MS04-P11

Size exclusion chromatography as a lab-based indicative for protein self-assembly prior to nucleation

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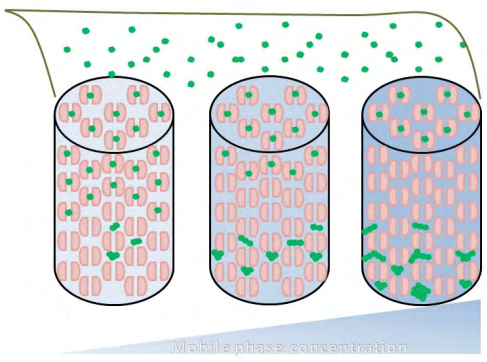
The potential use of size exclusion chromatography (SEC) as a lab-based scanning methodology to monitor protein self-assembly prior to nucleation is the subject of this study. The effect of the mobile phase ionic strength and protein concentration on the output of SEC experiments was exploerd using high-resolution size exclusion chromatography (SEC) coupled with static light scattering (SLS) analyses. SLS is capable of monitoring abrupt changes in the molar masses and the results highlight the effect of small changes in the mobile phase composition on the estimation of molar masses calculated from retention time-based calibration curve compared with those obtained from SLS analysis. By comparing the SLS data with the SEC chromatograms, we show that SEC can provide helpful information on the protein aggregation state as macromolecules approach known precipitation points in their phase diagrams. This study implies that SEC can be usefully used to study phase diagrams of different proteins.



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Adawy, A & Groves, M.R. (2017) Crystals 7(11), 331; doi:10.3390/cryst7110331

Keywords: size exclusion chromatography, static light scattering, protein nucleation

MS04-P12

Site-Specific Analysis of UL144 Protein from Human Cytomegalovirus Highlights its Role in Virus-Mediated Immune Evasion

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Human cytomegalovirus (HCMV, HHV-5) causes a spectrum of disease syndromes in children and adults. HCMV is a cause of mononucleosis in immunocompetent individuals and is a well-known cause of serious morbidity and sometimes fatal infections in immunocompromised patients, especially recipients of solid-organ or hematopoetic cell allografts and individuals with advanced AIDS. HCMV molds cell functions to support its replication and displays tropism for differentiated human cells that are critical for its life cycle. HCMV destroys infected cells by active lytic replication. The virus genes block apoptosis, interfere with the expression of immune recognition molecules on the surface of infected cells to avoid lysis by natural killer or cytotoxic T cells, and inhibit the antiviral effects [1]. The large size of the HCMV dsDNA genome, allows this virus to dedicate many genes to viral fitness; a number of these genes thwart the host inflammatory, innate, and adaptive immune responses. In clinical HCMV isolates, considerable genetic polymorphism exists and correlates with its clinical presentation. Of the viral genes, UL144 is particularly notable because of its role in modulating the host immune response. UL144 is found exclusively in clinical HCMV strains and encodes a structural homologue of the herpesvirus entry mediator. It has been proposed that UL144 plays a role in virus-mediated immune evasion by transmitting inhibitory signals to downregulate T-cell responses [2].

Sequence of HCMV UL144 shows 10 N-linked glycosylation sites located in extracellular part of the gene. Many of them are not present in other viral species that suggest that potential glycosylation is important only in humans and may play a role in ligand-binding recognition. Here, we present the characterization of such recombinant HCMV UL144 glycoprotein isolated from baculovirus-insect system. By liquid chromatography-tandem mass spectrometry (LC-MS/MS) we have identified up to 6 peptides of HCMV UL144 genes that have covered the most of the desired sequence. Intact protein analysis using a combination of LC and MS determined the accurate masses of these proteins (glycan-deficient mutein and native wild-type) and the relative abundance of their isoforms. We have also analyzed the glycan profiles and identified the most glycosylated UL144 species that showed mass of 20267,4 Da. As glycosylation plays an important role in receptor-ligand recognition we have performed the SPR binding studies to UL144 known cellular ligands that have show the importance of UL144 glycosylation in HCMV immune recognition.

References:

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Keywords: immunomodulation, HCMV UL144, glycosylation

MS04-P13

Structure-based cofactor binding properties of dihydrodipicolinate reductase (PaDHDPR) from Panisporosarcina sp. TG-14

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Dihydrodipicolinate reductase (DHDPR) is a key enzyme for the Diaminopimelate (DAP) and Lysine synthesis pathway by reducing the dihydrodipicolinate (DHDP) into tetrahydrodipicolinate (THDP) using NAD(P)H as a cofactor. It is known that DHDPR uses both NADPH and/or NADH as cofactor but the structural basis of its cofactor specificity is still unclear. In this study, we found that PaDHDPR from Paenisporosarcina sp. TG-14 has a strong preference for NADPH compared with NADH based on isothermal titration calorimetry and enzyme activity assay. To better understand the enzymatic mechanism of PaDHDPR in molecular level, the three crystal structures of unliganded, DPA (dipicolinate; a competitive inhibitor of DHDPR) bound and ternary (NADPH and DPA) complexed PaDHDPR were determined at 1.8, 1.8 and 2.1 Å resolutions, respectively. Comparison of these PaDHDPR structures with previously reported other DHDPR structures reveals that only ternary complex PaDHDPR structure shows completely closed conformation suggesting both substrate and nucleotide co-factor are essential for the domain movement. Moreover, NADPH binding induced local conformation changes in the N-terminal long loop region (residues 34-59) in PaDHDPR and the His35 and Lys36 residues located in this loop region make strong interaction network with the 2′-phosphate group of NADPH. These specific interactions may allow PaDHDPR to have a strong preference to NADPH as co-factor compared with NADH. In addition, activity assay using H35A and K36A mutants showed significantly reduced NADPH binding and enzymatic activity confirming the importance of these two residues. Collectively, these data provide detailed structural insights into pivotal conformational changes in PaDHDPR for enzymatic catalysis and the cofactor selectivity of this important bacterial enzyme.

References:

Lee, C. W., Park, S.-H., Lee, S. G., Park, H. H., Kim, H. J., Park, H., Park, H. & Lee, J. H. (2018). Scientific Reports.8: 7936

Keywords: dihydrodipicolinate reductase, Panisporosarcina sp. TG-14, psychrophile