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Study of low molecular weight effectors on the binding between cell membrane receptor IGF-1R and its substrate protein IRS-1 by SPR biosensor

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Abstract

Surface plasmon resonance (SPR) biosensor technique was used to study the effects of Mg^{2+}, Mn^{2+}, ATP, genistein, and quercetin on the binding kinetics between insulin-like growth factor-1 receptor (IGF-1R) and its intracellular substrate protein insulin receptor substrate-1 (IRS-1). IGF-1R was captured from cell lysates using anti-IGF-1R (α-subunit) monoclonal antibodies immobilized on biosensor chip surface, which retained the binding capability with its intracellular substrate. With IGF-1 stimulation, the association rate of IRS-1binding to IGF-1R increased and the affinity constant (1.37×10^9 M) is about 10 times higher than that without IGF-1 stimulation (1.27×10^8 M). The association and dissociation rates of IRS-1 binding to phosphorylated IGF-1R in the presence of either or both of Mg^{2+} and Mn^{2+}, and in the absence or presence of ATP, genistein, and quercetin, were determined from the real-time binding and dissociation curves and the results indicate that ATP, genistein, and quercetin reduced the affinity constants of IRS-1 and IGF-1R by 4.7-, 6.0-, and 6.6-folds, respectively, in the presence of Mg^{2+} and Mn^{2+}. Mn^{2+} slightly increased the affinity mainly due to the increased association rate, while Mg^{2+} and Mn^{2+} together showed no effect on the affinity. In addition, ATP alone did not affect the binding affinity. The results demonstrate that SPR biosensor could be used as a quantitative technique for studying effects of small molecules on the binding kinetics and affinity of receptor–cellular substrate. The method developed in the study may also be used in other biosensor techniques for studying the mechanism of molecule interactions.

Keywords: Surface plasmon resonance biosensor; Insulin-like growth factor-1 receptor; Insulin receptor substrate-1; Mg^{2+} and Mn^{2+}; ATP; Tyrosine kinase inhibitors

1. Introduction

The insulin-like growth factor receptor-1 (IGF-1R) signaling pathway is essential for normal growth, development, and differentiation. It plays an important role in transformation, proliferation, preventing apoptosis, and maintaining the malignant phenotype of tumor cells [1–4]. IGF-1 binds to the extracellular domain of IGF-1R and initiates a conformational change in the quiescent receptor that is transmitted to the intracellular beta subunit. The tyrosine kinase activity of IGF-1R is thereby activated and results in trans-autophosphorylation of the β-subunit. This autophosphorylation of IGF-1R leads to tyrosine phosphorylation of cellular substrates such as insulin receptor substrate 1 (IRS-1) and src homology 2/α-collogen (SHC) [5–6]. The tyrosine-phosphorylated proteins in turn form complex with a variety of src homology 2 (SH2) domain-containing proteins and initiate specific signal pathways [7]. IRS-1 consists of an amino-terminal pleckstrin homology domain for binding IGF-1R, and a large domain containing many motifs for tyrosine phosphorylation and for binding of SH2-containing proteins [8].

The tyrosine-phosphorylated IGF-1R has two distinct but related functions: kinase activity and ligand receptor to provide binding sites for its substrate proteins [9]. It is well known that

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IGF-1R requires divalent cations Mg^{2+}, Mn^{2+} for maximum kinase activity and ATP for a phosphate donor. While Mg^{2+}, Mn^{2+}, and ATP were shown to affect substrates binding affinity of some ATP-binding enzymes through binding to their respective binding sites, it is not clear whether excess of Mg^{2+}, Mn^{2+}, and ATP may also affect the substrate binding activity of IGF-1R [10–16].

Inhibition of IGF-1R tyrosine kinase activity is a therapeutic strategy for many cancers [17]. Genistein is a 4′,5,7-trihydroxyisoflavone that specifically inhibits protein tyrosine kinase activity including phosphorylation and autophosphorylation of many tyrosine kinase receptor kinases (PTK) [18,19]. Quercetin, 3,3′,4′,5,7-pentahydroxyflavone, is also known to inhibit PTK but is less specific than genistein [20,21]. Both genistein and quercetin inhibit tyrosine receptor kinase activity by competing with ATP. They are located in the ATP binding pocket of most tyrosine kinases. However, it is not clear if these molecules exert similar effects on IGF-1R and its substrate binding.

Biosensor technology based on the surface plasmon resonance (SPR) technique has been used to measure the binding kinetics between a biomolecule in solution and its binding partner immobilized on a sensor surface in real time including protein–protein and protein–DNA interactions [22–25]. Proteins and small molecules interactions have been studied based on SPR biosensor technique by direct binding assay, a surface competitive assay or an inhibitor in solution assay [26,27]. In our previous studies, surface plasmon resonance biosensor was used to measure the binding kinetics of IGF-1 and IGF-1 binding protein [28]. In addition, an *in vitro* method for detecting IGF-1R and its intracellular substrate IRS-1 interaction based on the SPR biosensor technique was developed [29].

In this study, we used the SPR biosensor to study the effects of IGF-1 stimulated receptor phosphorylation, Mg^{2+}, Mn^{2+}, and ATP on the binding of IRS-1 to IGF-1R. We have also investigated the effects of genistein and quercetin on the substrate binding activity of phosphorylated IGF-1R. The kinetic data provide quantitative information on the effects of these different effectors on IGF-1R and IRS-1 interaction as well as the structural features of the IGF-1R kinase and its substrate binding mechanism.

2. Materials and methods

2.1. Equipments and materials

The SPR biosensor instrument (BIAcore X), sensor chips CM5 of research grade, HBS-EP buffer (10 mM Hepes, 150 mM NaCl, 3.4 mM EDTA, 0.05% P20, pH 7.4), and the amine coupling kit containing *N*-ethyl-*N*′-(3-diethylaminopropyl)carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), and ethanolamine hydrochloride were acquired from Amersham Pharmacia (Uppsala, Sweden). Mouse monoclonal antibody against the α-subunit of human IGF-1R [IGF-1R (Ab-1)] and Genistein (Antibiotic G418) were from Calbiochem of EMD Biosciences, Inc. (San Diego, CA, USA). Polycolonal antibodies against IGF-1R (β-subunit) and Protein A/G agarose were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Antibody against phosphotyrosine (PY20) was obtained from Southern Biotechnology Associates, Inc. (Birmingham, AL, USA). IRS-1 was from Upstate Biotechnology Inc. (Lake Placid, NY, USA). Dulbecco's modified Eagle's medium (DMEM) was obtained from Invitrogen (Carlsbad, CA, USA). Protease inhibitor cocktail tablets were obtained from Roche Applied Science Company (Basel, Switzerland). All other chemicals used were of analytical grade, and doubly distilled deionized water was used in all experiments.

2.2. Cell lines, cell lysates preparation and immunoblotting of receptor and its autophosphorylation

The NIH-3T3 cell line overexpressing IGF-1R was a generous gift from Professor Derek LeRoith (National Institute of Diabetes and Digestive and Kidney diseases, NIH, Bethesda, MA, USA). The cells were maintained in the DMEM medium supplemented with 10% FBS, 500 μg/ml G418, 100 U/ml penicillin, and 100 μg/ml streptomycin. Control NIH-3T3 cell line was maintained in the same medium without G418. The two cell lines were cultured in a humidified atmosphere of 95% air and 5% of CO_2 at 37 °C. The cells overexpressing IGF-1R were grown to 80% confluence in 100-mm plates and then serum-starved overnight in DMEM without FBS. Then the cells were incubated either with or without IGF-1 (100 ng/ml) for 1 min at 37 °C. IGF-1R and its autophosphorylation were determined by the immunoprecipitation and immunoblotting method as previously described [30]. Blots were then stripped of antibodies by incubating in 2% SDS, 10 mM β-mecaptoethanol, 62.5 mM Tris–HCl, pH 6.8, at 50 °C for 30 min, blocked, and reprobed with anti-IGF-1R β-subunit antibody and detected.

The membrane fraction of the receptor for the SPR assay was prepared based on previously reported protocols [31]. Briefly, the NIH-3T3 cell line overexpressing IGF-1R was grown to confluence in 150-mm plates and incubated either with or without IGF-1 (100 ng/ml) for 1 min at 37 °C as described above. The cells were then washed rapidly three times with chilled phosphate-buffered saline and then lysed with 0.6 ml extraction buffer (1% Triton X-100 in 20 mM HEPES, 150 mM NaCl, 1 mM EGTA, pH 7.4, with freshly added phosphatase inhibitor sodium orthovanadate (final concentration 1 mM), and protease inhibitors prepared as the instructions. The cells were scraped and collected in a microtube. The plates were then washed by addition of 0.3 ml extraction buffer. The combined lysate was cleared at 4 °C by sequential centrifugation at 15,294 × *g* for 15 min (Centrifuge 5810R, Eppendorf) and at 262,000 × *g* for 30 min (CS150GXL, Hitachi). The supernatants containing the membrane fraction of the cell lysate were used in subsequent binding experiments.

2.3. Immobilization of the antibody against IGF-1R (α-subunit)

All SPR measurements were performed on the BIAcore X apparatus (BIAcore AB, Uppsala, Sweden) at 25 °C. Immobi-