Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytical Biochemistry 336 (2005) 28–38
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Measurement of the carbohydrate-binding specificity of lectins by a multiplexed bead-based flow cytometric assay

Kazuo Yamamoto[a,b,*], Seiichiro Ito[c], Fumiko Yasukawa[c], Yukiko Konami[a], Naoki Matsumoto[a]

[a] *Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, 277-8562 Chiba, Japan*
[b] *CREST, JST, 332-0012, Saitama, Japan*
[c] *Hitachi Software Engineering Co., Ltd. 230-0045 Yokohama, Japan*

Received 5 May 2004
Available online 11 November 2004

Abstract

Carbohydrate binding underlies many cell recognition events. Here, we describe a multiplexed glyco-bead array method for determining the carbohydrate-binding specificities of plant lectins using a bead-based flow cytometric analysis. *N*-glycans including high mannose, hybrid, and complex types and *O*-glycans from glycoproteins were immobilized on multiplexed beads, and the specificities of 13 kinds of sugar chains were monitored within 2 h in a single reaction. This strategy is easy, rapid, reproducible, and suitable for small samples and allows the reliable and simultaneous elucidation of sugar-binding properties under identical conditions.

Keywords: Lectin; Sugar specificity; Multiplexed analysis; Fluorescent microspheres; Flow cytometry

A number of cellular recognition events are thought to involve the specific binding of a structure expressed on one cell surface to a particular receptor on another cell. Sugar moieties on the cell surface play very important roles in these cellular recognition events [1–3]. Therefore, it is important to understand the molecular bases for the specificity and affinity of these interactions, especially to assess the structures of sugar chains required for interaction with lectins, a class of proteins that bind sugars. For many years lectins have been used to isolate and purify glycoproteins and to determine the location of glycoconjugates in cells. In recent years, several lectins have been found to interact with high affinity with specific glycoprotein determinants. The intracellular functions of carbohydrates attached to proteins have been reported to include folding, quality control, sorting, and transport of glycoproteins within the secretory system, which are affected by glycosylation and subsequent modification by sugar trimming and addition [4], immune recognition and immune surveillance [1], and the regulation of intracellular signal transduction [5] by the addition of O-linked *N*-acetylglucosamine to cytosolic proteins.

The carbohydrate-binding specificity of lectins has been studied by conventional hapten inhibition of hemagglutination using various sugars and sugar derivatives as inhibitors. Other approaches for examining the specificities of lectins have also been described, including equilibrium dialysis [6], affinity chromatography on immobilized lectin columns [7,8], titration calorimetry using a microcalorimeter [9], and current surface plasmon resonance [10]. However, when the ligands of lectins involved in cellular recognition are sugar moieties attached to proteins and/or lipids and when only limited amounts of material are available, it is quite often

* Corresponding author. Fax: +81 4 7136 3619.
E-mail address: yamamoto@k.u-tokyo.ac.jp (K. Yamamoto).

0003-2697/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2004.09.030

extremely difficult to elucidate the specificities of these interactions in detail at the molecular level.

Biomedical research has evolved significantly in recent years, with high-throughput, quantitative screening of whole genomes complementing studies focused on a few genes or proteins [11,12]. Since the addition of carbohydrates generates additional variety among proteins and provides functions beyond those provided by the genetic sequence, it will also be necessary to develop a large-scale analysis of the glycome, with a focus on the detailed interactions between carbohydrates and lectins. Suspension arrays of microspheres analyzed using flow cytometry offer a sensitive, accurate, and reproducible approach to performing a few dozen analyses under the same conditions [13]. This technology employs encoded microspheres as array elements that bear specific ligand or receptor molecules. These arrays have a significant advantage over flat surface arrays, such as those used in gene expression with DNA tips, with regard to preparation and use. It is also easy to select ligands or receptors of interest without an expensive spotter and a high-quality scanner. The current generation of commercially available microsphere arrays includes a 100-element array, which is enough for the analysis of interactions between carbohydrates and lectins.

In the present study, we prepared arrays of microspheres bearing Asn- and Ser/Thr-linked glycopeptides derived from several glycoproteins and successfully demonstrated their binding to plant lectins to identify the wide range of specificity and affinity of these lectins. This method provides a specific, sensitive, simple, reproducible, multiplexed, and rapid assay compared to conventional systems and is likely to become the primary assay platform in the future.

Materials and methods

Lectins

The biotin-labeled lectins; *Ricinus communis* agglutinin (RCA), wheat germ agglutinin (WGA), *Phaseolus vulgaris* leukoagglutinin (L-PHA), *P. vulgaris* erythroagglutinin (E-PHA), *Ulex europaeus* agglutinin (UEA-I), *Dolichos biflorus* agglutinin (DBA), *Lens culinaris* agglutinin (LCA), *Lotus tetragonolobus* agglutininn (LTA), *Glycine max* (soybean) agglutinin (SBA), peanut agglutinin (PNA), *Macckia amurensis* leukoagglutinin (MAL), *Agaricus bisporus* agglutinin I (ABA), *Datura stramonium* agglutinin (DSA), and *Sumbucus sieboldiana* lectin (SSA) were purchased from Seikagaku Kogyo (Tokyo, Japan).

Narcissus pseudonarcissus agglutinin (NPA), *Cytisus scoparius* agglutinin II (CSA), and *Galanthus nivalis* agglutinin (GNA) were purchased from EY Laboratories (San Mateo, CA, USA).

Microspheres

Polystyrene microspheres (beads, 5.5 μm in diameter) with a carboxylated surface and different ratios of red and orange fluorescence were purchased from the Luminex (Austin, TX, USA). Colored beads labeled 151, 155, 157, 160, 163, 169, 176, 177, 189, 191, 192, 193, 195, and 197 were used.

Glycopeptides

Hen egg ovalbumin was obtained from Sigma (St. Louis, MO, USA). Porcine thyroglobulin was purified from porcine thyroid glands as described previously [14]. High-mannose-type glycopeptides derived from porcine thyroglobulin were purified as previously described [15]. Bovine thyroglobulin was purchased from Sigma. Purification of complex-type glycopeptides from bovine thyroglobulin was performed according to the method of Cummings and Kornfeld [16]. Hybrid-type and high-mannose-type glycopeptides from hen egg ovalbumin were obtained according to the methods of Yamashita et al. [17] and Tai et al. [18,19], respectively. Mucin-type glycopeptides from porcine submaxillary mucin and from bovine submaxillary mucin were purified by the method of Sueyoshi et al. [20], and the structures of these glycopeptides were determined as reported previously [21].

Coupling of glycopeptides to microspheres

For the coupling of glycopeptides to microspheres, 2.5×10^6 carboxylated microspheres in 100 μl of 50 mM 2-(*N*-morpholino)-ethanesulfonic acid (Mes) (Dojindo Laboratories, Kumamoto, Japan) were mixed with glycopeptides (50 nmol in 100 μl of 50 mM Mes). Approximately 10^8 coupling sites are present on each microsphere and 100-fold excess of glycopeptides was used in each reaction; 10 μl of freshly made 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; 20 mg/ml in water) (Pierce Biotechnology, Rockford, IL, USA) was added three times with a 30-min incubation in the dark after each addition. Three additional fresh 10-μl aliquots of EDC were added and incubated for 30 min with occasional sonication to keep the microspheres unclamped and in suspension. After the coupling of the glycopeptides, the microspheres were washed with 1 ml of 150 mM Tris–HCl, pH 8.0, containing 0.02% Tween 20, suspended in filter-sterilized 150 mM Tris–HCl, pH 8.0, containing 0.02% Tween 20, and stored in the dark at 4 °C.

To measure the actual glycopeptide content of each microsphere, N-linked high-mannose-type and hybrid-type glycopeptides (glycopeptides 3–11) attached to 1.25×10^6 microspheres were treated with 600 units of endo-β-*N*-acetylglucosaminidase H (endo-H; New