Electrochemical detection of *Arachis hypogaea* (peanut) agglutinin binding to monovalent and clustered lactosyl motifs immobilized on a polypyrrole film†

Marie-Pierre Dubois,[ab] Chantal Gondran,[b] Olivier Renaudet,[c] Pascal Dumy,[c] Hugues Driguez,[a] Sébastien Fort*[a] and Serge Cosnier*[b]

Received (in Cambridge, UK) 12th May 2005, Accepted 13th June 2005
First published as an Advance Article on the web 14th July 2005
DOI: 10.1039/b506699a

Direct detection of peanut agglutinin/lactose interactions was realized by an electrochemical approach based on a polypyrrole coated electrode displaying pendant carbohydrates.

Molecular recognition between carbohydrates and proteins displayed on the cell surface of living organisms plays key roles in many biological processes such as leukocyte adhesion to platelets and endothelium, cancer metastasis or microorganism adhesion to host cells.[1] Studying carbohydrate–protein interactions is valuable to understand how cell surface biology is widely mediated by low affinity multivalent associations.[2] It should also benefit the development of therapeutic substances that would mimic or interfere with the recognition processes. In this perspective, the development of carbohydrate microarrays has gained much attention over the last five years.[3] Immobilization of saccharidic structures onto various solid supports (microplates, glass slides, gold surfaces…) furnished efficient tools to investigate the specificity of many carbohydrate-binding proteins. However most of the sensors developed so far rely on the indirect detection of labeled proteins (with a fluorescent tag or a biotin motif). Since high sensitivity in the detection and spatial addressing are required for high throughput diagnostic applications, we turned our attention to carbohydrate immobilization *via* electropolymerized polypyrrole films that may allow direct detection of carbohydrate-protein interactions by electrochemical methods. Electrochemical transduction ensures attractive advantages such as its ease of use in turbid samples, portability, low cost and compatibility with bulk manufacturing procedures.[4] Moreover, among the conventional procedures of surface functionalization with molecular reagents, only the electrochemical polymerization of organic polymers allows the reproducible functionalization of conductive micro-surfaces with a precise spatial resolution. In addition, the quality of the electrogenerated polymer films (absence of defects and chemical stability) constitutes an attractive advantage for the regularity at the molecular level of additional functionalization of the electrode *via* the polymer film. In particular, the combination of electrochemical addressing with high affinity avidin–biotin interactions provided an efficient method for biomolecule immobilization with a high degree of control over the molecular architecture. The latter was based on the electrogeneration of biotinylated films that allow the subsequent attachment of biotinylated molecules (enzymes, antibodies, bacteria) through the formation of avidin bridges.[5–7] Herein we report for the first time the immobilization of biotinylated-carbohydrate structures onto biotinylated polypyrrolic films for the electrochemical detection of lectin binding without a labeling step.

Biotin–lactosyl conjugates **1** and **2** were designed and synthesized (see ESI†) as recognition element of the lectin binding detection system depicted in Fig. 1. Lactosyl moiety was chosen as a simple ligand for the detection of peanut (*Arachis hypogaea*)

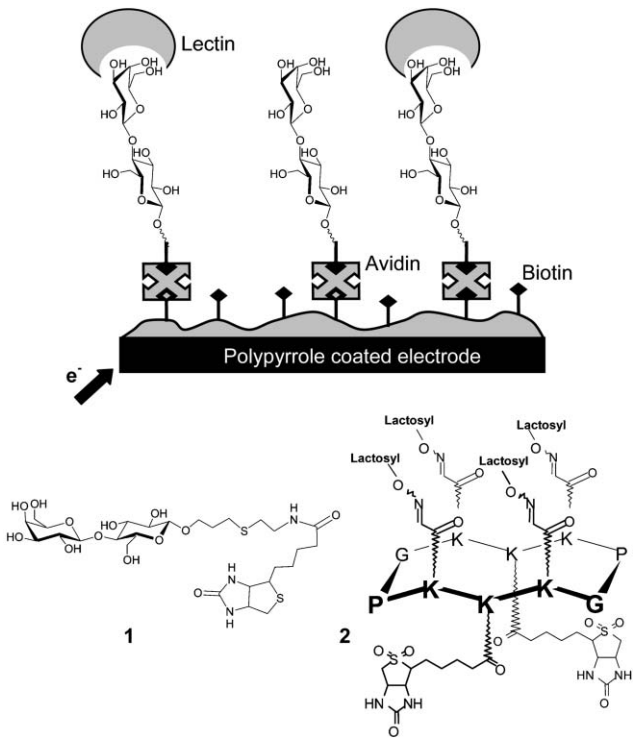

Fig. 1 Schematic representation of the electrochemical PNA lectin detection system with the structures of immobilized lactosyl conjugates.

[a]*Centre de Recherches sur les Macromolécules Végétales (CERMAV), Institut de Chimie Moléculaire de Grenoble FR-CNRS 2607, BP 53, F-38041, Grenoble Cedex 9, France. E-mail: sebastien.fort@cermav.cnrs.fr; serge.cosnier@ujf-grenoble.fr*
[b]*Laboratoire d'Electrochimie Organique et Photochimie Redox (LEOPR), Institut de Chimie Moléculaire de Grenoble FR-CNRS 2607, BP 53, F-38041, Grenoble Cedex 9, France*
[c]*Laboratoire d' Etudes Dynamiques et Structurales de la Sélectivité (LEDSS) Institut de Chimie Moléculaire de Grenoble FR-CNRS 2607, BP 53, F-38041, Grenoble Cedex 9, France*

† Electronic supplementary information (ESI) available: synthesis of **1** and **2**, preparation and characterization of the detection system. See http://dx.doi.org/10.1039/b506699a

agglutinin (PNA). The PNA lectin is a homotetrameric protein with specificity for the tumour associated T-antigenic disaccharide Galβ-1,3-GalNAc and lactose Galβ-1,4-Glc to a lesser extent. Its interaction with lactose has been well documented since the first crystal structure of a complex PNA/lactose[8] in 1982, hence it constitutes a good model for the design of a sensing device that could be used for high throughput screening of new ligands or inhibitors. Comparison of the monovalent and the "Regioselectively Addressable Funtionalized Template" (RAFT)[9]-clustered presentation of the carbohydrate structure on the surface of the electrode were expected to give information about multivalent requirement for a strong lectin adhesion. With the aim of designing biosensing devices for the reagentless detection of lectin, exploitation of a multivalent effect would enhance the binding affinity of the immobilized carbohydrate ligand to protein and hence the biosensor sensitivity. A copolymer film poly-(pyrrole–biotin)[5] and poly(pyrrole–ammonium tetrafluoroborate)[10] was electrogenerated on glassy carbon rotating disk electrodes (see ESI†). The biotin groups allow the anchoring of the carbohydrates by affinity interactions while the ammonium groups confer a hydrophilic character to the resulting film. This improves the copolymer swelling in aqueous solutions and hence its permeability that is a key parameter for electrochemical transductions. The copolymer film was successively coated with an avidin monolayer ($10.5.10^{-12}$ mol cm^{-2}),[11] rinsed with a phosphate buffer solution and incubated with a carbohydrate–biotin conjugate solution (20 μL of a 3 μM solution *i.e.* 6.10^{-11} mol). The construction of the assembly was sequentially analyzed by an electrochemical approach based on permeability measurements[12] with Ru(III)hexamine trichloride[13] as electroactive probe *via* its reduction on the underlying carbon surface at −0.4 V (Fig. 2A and 2B). In the Koutecky–Levich plot representation[12] (Fig. 2C), the intercept, that is inversely proportional to the permeability, increased significantly once the avidin layer was deposited. In contrast, the subsequent anchoring of biotinylated carbohydrates induced a slight permeability increase for both modified electrodes. The steric bulkiness of monovalent and clustered carbohydrate structures indeed is negligible compared to that of the avidin molecule. On the other hand, the highly hydrophilic character of both carbohydrates may lead to a less compact and hence more permeable avidin layer. The lactose functionalized electrodes were further incubated with the *Arachis hypogaea* lectin (PNA) at 10 μg mL^{-1} and washed carefully. As expected, binding of the lectin was indicated on both assemblies by a decrease in Ru complex permeation due to additional steric hindrances brought by the protein anchoring. A lower permeability value ((7.8 ± 0.4)10^{-5} cm s^{-1}) was observed with the RAFT-lactosyl conjugate than with regular lactose ((2.9 ± 0.4)10^{-4} cm s^{-1}). It should be noted that the attachment of biotinylated lactoside **1** and RAFT–lactosyl **2** occurred on a compact avidin layer providing solely one biotinylated derivative per avidin molecule liable to develop protein–ligand interactions.[5] Consequently, the higher amount of immobilized lectin with RAFT–lactosyle **2** than with lactoside **1** reflects a stronger protein ligation due to a possible cooperative multivalent effect of the four lactosyl units displayed by **2**.

The reagentless detection of the recognition event with the PNA lectin was also analyzed by Electrochemical Impedance Spectroscopy (EIS). The binding of the reacting partner is then verified through the detection of either a shift in impedance, in

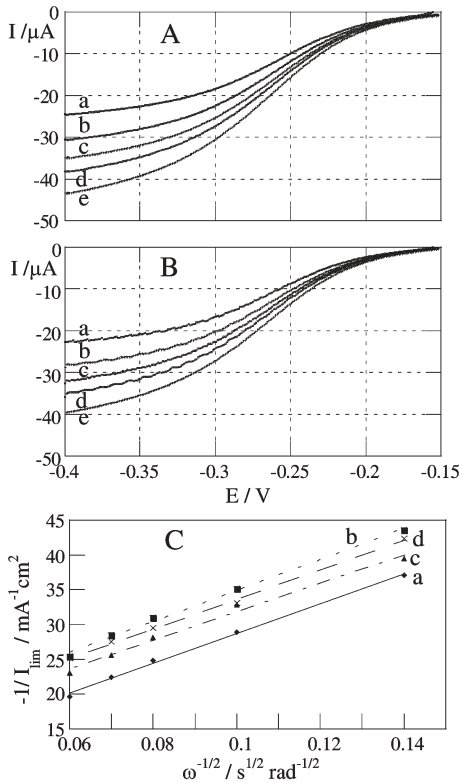

Fig. 2 Rotating-disk electrode voltammograms of assembly after RAFT–lactosyl **2** deposition (A) and after incubation with PNA lectin (B): (a) 500, (b) 1000, (c) 1500, (d) 2000, (e) 3000 r.p.m; Koutecky–Levich plots for each step of construction in presence of 2 mM of Ru(III)hexaamine chloride in phosphate buffer (C). a) copolymer film; b) assembly after avidin layer deposition; c) assembly after RAFT–lactosyl **2** deposition and d) after incubation with PNA lectin.

capacitance or in admittance.[14] Formation of the complex on a conductive surface alters the capacitance and the resistance at the surface electrolyte interface. Our EIS data were obtained at a frequency varying from 5 Hz to 50 kHz (Fig. 3). The Nyquist plots of the impedance exhibits the typical shape of a modified electrode.[15] Two characteristic parts of the spectra can be marked out: a semi-circle located in the range of high frequencies and a nearly vertical capacitive response at low frequencies. The semi-circle corresponds to a parallel $R_{ct}//C_{dl}$ element. The presence of such an element is attributed to a contribution of charge transfer resistance R_{ct} at modified electrode/solution interface and the necessity to charge double layer capacity C_{dl} of this interface. The intercept of the semi-circle with the Re (Z) axis at high frequencies ($\omega \rightarrow \infty$) is equal to the ohmic resistance of the solution and film. As can be seen in Fig. 3A and 3B, the lectin–lactosyl interaction modifies the impedance response in the high frequency domain (50 kHz to 400 Hz). The lectin interaction increases the ohmic resistance ($\omega \rightarrow \infty$): $+110 \pm 2\ \Omega$ for the biotin–lactosyl construction and $+ 190 \pm 8\ \Omega$ for the RAFT construction. The lectin binding increases also the charge transfer resistance: $+1200 \pm 100\ \Omega$ for the biotin-lactosyl construction and $+ 6900 \pm 500\ \Omega$ for the RAFT construction whereas the depletion angle of parallel $R_{ct}//C_{dl}$ element decreases. As previously observed with permeation measurements, it clearly appears that the better fixation of the PNA occurred onto the RAFT–lactosyl

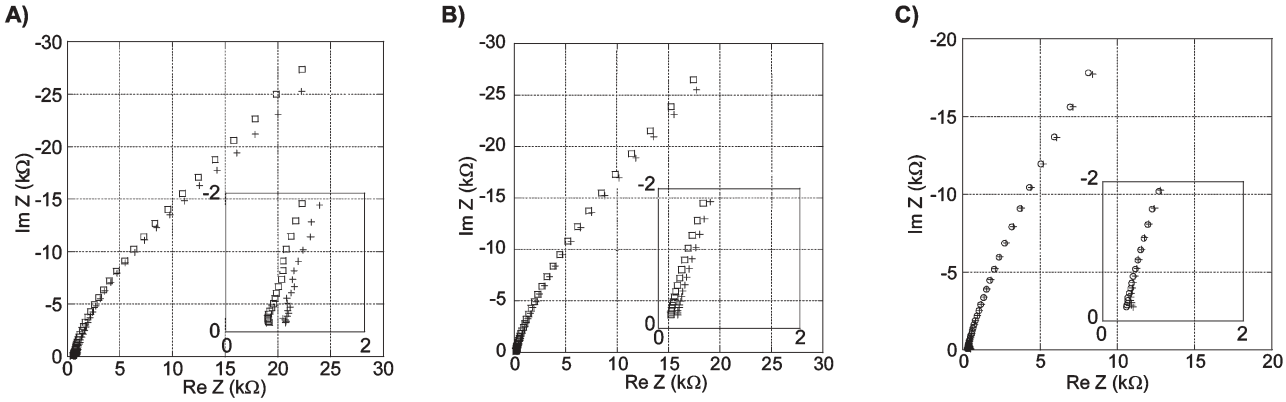

Fig. 3 Impedance spectrum between 50 kHz and 5 Hz, and zoom between 50 kHz and 400 Hz. A) RAFT–lactosyl **2** construction, B) Biotin–lactoside **1** construction, C) Avidin construction. □ Lactose modified electrode, ○ Avidin modified electrode, + After lectin incubation. The working electrodes were glassy carbon rotating disks (diameter 3 mm).

construction. Non specific adsorption of the lectin was excluded since control experiments carried out with omitting lactosyl immobilization step did not reveal any binding either by amperometric or impedance spectroscopy measurements (Fig. 3C).

As a proof of the concept, the affinity interactions of lactosyl-electrodes were investigated with a horseradish peroxidase PNA labeled lectin. The lectin binding was thus evaluated through the amperometric detection of the enzymatically oxidized hydroquinone in the presence of hydrogen peroxide, the system being potentiostated at 0 V.[6] The current intensity for the quinone reduction which is related to the amount of anchored lectin, was found 60 times higher for the RAFT–lactosyl **2** than for lactoside **1** (*e.g.* 6.1 $\pm$ 0.1 μA cm^{-2} and 0.10 $\pm$ 0.03 μA cm^{-2} respectively). This improvement can be regarded at least as a 15 fold increase per lactosyl unit illustrating the better affinity of the tetravalent presentation of the carbohydrate for the PNA lectin. Such effect was previously observed with Concanavalin A, an other tetrameric lectin, when a RAFT–mannose conjugate was used.[9b]

In conclusion, we have demonstrated that polypyrrole conducting films can be efficiently functionalized with carbohydrate structures by means of biotin–avidin bridges for the detection of carbohydrate binding protein. PNA lectin binding to lactose was qualitatively demonstrated with permeation and impedance spectroscopy measurements. In addition amperometric detection was quantitatively achieved with a peroxidase-labeled PNA highlighting the beneficial effect brought by the RAFT clustered presentation of lactosyl residues. This work demonstrates for the first time the use of a full electrochemical approach for the direct detection of carbohydrate–protein interactions without an additional labeling step. It is expected that the combination of such electrochemical approach with the RAFT clustered presentation may open a new way for the design of carbohydrate microarrays.

We thank l'Institut de Chimie Moléculaire de Grenoble for financial support and the MNERT for grant No. 11057-2003 to M-P. D.

Notes and references

1 A. Varki, *Essentials of glycobiology*, (Eds. A. Varki, R. Cummings, J. Esko, H. Freeze, G. Harth and J. Marth), Cold Spring Harbor Laboratory Press, New York, 1999.
2 M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem. Int. Ed.*, 1998, **37**, 2754.
3 (*a*) R. Jelinek and S. Kolusheva, *Chem. Rev.*, 2004, **104**, 5987; (*b*) D. M. Ratner, E. W. Adams, M. D. Disney and P. H. Seeberger, *ChemBioChem*, 2004, **5**, 1375.
4 S. Cosnier, *Anal. Bioanal. Chem.*, 2003, **377**, 3, 507.
5 C. Mousty, J.-L. Bergamasco, R. Wessel, H. Perrot and S. Cosnier, *Anal. Chem.*, 2002, **73**, 2890.
6 R. E. Ionescu, C. Gondran, L. A. Gheber, S. Cosnier and R. S. Marks, *Anal. Chem.*, 2004, **76**, 6808.
7 S. Da Silva, L. Grosjean, N. Ternan, P. Mailley, T. Livache and S. Cosnier, *Bioelectrochemistry*, 2004, **63**, 297.
8 D. M. Salunke, M. Islam Khan, A. Surolia and M. Vijayan, *J. Mol. Biol.*, 1982, **154**, 177.
9 (*a*) P. Dumy, I. M. Eggelston, S. Cervigni, U. Sila, X. Sun and M. Mutter, *Tetrahedron Lett.*, 1995, **36**, 1255; (*b*) O. Renaudet and P. Dumy, *Org. Lett.*, 2003, **5**, 3, 243.
10 L. Coche-Guerente, A. Deronzier, B. Galland, J.-C. Moutet, P. Labbe, G. Reverdy, Y. Chevalier and J. Amhrar, *Langmuir*, 1994, **10**, 602.
11 The avidin content was deduced from a control experiment by estimation of the enzyme activity of the polymeric film after the anchoring of an avidin-conjugated alkaline phosphatase with *p*-nitrophenylphosphate as enzyme substrate. J. C. Say, R. P. M. Furriel, P. Ciancaglini, J. A. Jorge, M. Lourdes, T. M. Polizeli, J. M. Pizauro, H. F. Terenzi and F. A. Leone, *Phytochemistry*, 1996, **41**, 71.
12 (*a*) D. A. Gough and J. K. Leypoldt, *Anal. Chem.*, 1980, **52**, 1126; (*b*) J. M. Saveant, *J. Electroanal. Chem.*, 1991, **302**, 91.
13 E. Höfer, E. Steckhan, B. Ramos and W. R. Heineman, *J. Electroanal. Chem.*, 1996, **402**, 115.
14 (*a*) E. Katz and I. Willner, *Electroanalysis*, 2003, **15**, 913; (*b*) A. Sargent, T. Loi, S. Gal and O. A. Sadik, *J. of Electroanal. Chem.*, 1999, **470**, 144.
15 (*a*) E. G. Tolstopyratova, S. N. Sazonova, V. V. Malev and V. V. Kondratiev, *Electrochim. Acta*, 2005, **50**, 1565; (*b*) M. D. Levi, Y. Gofer and D. Aurbach, *Russ. J. Electrochem.*, 2004, **40**, 310; (*c*) R. P. Buck, *J. Electroanal. Chem.*, 1988, **258**, 1.

