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POSTERS

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Indexing

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P.09-153-Wed
Intermediates of orange carotenoid protein photocycle

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The 35-kDa Orange Carotenoid Protein (OCP) is responsible for photoprotection in cyanobacteria. It acts as a light intensity sensor and efficient quencher of phycobilisome excitation. Photoactivation triggers large-scale conformational rearrangements to convert OCP from the orange OCPO state to the red active signaling state, OCPR, as demonstrated by various structural methods. Such rearrangements imply a complete, yet reversible separation of structural domains and translocation of the carotenoid. In this study, we took advantage of single 7 ns laser pulses to study carotenoid absorption transients in OCP on the timescale from 100 ns to 10 s, which allowed us to detect a red intermediate state preceding the red signaling state, OCPR. In addition, time-resolved fluorescence spectroscopy and the assignment of carotenoid-induced quenching of different tryptophan residues derived thereof revealed a novel orange intermediate state, which appears during the relaxation of photoactivated OCPR to OCPO. Our results show asynchronous changes between the carotenoid- and protein-associated kinetic components in a refined mechanistic model of the OCP photocycle, but also introduce new kinetic signatures for future studies of OCP photoactivity and photoprotection. This work was supported by a grant from the Russian Science Foundation No. 17-74-30019.

P.09-154-Mon
Digestomics of cow's milk: casein-derived digestion-resistant peptides aggregate into functional complexes

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Allergy to cow's milk proteins is commonly reported in infants, with majority of them successfully outgrowing it by the end of childhood. Safer alternatives for introduction of milk into children's diet have been investigated and milk proteins hydrolysates with fragments less than 3 kDa are considered as hypoallergenic. The aim of this study was to identify digestion products of major milk allergens and to examine the IgE reactivity and allergenicity of short digestion-resistant peptides (SDRP) released by pepsin digestion of whole milk. Here, raw milk was subjected to simulated gastric digestion and analyzed by electrophoresis and Western blotting. In gastric digests α-lactalbumin and β-lactoglobulin were present mostly as intact proteins. High-resolution mass-spectrometry peptidomics analysis of SDRP fractionated from digests revealed that majority of the digestion-resistant peptides originated from caseins (97% of peptides). SDRP mostly overlapped with the known IgE epitopes of cow's milk allergens, with the average peptide length of 10.6 ± 3.5 amino-acids. The ability of SDRP to compete for IgE binding with individual milk allergens and the mixture of the milk proteins was demonstrated. Since thirty amino acids has been suggested as a minimal length of a peptide able to cross-link two IgE molecules on the surface of mast cells and provoke an allergic reaction, it was unexpected that SDRP of induced allergic *in vivo* responses (positive skin-prick tests) in 4 out of 5 milk-allergic subjects. Hence, aggregation ability of the SDRP was assessed and confirmed by size-exclusion chromatography. Our results prove that short digestion-resistant peptides mainly corresponding to the continuous epitopes of milk proteins induce an allergenic *in vivo* response due to aggregation.

P.09-155-Tue
Antineurocytoskeletal antibodies and their immune complexes in patients with neurodegenerative diseases

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Neurocytoskeletal proteins, such as neurofilaments (Nf), may be released from neurons during a neurodegenerative process and induce the synthesis of specific autoantibodies. These antibodies could form immune complexes with corresponding antigens. The autoantibodies are present both in a free form and bound in immune complexes. An analysis of free antibodies alone cannot give the information on their total production. The aim of our study was to introduce and optimise the ELISA (Enzyme-Linked Immuno-Sorbent Assay) method for the determination of specific immune complexes of IgG antibodies against the heavy subunit of neurofilament (NfH) with the corresponding neurofilament subunit. Levels of anti-NfH antibodies and immune complexes determined by our in-house ELISA method were expressed in the same arbitrary units of concentration using the commercial calibrator for immunoglobulins. We evaluated this method on the pilot groups of patients in serum and cerebrospinal fluid (CSF) samples. Simultaneous determination of free antibodies against the heavy subunit of neurofilament and corresponding immune complexes have been performed in patients with mild cognitive impairment (MCI), Alzheimer′s disease (AD) and age-matched control subjects. Levels of free antibodies and antibodies bound in immune complexes were significantly lower in patients with MCI than in the patients with AD and control subjects. Significant differences between levels of free antibodies and antibodies bound in the immune complexes were observed especially in CSF. Our ELISA method is suitable for analysis of both serum and CSF. The parallel analysis of free anti-Nf antibodies and their immune complexes could be evaluated together and provide more complex information about autoantibody response against neurocytoskeletal proteins in neurodegenerative diseases. The study is supported by PROGRES Q25/LF1 and RVO-VFN64165.