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Isoelectric focusing of steroid hormone receptors in slabs of polyacrylamide gel

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Summary

Isoelectric focusing in 1–2 mm thick slabs of polyacrylamide gel is a versatile and specific method for quantitation and characterization of estrogen, mineralocorticoid, progestin, and glucocorticoid receptors. This method has been used for routine measurements of estrogen receptor in human breast cancer in Stockholm since 1976. It is also used for quantitation of estrogen receptor in fine needle biopsies from breast cancer tissue. Isoelectric focusing in gel slabs allows rapid separation (1.5 hr) of receptors from other non-receptor proteins that bind steroid hormones, such as transcortin and sex hormone-binding globulin. The tritium-labeled steroid–receptor complex is localized by slicing the gel followed by radioactivity analysis in a liquid scintillation counter. The isoelectric point of the tritium peak is determined with a surface electrode. The isoelectric point serves as a qualitative control. Thus, only one analysis is needed for each receptor quantitation, provided that a saturating concentration of tritiated ligand is used for receptor binding. This means that a minimal amount of tissue is needed for one receptor analysis with isoelectric focusing.

Introduction

The positive correlation between presence of estrogen and progesterone receptors and the prognosis of the disease as well as the response to endocrine treatment of human breast cancer has created a demand for simple and specific methods for routine steroid receptor analysis in clinical samples. We have found isoelectric focusing in thin slabs of polyacrylamide gel to be a useful method to serve this purpose. Below follows a short description of this method as it may be applied for analysis of estrogen, progestin, glucocorticoid, and mineralocorticoid receptors.

Abbreviation: RU 26988 = 11β, 17β-dihydroxy-17α-(1-pro- pionyl)-androst-1,4,6-trien-3-one.

A more detailed description of each method has been published (or is in press) elsewhere and the references are given below.

General methods

Tissue samples are homogenized with a polytron (Kinematica, Lucerne, Switzerland), or if sufficiently soft tissues, with a loose fitting Potter Elvehjem teflon/glass homogenizer. Samples are then centrifuged at about 100,000 × g for cytosol preparation. The cytosol is labeled with a saturating concentration of the appropriate tritiated ligand (see below). In indicated cases a limited trypsin digestion step is then performed by incubating the cytosol with the given amount of trypsin for 30 min.
at 10°C. The amount of added trypsin is related to the approximate protein concentration in the cytosol measured as difference in absorbance at 280 and 310 nm and expressed as A_{280-310} nm. Each sample is then treated with dextran-coated charcoal (1% (w/v) charcoal and 0.1% (w/v) dextran T500, final concentrations), to remove excess of unbound steroid. The supernatant is taken for analysis. For preparation of gel see (1, 2). Gels are commercially available (LKB-Produkter, Bromma, Sweden).

Isoelectric focusing may be performed, e.g., with an LKB Multiphor connected to a constant power supply and a cooling system (LKB-Multitemp).

Double layers of electrode strips soaked in 1 M sodium hydroxide (cathode) or 1 M phosphoric acid (anode) are used. The gel is prefocused for 30 min at 30 mA and 30 W (ready-made gel) or 30 min at 20 mA and 20 W (self-made gel).

Sample frames of acrylic plastic are used in order to apply up to 0.3 ml of sample solution on the gel. Inner diameter is about 7 x 10 mm and height 3 mm. After prefocusing the gel, the sample frames are placed on the gel surface 0.8-1 cm from the cathode electrode strip where the final pH is about 8-8.5. (The hormone-receptor complex is dissociated or destroyed in the acid pH near the anode.) Ferritin and hemoglobin dissolved in water are placed between the sample frames as small drops directly on the gel surface (or may be added in the sample solution as internal markers). Ferritin is focused as a yellow band around pH 4.5 and hemoglobin as a brown band around pH 7.6.

Each sample is pipetted into a sample frame. Usually eight samples are analyzed simultaneously. If enough tumor material is available, it is convenient to place a sample volume containing 0.5–1 A_{280-310} nm units of cytosol protein in each frame. This amount of cytosol protein is enough to detect low amounts of receptor (0.01 fmol/μg DNA). The risk of overloading the gel is small (see however about progesterone receptor below).

The power unit is set at 20 mA, 20 W and 1,200 V and focusing is continued for 1.5 hr.

For further details concerning isoelectric focusing and gel fractionation see (1, 2).

Receptor quantitation

The radioactivity in the vials is measured and the efficiency calculated according to the external standard technique. Routinely only a 5-cm long gel strip between pH 8 and pH 4.7 is fractionated and assayed for radioactivity.

Results and specific methods

Estrogen receptors in human breast cancer tissue (1, 2) or rat liver (3) may be quantitated by the above described method. For further experimental details see above given references. A sample containing 10 μg of DNA is sufficient for an estrogen receptor analysis. This sensitivity makes it possible to use fine needle aspiration biopsy to collect a sample from a human breast cancer nodule (4).

As illustrated in Fig. 1, the non-trypsinized tumor cytosol is usually focused as a double peak with maxima at pH 5.9–6.1 and 6.5–6.7; both these peaks disappear when a 100-fold excess of unlabeled diethylstilbestrol is present in the incubation medium. After trypsin digestion (Fig. 1B), only one

![Graph](image-url)

Fig. 1. Isoelectric focusing of human breast cancer cytosol labeled with 5 nM [3H]-estradiol with (O—O) or without (●—●) a 100-fold excess of unlabeled diethylstilbestrol; (A) untreated, and (B) treated with 5 μg of trypsin for 30 min at 10°C prior to isoelectric focusing. The arrow marks the sample application point; (x—x) signifies pH.