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Binding of D-mannose-containing glycoproteins to D-mannose-specific lectins studied by surface plasmon resonance

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ABSTRACT

Binding of selected glycoproteins containing α-D-mannose sites (invertase, glucoamylase, transferrin, glucose oxidase, and albumin α-D-mannopyranosylphenyl isothiocyanate) with two lectins selective for α-D-mannose branching glycans, concanavalin A (ConA), and Lens culinaris agglutinin (LCA) was studied on lectin biochips by microfluidic surface plasmon resonance (SPR). Lectin-containing biochips were prepared by covalent immobilization of lectins on a flat carboxymethylated gold surface. Both measurement as well as regeneration conditions were optimized. The determined dissociation constants of lectin–glycoprotein interactions were 10^{-5} to 10^{-7} mol/l. Dissociation constants K_D of studied bindings were estimated by the steady state specific binding models based on both a binding to one site and the multiple binding sites model using Hill slope.

1. Introduction

Discovering of the importance of protein glycosylation is associated with advances in glycobiology and is progressing very quickly. Glycosylation is perhaps the most extensive and complex form of post-translational modification of proteins and most of proteins in organism are glycosylated. Both N-linked and O-linked glycoproteins, in the form of glycopeptides, glycolipids, glycosaminoglycans, or other glycoconjugates, play an important function in most of biological processes. It is now known that glycoproteins play a key role in regulation of cell development, cell differentiation, immune response, inflammations, fertilization, pathogen–host interactions and other [1,2] and changes in protein glycosylation are associated with the development of many diseases as cancer, rheumatoid arthritis, multiple sclerosis, Alzheimer's disease, diabetes, etc. Scientists involved in glyco-related research have keen interest in the appropriate analytical methods for determination of carbohydrates having a large potential in medicine with special interest to biomarker research area, drug discovery and biotechnology [3,4]. Among the most sensitive, reproducible and reliable techniques allowing the determination of sugar units in glycoproteins are mass spectroscopy in tandem arrangement, MS with electrospray ionization, and MALDI-ToF MS. However, MS techniques are expensive, time consuming, and need highly qualified operator. Moreover, usually the step of glycans release from glycoprotein and their separation by one of the separation techniques (e.g. HPLC, high performance anionic exchange chromatography, capillary affinity electrophoresis) has to be included [5,6].

An alternative for glycoprotein analysis is the use of lectins in combination with any of biorecognition techniques. Lectins are special proteins specifically interacting with certain glycan structural motifs. Use of lectins in the glycoprotein analysis may either eliminate the need for glycans release and separation or in combination with some of identification techniques (e.g. MS) it can improve analytical performance significantly. The lectin–glycoprotein interactions are studied with various techniques as isothermal titration calorimetry, lectin affinity chromatography, enzyme-linked lectin assay, as well as with modern bioanalytical methods such as surface plasmon resonance (SPR), microarray and flow cytometry [7,8]. However, it is difficult to compare K_D values measured by various methods one other because the experimental parameters of these methods differ and influence measured K_D.

For examination of glycoprotein–lectin binding the multivalency of binding partners plays an important role. Glycoproteins often contain few or several sugar epitopes that can bind to the sugars-recognizing substances as lectins, what influences determination of the binding affinities on the surface-based glycoconjugate–lectin interaction. The multivalency is the reason

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of high specificity and affinity of interaction glycoprotein–glycan binding protein in biological processes in spite of the relatively weak affinity and specificity of the interaction between single binding site of glycan binding protein with single glycan epitope. This phenomenon first described in 1995 [9] was named "cluster effect" [10]. The binding affinity of a glycan towards a lectin can be even powered by the presence of a second sugar due to secondary interaction of this second glycan structure (that itself is not a ligand for this lectin) if a certain density of glycan structure which is ligand for this lectin is achieved, referred to as "heterocluster effect" [11]. However, the effects of clustered epitopes on the affinity of ligand–receptor interactions in many cases are still not very well understood [12].

Mannose-containing glycoproteins are typical natural multivalent glycoconjugates and α-D-mannose is one of the carbohydrate units often built in the glycoproteins. D-Mannose can be linked to the protein through both N-linkage (in the different structures, as oligomannose, in complex form, and in hybrid form) and O-linkage. Determination of glycoprotein mannosylation is of great importance, such as for example relationship between liver cancer and changes in α-fetoprotein mannosylation detected by two α-D-mannose binding lectins, ConA and LCA [13].

In our previous works, we have studied interaction of ConA with some natural and synthetic glycosylated proteins by SPR [14,15]. Label-free SPR offers a real-time detection providing both equilibrium and kinetic data and is very popular method for monitoring of biomolecular interactions including lectin–glycoconjugate binding. In this study, we have measured by SPR interactions of two D-mannose-binding lectins (ConA and LCA) with 5 D-mannose-containing glycoproteins (invertase, glucoamylase, transferrin, glucose oxidase, and albumin α-D-mannopyranosylphenyl isothiocyanate) on a biochip with low surface density of lectins to suppress the clustering effect. Dissociation constants of binding (K_D) have been calculated according to two steady state specific binding models. The first one was based on fitting to a 1:1 binding model, the second was based on fitting to multiple binding sites model using Hill slope.

2. Materials and methods

2.1. Lectins and glycoproteins

Lectin ConA was commercially available from Sigma–Aldrich, lectin LCA was from Biogema Košice, Slovakia. The glycoproteins invertase (INV), glucoamylase (GA), transferrin (TRF), glucose oxidase (GO), and bovine albumin α-D-mannopyranosylphenyl isothiocyanate (mannosylated albumin, BAM) as well as methyl-α-D-mannopyranose were purchased from Sigma–Aldrich.

2.2. SPR assay and biochip preparation

Measurements were carried out on the Biacore 3000 instrument (GE Healthcare, Uppsala, Sweden). To avoid non-specific interactions with dextran matrix and to achieve a low level of lectin immobilization, the C1 sensorchip (GE Healthcare) was used. Lectins were covalently attached via primary amines of lysine side chains and N-terminus, using EDC/NHS chemistry according manufacturer instructions. Briefly, free carboxylic groups on the chip surface have been modified by 7 min injection of freshly prepared aqueous solution of 0.2 M EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimid hydrochloride) and 0.05 M NHS (N-hydroxysulfoimid succinic acid) (GE Healthcare) at a flow rate 5 μl/min. Stock solutions of lectins [1 μg/ml in 10 mM MES buffer (Sigma) pH 6.1, 150 mM NaCl (Sigma), 1 mM $CaCl_2$, and $MnCl_2$ (Merck)] were 10-fold diluted in 10 mM acetate buffer pH 4.5 (GE Healthcare) supplemented with 1 mM Ca^{2+} and Mn^{2+} ions. Diluted lectins were injected onto the surface of activated biochip at a flow rate of 5 μl/min, contact time was minimalized to achieve maximal response of 500 RU (response units). Free active groups were subsequently blocked by injection of 1 M ethanolamine pH 8.0 (GE Healthcare) for 7 min. The reference biochip flow cell has been modified by the same procedure as analytical flow cell, excepting lectin injection. The glycoprotein–lectin interactions were observed at a flow rate of 50 μl/min in the buffer containing 10 mM HEPES (Sigma) pH 7.4 supplemented with 150 mM NaCl and 0.005% surfactant P-20 (GE Healthcare). Glycoproteins were serially diluted in the range of 22–1800 μg/ml and injected for 4 min. The dissociation of glycoproteins was followed for further 6 min. For the regeneration, two consecutive injections of 0.8 M methyl-α-D-mannopyranose in water (156 s and 120 s) were used for displacement of glycoproteins, followed by an injection of 1 mM Ca^{2+} and Mn^{2+} in MES buffer pH 6.1 (see above) for 60 s to facilitate recovery of the active structure of immobilized lectins. The recorded sensograms were evaluated with the BIA evaluation software 4.1 (Biacore AB).

2.3. K_D calculation

Two different steady state binding models for K_D estimation from SPR steady state response have been used. Model 1:1 (specific binding to one site) used Eq. (1) for fitting the binding isotherm. Model with Hill slope considering multiple specific binding sites fitted binding isotherms in accordance with Eq. (2) and was successfully used for study of multivalent carbohydrate–lectin interaction by ITC [16].

$$RU = RU_{max} \cdot \frac{c}{K_D + c} \quad (1)$$

$$RU = RU_{max} \cdot \frac{c^h}{{K_D}^h + c^h} \quad (2)$$

RU is the differential steady state SPR response in Resonance Unit; RU_{max} is the differential SPR response for maximum specific binding extrapolated to saturation concentration of glycoprotein (ligand); c is glycoprotein concentration; K_D is the glycoprotein concentration needed to achieve a half-maximum binding at equilibrium, if $h = 1$, K_D this is the equilibrium dissociation constant; h is the Hill slope, if $h > 1$ lectin or glycoprotein has multiple binding sites with positive cooperativity, if $h < 1$ there is negative cooperativity.

3. Results and discussion

To evaluate the applicability of SPR method for qualitative and quantitative assessment of the presence of glycoproteins in the sample, we have to optimize both the lectin biochip preparation and measurement conditions and to determine the dissociation constants K_D of lectin–glycoprotein interactions. However, the multivalent character of glycoconjugate–lectin binding influences the study of this interaction. Generally, due to the avidity effect, the value of K_D of carbohydrate–lectin interaction decreases with the increasing number of sugar epitopes on glycoprotein, and, similarly, the variation in surface concentration of lectin onto biochip could lead to changes in the interaction and hence to changes in K_D due to the clustering effect. The binding to surface-based multivalent ligand–receptor interaction can be significantly different from those involving disperse soluble species [17]. In order to minimize the clustering caused by crosslinking of sugar binding sites on neighbor lectin molecules on the chip surface by glycans, the lectin biochips with relatively low density of lectin surface loading were prepared. Used chip (Sensor Chip C1) has a sur-