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Measuring the affinity of a radioligand with its receptor using a rotating cell dish with in situ reference area

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Abstract

This report describes a semi-automated method for the measurement of affinity of radiolabeled ligands interacting with cell-surface receptors on intact cancer cells. The method saves labor time and reagents compared to common manual measurements. A complete affinity measurement can be performed in one cell dish by using a target cell area and a reference area and repeatedly measure the differential activity (i.e. target activity—reference activity). The affinities obtained for different ligand–receptor interactions agreed with affinities reported in the literature.

Keywords: Radioligand; Affinity; Cell-based; Interaction

1. Introduction

The determination of the affinity of a radiolabeled ligand binding to its cell-surface receptor is a common measurement in different fields in biology and biochemistry, including cancer research (Smith-Jones et al., 1999; Sundberg et al., 2003a), pharmacology (Li et al., 1997), and drug discovery (Culliford et al., 2002). Apart from industrial scale, automated high-throughput methods (Minor, 2003), the methods for measuring the affinity are often manual and labor intense. In this paper, a simplified and partly automated method for the small-scale determination of affinity is described.

In a typical affinity measurement, target cells are grown in several separate containers (cell dishes or wells in a microplate) and to each container different known amounts of radiolabeled ligand are added. After incubation, the containers are washed, the activity of each container is measured, and the number of cells present in each container is determined. The affinity of the interaction can be derived by Scatchard analysis or non-linear fits (see e.g. Smith-Jones et al. (1999) or Klein et al. (2004)). A typical affinity measurement includes handling of a large number of containers (tens per tested ligand) and requires that the number of target cells per container is approximately the same at the time of ligand addition.

The method for affinity determination could be significantly simplified by using the following strategy. Take one container, typically a circular cell dish, and include an active area (where the target cells are grown) and a reference area (lacking target cells) within the container. Then stir the liquid in the container in order to get a homogenous liquid over both the active area and the reference area. Next, detect the activity of the active area and the reference area separately and use the difference of the activities as output signal.

By repeatedly measuring the differential signal at increasing ligand concentrations, a binding level versus

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ligand concentration curve is obtained from which the affinity can be derived. Measurements performed according to this strategy have several advantages compared to manual measurements. Fewer containers need to be handled, smaller amounts of reagents will be consumed and there is no need to count the number of cells in the containers. In particular, less radioactive material is required, which is beneficial from a safety and environmental perspective.

The main drawbacks of the method are that the measurement has to be performed in increasing concentration order and that the preparation of the containers becomes more complicated, because target cells must be present in a defined active area only.

This paper describes affinity measurements of radiolabeled ligands binding to cell-surface receptors. The measurements were performed according to the simplified strategy using a prototype instrument.

2. Materials and methods

2.1. Cells and reagents

Procedures and reagents not specified in this report followed Sundberg et al. (2003a). To obtain a well-defined active area with target cells, a 0.5–2 ml aliquot of A431 (ATCC, CLR 1555, Rocksville, MD, USA), U343 (MGaCl2:6, Rocksville, MD, USA) or SKOV-3 (ATCC, HTB-77, Rocksville, MD, USA) cell suspension containing approximately 10^5 cells was deposited in a local area ($\sim$5 cm^2 large) near the edge of an 8 cm cell dish as indicated in Fig. 1. The dish was maintained slightly tilted during a few hours to ensure that the cells attached firmly to the dish prior to filling it with cell culture medium. The dishes were incubated at least over night to allow the cells to attach firmly to the cell dish. A431 cells are known to have approximately 2×10^6 epidermal growth factor receptors (EGFR) per cell (Sundberg et al., 2003b), U343 approximately 1×10^6 EGFR per cell (Sundberg et al., 2003b), and SKOV-3 approximately 10^5–10^6 HER2 receptors per cell (Xu et al., 1999).

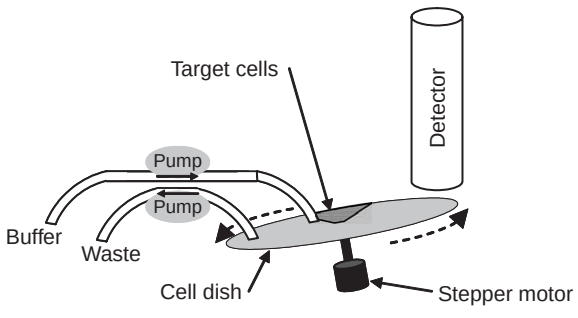

Fig. 1. Schematic of the prototype instrument.

Human epidermal growth factor (EGF) and an irrelevant antibody (anti-A33, kindly donated by the Ludwig Institute, New York, USA) was labeled with ^{125}I using the Chloramine-T method as described by Sundberg et al. (2003a). Typically, 3 μg EGF was labeled with 3 MBq ^{125}I. The affibody $Z_{HER2/neu:4}$ (Z_{HER2}), known to bind to the HER2/neu receptor, was labeled with ^{125}I using an indirect method with *N*-succinimidyl-*p*-(trimethylstannyl)benzoate (SPMB) as labeling precursor, essentially as described by Wikman et al. (2004).

The affinity of the EGF–EGFR interaction on A431 cells interaction was measured in PBSM (1000 ml PBS (1.5 mM $NaH_2PO_4 \times H_2O$, 8.1 mM $Na_2HPO_4 \times 12$ H_2O, 2.7 mM KCl, 0.14 M NaCl, pH 7.5) + 50 ml HAMS-F10 medium (Kebo, Sweden)). The affinity of the EGF–EGFR interaction on U343 cells and Z_{HER2}–HER2 interaction on SKOV-3 cells was measured in HAMS-F10 medium (Kebo, Sweden).

2.2. Device for affinity determination

The affinity of the interaction of EGF with EGFR was measured in a prototype instrument outlined in Fig. 1. The dish was placed on a sloping support that could be rotated using a stepper motor. A scintillation detector (a modified series 900, Mini-Instruments, Essex, UK) was placed above the highest point of the rotating support, collimated so that only approximately 10 cm^2 of the cell dish was visible for the detector window. The detector was modified to allow for computerized, time-resolved measurement of radiation. Means for liquid transfer to and from the cell dish was installed as described in Fig. 1. The stepper motor, the detector and the pumps were controlled by an IBM compatible computer connected to a LabJack measurement and automation device (LabJack Corp., Lakewood, CO). The obtained results were analyzed using MATLAB 6.5 (The Mathworks Inc., Natick, MA). All equipments were placed in a cold room (temperature 4–8 °C) during operation to avoid internalization of bound ligands.

In preparation for each measurement of ^{125}I-EGF binding to EGFR on A431 cells, the cell dish was rinsed with PBSM. After the rinsing procedure, approximately 1 ml PBSM was left in the cell dish. The liquid accumulated in the lower end of the cell dish and was not visible for the detector. The measurement was then performed in the following manner:

1. A small amount of 0.5 μM ^{125}I-EGF was added to the PBSM present in the cell dish.
2. The PBSM + ^{125}I-EGF liquid was incubated for 3 min. During this time, the cell dish rotated slowly ($\sim$10 rounds per minute).
3. The activity along the perimeter of the cell dish was measured at 24 positions per round, 4 rounds per