THE USE OF CYTOSTAR-T™ SCINTILLATING MICROTITRE PLATES FOR MONITORING RECEPTOR BINDING USING LIVE CELLS

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Introduction

The binding of a ligand to a receptor often represents the first step in a complex cascade of biochemical events associated with a signal transduction pathway. Consequently the receptor provides an attractive accessible target for intervention by therapeutic agents.

Receptor binding assays have been extensively used to characterise and classify receptors and to identify receptor agonists and antagonists as potential drugs. Classically these assays have utilised radiolabelled ligands and membrane preparations derived by disruption of cells or tissues containing the receptor of interest(1). As this approach generally results in loss of cellular integrity, certain aspects of the regulation of receptor expression cannot be studied. Furthermore, cells which normally grow in a well defined orientation often lose phenotypic characteristics when dissociated.

The Cytostar-T plate enables the study of cell cultures which normally grow as an adherent monolayer on culture treated plastic or on a layer of extracellular matrix proteins. This system is therefore potentially suitable for carrying out ligand binding assays in a homogenous format without disturbing the equilibrium between bound and free ligand, and whilst maintaining the cells in a more physiological environment.

Experimental

The interaction of [125I]EGF (AmershamTM, IM196 >750Ci/mmol) with EGF-R expressed by the A431 human cell line was studied as a model system to investigate the performance of Cytostar-T plates in the measurement of kinetic events in comparison with SPA and filtration techniques.

The binding of [125I]EGF to A431 cells measured by Cytostar-T plate and filtration. For Cytostar-T assay 80% confluent A431 cell monolayers (Cytostar-T) were incubated at 37°C for 2 hours in assay buffer (20mM HEPES, 2mM CaCl₂, 0.1% BSA) containing [125I]EGF (100nM final concentration) and unlabelled EGF at a concentration range of 0.1-50nM. Plates were counted on a Wallac 1450 MicroBeta™ scintillation counter. For filter assay 9µl of aliquots of A431 cell fractions were incubated at 37°C for 2 hours in assay buffer containing [125I]EGF (100nCi/ml final concentration) and unlabelled EGF at a concentration range of 0.1-50nM. Reactions were loaded onto Whatman GF/C filters, washed through with assay buffer and counted in liquid scintillant on an LKB Rackbeta 1209 counter. Results are means (n=3).

The association and dissociation constants of [125I]EGF binding to EGF-R expressed by A431 cells were determined for each assay system. Dissociation curves were achieved by the addition of an excess of unlabelled EGF. The Cytostar-T plate and SPA assay techniques allowed a single set of assay wells to be monitored continuously over time. Figure 3 shows typical data obtained by the Cytostar-T plate technique and kinetic parameters are summarised in Table 2.

Equilibrium saturation binding experiments were performed using SPA and Cytostar-T. The Kᵦ and Kᵦᵣ values are summarised in Table 1.

Conclusions

The results of this study show that the kinetic parameters obtained for the EGF-R on A431 cells by Cytostar-T plate are compatible with those obtained by SPA and filtration methods. Total cpm measured are lower on Cytostar-T compared to SPA due to differences in counting efficiency but competition curves and IC₅₀ values were virtually identical across the assay methodologies. Association/dissociation rates and saturation binding curves were also similar. The slightly higher Kᵦ and Kᵦᵣ values obtained with Cytostar-T compared with SPA and filtration were probably due to the influence of post-binding events that occur in the viable cell(2).

It can be concluded from this study that both Cytostar-T plates and SPA beads have the sensitivity required to measure kinetic events without causing interference with the receptor/ligand interaction. The advantages of Cytostar-T plates and SPA beads over traditional methods is significant in that the technology is able to measure real-time binding events, allowing the researcher to make considerable savings in both labour and reagents. The Cytostar-T scintillating microplates have the additional advantage in that cells can be assayed in a more physiological environment, allowing the continuous monitoring of biochemical and morphological events over short or extended time periods without any disruption of the cells.

References