Abstract:

Synthesis of immunofluorescence probes and affinity surfaces for selective and sensitive analysis of biologically important molecules

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We will report a several methods for synthesis of immunofluorescence probes and affinity surfaces. We used quantum dots as a fluorescence affinity surface. Quantum dots (QDs), semiconductor inorganic nanocrystals, show a great potential as photoluminescent probes at cellular level for in vivo immunolabelling or cellular tracking. The main advantages of quantum dots, when compared with the conventional organic fluorescent dyes, are practically no photobleaching, wide excitation and narrow emission spectra and size dependent emission maximum wavelengths. Typical materials for the preparation of quantum dots are elements of II – VI or III – V group. We have prepared water soluble QDs in the size range from 2.8 to 4.4 nm by the chemical reaction between $CdCl_2$ and NaHTe with 3-mercaptopropionic acid (MPA) as a hydrophilic surface ligand. To reduce their cytotoxicity, QDs are coated by two epitaxial layers of CdS and ZnS.

For the preparation of conjugates, it is possible to use a lot of conjugation procedures with variety of cross-linkers. Their role is to bind different functional groups attached to QDs with those present in biologically important molecules. We have used the conjugation reaction via zero-length cross-linkers such as 1-ethyl-3-(3-dimethyl-3-aminopropyl) carbodiimide hydrochloride and *N*-hydroxysulfosuccinimide for bonding carboxylated QDs and proteins (especially antibodies). The formation of conjugate is checked by capillary zone electrophoresis with laser-induced fluorescence detection. We have found optimal conditions for the separation of the conjugate, immunocomplex and QDs.

Non-coated and double coated QDs are conjugated with various proteins such as antibody to proliferating cell nuclear antigen (anti-PCNA), anti-chicken egg albumin, anti-CD3 membrane protein and annexin V. Antibodies labelled with QDs are applied as fluorescent probes for the detection of various antigens in cancer cells and apoptotic mouse intestine and human lymphocyte cells. Epifluorescence microscopy with excitation by using mercury arc lamp (460-550 nm) and total internal reflection

fluorescence microscopy with Nd:YAG laser (532 nm) are used as a high sensitivity detection instrumentation.

Finally, we will report about Caspase-3 determination. Caspase 3, a cysteine-aspartic acid protease activated in the apoptotic pathway, plays an essential role in the programmed cell death. Moreover, failure of apoptosis is one of the main contributions to tumor development. Under normal circumstances, caspases recognize tetra-peptide sequences Asp-x-x-Asp on their substrates and hydrolyze peptide bonds after aspartic acid residues. It is known from literature that in one apoptotic cell about 1.6×10^{-19} mol of Caspase can be activated. Various techniques for the determination of Caspase 3 in free cells or tissue samples are commercially available. While the Caspase concentration is determined by Enzyme-Linked Immunosorbent Assays (ELISA) with colorimetric detection, its activity is usually assayed by a chemiluminescence reaction. The best commercial methods reach the limit o detection higher than 1pg of Caspase 3.

We have developed a special device for the determination of Caspase concentration or activity in microdissected cells: (i) The concentration is determined by ELISA assay with chemiluminescence detection. In this technique, a monoclonal antibody immobilized on the device wall interacts with a Caspase 3 epitope. Then, a polyclonal antibody labeled by horse radish peroxidase binds another Caspase epitope. The presence of this antigene is detected as a chemiluminescence signal after the addition of a mixture of luminol with hydrogenperoxide. (ii) The activity of Caspase 3 is determined by the system based on Luciferin/Luciferase chemiluminescence reaction. The luciferin modified with tetrapeptide sequence (DEVD) specific to the recognition for Caspase-3 is cleaved to form free luciferin, which immediately reacts with luciferase to produce light. Our detection device consists of a microfluidic chamber held inside a housing of photomultiplier tube (Hamamatsu R446) with spectral response 185-870 nm. The cell is accessible by a Hamilton syringe fixed in a light-proof cassette. The sensitivity of the device proved to be by one order of magnitude better than the commercially available technologies.