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Discrimination of neutral oligosaccharides through a nanopore

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ABSTRACT

The detection of oligosaccharides at the single-molecule level was investigated using a protein nanopore device. Neutral oligosaccharides of various molecular weights were translocated through a single α-hemolysin nanopore and their nano-transit recorded at the single-molecule level. The translocation of maltose and dextran oligosaccharides featured by 1 → 4 and 1 → 6 glycosidic bonds respectively was studied in an attempt to discriminate oligosaccharides according to their polymerization degree and glycosidic linkages. Oligosaccharides were translocated through a free diffusion regime indicating that they adopted an extended conformation during their translocation in the nanopore. The dwell time increased with molecular mass, suggesting the usefulness of nanopore as a molecular sizing device.

1. Introduction

Among all the biopolymers, carbohydrates offer the largest potential of information owing their incomparable variety of combinations and regioselective modifications of their constitutive monosaccharides [1]. It is now recognized that carbohydrates are involved in many crucial biological processes, mediating biological mechanisms such as growth control, apoptosis, cell differentiation and proliferation, as well as physiopathologic disorders like tumoral metastasis, autoimmune diseases and inflammation [2,3]. However these mechanisms are still poorly understood, mainly because of the high structural diversity and the complex dynamic properties of polysaccharides [4]. Structural analysis of carbohydrates present in living systems is recognized as one of the most challenging task of glycosciences, given the structural complexity related to the monosaccharide composition, the different isomeric forms, the various degree of branching and polymerization, and the difficulty in detection [5,6]. The huge structural diversity of carbohydrates has given rise to the concept of isomer barrier that represents a major hindrance to the structure–activity relationship establishment [7].

New approaches based on single-molecule detection are currently arousing great interest in biology as it allows the direct observation and nanomanipulation of biomolecules [8,9]. Among them, the imaging techniques such as atomic force microscopy and single molecule fluorescence spectroscopy, the force techniques such as AFM-based force spectroscopy and the optical traps and magnetic tweezers experiments [8,10] have been successfully applied to the study of the interaction of DNA and proteins [11,12]. Comparatively few data have been reported about carbohydrates and their interaction studied by these single molecule approaches [13,14], partly because of the difficulties in manipulation of such structurally heterogeneous and polydisperse biopolymers. Most of the studies concerning the analysis of carbohydrates at the single-molecule level were performed by atomic force microscopy and imaging of individual polysaccharide molecules adsorbed on a surface [9,15]. Recently, nanometer-scale protein channels have been introduced as ultrasensitive biosensors allowing the detection and the characterization of various analytes at the single-molecule level [16]. The method is based on the measurement of fluctuations in ionic current passing through the pore under an applied potential. Indeed, a change in ionic current is detected when an analyte is translocated through the pore, leading to a transient current blockade. The extent of the current decrease and its duration are related to structural features of the analytes [17]. The α-hemolysin (α-HL) protein is widely used to prepare such nanometer-sized pore because of its property of self-assembling into a transmembrane heptameric pore of a well-suited diameter (1.5 nm) in lipid bilayer [18]. Besides, the α-HL nanopore remains open at neutral pH and high ionic strength [17]. This approach has been previously demonstrated for the detection of peptides and proteins [19,20], nucleic acids [21] and neutral polymers [22]. As regards nucleic acids, it is worth mentioning that it allowed the discrimination between single-stranded DNA and double-stranded DNA [17], different homopolymeric RNA and DNA [23,24], and

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DNA strands of various length [25], opening up the way for ultra-rapid DNA sequencing [26].

The nanopore detection and characterization represent thus a promising method for deciphering information encoded in linear polymers. Therefore, this method should be of great interest for the characterization of polysaccharides, although it has been not applied to this class of biopolymer to date. In the present work, we report on the nanopore detection of individual oligosaccharide molecules and the capability of this new approach to discriminate oligosaccharides according to their polymerization degree and glycosidic linkages. For that purpose, maltose and dextran oligosaccharides exhibiting $1 \rightarrow 4$ and $1 \rightarrow 6$ glycosidic bonds respectively were translocated through a α-HL nanopore and the characteristics of their nano-transit were recorded.

2. Materials and methods

2.1. Chemicals and reagents

Diphytanoyl phosphatidylcholine-lecithine was purchased from Avanti Polar Lipids. Decane 99% was obtained from SDS (France). α-hemolysin from *Staphylococcus aureus*, maltose and dextran oligosaccharides were from Sigma–Aldrich (St. Louis, MO, USA) All chemicals were of analytical grade. All buffers and solutions were prepared using ultra-pure water (MilliQ, Millipore, Bedford, MA, USA).

2.2. Single α-HL nanopore formation

Membrane lipid bilayer was formed according to a previously described method [27]. Briefly, a film of a 1% of diphytanoyl phosphatidylcholine-lecithine in decane was spread over an aperture 90 μm in diameter size perforated in a polysulfone plate (Warner Instruments, USA). The planar bilayer was formed upon thinning of the decane film at room temperature. The polysulfone plate was then inserted in a device divided in two compartments, cis and trans. Both compartments were filled with 1 mL of 5 mM Hepes, 1 M KCl pH 7.5. The formed lipid membrane was of 60 ± 1 pF capacitance. The formation of unitary protein nanopore was initiated by the addition of 10 μL of 0. 3 μM α-hemolysin prepared in 5 mM Hepes, 1 M KCl pH 7.5 to the cis compartment.

2.3. Detection and recording of the oligosaccharide transit in α-HL nanopore

Both cis and trans compartments were filled with 1 mL of 5 mM Hepes, 1 M KCl pH 7.5. For the nano-transit experiment, 10 μl of 20 mM oligosaccharide in 5 mM Hepes, 1 M KCl pH 7.5 were added to the cis compartment. A 100 mV membrane potential was applied using Ag–AgCl electrodes in contact with the electrolyte within each compartment. The cis and trans compartments were connected to ground and the to the head-stage of a membrane amplifier respectively. The ionic current through a single α-HL nanopore was measured by a BLM 120 patch-clamp amplifier (Bio-logic, France), filtered with a five-pole output filter at 10 kHz, and sampled at 50 kHz by a 16 bit acquisition card (PCI-6014 model, National Instrument, USA). Current traces were analyzed with Igor Pro software (WaveMetrics, USA).

3. Results and discussion

In a typical single nanopore experiment, an ionic current of 99 ± 6.5 pA (V = +100 mV, cathode at the cis side) was recorded in the absence of oligosaccharides in the cis and trans compartments, corresponding to an unoccupied pore. The addition of oligosaccharides in the cis compartment resulted in transient current decreases due to interaction of the sugars with the nanopore. The current blockades were characterized according to the following parameters, duration, frequency and amplitude, which were determined for oligosaccharides of different molecular weights ranging from 504 to 10 300 g mol^{-1} (Fig. 1).

3.1. Dwell time

The durations of the current blockades were distributed in two distinct populations, the short-duration events τ_s of 50–90 μs and the long-duration events τ_l of 130–700 μs (33% of the total). The shorts events that accounted for 67% of the total were attributed to oligosaccharides plugged at the pore entrance and failing to translocate through the α-HL pore [28]. These short events showed almost no dependence over oligosaccharide molecular weight (Fig. 2A). Conversely, the duration of the long events showed a significant increase with the oligosaccharide molecular weight (Fig. 2B), ranging from 130 μs for the trisaccharide maltotriose (MW 504.44 g mol^{-1}) to 730 μs for dextran (Mw 10,300 g mol^{-1}). Being neutral, the oligosaccharides are expected to enter the pore according to a diffusion-controlled motion. Accordingly, the dwell time τ_L can be described by the Fick's law of diffusion $\tau_L = L^2/4D$, where L is the pore length and D the diffusion coefficient of the oligosaccharide. Since D is also related to the oligosaccharide hydrodynamic radius R_h according to the Einstein–Stockes equation: $D = k_B T/(6\pi\eta R_h)$ (k_B the Boltzmann's constant, T the temperature, η the viscosity), then the dwell time is also function of the oligosaccharide hydrodynamic radius. Consequently, as R_h follows a square root law of the molecular weight the dwell time should follow a power law of the molecular weight $\tau_L \approx M_W^{\alpha}$ $\alpha = 0.5$. Indeed, the variation of the long event duration provided an excellent power law fit with the molecular weight, with two slightly different behaviors between oligosaccharides below 2000 g mol^{-1} $\tau_{L(\mu s)} \alpha M_W^{(0.55\pm0.1)}$ and above this molecular weight $\tau_{L(\mu s)} \alpha M_W^{(0.6\pm0.1)}$. Therefore, except for the smallest oligosaccharide maltotriose whose detection is likely biased by the electronic filtering as previously reported [29], these results indicate a molecular weight-dependent diffusive behaviour of the oligosaccharides in the nanopore. The hydrodynamic radius R_h of the tested oligosaccharides was estimated based on previously reported R_h determination [30]. Up to 2000 g mol^{-1}, oligosaccharides have R_h below or near the average radius (1 nm) of the α-HL nanopore. Therefore, being smaller than the pore inner size, these low molecular weight oligosaccharides follow a free diffusive behaviour in the nanopore. Given the power law determined for the oligosaccharides of molecular weight above 2000 g mol^{-1}, *i.e.* larger that the pore size, these heavier oligosaccharides were likewise translocated trough the pore following a free diffusion behavior. However, slight friction inside the nanopore cannot be excluded as suggested by the short deviation of the power law compared to an ideal free diffusion. This result suggested that the nanopore confinement constrained the oligosaccharides to adopt a linear extended conformation during their translocation in the nanopore.

3.2. Event frequency

The event frequency (blockade/s) is linked to the interaction of oligosaccharides with the pore entrance and depends on the concentration of the probed molecules in the cis compartment. Here, the different oligosaccharides were tested at the same concentration (200 μM) in the cis compartment. Nevertheless, a decrease of the average event frequency was observed with the increase of the oligosaccharide molecular weight (Fig. 3). Two frequency regimes could be distinguished, one showing a higher translocation frequency for molecular weight below 1500 g mol^{-1},