Chemistry & Biology, Vol. 9, April, 2002, PII S1074-5521(02)00133-3

Carbohydrate Analysis Prepares to Enter the "Omics" Era

In this issue, Houseman and Mrksich describe a carbohydrate array preparation method that can be used to analyze protein-carbohydrate interactions and to characterize the substrate specificity of a carbohydrate-modifying enzyme. Carbohydrate chips were prepared by a novel procedure that allows the covalent attachment of carbohydrate-diene conjugates to a specially engineered monolayer surface. The surface presents a precisely controllable ratio of reactive benzoquinone and inert ethylene glycol groups. Nonspecific adsorption of proteins to the surface is extremely low, and the surface is compatible with popular detection techniques. The immobilization technique was demonstrated to be compatible with recently developed automated solid phase carbohydrate synthesis methods, paving the way for the development of highly complex carbohydrate arrays.

Biomolecular microarrays are becoming increasingly valuable research tools for the study of biological processes. DNA microarray technology is by far the most advanced and has been extensively utilized to study gene expression [1] and, more recently, to directly identify transcription factor binding targets [2]. Recently, protein microarrays have been used for the large-scale analysis of protein activities and function [3]. Carbohydrate-containing macromolecules are important for many biological processes, such as cell-cell recognition, adhesion, and host-pathogen interactions. Recent reports have demonstrated the feasibility and utility of carbohydrate arrays. Such reports include studies by Wang et al., who printed 48 carbohydrate-containing molecules onto nitrocellulose-coated glass slides and used them to detect antibodies in human serum with anti-carbohydrate activity [4]. On pages 443–454 of this issue, Houseman and Mrksich describe the construction of carbohydrate arrays that make use of a specially designed immobilization surface. These "carbochips" were used to quantitatively analyze protein-carbohydrate interactions and β-1,4-galactosyltransferase substrate specificity.

Microarrays are constructed from two essential components: the immobilization substrate and the biomolecules that are to be immobilized. Of course, it is critical that the immobilization surfaces be compatible with the samples that are to be arrayed. An optimal substrate would allow the permanent and quantitative immobilization of target molecules in a defined orientation while minimizing nonspecific adsorption. Although many immobilization substrates exhibit some of these qualities, none combine all of them. Additionally, all commonly used immobilization substrates require some form of blocking step after samples have been arrayed, and the efficiency of this step is crucial to the success of the subsequent assay. Blocking efficiency is especially important for minimizing background when high-complexity samples such as serum are applied to the array. Although commonly used blocking reagents such as bovine serum albumin have been used effectively, maximal signal-to-noise ratios can only be achieved through improved blocking protocols.

The ability to control the density and orientation of arrayed molecules may be especially important in the case of carbohydrates, whose binding properties can vary with the orientation and density of carbohydrate groups. With this in mind, Houseman and Mrksich developed a protocol that allows the quantitative covalent attachment of homogeneously oriented carbohydrate molecules to a specially modified glass surface. Gold-coated glass slides were treated with a mixture of two alkanethiols: one modified with an inert ethylene glycol group, the other modified with a hydroquinone group. The hydroquinone groups were oxidized to benzoquinone groups and used to immobilize carbohydrate-diene conjugates through a Diels-Alder reaction. The density of immobilized carbohydrates can be precisely controlled by varying the relative concentration of the reactive hydroquinone-substituted alkanethiol. After arraying the carbohydrates, remaining benzoquinone groups were blocked by addition of an ethylene glycol-diene conjugate. Carbohydrates immobilized in this manner retained binding specificity for several tested lectins and served as effective substrates in a glycosyltransferase assay. Significantly, the background, or "noise," in these assays appears to be very low. Indeed, when quantitative binding tests were performed with the "sticky" protein fibrinogen, nonspecific adsorption to the immobilization substrate was almost nonexistent. Thus, the ethylene glycol-presenting monolayer seems to be very effective at minimizing nonspecific interactions. It will be interesting to see how this immobilization substrate performs when challenged with a more complex sample, such as an extract or serum. Significantly, these authors have also used the same protocol to create peptide arrays [5], suggesting that this immobilization surface may be adaptable for use with a wide range of biomolecules.

In addition to the immobilization surface, the second essential component of a microarray is the molecules to be arrayed. Although valuable as a proof of principle, the carbohydrate arrays that both Wang et al. [4] and Houseman and Mrksich describe contain only small numbers of arrayed molecules. To unlock the full potential of microarray technology, large, high-quality collections of biomolecules must be available. Methods for creating large collections of DNA molecules are well known, and recently, methods have been described that allow the rapid purification of large numbers of proteins [3]. Significantly, Houseman and Mrksich have demonstrated that their immobilization surface is compatible with a recently described automated solid phase oligosaccharide synthesis method [6]. The combination of these two technologies should allow the creation of high quality "carbochips" that present a large number of complex and diverse carbohydrate structures.

Scott Bidlingmaier and Michael Snyder
Molecular, Cellular, and Developmental Biology
Yale University
New Haven, Connecticut 06520-8103

Selected Reading

1. Schena, M., Shalon, D., Davis, R.W., and Brown, P.O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science *270*, 467–470.
2. Iyer, V.R., Horak, C.E., Scafe, C.S., Botstein, D., Snyder, M., and Brown, P.O. (2001). Genomic binding sites of the yeast cell-cycle transcription factors SBF and MBF. Nature *409*, 533–538.
3. Zhu, H., Bilgin, M., Bangham, R., Hall, D., Casamayor, A., Bertone, P., Lan, N., Jansen, R., Bidlingmaier, S., Houfek, T., et al. (2001). Global analysis of protein activities using proteome chips. Science *293*, 2101–2105.
4. Wang, D., Liu, S., Trummer, B.J., Deng, C., and Wang, A. (2002). Carbohydrate microarrays for the recognition of cross-reactive molecular markers of microbes and host cells. Nat. Biotechnol. *20*, 275–281.
5. Houseman, B.T., Huh, J.H., Kron, S.J., and Mrksich, M. (2002). Peptide chips for the quantitative evaluation of protein kinase activity. Nat. Biotechnol. *20*, 270–274.
6. Plante, O.J., Palmacci, E.R., and Seeberger, P.H. (2001). Automated solid-phase synthesis of oligosaccharides. Science *291*, 1523–1527.