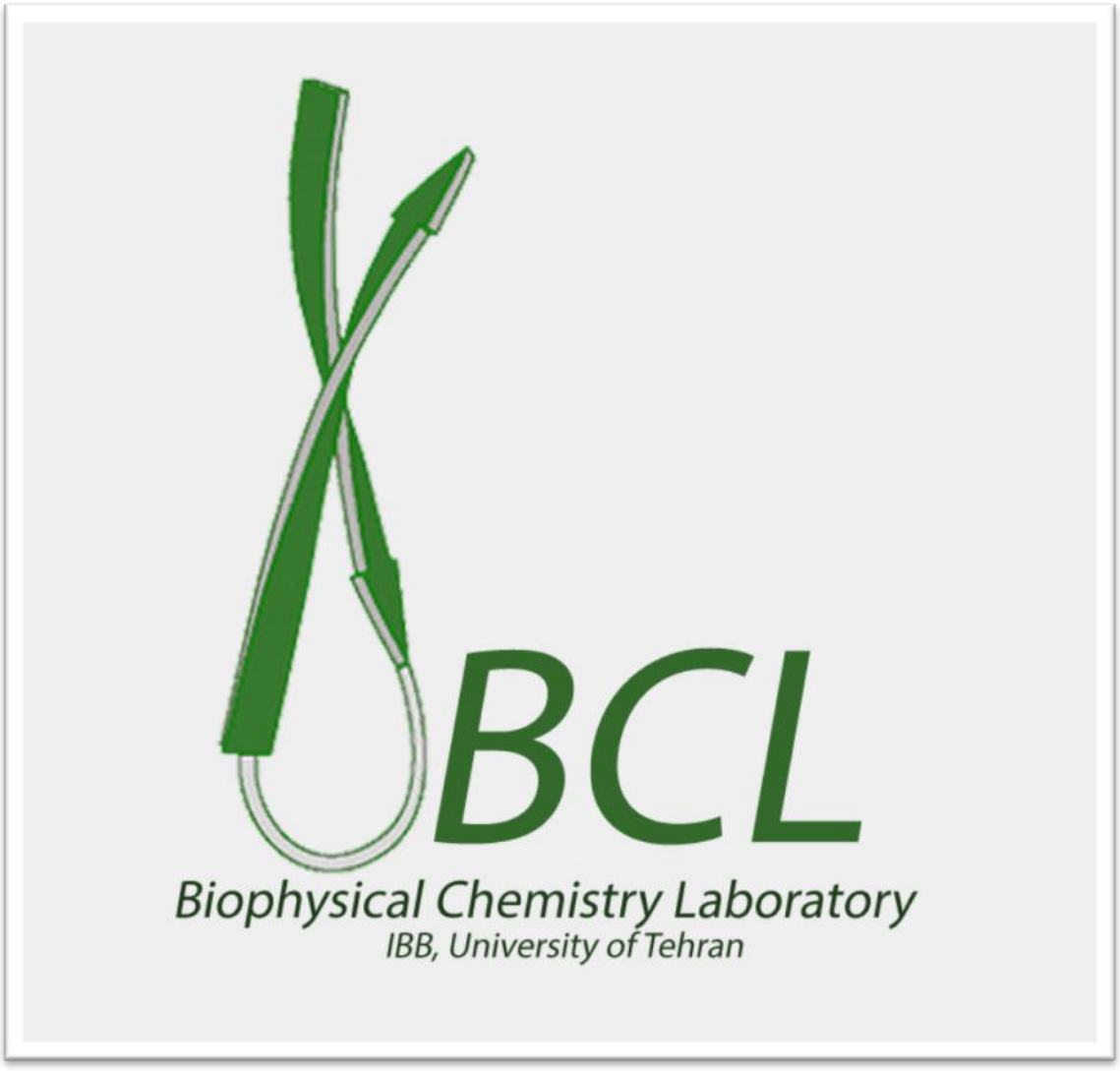
BIOPHYSICAL CHEMISTRY LAB

ANNUAL REPORT 2015



Institute of Biochemistry and Biophysics
Tehran, Iran

http://bcl.ut.ac.ir/

CONTENTS

FOREWORD

The Biophysical Chemistry Laboratory (BCL) was established in 1986 in the Institute of Biochemistry and Biophysics (IBB) as a main base and the mother of Biophysical Chemistry in Iran. BCL is famous worldwide in the research area of Thermodynamics of Protein Denaturation and Biomacromolecular Interaction.

This laboratory enjoys from advanced facilities and it is equipped with advanced apparatuses for the research. LBC is equipped with Nano Differential Scanning Calorimeters (DSC); Nano Isothermal Titration Calorimeters (ITC); modern Circular Dichroism (CD) Spectropolarometry, Sensitive Densitometer and Tensiometer; Fluorescence and Uv-vis Spectrophotometers, Microviscometers and Biochemical and Biophysical methods as well as and Computational facilities.

BCL is an appropriate place for the promotion of the research and science in the field of Biochemistry, Nanobiophysics, Biotechnology and Biophysical Chemistry. BCL is a suitable laboratory for training PhD students and postdoctorate researchers, associate researchers and sabbatical leaves for faculty members at national and international levels. Faculty members, postdoctorates, students and foreign research associates using the facilities of this laboratory which have published hundreds of full research articles in international prestigious journals. BCL is an appropriate laboratory for supporting and promoting the research of scientists and researchers at national and international levels.

BCL is advanced nano-laboratory with accurate equipment, skilled technicians and capable of serving linked with Nanotechnology Laboratory Network (LNN). BCL is accessible to LNN and it is ready to make a good service in various aforementioned area. UNESCO Chair on Interdisciplinary Research in Diabetes and Center of Excellence in Biothermodynamics are lined with BCL.

Biophysical Chemistry Lab

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- M.Khalesi

STUDENTS (Year 2015)

Doctor of Philosophy (PhD)	Master of Science (MSc)
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❋M.Valipour	☆E.Vahdat
❋E.Najd Gerami	☆B.Babaee
❋J.Rafiee	☆E.Hosseini
❋R.Karamzadeh	☆N.Pishkari
❋M. Qafary	

Alumni (Year 2014)

PhD

Name	Topic of Thesis
L.Fotouhi	Study of heme degradation and ROS production upon interaction of hemoglobin with ionic surfactant
M.Mazaheri	The study of effect of aflatoxin M1 and Lead ions on protein (BSA, HSA and β-Lg) fibrillation in vitro
A.H.Keyhan	The biophysical chemistry study on the structure and function of choline oxidase upon the effect of ionic liquids using carbon nanotube/ionic blue nanocomposites liquids/prussian
A. Khatibi	The effects of biocompatible mesoporous silica nanoparticles on the reversibility, structural stability and activity of human carbonic anhydrase II

MSc

Name	Topic of Thesis
M.R. Dashti	Study of fibrillation and reactive oxygen species on structure and function of hemoglobin upon interaction with ribose, fructose and potassium sorbate

Technolgies:

Biophysical Chemistry Kits

Today, with the increase in production and a variety of processed foods consumed meat species, the identification of the mixed meat products should be concerned by precise technique. The companies producing meat products require the governor to set up laboratories to be able to offer their products and goods produced permission to enter the competition in the global market.

For this purpose, the detection methods and identify the species that have been used in meat production, can solve the problems in this area. In the laboratory of Biophysical Chemistry (BCL) at IBB, designed and made a new kit as BCL-Kit. The new biological methods that are used to detect meat, genetic methods were used in this kit, as well as intended for the qualitative detection of species content in raw, processed and mixed meats such as hamburger creates an even, sausage, barbecue, etc.

This product was applied to identify nine meats (cattle, sheep, chicken, buffalo, dog, cat, pig, camel, and donkey) for products and polymerase chain reaction (PCR) use the highly conserved regions of cytochrome b gen as a target sequence.

For the identification of species, it is preferable to detect DNA and extracted it from muscle.

DNA and polymerase chain reaction (PCR) use the highly conserved regions of cytochrome b gen as a target sequence. It can detect based upon PCR amplification of mitochondrial genes for species-specific detection with agarose gel electrophoresis.

The additional instructions provided inside this kit that will help the cosume for identification of meat species.

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Species Specific Identification of Meat Derivative Products by Molecular Genetic Approach (Kit)

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Benefits of walnut peptides in food industries as a nutraceutical

Walnut is a plant with economic value. In addition of food consumption, walnut is also used in traditional medicine and its healing properties have been known since many time ago. Walnut is mainly composed of protein and fatty acids. The seeds are a rich source of protein (10 to 20% of weight). Walnut contain 18 different amino acids that 8 of them are essential amino acids. Walnut also is a good source of arginine and lysine. The low proportion of lysine to arginine in walnut play an important role in serum cholesterol level. In addition, walnut has a significant amount of the amino acid tyrosine that is a semi-essential amino acid. Therefore walnut seeds can be considered as a complementary source for other foods.

Bioactive peptides normally are inactive and hidden inside the natural protein. Enzymatic hydrolysis can lead to release of these peptides. These peptides are protein fragments that have a lot of positive impact on the status and functions of the body. Bioactive peptides derived from hydrolysis of walnut protein by digestive enzymes are very important in food, pharmaceutical and nutrition industries. Because of many physiological functions, including antioxidant, anticancer and antihypertensive activity of these peptides, they can be used as ingredients in foods product, dietary supplements and nutraceutical.

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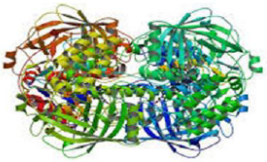
Catalase is an antioxidant enzyme with a lot of important roles in various industries. It can effectively catalyzes the transformation of residual hydrogen peroxide into oxygen and water. It can be widely used in food, textile, paper, electronics, and other industries to remove hydrogen peroxide, therefore it can lead to a significant saving of water resources and reduce energy consume.

APPLICATION FIELD & EFFICACY:

--**Food Industry:** it can remove residual hydrogen peroxide after :
using it as bleacher, preservative in food processing, eliminating the special odor of hydrogen peroxide caused by ultraviolet irradiation in production of milk, cheese and other products and leavening agent of baking food.
--**Textile Industry:** to remove hydrogen peroxide after bleaching and before dyeing, saving water, energy and time, not damaging the fibers and dyestuffs., and do not pollute the environment;
--**Papermaking Industry:** monitoring and optimizing the amount of hydrogen peroxide in bleaching process, degrading residual hydrogen peroxide after the bleaching;
--**Electronics Industry:** to remove hydrogen peroxide after eroding germanium, silicon transistors and semiconductor components; saving water, energy and time.

According to our research, the required amount of catalase enzyme can be significantly reduced by using an enzyme activator that effectively lead to low costs.

Catalase



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Reducing the cost of catalase in industry by enzyme activation

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PUBLICATIONS 2015

1- L. Fotouhi, S. Yousefinejad, N. Salehi, A.A. Saboury, N. Sheibani, A.A. Moosavi-Movahedi "Application of merged spectroscopic data combined with chemometric analysis for resolution of hemoglobin intermediates during chemical unfolding" Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 136, 1974-1981 (2015)

2-A. A. Moosavi-Movahedi, F.Ghamari, S.M. Ghaffari, M.Salami, F. Farivar, F. Moosavi-Movedi, A. Johari and A. L. N. Aminin "Natural peptides anti-glycation effect in the presence of Aloe vera phenolic components on human serum albumin" RSC Advances 5, 248-254 (2015)

3- S. Yousefinejad, M. Bagheri, and A.A. Moosavi-Movahedi "Quantitative Sequence-Activity Modeling of Antimicrobial Hexapeptides Using a Segmented Principal Component Strategy: An Approach to Describe and Predict Activities of Peptide Drugs Contain L/D and Unnatural Residues" Amino Acids 47, 125-134 (2015)

4-B. Delavari, A.A. Saboury, M. S. Atri, A. Ghasemi, B. Bigdeli, A. Khammari, P. Maghami, A. A. Moosavi-Movahedi, T. Haertlé, B. Goliaei " Alpha-lactalbumin: a new carrier for vitamin D3 food enrichment" Food Hydrocolloids 45, 124-131 (2015)

5-R.Yousefi, R.Mohammadi, A.Taheri-Kafrani , M.B. Shahsavani, M. Dadkhah-Aseman, S.M. Nabavizadeh , M. Rashidi , N. Poursasan , A.A. Moosavi-Movahedi " Study of the interaction between two newly synthesized cyclometallated platinum (II) complexes and human serum albumin: Spectroscopic characterization and docking simulation" Journal of Luminescence 15, 139-146 (2015)

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7-Z. Moosavi-Movahedi, H. Gharibi, H. Hadi-Alijanvand, M. Akbarzadeh,M. Esmaili, M. S. Atri, Y. Sefidbakht, M. Bohlooli, K. Nazari, S. Javadian, Jun Hong, A. A. Saboury, N. Sheibani and A. A. Moosavi-Movahedi "Caseoperoxidase, Mixed β-Casein-SDS-Hemin-Imidazole Complex: A Nano Artificial Enzyme" Journal of Biomolecular Structure and Dynamics 33(12), 2619-2632 (2015)

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9-M. Jaafari, M. R. Ashrafi Kooshk, S.M. Asghari, A.A. Moosavi-Movahedi, S. Ghobadi and R. Khodarahmi "Direct evidence for non specific peroxidase activity of ''ferritin–heme" complex: possible role in the development of neurodegenerative diseases" Journal of the Iranian Chemical Society 12, 779-790 (2015)

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12-H. Rahaman, Md. K. Alam Khan, Md. Imtaiyaz Hassan, A. Islam, A.A. Moosavi-Movahedi and Faizan Ahmad "Heterogeneity of equilibrium molten globule state of cytochrome c induced by weak salt denaturants under physiological condition" PLOS ONE, e0120465 (2015)

13-M. Shamsipur, F. Molaabasi, M. Shanehsaz and A.A. Moosavi-Movahedi "Novel blue-emitting gold nanoclusters confined in human hemoglobin, and their use as fluorescent probes for copper (II) and histidine" Micorchim Acta 182(5-6),1131-1141(2015)

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15-M. Sabokdast, M. Habibi-Rezaei, N. Poursasan, F. Sabouni, M. Ferdousi, E. Azimzadeh-Irani and A. A. Moosavi-Movahedi "Insulin glycation coupled with liposomal lipid peroxidation and microglial cell death" RSC Advances 5, 33114-33122 (2015)

16-M. S. Atri, A.A. Saboury, A.A. Moosavi-Movahedi, K. Kavousi, Sh. Ariaeenejad "Effects of zinc binding on the structure and thermal stability of camel alpha-lactalbumin" Journal of Thermal Analysis and Calorimetry 120(1),481–488 (2015)

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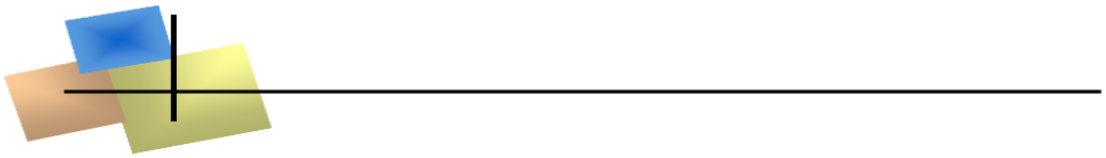
19-A. A. Alizadeh, M. Hamzeh-Mivehroud, M. Farajzadeh, A. A. Moosavi-Movahedi and S. Dastmalchi "A simple and rapid method for expression and purification of functional TNF-α using GST fusion system" Current Pharmaceutical Biotechnology 16(8),707-715 (2015)



20-M. Mazaheri , A.A. Moosavi-Movahedi, A.A. Saboury , F. Khodagholi , F.Shaerzadeh , N. Sheibani "Curcumin protects β-lactoglobulin fibril formation and fibril-induced neurotoxicity in PC12Cells" PLOS ONE DOI:10.1371/journal.pone.0133206 (2015)

21-M. Ansari, M. Habibi-Rezaeib, S. Salahshour-Kordestani, A. A. Moosavi Movahedi and N. Poursasand "Prevention of serum albumin glycation/fibrillation by cyclodextrin functionalized magnetic nanoparticles" Protein & Peptide Letters 22, 594-600 (2015)

22-S. Zolghadri, A.A. Saboury, M. Sadat Atri and A.A. Moosavi-Movahedi "Differential propensity of citrate- and polyethylene glycol-coated silver nanoparticles to bovine hemoglobin" Toxicology and Industrial Health 31(8),721–726 (2015)

23-M. Valipour, P. Maghami, M. Habibi-Rezaei,M. Sadeghpour, M. A. Khademian, K. Mosavi, N. Sheibani and A.A. Moosavi-Movahedi "Interaction of insulin with methyl tert-butyl ether promotes moltenglobule-like state and production of reactive oxygen species" International Journal of Biological Macromolecules 80, 610-614(2015)

24- G.H. Hakimelahi, F.-Y. Tsai, A.A. Moosavi-Movahedi and B. Golzarroshan "Therapeutic Direction and Issues Regarding HBV Infection" Biomacromol. J. (BMMJ) 1(1), 1-18 (2015)

25-A. Broumand, Z. Emam-Djomeh, F. Khodaiyan, S. Mirzakhanlouei, D. Davoodi, A.A. Moosavi-Movahedi "Nano-web structures constructed with a cellulose acetate/lithium chloride/polyethylene oxide hybrid: modeling, fabrication and characterization" Carbohydrate Polym 115, 760-7 (2015)

26-E. Mahmoodi-Khaledi, N. Kashef, M. Habibi-Rezaei and A.A. Moosavi-Movahedi "In vitro characterization of antibacterial potential of Iranian honey samples against wound bacteria" Eur Food Res Technol 241(3), 329-340 (2015)

27-K. Khoshaman, R. Yousefi, A. M. Tamaddon , L. Saso and A.A. Moosavi-Movahedi "The impact of Hydrogen peroxide on structure, stability and functional properties of Human R12C mutant αA-crystallin: The imperative insights into pathomechanism of the associated congenital cataract incidence" Free Radical Biological Medicine 89, 819-830 (2015)

28-M. Sabokdast, M. Habibi-Rezaei, A.A. Moosavi-Movahedi, M. Ferdousi, E. Azimzadeh-Irani, N. Poursasan "Protection by beta-Hydroxybutyric acid against insulin glycation, lipid peroxidation and microglial cell apoptosis" Daru Journal of Pharmaceutical Sciences 27;23:42. Epub (2015). doi:10.1186/s40199-015-0126-5
see: http://www.darujps.com/content/23/1/42

Plenary Speaks in Conferences (2015)

1- A.A.Moosavi-Movahedi " Biophysics and Metaphysics of light" in recognition of the International Year of Light (IYL 2015), the is Symposium on Light and Biology was held in Znjan, October 29-30, 2015 [Key Speaker]

2- A.A.Moosavi-Movahedi " Heat and Light" in recognition of the International Year of Light (IYL 2015), the is Symposium on Heat and Light was held at University of Tehran, Institute of Biochemistry and Biophysics,in Tehran, November 11, 2015 [Key Speaker]

3- A.A.Moosavi-Movahedi " Diabetes and Biomolecular Science" in recognition of Diabetes week in Iran, the is Symposium on Diabetes and Biomolecular Science was held at University of Tehran, Institute of Biochemistry and Biophysics,in Tehran, November 25, 2015 [Key Speaker]

Selected Papers (year 2015)

RSC Advances

PAPER

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Natural peptide anti-glycation effect in the presence of *Aloe vera* phenolic components on human serum albumin

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The Maillard reaction, non-enzymatic glycation after a complex series of reactions that involve reducing-sugars and proteins, produces a large number of end-products that are known as advanced glycation end-products (AGEs). AGEs are related to the pathogenesis of many diseases such as diabetes, inflammatory arthritis and cataracts. Today there is an increasing demand for natural AGE inhibitors for curing diabetes that have fewer side effects compared to synthetic drugs. The aim of this study was to explore the anti-glycation effect of aloin, in the presence and absence of casein-derived peptides, on human serum albumin (HSA). UV-visible and fluorescence spectroscopy were used to explore the number of free lysine residues, AGE formation and fibril formation during the HSA glycation. According to trinitrobenzenesulfonic acid and fluorescamine assay results, the presence of aloin and peptides reduced the number of glucose-attached lysine residues. Moreover AGE specific fluorescence and thioflavin T fluorescence decreased in the presence of aloin and peptides, indicative of a decrease in the formation of AGEs and fibrils respectively during the glycation of HSA. According to this study, aloin and camel casein derived peptides showed a synergic anti-glycation effect and inhibited the formation of fibrils and AGEs during the HSA glycation. This effect can be related to the antioxidant activity of aloin–peptide complex.

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Introduction

It has been reported that 366 million people had diabetes in 2011, and it is estimated that this number will increase to 552 million by 2030.[1] In diabetes, either insulin deficiency or insulin resistance triggers an abnormal increase in blood glucose, known as hyperglycemia. High blood glucose concentrations can cause a number of harmful health effects, such as an increased risk of heart disease, strokes, kidney disease, and blindness.[2–4] The long-term effects of diabetes mostly can be caused by protein glycation.

Protein glycation is a non-enzymatic reaction that starts with the nucleophilic attack of a reducing sugar with primary amine groups on proteins to form a Schiff base.[5] A slow rearrangement of this intermediate product can create more stable Amadori products, or ketoamine. Furthermore, modifications of these glycation products, such as rearrangement, oxidation, polymerization and cleavage, produces irreversible conjugates known as advanced glycation end products (AGEs).[6]

Serum albumin, with its long half-life (about 21 days) and high concentrations in the circulatory system, is highly sensitive to glycation.[7] High concentrations of glycated albumin in cases of diabetes mellitus can trigger many metabolic disorders such as retinopathy, nephropathy, neuropathy and coronary artery disease.[8,9] Today there is an increasing demand for plant based drugs for diabetes that are natural, less toxic, and cost-effective.[10] Previous studies have shown that *Aloe vera* gel and phytosterols derived from *Aloe vera* gel are useful for the treatment of type 2 diabetes mellitus.[11]

Aloe vera L. (syn.: *Aloe barbadensis* Miller; Hindi: Ghikanvar; AV), belongs to the family Liliaceae (sub-family of the Asphodelaceae), native to North Africa and cultured in warm climatic areas.[12] *Aloe vera* (AV) has a high content of phenolic compounds, glycosides (aloins), 1,8-dihydroxyanthraquinone derivatives (aloe-emodin), beta-1,4 acetylated mannan, mannose-phosphate and alprogen glucoprotein.[13] Leaf exudates and mucilaginous gel of aloe have anti-inflammatory,[14] anti-cancer,[15] antioxidant,[16] cytoprotective, cardiac stimulatory and immunomodulatory activities.[17] They have also been reported to have antidiabetic and hypoglycemic properties.[18–20] Little is known about the aloe ingredients responsible for all of these

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ORIGINAL ARTICLE

Quantitative sequence–activity modeling of antimicrobial hexapeptides using a segmented principal component strategy: an approach to describe and predict activities of peptide drugs containing L/D and unnatural residues

Saeed Yousefinejad · Mojtaba Bagheri · Ali Akbar Moosavi-Movahedi

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Abstract The treatment of infections caused by multi-drugs resistant bacteria and fungi is a particular challenge. Whereas cationic antimicrobial peptides (CAPs) are considered as promising drug candidates for treatment of such superbugs, recent studies have focused on design of those peptides with increased bioavailability and stability against proteases. In between, applications of the quantitative structure–activity relationship (QSAR) studies which provide information on activities of CAPs based on descriptors for each individual amino acid are inevitable. However, the satisfactory results derived from a QSAR model depend highly on the choice of amino acid descriptors and the mathematical strategy used to relate the descriptors to the CAPs' activity. In this study, the quantitative sequence–activity modeling (QSAM) of 60 CAPs derived from O-W-F-I-F-H(1-Bzl)-NH_2 sequence which showed excellent activities against a broad range of hazardous microorganisms: e.g., MR*SA*, MR*SE*, *E. coli* and *C. albicans*, is discussed. The peptides contained natural and non-natural amino acids (AAs) of the both isomers D and L. In this study, a segmented principal component strategy was performed on the structural descriptors of AAs to extract AA's indices. Our results showed that constructed models covered more than 82, 94, 80 and 78 % of the cross-validated variance of *C. albicans*, *MRSA*, *MRSE* and *E. coli* data sets, respectively. The results were also used to determine the important and significant AAs which are important in CAPs activities. According to the best of our knowledge, it is the first successful attempt in the QSAM studies of peptides containing both natural and non-natural AAs of the both L and D isomers.

Keywords Cationic antimicrobial peptides · Superbugs · Chemoinformatic · Quantitative sequence–activity modeling · Amino acid descriptors

Introduction

The fact of increasing use of antibiotics in immunosuppressant patients has resulted in the prevalence and drug resistance of bacterial/fungal superbugs (Guilhelmelli et al. 2013; Mayer et al. 2013). Cationic antimicrobial peptides (CAPs) as a wide category of compounds with their primitive defense mechanism could be an effective immune wall against the superbugs-associated infections (Zasloff 2002). CAPs are found in a wide range of eukaryotic organisms, which mostly show action by damaging bacterial/fungal cell membrane (Dathe and Wieprecht 1999; Bagheri et al. 2011).

Design and introduction of new CAPs with better therapeutic activity are always demanding and are in progress (Reddy et al. 2004; Junkes et al. 2011). On the other hand, because of growing emergence of bacterial/fungal superbugs some efforts have been started to introduce suitable CAPs as a new generation of antibiotics.

Some methods have been proposed to help the design of peptides and also analog peptide libraries with desired

Electronic supplementary material The online version of this article (doi:10.1007/s00726-014-1850-8) contains supplementary material, which is available to authorized users.

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Alpha-lactalbumin: A new carrier for vitamin D_3 food enrichment

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ABSTRACT

Vitamin D is a fat-soluble regulatory vitamin maintaining blood calcium and phosphorus concentrations within a narrow physiological range. Binding to an appropriate delivery system can enhance vitamin D_3 solubility, simplify its transport, protect it from degradation and increase its bioavailability. Alpha-lactalbumin as a milk protein is a good candidate for a vitamin encapsulation. Binding properties and conformational change of bovine apo alpha-lactalbumin upon interaction with vitamin D_3 were investigated by calorimetry, spectroscopy and by molecular docking. Tryptophan fluorescence quenching indicates that the protein conformation changes in the presence of vitamin D_3. However, according to far UV CD results, the secondary structure of the protein was altered in the presence of vitamin D_3. The molecular modeling showed that Van der Waals interactions, hydrogen bond and hydrophobic interactions play a major role in the binding of vitamin D_3 to the alpha-lactalbumin hydrophobic pocket. The particle size of alpha-lactalbumin and vitamin D_3 complex is much larger than the native protein. Surprisingly, in the presence of vitamin D_3, the thermal stability of the protein decreases. The binding constant and standard Gibbs free energy change (ΔG°) of binding vitamin D_3 to the protein obtained from ITC are 3.66×10^5 M^{-1} and −7.6 kcal mol^{-1}, respectively, what agrees with results obtained by measurement of fluorescence and by molecular docking. The formed complex is a suitable candidate in order to enrich the low-fat food and non-alcohol drinks. According to the results, alpha-lactalbumin can be introduced suitable carrier for vitamin D_3.

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1. Introduction

Fat-soluble vitamin D is not classified as an essential nutrient because it can be made by the body during exposure of the skin to sun ultraviolet rays. 7-Dehydrocholesterol converts to previtamin D_3 during exposure to ultraviolet radiation in the skin and then it is converted to vitamin D_3 through thermal isomerization. Finally, vitamin D_3 is hydroxylated to 25-hydroxyvitamin D (25-OHD) in the liver. Serum concentration of 25-OHD is considered as a clinical measure of vitamin D status (Wagner, Sidhom, Whiting, Rousseau, & Vieth, 2008).

One of the most important biological functions of vitamin D_3 is to maintain blood calcium and phosphorus concentrations within a narrow physiological range (Forrest, Yada, & Rousseau, 2005). Recent evidence suggested that vitamin D has a protective effect against a variety of diseases, including multiple sclerosis (Kimball, Ursell, O'Connor, & Vieth, 2007; Mahon, Gordon, Cruz, Cosman, & Cantorna, 2003; Munger, Levin, Hollis, Howard, & Ascherio, 2006), diabetes (Casteels et al., 1998; Chiu, Chu, Go, & Saad, 2004; Hyppönen, Läärä, Reunanen, Järvelin, & Virtanen, 2001), cardiovascular disease (Lind et al., 1995; Wang et al., 2008), microbial infections (Liu et al., 2006; Martineau et al., 2007; Zasloff, 2006), metabolic syndrome, hypertension, bone diseases and

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Caseoperoxidase, mixed β-casein–SDS–hemin–imidazole complex: a nano artificial enzyme

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A novel peroxidase-like artificial enzyme, named "caseoperoxidase", was biomimetically designed using a nano artificial amino acid apo-protein hydrophobic pocket. This four-component nano artificial enzyme containing heme–imidazole–β-casein–SDS exhibited high activity growth and k_{cat} performance toward the native horseradish peroxidase demonstrated by the steady state kinetics using UV–vis spectrophotometry. The hydrophobicity and secondary structure of the caseoperoxidase were studied by ANS fluorescence and circular dichroism spectroscopy. Camel β-casein (Cβ-casein) was selected as an appropriate apo-protein for the heme active site because of its innate flexibility and exalted hydrophobicity. This selection was confirmed by homology modeling method. Heme docking into the newly obtained Cβ-casein structure indicated one heme was mainly incorporated with Cβ-casein. The presence of a main electrostatic site for the active site in the Cβ-casein was also confirmed by experimental methods through Wyman binding potential and isothermal titration calorimetry. The existence of Cβ-casein protein in this biocatalyst lowered the suicide inactivation and provided a suitable protective role for the heme active-site. Additional experiments confirmed the retention of caseoperoxidase structure and function as an artificial enzyme.

Keywords: caseoperoxidase; biomimetic; artificial enzyme; horseradish peroxidase (HRP); camel β-casein; SDS monomer; imidazole; heme

1. Introduction

The term biomimetic chemistry describes a novel chemistry that is inspired by a living system (Breslow, 2005; Marchetti & Levine, 2011). Artificial enzymes can be considered a part of biomimetic chemistry, which attempts to improve the performance of chemical reactions and catalysts by imitating enzymatic processes (Silavi, Divsalar, & Saboury, 2012; Yatsimirsky, 2004). Robust biocatalysts may be formed by encapsulating a prosthetic group in hydrophobic pocket-like micelles (Moosavi-Movahedi et al., 2008; Wang et al., 2010), vesicles (Murakami, Hisaeda, & Ohno, 1984, 1987, 1990, 1991) and macrocyclic compounds (Bender & Komiyama, 1978; Diederich, 1987; D'Souza & Bender, 1978; Murakami, Kikuchi, Hisaeda, & Hayashida, 1996; Tabushi & Yamamura, 1983) that mimic the polypeptide envelope protecting the catalytic center of the natural enzymes. Artificial enzymes could be constructed from native proteins as host for prosthetic active sites to simulate the catalytic functions exhibited by natural enzymes (Grinbergs, O'Brien, & Hrkal, 1999; Monzani et al., 2001; Quilez, Lauzon, Desfosses, Mansuy, & Mahy, 1996).

The heme group plays a fundamental role in the activation of hemoproteins and hemoenzymes, such as horseradish peroxidase (HRP). The heme moiety is protected by the hydrophobic surrounding to avoid its reduction into inactive oxo-complexes. In our previous studies, we have designed and constructed various peroxidase-like artificial enzymes based on several existing and effective HRP parameters. These included the existence of axial histidinated heme complex (Moosavi-Movahedi et al., 2008), proper hydrophobic environment surrounding the heme-based active site as micelles (Moosavi-Movahedi et al., 2008), reverse micelles (Farivar et al., 2010), assembled nafion (Hong et al., 2012), and the presence of negative and positive surface charged hydrophobic pockets as vesicular peroxidase

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RESEARCH ARTICLE

Heterogeneity of Equilibrium Molten Globule State of Cytochrome *c* Induced by Weak Salt Denaturants under Physiological Condition

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Abstract

While many proteins are recognized to undergo folding via intermediate(s), the heterogeneity of equilibrium folding intermediate(s) along the folding pathway is less understood. In our present study, FTIR spectroscopy, far- and near-UV circular dichroism (CD), ANS and tryptophan fluorescence, near IR absorbance spectroscopy and dynamic light scattering (DLS) were used to study the structural and thermodynamic characteristics of the native (N), denatured (D) and intermediate state (X) of goat cytochorme *c* (cyt-*c*) induced by weak salt denaturants (LiBr, LiCl and $LiClO_4$) at pH 6.0 and 25°C. The LiBr-induced denaturation of cyt-*c* measured by Soret absorption ($\Delta\varepsilon_{400}$) and CD ($[\theta]_{409}$), is a three-step process, $N \leftrightarrow X \leftrightarrow D$. It is observed that the X state obtained along the denaturation pathway of cyt-*c* possesses common structural and thermodynamic characteristics of the molten globule (MG) state. The MG state of cyt-*c* induced by LiBr is compared for its structural and thermodynamic parameters with those found in other solvent conditions such as LiCl, $LiClO_4$ and acidic pH. Our observations suggest: (1) that the LiBr-induced MG state of cyt-*c* retains the native Met80-Fe(III) axial bond and Trp59-propionate interactions; (2) that LiBr-induced MG state of cyt-*c* is more compact retaining the hydrophobic interactions in comparison to the MG states induced by LiCl, $LiClO_4$ and 0.5 M NaCl at pH 2.0; and (3) that there exists heterogeneity of equilibrium intermediates along the unfolding pathway of cyt-*c* as highly ordered (X1), classical (X2) and disordered (X3), i.e., $D \leftrightarrow X3 \leftrightarrow X2 \leftrightarrow X1 \leftrightarrow N$.

Introduction

The folding from the readily synthesized unfolded protein at ribosome to the native active state is remarkably fast despite the astronomical number of possible conformations available to polypeptides. All the proposed mechanisms for protein folding, i.e., the framework model, the

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ORIGINAL PAPER

Novel blue-emitting gold nanoclusters confined in human hemoglobin, and their use as fluorescent probes for copper(II) and histidine

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Abstract We report on the synthesis and characterization of highly fluorescent gold nanoclusters (Au-NCs) from a gold precursor using a single-step chemical reduction in the presence of human adult hemoglobin (Hb). The conformational changes of Hb before and after cluster formation were studied by various spectroscopic techniques. The Au-NCs in Hb display blue fluorescence with a peak centered at 450 nm (photoexcited at 365 nm) and a quantum yield of 2.8 %. The Au-NCs exhibit excellent photostability and long-term stability, and can be applied in the pH range 5–12 even in the presence of high electrolyte concentrations. The Au-NCs in Hb can act as a highly sensitive and selective fluorescent turn-off probe for Cu(II) ion. The observed reversible fluorescence recovery of Hb-AuNCs/Cu(II) aggregates was exploited to develop a selective and sensitive turn-on fluorescence assay for His. Under optimized conditions, the probe gives a fluorescent response that is linear in the 0.1 to 20.0 μM concentration range of Cu(II), with a limit of detection of 28 nM. The probe for His, in turn, has a linear range in the 1–21 μM concentration range, and the limit of detection is 0.6 μM.

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Keywords Hemoglobin-capped gold nanoclusters · Fluorescent nanocluster · Off-on fluorescence switch · Copper(II) detection · Histidine

Introduction

Metal nanoclusters (NCs) composed of very few atoms with an ultra small size ranging from subnanometer to approximately 2 nm and exhibiting strong fluorescence emission have become one of the important nanomaterials in current nanoscience [1–7]. Among these, the gold nanoclusters (AuNCs) have been extensively studied because of their intrinsic characteristics such as ease of preparation, chemical stability, good water solubility, and low toxicity [2–6].

The fluorescence of AuNCs correlates not only with the metal quantization effect but also with the surface ligands or scaffolds [1–6]. The first fluorescent AuNCs synthesized with a protein bovine serum albumin (BSA) as both template and reducing agent were reported by Xie et al. in 2009 [2]. An interesting protein candidate capping agent in the synthesis of novel AuNCs is human adult hemoglobin (Hb), known as a major protein component in erythrocytes, which acts a carrier of oxygen and also aids the transport of carbon dioxide and regulates the pH of blood [8]. Since Hb is an important functional protein for reversible carrying and storage of oxygen, and a protein with high α-helical content, its potential use in the synthesis of new AuNCs was a main focus of this study.

Although copper plays a prominent role in regulating many biological processes [9], its increased level in body is deleterious to vital biological organs such as liver and kidneys [10]. The free Cu^{2+} ions are known to induce toxicity to cells and possess a strong affinity towards the histidine-rich regions of the prion protein (PrP) causing misfolding and protein fibrilization [11]. Since current atomic and molecular spectroscopic techniques for the detection of Cu^{2+} ions require expensive instruments and/or

PLOS ONE

RESEARCH ARTICLE

Curcumin Protects β-Lactoglobulin Fibril Formation and Fibril-Induced Neurotoxicity in PC12Cells

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Abstract

In this study the β-lactoglobulin fibrillation, in the presence or absence of lead ions, aflatoxin M1 and curcumin, was evaluated using ThT fluorescence, Circular dichroism spectroscopy and atomic force microscopy. To investigate the toxicity of the different form of β-Lg fibrils, in the presence or absence of above toxins and curcumin, we monitored changes in the level of reactive oxygen species and morphology of the differentiated neuron-like PC12 cells. The cell viability, cell body area, average neurite length, neurite width, number of primary neurites, percent of bipolar cells and node/primary neurite ratios were used to assess the growth and complexity of PC12 cells exposed to different form of β-Lg fibrils. Incubation of β-Lg with curcumin resulted in a significant decrease in ROS levels even in the presence of lead ions and aflatoxin M1. The β-Lg fibrils formed in the presence of lead ions and aflatoxin M1 attenuated the growth and complexity of PC12 cells compared with other form of β-Lg fibrils. However, the adverse effects of these toxins and protein fibrils were negated in the presence of curcumin. Furthermore, the antioxidant and inhibitory effects of curcumin protected PC12 cells against fibril neurotoxicity and enhanced their survival. Thus, curcumin may provide a protective effect toward β-Lg, and perhaps other protein, fibrils mediated neurotoxicity.

Introduction

Amyloid fibrils are fibrillary protein aggregates that are implicated in a variety of human diseases [1–4]. Some researchers have shown that the amyloid fibril-forming propensity is a generic property of all polypeptides [5]. Certain metal ions contribute to pathogenesis of these degenerative diseases [6]. The generally accepted argument on the role of divalent metals in protein aggregation is based on their ability to act as bridges, as well as to provide an

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Therapeutic Direction and Issues Regarding HBV Infection

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ABSTRACT

With up to 400 million affected people worldwide, chronic hepatitis B virus (HBV) infection is still a major health care problem. During the last decade, several novel therapeutic approaches have been developed and evaluated. In most regions of the world, interferon-α (IFN-α), and nucleos(t)ide analogues are currently approved. Despite major improvements, none of the existing therapies is optimal since viral clearance is rarely achieved. HBV establishes a stable nuclear covalently closed circular DNA (cccDNA). Interferon-α treatment can clear HBV but is limited by systemic side effects. Up-regulation of APOBEC3A and APOBEC3B enzymes by use of IFN-α or lymphotoxin-β (LT βR) was found to result in cytidine deamination, apurinic/apyrimidinic site formation, and finally cccDNA degradation that prevented HBV reactivation, while genomic DNA was found to remain intact. As such, development of new therapeutics in combination with existing antivirals, may cure hepatitis B. With respect to the selectivity observation on the activation of LTβR, however, more studies are necessary on the potential utility of LTβR agonists for clearance of cccDNA in chronic hepatitis B (CHB). HBV is a DNA virus that can integrate DNA into host genome thereby increases the yield of trans-activator protein HBxAg that may deregulate many pathways involving in metabolism of cells causing Hepatocellular Carcinoma (HCC) development. This review aimed at therapeutic direction and issues regarding HBV infection.

Keywords: Hepatities B, Interferon, lymphotoxin-β, Antiviral, Carcinoma, cccDNA

INTRODUCTION

Chronic hepatitis B (CH-B) is characterized by inflammatory liver disease of variable severity driven by persistent replication of the hepatitis B virus (HBV) [1]. In the HBV life cycle, the DNA containing nucleocapsids fulfill two functions. First, they can be either re-imported into the nucleus to form additional cccDNA or second, they can be enveloped for secretion *via* the endoplasmic-reticulum (ER). After budding into the ER lumen, the envelope proteins are secreted by the cell either as small, non-infectious subviral spherical or filamentous particles (SVPs) of 22 nm diameter or as infectious virions of 42 nm (Dane particles). Usually, the non-infectious SVPs are produced in a 1,000 to 1,000,000-fold excess over virions [1]. The development of a safe and effective hepatitis B surface antigen recombinant vaccine was an important milestone towards achieving control of CH-B, and its widespread implementation has dramatically reduced the incidence of infection [2]. However, for those chronically infected with HBV, antiviral chemotherapy represents the best prospect of controlling active replication and thereby preventing life-threatening hepatic disease [3].

Existing therapies either approved or in clinical trial, still have the disadvantage of low response rates and selection of resistance [4]. The complex interplay between the HBV-infected hepatocyte and the host immune response greatly influences the clinical course of disease and, consequently, strategies for clinical management [5]. As morbidity and mortality in CH-B are linked to the development of cirrhosis and hepatocellular carcinoma, the goals of antiviral therapy are to induce disease remission, to arrest disease progression to cirrhosis, and to block the liver failure and/or hepatocellular carcinoma [6].

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The impact of Hydrogen peroxide on structure, stability and functional properties of Human R12C mutant αA-crystallin: The imperative insights into pathomechanism of the associated congenital cataract incidence

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ABSTRACT

The oxidative stress in eye lens which occurs during inflammation and under chronic hyperglycemia has been already indicated in the pathogenesis of cataract disorders. The aim of this study was to examine structural and functional properties of R12C mutant αA-Crystallin (αA-Cry) in the presence of hydrogen peroxide. The study was done using different spectroscopic techniques and gel mobility shift assay. According to results of our study, H_2O_2 oxidation strongly compromises the chaperone function of the R12C mutant but not of wild-type αA-Cry. Also, it affects the structural properties of both wild-type and mutant proteins, albeit to different degree. The H_2O_2 exposure promotes extensive disulfide mediated oligomerization of the R12C mutant but not of the wild-type as revealed by gel mobility shift assay and dynamic light scattering. Moreover, in the presence of hydrogen peroxide, the mutant protein demonstrates severe conformational and protease instability and increased amyloidogenic propensity. The obtained results suggest that incubation of R12C mutant recombinant αA-Cry with hydrogen peroxide accelerates the molecular events which have been already implicated in the pathomechanism of cataract development. Taken together these results suggest that individuals carrying the R12C mutation are at an increased risk to develop early-onset cataract under condition of oxidative stress.

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1. Introduction

The vertebrate lenses comprise of three major classes of long-lived proteins termed as α-, β- and γ-Crystallins (Crys). These proteins display both structural and functional characteristics and possess limited turn over during the life span [1–3]. While β- and γ-Crys play largely structural role, α-Cry displays prominent chaperoning ability which is highly important for the maintenance of lens transparency [2,4]. Also, this protein indicates antiapoptotic activity and copper sequestration ability, as the later attenuates the harmful effects of copper-catalyzed generation of reactive oxygen species (ROS) in the lenticular tissues [5,6]. α-Cry is composed of two protein subunits of 57% sequence identity, the 173 residues αA- and the 175 residues αB-Cry [3,4,7]. Each α-Cry subunit consists of three domains, a flexible N-terminal domain, a middle α-Cry domain resembling the β-sandwich of the immunoglobulin fold, and a flexible C-terminal extension which is important for the interaction with client proteins [8]. These protein subunits with a molecular mass of about 20 kDa are capable to form both homo and hetero oligomeric assemblies. In the most of vertebrate lenses, the hetero oligomers usually constitute 3:1 ratio of αA and αB subunits with the size range between 300 and 1000 kDa [9,10]. Several factors including genetic mutations, accumulation of post-translational modifications, environmental stresses and a number of diseases could interrupt the native structure of α-Cry and subsequently compromise its chaperone function. Therefore, under the above mentioned conditions the formation of insoluble protein aggregates in the lenticular tissues eventually leads to blurred vision and cataract incidence [2,3,7]. While genetic mutations in crystallin genes are associated with different types of congenital and early-onset cataracts, post translational modifications believed to be cause of age-related

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