Biomeditsinskaya Khimiya

ISSN 2310-6905 (online), ISSN 2310-6972 (print)

2010 volume 56 issue 1

1. Kaysheva A.L., Ivanov Yu.D., Zgoda V.G., Frantsuzov P.A., Pleshakova T.O., Krohin N.V., Ziborov V.S., Archakov A.I.

Visualization and identification of hepatitis c viral particles by atomic force microscopy combined with ms/ms analysis.

Possibility of detection and identification of hepatitis C viral particles with mass spectrometry (MS) in combination with atomic force microscopy (AFM) had been investigated. AFM/MS approach is based on two technologies: 1. AFM-biospecific fishing that allows to detect, concentrate from solution and to count protein complexes on a surface of AFM-nanochip; 2. mass spectrometric identification of these complexes. AFM-biospecific fishing of HCVcoreAg from solution was carried onto surface of AFM-nanochips with immobilized anti-HCVcoreAg. It was shown that HCVcoreAg/anti-HCVcoreim complexes were formed onto AFM-nanochips in quantity sufficient for mass spectrometric identification. Thus, AFM/MS approach allows to identify fragments of hepatitis C virus fished onto a surface of AFM-nanochip from serum.

DOI: 10.18097/pbmc20105601026

2. Ivanov A.V., Kopylov A.T., Zgoda V.G., Toropygin I.Yu., Khrjapova E.V., Ivanov Yu.D.

Mass-spectrometric identification of interaction sites for cytochrome P450 2b4/nadph cytochrome P450 reductase.

We determined the interaction sites of the cytochrome P450's protein-partners: 2B4 (d-2B4) and NADPH-cytochrome P450 of reductase (d-Fp). While in operation, these proteins are forming the complexes. We used 4-4'-dithio(bisphenyl)azide linker for non-specific covalent coupling of d-2B4 complexes with d-Fp in Emulgen-913 - monomerized system. Covalently-linked peptides in this complex were identified with ESI-MS/MS. Several sites of these proteins' binding with each other were revealed. Based on them, a model of intermolecular protein interactions was created. The model includes 5 cross-linker-stabilized contact sites of d-2B4 with d-Fp involving the following peptides of d-2B4 and d-Fp: 1) d-2B4423-433 Ð¸ d-Fp 102-109; 2) d-2B4324-336 Ð¸ d-Fp570-585; 3) d-2B4327-336 Ð¸ d-Fp452-464; 4) d-2B4 192-197 Ð¸ d-Fp456-464; 5) d-2B4 134-139 Ð¸ d-Fp406-425.Herein, in the latter two cases, the peptides of d-Fp are located in their inter-domain slit and stabilize protein-protein complex via nanoprobe cross-linker; therefore, the formation of d-2B4/d-Fp complexes in these sites may involve aminoacid residues d-Fp456-464 and d-Fp406-425 surrounding inter-domain slit.

DOI: 10.18097/pbmc20105601040

3. Shumyantseva V.V., Suprun E.V., Bulko T.V., Dobrinina O.V., Archakov A.I.

Sensor systems for medical application based on hemoproteins and nanocomposite materials.

Recent advances in nanotechnologies stimulate the development of sensor systems based on nanocomposite materials. This review discusses the prospects and challenges of sensors coupled with functionally important for medicine hemoproteins and nanoscale materials. Authors summarized their own experimental results and literature data on hemoprotein-based sensor systems. Mechanisms and the main function principles of electrochemical nanosensors are also discussed.

DOI: 10.18097/pbmc20105601055

4. Rakhmetova S.Yu., Radko S.P., Gnedenko O.V., Bodoev N.V., Ivanov A.S., Archakov A.I.

Photoaptamer heterodimeric constructs as a new approach to enhance the efficiency of formation of photocrosslinking with a target protein.

Using two DNA aptamers selectively recognizing anion-binding exosites 1 and 2 of thrombin as a model, it has been demonstrated that their conjugation by a poly-(dT)-linker (ranging from 5 to 65 nt in length) to produce aptamer heterodimeric constructs results into affinity enhancement. The apparent dissociation constant (Kdapp) measured at the optical biosensor Biacore-3000 for complexes of thrombin with the heterodimeric constructs reached minimum values (Kdapp = 0,2-0,4 nÐœ) which were approximately 30-fold less than for the complexes with the primary aptamers. A photoaptamer heterodimeric construct was designed connecting photoaptamer and aptamer sequences with the poly-(dT)-linker of 35 nt long. The photoaptamer used could form photo-induced cross-links with the exosite 2 of thrombin and the aptamer used could bind to the exosite 1. The measured value of Kdapp for the photoaptamer construct was approximately 40-fold less than that for the primary photoaptamer (5,3 and 190 nM, respectively). Upon exposure to the UV radiation at 308 nm of the equimolar mixtures of thrombin with the photoaptamer construct, the equal yield of the crosslinked complexes was observed at concentrations which were lower by two orders of magnitude than in the case of the primary photoaptamer. It was found that concurrently with crosslinking to thrombin a photo-induced inactivation of the photoaptamer occurs presumably due to formation of the intermolecular crosslinking.

DOI: 10.18097/pbmc20105601072

5. Lisitsa A.V., Shilov B.V., Evdokimov P.A., Gusev S.A.

Knowledgebases in postgenomic molecular biology.

Knowledgebases can become an effective tool essentially raising quality of information retrieval in molecular biology, promoting the development of new methods of education and forecasting of the biomedical R&D. Knowledge-based technologies should induce "paradigm shift" in the life science due to integrative focusing of research groups towards the challenges of postgenomic era. This paper debates concept of the knowledgebase, which exploits web usage mining to personalize the access of molecular biologist to the Internet resources.

DOI: 10.18097/pbmc20105601082

6. Veselovsky A.V., Sobolev B.N., Zharkova M.S., Archakov A.I.

Computer-based substrate specifity prediction for cytochrome P450.

Cytochrome P450 is important class of enzymes metabolizing numerous drugs. The composition and activity of these enzymes are determined the drug distribution in organism, its pharmacological and toxic effect. Thus the prediction of the behaviour of compounds in organism is essential for discovery and development of new drugs in the early stages of this process. The different isoforms of cytochrome P450 can oxidized wide range of chemical compounds and their substrate specifity do not correlate with their taxonomical classification. The main methods of cytochrome P450 substrate specifity prediction is reviewed. These methods divided based on primary informations that used: prediction based on amino acid sequences, ligand-based (pharmacophore and QSAR models) and structure-based (molecular docking, affinity prediction) methods. The common problem of cytochrome P450 substrate prediction and advantage and disadvantages of these methods are discussed.

DOI: 10.18097/pbmc20105601090

7. Ipatova O.M., Torkhovskaya T.I., Medvedeva N.V., Prozorovskiy V.N., Ivanova N.D., Shironin A.V., Baranova V.S., Archakov A.I.

Bioavailability of oral drug formulations and methods for its improvement.

The recent studies in nanotechnology resulted in the development of novel formulations with improved bioavailability. This is especially important for oral administered drugs as the most convenient formulations. The current review deals with the processes occurring at the gastro-intestinal (GI) tract and their influence on the drug form. The increase of bioavailability of the drug may be achieved through designing novel formulations according to the specific drug properties. They include capsules that release pharmaceutical agents at various parts of the GI tract, floating systems that prolong the presence of the drug in the GI tract, dispersed forms with surface-active soluble polymers, micelles that carry poor-soluble drugs inside their non-polar core, agents that facilitate tight junction opening, such as caprate and chitosan, and lipid-based formulations. The own data show the stimulating influence of phospholipid nanoparticles on peroral absorption of drug indomethacin in rats and on passage of transport marker and drugs through Caco-2 cell monolayer in vitro. The review summarizes current understanding of factors that influence the bioavailability of the oral drug forms, currently used models for pharmacokinetic studies, and various approaches to developing novel pharmaceutical forms that increase the bioavailability of the drugs.

DOI: 10.18097/pbmc20105601101

8. Belyaeva N.F., Kashirtseva V.N., Medvedeva N.V., Khudoklinova Yu.Yu., Ipatova O.M., Archakov A.I.

Zebrafish as a model system for biomedical studies.

Zebrafish (Danio rerio) are now firmly established as a powerful research model for many areas of biology and medicine. Here, we review some achievements of zebrafish - based assays for modeling human diseases and for drug discovery and development. For drug discovery, zebrafish are especially valuable in the earlier stages of research as they provide a model organism to demonstrate a new treatment's efficacy and toxicity before more costly mammalian models are used. This review provides examples of compounds known to be toxic to humans that have been demonstrated to functional similarly in zebrafish. Major advantages of zebrafish embryons are that they are readily permeable to small molecules added to their incubation medium and the transparent chorion enables the easy observation of development. Assay of acute toxicity (LC50 estimation) in embryos can also include the screening for developmental disorders as an indicator of teratogenic effects. We used zebrafish for toxicity testing of new drugs on the base of phospholipid nanoparticles. The organization of the genome and the pathways controlling signal transduction appear to be highly conserved between zebrafish and humans that allow using zebrafish for modeling of human diseases some examples of which are illustrated in this paper.

DOI: 10.18097/pbmc20105601120

9. Petushkova N.A., Lisitsa A.V., Pozdnev V.F., Karuzina I.I.

Fluorescence-based determination of enzyme activity of recombinant CYP51b1 (sterol 14Î±-demethylase) with coumarin derivatives.

The current investigation was undertaken with the aim to carry out an in vitro evaluation of the ability of coumarin derivatives as probe substrates to predict the activity of CYP51b1. The results obtained indicate that 7-aminocoumarin-4-acetic acid (ACAC) can be used to determine the recombinant CYP51b1 activity. Determination of CYP51b1 activity with ACAC is based on the direct registration of fluorescence increasing at 30Â°C. It was found also that BMR in a simple soluble model system can be used as an electron donor for CYP51B1. Fluorescence-based assay is highly sensitive and can be used for the screening of sterol 14alpha-demethylase inhibitors.

DOI: 10.18097/pbmc20105601132

10. Buneeva O.A., Gnedenko O.V., Fedchenko V.I., Ivanov A.S., Medvedev A.E.

Interaction of human cytokeratins with isatin analogues.

Using an optical biosensor Biacore 3000 the interaction of human recombinant cytokeratins (CK) with isatin analogues (5-aminocaproyl-isatin and 5-aminoisatin) immobilized on the CM-5 chip has been investigated. CK-14 effectively interacted with 5-aminocaproyl-isatin immobilized on the carboxymethyl dextran chip surface, but not with a "shorter" analogue (5-aminoisatin). In contrast to CK14 CK8 effectively interacted only with 5-aminoisatin. In both cases cytokeratin binding with the immobilized isatin analogues was characterized by rather high affinity (Kd of 0.7 Î¼M for the pair CK14/immobilized 5-aminocaproylisatin and 1.7 Î¼M for the pair CK8/immobilized 5-aminoisatin). CK20 did not interact with both immobilized isatin analogues. Taking into consideration non-specific binding of mouse CK14 and rat CK8 with 5-aminocaproyl-Sepharose we have performed comparative analysis of amino acid sequences of human, mouse, and rat CK8 and CK14. The data obtained suggest that in the case of human, mouse, and rat CK14 the N-terminal domain is the most variable amoung these species, whereas the major differences between amino acid sequences of human, mouse, and rat CK8 have been found both in N-terminal and C-terminal regions.

DOI: 10.18097/pbmc20105601138