α -lytic protease, carboxypeptidase Y, cathepsins L, and all thermolysin-like, neutral zinc-metalloproteases (TNPs) identified so far. In previous studies, the gene of a novel haloalkaline zinc-metalloprotease (SVP2) from the moderately halophilic bacterium, *Salinivibrio proteolyticus* has been cloned, sequenced, and classified as a member of family M4, which includes a large group of TNPs that are produced by both Gram-positive and Gram-negative bacteria. Newly synthesized SVP2 precursor undergoes several proteolytic processing events to yield mature SVP2, from which the N-terminal propeptide usually comprising 199 amino acids are removed. To define the role of the propeptide of SVP2 in its processing and folding, we constructed a new vector with truncated form of nucleotide sequence of SVP2 precursor gene (Δ N-SVP2), and the effects of this propeptide deletion on casienolytic activity, processing, stability, and accumulation inside and outside of the cell were examined. Our results indicated that, although the N-terminal propeptide of SVP2 precursor shares 48% identity with that of other TNPs, which assists the refolding of protease, inhibits the folded protein to process its C-terminal propeptide, and shows a stronger inhibitory activity toward mature proteases, the SVP2 propeptide does not reveal similar functions, suggesting it makes different structure upon folding.

Key words: chaperoning, thermolysin-like metalloprotease, protein folding.

Abstract No.31

Analysis of pH Dependence Activity of Alcohol Dehydrogenase

M. Saifi Abolhassan, F. Khorsand, M. Islami, S.Z. Moosavi-Nejad

Department of Biology, Faculty of Basic Science, Alzahra University,
Tehran, Iran

The activity of an enzyme is profoundly affected by pH. The effect of pH can be irreversible inactivation, occurring at extreme pH extents or reversible inactivation due to pH effects on kinetic parameters of enzymatic reaction. The essence of studying pH effects on enzyme is undoubtedly due to the vast application of them in industry undeniable. The biotechnological potentials of alcohol dehydrogenases encouraged us to investigate the effects of pH on its activity and stability. In this study, the effects of pH on PQQ dependent alcohol dehydrogenase purified from *Acetobacter* sp. Strain SSM15 was investigated. Enzyme assay was colorimetrically performed in McIlvaine buffer with ethanol as substrate at 25 °C. According to the log v_{max} versus pH graph, the optimum pH of ADH activity was achieved. The pK_{es} values, related to the protonation constant of the enzyme-substrate complex, were determined from the slope of the graph which is in concert with the data reported from its structural studies. The pK_{es1} and pK_{es2} were calculated 4.1 and 7.2, respectively. The pH profile of the enzyme stability was obtained by enzyme incubation in different pH for 24 hr. Concerning the pH dependence of enzyme activity, the enzyme can be considered as a diprotic type, which is reactive in monoionized form.

Key words: Alcohol dehydrogenase, PQQ, pH effects, pK_{es} , Activity, Stability.

Abstract No.32

Prediction of residues on bovine carbonic anhydrase as binding sites in reacting with sodium dodecyl sulfate based on molecular dynamics and docking simulations

S. Ebadif, M. R. Housaindokht, M. R. Bozorgmehr*

1- Department of chemistry, Faculty of science, Islamic Azad
University, Mashhad branch, Mashhad, Iran

2- Department of chemistry, Faculty of science, Ferdowsi University of
Mashhad, Mashhad, Iran

The main objective of this study is to specify binding site and define the binding affinity of sodium dodecyl sulfate (SDS) into bovine carbonic anhydrase (BCA) using the P_i values of residues and the free binding energy. P_i values obtain by the simulation procedure. The

residue I with $P_i > 1$ has been considered to have affinity toward the SDS ligand, while with $P_i < 1$ it has no affinity. So, the calculated P_i values for BCA residues and docking simulation to be used to prove that. In the docking scheme, the SDS ligand docked into each of the residues with $P_i > 1$, which are located on different regions of the surface of BCA. Moreover, the Blind Docking method was employed, the results of which were in good agreement with the above docking.

Key words: bovine carbonic anhydrase, sodium dodecyl sulfate, molecular dynamics, docking simulations.

Abstract No.33

Investigation of the association behaviors between lomefloxacin and human serum albumin: A fluorescence spectroscopic study

R. Assaran Darban^{1,2}, M. Saleh-Moghadam¹, J. Chamani²

1. Razavi Khorasan Payame Noor University, Mashhad, Iran
E-mail: mrasaran78@yahoo.com, 2. Department of Biology, Faculty of Sciences, Islamic Azad University-Mashhad Branch, Mashhad, Iran

Protein plays an important role in life processes and investigation of the interaction between small molecules and protein has been the focus of extensive research in recent years. The binding between probes and proteins is of ever increasing importance in the pharmaceutical industry, sensor and scientific communities. Albumins, the most abundant protein in the plasma, are characterized by a low content of tryptophan and methionine, a high content of cysteine and preponderance of charged acidic and basic amino acids. The interaction between lomefloxacin and human serum albumin (HSA) has been studied by steady fluorescence spectroscopy. The binding of lomefloxacin to HSA quenches the tryptophan residue fluorescence and the results show that both static and dynamic quenching occur together with complex formation. The binding constant and binding sites of lomefloxacin to HSA at pH 7.4 are calculated and showed two set of binding sites, according to the double logarithm regression curve. In addition, the distance between the lomefloxacin and HSA is estimated to be 1.32 nm using Foster equation on the basis of the fluorescence energy transfer. On the other hand the fluorescence spectra show that the microenvironment of the tryptophan and tyrosine residues has obvious changes, which obeys the phase distribution model. Finally, the thermodynamic data show that lomefloxacin molecules enter the hydrophobic cavity of HSA via hydrophobic and electrostatic interactions. The interaction between

lomefloxacin and HSA induced an obvious reduction of the protein alpha helix and beta sheet structures.

Key words: lomefloxacin, human serum albumin, fluorescence spectroscopy.

Abstract No.34

Binding of curcumin to beta casein, a route to make a functional food

Mansoor Esmail¹, S.Mahmoud Ghaffari¹, Zainab Moosavi-Movahedi², Ahmad Sharifzadeh¹, Reza Yousefi³, Mohammad Farhad¹, Ali Akbar Moosavi-Movahedi⁴

1- Institute of biochemistry and biophysics, Tehran University, Tehran, Iran, 2- Department of chemistry, faculty of science, Tarbiat modares university, Tehran, Iran, 3- Department of Biology, faculty of Sciences, University of Shiraz, Shiraz, Iran, 4- ENT-HNS Research Center of Iran University of Medical Science, Tehran, Iran.

International food information council (IFIC) defines the functional food as "foods that provide health benefits beyond basic nutrition". Recently a world wide attempts is performed to devise novel encapsulation materials for multi-target drugs with low solubility and hence their incorporation into food. In this regard physico-chemical properties of biopolymers such as carbohydrates and proteins render them a good candidate to achieve this purpose. In this study we speculated the virtue of the interaction between curcumin, a potent anticancer and anti inflammatory natural polyphenol, and beta-casein using U.V-Vis and fluorescence spectroscopies at different temperatures. Obtained binding isotherm plots revealed that hydrophobic interactions are the main factors contributed in beta-casein upon interaction with curcumin. Augmenting the temperatures up to 37°C increased the number of bound curcumin up to 4 times. ANS fluorescence explained this phenomenon very well since surface hydrophobicity is enhanced at upper temperatures. These findings imply that casein as a natural biopolymer from milk can be a good matrix for increasing the solubility of curcumin in pharmacology.

Key words: curcumin, beta-casein, solubility, hydrophobic surface, functional food.