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Autoradiography: Detection and Analysis of Radioactive Entities

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Abstract

Autoradiography is a specific biological tool used to detect radioactive materials by using X-ray photographic films. A technically simple technique to be used for characterizing receptors and localizing their positions in the tissues. Moreover its detection sensitivity could be enhanced using fluorography by transforming radioactive emissions into light.

Keywords: Fluorography; Photographic emulsion; Silver halide

Introduction

Autoradiography is employed for the detection of materials that possess radioactive properties. By using X-ray films, autoradiography determine the relative positions and intensities of radiolabeled bands in a gel or blot. In 1867 the first autoradiography was observed accidently when an emulsion of silver chloride and iodide turns black by uranium salts [1]. With the advent of photographic emulsions and photographic films after World War II, autoradiography was used as a biological technique for the detection of radioactive substances or materials labelled with radioactive isotopes [2].

Mechanism

Penetration of negatively charged beta particles emitted by radioactive salts through silver halide film emulsion causes activation of silver present in the emulsion. Activated silver crystals are very unstable therefore quickly reduced to black silver particles which is easily detectable. Autoradiography sensitivity is improved by carrying the detection process at 70°C and preflashing the film before use. Preflashing needs only one hit per crystal deposited to increases sensitivity [3]. Autoradiography detection limits vary for different radioisotopes as given in the table below (Table 1) [4,5].

Sequential steps of autoradiography

- Brief exposure of living cells to a pulse of specific radioactive material for a variable time.
- Preparation of samples are for microscopy either light or electron.
- Dissection of samples into sections for coverage with thin film of photographic emulsion which are then incubated in the dark for few days for radioactive decay. The exposure time depends on isotope activity, temperature and the background radiation.
- Development of photographic emulsion.
- Toluidine blue is used for counter staining to reveal tissue histology. Instead Osmium or dipping emulsion can be used for pre-staining of the entire tissue before exposure to the photographic emulsion to avoid for individual post- staining each slide.
- Microscopy either light or electron is used to determine the relative position of the silver particles.
- Generation of records in the form of autoradiographs [6,7].

Isotope	Count per minute (CPM) for Detection	Energy per Emission (MEV)
^{3}H	$>10^{7}$	0.0055
^{14}C	2000	0.050
^{35}S	1000	0.167
^{32}P	100	0.70
^{125}I	10	Gamma

Table 1: Autoradiography detection limit.

Fluorography

Autoradiography sensitivity is greatly enhanced through fluorography which transforms radioactive emissions into light which efficiently penetrates the film to be readily detected [8]. A number of phosphor compounds absorb energy from beta particles and re-emit it as light e.g. Autofluor [9,10].

Advantages

- Technically easy not much expertise required,
- Highly specific detection tool,
- Unlike tissue bath preparations, pharmacologically characterize and localize receptors in tissues,
- Enables characterization of receptors in different tissues in different animals or brain regions [11,12].

Disadvantages

- Lack of assessment criteria to determine whether the binding site really corresponds to an actual receptor,
- Non-physiological significance of high affinity radiolabelled receptor,
- Non-specificity of ligands can easily cause misinterpretation of results [13].

Autoradiography practical applications

Autoradiography provides qualitative as well as quantitative

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information regarding a specimen. Some of the following applications of this technique are given below:

- Autoradiography is used to determine receptor distribution and localization while studying neurodegenerative disorders [14].
- Application of autoradiography in electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets during blotting [15].
- To study cytogenesis of the forebrain [16].
- Applications in radiopharmaceutical research [17].
- Applications in radioimmunoelectroosmophoresis to study viruses [18].
- In imaging and analyzing rock porosity [19].
- In matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI), and secondary ion mass spectrometric imaging (SIMS-MSI) for pharmaceutical discovery and development [20].
- In whole body imaging [21].
- Tool for genetic studies [22].
- For comparison of complex mixtures of proteins [23]
- Applications in microbial ecology [24].
- Determining gross absorption and utilization of foliar applied nutrients etc. [25].

Conclusion

Today, autoradiography is employed as an important detection tool for the identification of different target receptors in various tissues to provide us with a better understanding of molecular pharmacological pathways.

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