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Nanoparticle-Based Sensing of Glycan−Lectin Interactions

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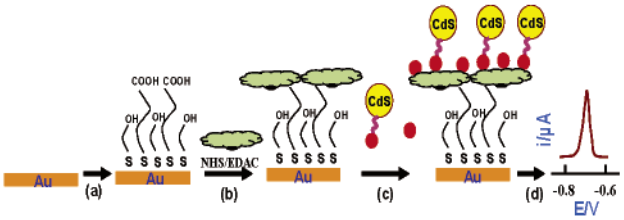
Glycosylation is one of the most crucial post-translational modifications in eukaryotic organisms; every cell is covered with glycan moieties. The glycosylation profile (glycosignature) on the cell surface is dynamic and its alterations are indicative of changes in cellular environment and physiology. Most chronic and immunological diseases and their progression are accompanied by corresponding glycosignature changes of affected cells and circulatory proteins. The alteration in glycosignatures associated with malignant transformation, tumor progression, and metastasis is very well documented.[1] For example, in breast cancer cells, extension of O-linked glycosylation of MUC1 protein is defective and aberrant, resulting in increased expression of truncated O-linked glycans, known as T (β-D-Gal-[1→3]-D-GalNAc-α−Ser/Thr) and Tn (GalNAc-α−Ser/Thr) antigens.[2]

Lectin−sugar interactions play crucial roles in a variety of biological processes, including fertilization, cell migration, cancer, and host−pathogen interactions.[3] The detection of glycans in disease states and related lectin−sugar interactions has thus received considerable attention recently.[4] Existing methods for probing lectin−carbohydrate interactions (reviewed recently by Jelinek and Kolusheva[4]) are tedious, requiring extensive instrumental setup and technical expertise. Accordingly, there are critical needs for developing effective new glycotechnologies and biosensors that are sensitive, rapid, simple, reliable, and cost-effective.

Here we present the first report on nanoparticle-based biosensing of sugars based on their interaction with surface-functionalized lectins. Nanotechnology is opening new horizons for highly sensitive bioaffinity and biocatalytic assays and for novel biosensor protocols that employ electronic, optical, or microgravimetric signal transduction.[5−7] Nanoparticles, such as colloidal gold or inorganic nanocrystals, offer considerable promise as quantitation tags for biological assays owing to their substantial amplification and unique coding capabilities. Biomolecule-nanoparticle hybrid systems have, thus, been used in biosensors for monitoring the biomolecular interactions of nucleic acids and proteins but have not been applied to sugar−lectin interactions. Electrochemical readout offers attractive advantages of miniaturization and low-cost (for meeting the demands of point-of-care diagnostics) and elegant ways for interfacing biorecognition events and signal transduction.[6,8] Given that nanoparticle-based biosensors in the field of glycobiology represent a completely unexplored field, we expect many exciting biomedical opportunities soon.

Our novel bioassay (Scheme 1) involves the immobilization of the lectin, the carbohydrate recognition element, onto the gold surface (in connection to a mixed self-assembly monolayer and EDAC/NHS coupling; a and b), competition between a nanocrystal (CdS)-labeled sugar and the target sugar for the carbohydrate binding sites on lectins (c), and monitoring the extent of competition through highly sensitive electrochemical stripping detection of the captured nanocrystal (d). EDAC/NHS coupling was used also for

Scheme 1. Operation of the Nanoparticle-Based Bioelectronic Sensor for Glycans Involving Competition of the Tagged Sugar with the Target Analytes for the Binding Sites of the Immobilized Lectin[a]



[a] (a) Mixed self-assembled monolayer on the gold substrate; (b) covalent immobilization of the lectin; (c) addition of the tagged and untagged sugars; (d) dissolution of the captured nanocrystals, followed by their stripping-voltammetric detection at a mercury-coated glassy carbon electrode. See Supporting Information for full details.

conjugating the CdS tracer (capped with carboxy-terminal alkylthiol) to the 4-aminophenyl-β-D-galactopyranoside sugar. The utility of CdS inorganic nanocrystal tags for amplified and multiplexed electrochemical bioaffinity assays of proteins[8] or DNA[9] has been demonstrated. Unlike these earlier two-step sandwich bioassays, the present protocol relies on a one-step competitive assay (in connection to a nanocrystal-tagged sugar), which is more suitable for monitoring small sugar molecules and lectin−sugar interactions. The lectin−sugar recognition event thus yields a distinct cadmium stripping voltammetric current peak, whose size decreases upon increasing the level and affinity of the target glycan.

The assay has been optimized and tested using a model system involving a surface-bound pure *Arachis hypogaea* (peanut agglutinin, PNA) lectin and various analytes, including the cancer-associated T antigen (β-D-Gal-[1→3]-D-GalNAc disaccharide). This protocol exhibits excellent discrimination between target and nontarget sugars. Figure 1 displays the voltammetric response for several target sugars with varying affinity to PNA (b−d) and nontarget (e and f) sugars, along with the response without added sugar (a). As expected for a competitive assay, the cadmium stripping peak decreases in the presence of the target monosaccharide *N*-acetyl-D-galactosamine (GalNAc) (b), D-galactose (Gal) (c), and the disaccharide β-D-Gal-[1→3]-D-GalNAc (d), in comparison to the response without the sugar (a), reflecting the smaller fractions of captured nanocrystals. The trend in sensitivity, β-D-Gal-[1→3]-D-GalNAc > Gal > GalNAc, is consistent with the reported relative affinity of these carbohydrate moieties to PNA lectin.[10] In contrast, no change in the response is observed in the presence of a large (25-fold) excess of the nontarget glucose and mannose (e) and (f), respectively, versus (a). Such attractive behavior reflects also the absence of nonspecific adsorption effects and is attributed to the high-density mixed monolayer on the gold surface, including the blocking action of its hydrophilic 6-mercapto-1-hexanol component. Lectin arrays should be useful for distinguishing among individual sugars.

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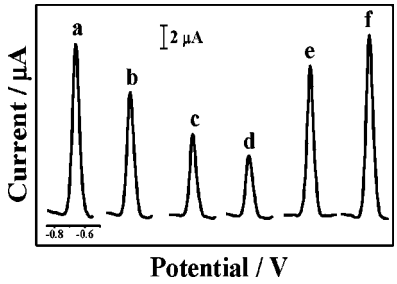

Figure 1. Square-wave voltammetric stripping signals in the presence of (a) "control" solution (no target), (b) 11.1 μM GalNAc, (c) 11.1 μM Gal, (d) 11.1 μM β-D-Gal-[1→3]-D-GalNAc, (e) 277 μM glucose, and (f) 277 μM mannose. Incubation time, 60 min. Dissolution of the QDs (conjugated to the lectin-bound sugar molecules) was carried out by adding 100 μL nitric acid (0.1 M) and incubating for 60 min. The resulting solution was transferred to the electrochemical cell containing 300 μL of acetate buffer (0.1 M, pH 5.3) and 10 ppm Hg^{2+}. Electrochemical stripping detection proceeded using an 8 min deposition at −1.1 V and scanning the potential to −0.2 V using an amplitude of 25 mV, a potential step of 4 and a frequency of 25 Hz. Concentration of the tagged sugar [CdS−(4-aminophenol-β-D-galactopyranoside)], 800 μg L^{-1}.

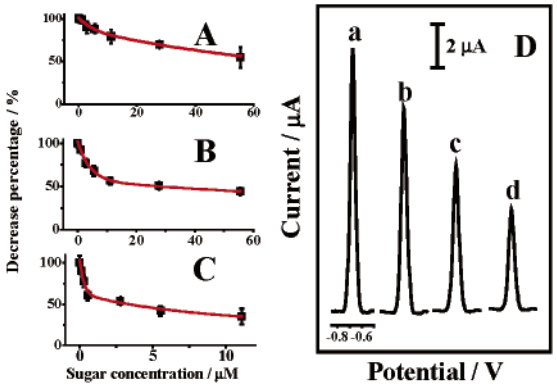

Figure 2. Corresponding calibration plots of (A) GalNAc, (B) Gal, and (C) β-D-Gal-[1→3]-D-GalNAc. (D) Square-wave voltammetric stripping signals in the presence of (a) 0.0, (b) 0.277, (c) 2.77, and (d) 11.1 μM β-D-Gal-[1→3]-D-GalNAc. Other conditions, as in Figure 1.

Figure 2D displays typical voltammograms for different concentrations of the target β-D-Gal-[1→3]-D-GalNAc glycan over a 0.277−11.1 μM range (b−d), along with the "control" response without the target (a). Distinctly smaller cadmium stripping peaks, corresponding to smaller levels of the captured CdS-tagged sugar, are observed in the presence of increasing concentrations of β-D-Gal-[1→3]-D-GalNAc. The corresponding calibration plot (over a wider concentration range of 0.111 to 11.1 μM) is characteristic of competitive assays, with a fast decrease of the peak current up to 1.11 μM and a slower one thereafter (Figure 2C). These data indicate a detection limit of 0.1 μM, which corresponds to 38.3 ng mL^{-1} or to 10 pmole in the 100 μL sample.

An even lower detection limit is expected in connection to a catalytic enlargement of the CdS tracer.[11] Also shown in Figure 2 are calibration curves for GalNAc (A) and Gal (B) recorded in similar competitive assays over the 1.11−55.5 μM range. Both sugars display analytically useful signals, with the Gal peak decreasing faster with concentration compared to GalNAc. Detection limits of 1 μM Gal and 2.7 μM GalNAc, corresponding to 100 and 270 pmoles, respectively, in the 100 μL sample, can thus be estimated, with lower ones are expected in connection to advanced nanoparticle-amplification schemes.[6] The trend in sensitivity is consistent with that observed in Figure 1. The current response at the 11.1 μM level decreases by 20, 45, and 65% for GalNAc, Gal, and β-D-Gal-[1→3]-D-GalNAc, respectively. The reported bioassay offers a good reproducibility, as is evident from the relative standard deviation of 5.7% for a series of six repetitive measurements of 27.7 μM GalNAc (not shown).

The optimal concentration of the CdS-tagged sugar was determined using different concentrations over the 0.2 and 1.5 μg mL^{-1} range. The response increases rapidly with the concentration of the tagged sugar up to 0.8 μg mL^{-1} and levels off thereafter (Figure 1, SI). Another parameter that affects the efficiency and sensitivity of the new glycan sensor is the incubation time (during the competition between the tagged and target sugars). This was evaluated over the 20−120 min range (Figure 2, SI) in the absence (a) and presence (b) of the target sugar. In both cases, the cadmium peak increased in an almost linear fashion up to an incubation time of approximately 80 min and leveled off thereafter.

In conclusion, we have demonstrated for the first time the use of nanoparticle tracers for monitoring lectin−sugar interactions and for bioassays of disease-related glycan markers. The glyconanosensors reported here for electrochemical biosensing of free glycans can be readily expanded for analogous measurements of glycoconjugates and for other nanoparticle-based transductions (e.g., fluorescence) of glycan−lectin interactions. Their clinical utility is currently under investigation in connection to relevant real samples. The coding capability of inorganic nanocrystals[8,9] should facilitate the simultaneous and selective detection of multiple glycan markers in connection with lectin-based carbohydrate arrays. These developments will allow decentralized testing for disease-related sugar markers, glycan profiles, and lectin−sugar interactions to be performed more rapidly, sensitively, inexpensively, and reliably and promise to bring substantial advances in the diagnosis of certain diseases, for example, cancer and immunological disorders. There is no doubt that nanoparticles have considerable promise in glycomics.

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Supporting Information Available: Related instrumentation, reagents, immobilization schemes, optimization, and procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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