Session II: Biological Applications

Fluorescent colorants in immunochemistry

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Prof. Sergei A. Eremin is Head of Research Group of Immunoassays, Department of Chemical Enzymology, Faculty of Chemistry, M.V.Lomonosov Moscow State University, Russia. Prof. Sergei A. EREMIN was born in 1954 and received his B.Sc. in Chemistry on 1976 at the Faculty of Chemistry, M.V.Lomonosov Moscow State University (MSU). From 1976 to 1979 he was Ph.D. student at the Department of Organic Chemistry, MSU. Prof. Eremin was awarded a Ph.D. degree in organic chemistry on 1982. The title of his Ph.D. Thesis was “Synthesis and properties of a-metallic ketons of cyclopentadienylcarbonyl derivatives of transition metals (Fe, Mo, W)”. In 2004 Prof. Eremin was awarded a D.Sc. degree in Biotechnology and Analytical Chemistry at MSU. The title of D.Sc. Dissertation was ”Fluorescence Polarization Immunoassay of physiology active compounds”.

Since 1979 up to now Prof. Eremin joined the Department of Chemical Enzymology, MSU as researcher, scientist, senior and leader scientist. He was Associate Professor of Moscow Institute of Fine Chemical Technology, Moscow, Russia. Since 2005 he is also Professor at Department of Toxicological Chemistry, Faculty of Pharmacy, I.M.Sechenov First Moscow State Medical University, Moscow, Russia. Prof. Eremin was supervisor of 11 Ph.D. and currently the supervisor of 4 Ph.D. students and several graduated students.

Current activity of his research group is focused on: the study of antigen - antibody interaction; investigation of influence of chemical structure of tracer on the sensitivity and specificity of immunoassay; development of novel immunochemical methods. The results of his research have been presented as oral report at many international conferences. He has published extensively on these topics and is co-author of more than 200 scientific publications in Russian and International Journal and several reviews and patents. Prof. Eremin is and was coordinator of several international research grants.

Prof. Eremin is a member of International Association of Environmental Analytical Chemistry and all Russia D.I.Mendeleev Chemical Society; he is member of editorial board of Russian Journal of Analytical Chemistry, Food and Agricultural Immunology, and International Journal of Food Science & Technology.

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Abstract:

Fluorescein and its analogs are widely used to label peptides, proteins, ligands and others biomolecules and applied for immunochemistry. These dyes are used as Fluorescein-labeled tracer for Fluorescence Polarization Immunoassay (FPIA). The FPIA is a homogeneous (without separation) competitive immunoassay method based on the increase in fluorescence polarization (FP) of fluorescent-labeled small antigens (tracer) when bound by specific antibody or another recognition elements (aptamer, MIP, receptor, peptide and etc.). If the sample contains the antigen as analyte, it will compete with the tracer for binding with antibody and the polarization signal will decrease in proportion to analyte concentration. The total time for performance of FPIA is a few seconds or minutes. The minimum detectable quantity (about 0.1 ng analyte) is comparable with chromatography and ELISA methods, although this may be limited by sample matrix interference. Because of its simplicity and speed, FPIA is readily automated and therefore suitable for high throughput screening (HTS) in a variety of application areas.

The synthesis of fluorescent-labeled antigen (tracer) can be achieved under gentle and simple conditions by using commercially available fluorescein derivatives such as fluorescein isothiocyanate (FITC), (aminoacetoamido)fluorescein, 4'-(amino-methyl)fluorescein hydrochloride, 5-(4,6-dichloro-triazinyl)aminofluorescein hydrochloride (DTAF) and carboxyfluorescein. Such tracers are stable on storage and benefit from the ability of fluorescein to fluoresce with high quantum yield while retaining the immunoreactivity of the antigen.

The FPIA requires specialized instrumentation for FP measurement and can be applied primarily for detection of small molecules.

The recent advantages and limitation for FPIA will be presented.