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A novel competitive capacitive glucose biosensor based on concanavalin A-labeled nanogold colloids assembled on a polytyramine-modified gold electrode

Mahmoud Labib[a,b], Martin Hedström[a], Magdy Amin[b], Bo Mattiasson[a,*]

[a] Department of Biotechnology, Lund University, Box 124, 221 00, Lund, Sweden
[b] Microbial Biotechnology Center, Faculty of Pharmacy, Cairo University, Kasr El-Aini, Box 115 62, Cairo, Egypt

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ABSTRACT

A highly sensitive competitive capacitive glucose biosensor was constructed based on gold nanoparticles, which were employed as a platform to immobilize concanavalin A (Con A). Gold nanoparticles were fixed on a gold electrode, on which a layer of polytyramine was preformed via electrochemical polymerization. The sensing mechanism is based on the competitive dissociation of a glucose polymer or a glycoconjugate from the glycoligand binding sites of immobilized Con A by the added glucose. To further improve the sensor response, several glucose polymers as well as a synthesized glycoconjugate using the periodate method, were screened. Consequently, dextran (MW 39 kDa) was selected and the feasibility of the proposed biosensor was evaluated for a competitive assay of glucose. Experimental results show that the biosensor responded linearly to glucose in the range from 1.0×10^{-6} to 1.0×10^{-2} M, corresponding to 0.18 μg mL^{-1} to 1.8 mg mL^{-1} of glucose with a detection limit of 1.0×10^{-6} M under optimized conditions. The studied biosensor exhibited a response time of about 15 min and a neglectable loss in sensitivity after 10 repeated analytical cycles.

1. Introduction

Highly sensitive glucose monitoring systems are continuously sought due to their potential applications in conditions where the glucose levels are very low such as in cell cultures and microbial fermentation processes [1,2]. Several biosensors, including turbidity sensors [3], viscometric affinity sensors [4], piezoelectric sensors [5], and surface plasmon resonance based sensors [6], were developed for glucose measurement. However, they are mostly confined to millimolar glucose concentrations, which restrict their applications for monitoring of very low glucose levels. Glucose sensors based on fluorescence spectroscopy have been investigated by many groups due to their high sensitivity and superior specificity [7,8]. However, the sensor performance suffers from problems caused by non-specific changes in fluorescence (photobleaching), which could compromise the sensors accuracy and reliability overtime. Furthermore, the measurements are functions of not only glucose concentration but also fluorophore concentration [9]. Among these methods, the electrochemical approach is more amenable due to its inherent rapidity, simplicity, low cost, and possible miniaturization. Most of the electrochemical glucose biosensors are based on the use of an enzyme such as glucose oxidase [10], glucose dehydrogenase [11], and glucokinase [12]. However, such enzyme sensors are prone to interference from electro-active species; usually suffer from drift, and lacks reproducibility [13]. Apart from enzymes, several ligands have been employed for glucose recognition, including boronic acid derivatives [14], periplasmic binding proteins [15], antibodies [16], and lectins such as human lectins [17] and the plant lectin, concanavalin A (Con A) [18]. However, intense research efforts to develop affinity sensors for glucose have been focused on Con A, since it had been first employed for glucose sensing by Schultz et al. in 1982 [19]. This, due to its inherent stability and activity in the physiological pH range [20]. Based on this system, several analytical techniques were developed for glucose measurement, including optical [21], electrochemical [22], and viscometry based assays [23].

Capacitive biosensors are attracting wide-spread attention in clinical, environmental and biotechnological applications. They are particularly sensitive electrochemical tools, which have been employed extensively for various applications, including antibody-based detection of proteins and polysaccharides [24,25]. Recently, we introduced a new concept, using the capacitive biosensor to quantitatively study low molecular weight analytes in a competitive manner [26]. It is generally accepted that small molecules introduced to the capacitive transducer *per se* are not able to increase the thickness of the dielectric layer in order to induce a

* Corresponding author. Tel.: +46 46 2228264; fax: +46 46 2224713.
E-mail address: Bo.Mattiasson@biotek.lu.se (B. Mattiasson).

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measurable signal. However, if binding to the ligand is followed by a sufficiently large conformational change, the means to directly analyze the low molecular compound arise [27].

It is well recognized that the performance of capacitive biosensors generally depends on how well the biorecognition element is attached to the transducer, which is known as "interfacial design" or "biomolecular immobilization". Consequently, to improve the biosensor performance, suitable immobilization methods must be explored. Electropolymerization, as a promising approach to prepare immobilization matrices for biosensors, has received attention in recent years [28,29]. Unlike the conventional strategies for immobilization, electropolymerization has no limitations in terms of the geometry and area of the electrode, and offers advantages with respect to thickness control and uniformity of the polymer film on electrode surfaces with more complex geometries [30]. The electropolymerization of tyramine has been shown to produce insulating films with reactive amines on solid electrodes [31]. Several advantages of insulating films are that they are considerably thinner than the typical conducting polymer films due to the self-limiting nature of the polymerization reaction and that the substrates can be coated without affecting their electronic properties. In addition, they are perm-selective, which might be useful in preventing interfering species from approaching or contaminating the electrode surface [32]. For these reasons, electropolymerized tyramine has been used as an immobilization matrix for various biomolecules and also for the construction of biosensors [33,34].

In recent years, fabrication of electrochemical sensors and biosensors based on gold nanoparticles has witnessed tremendous growth [35]. It has been demonstrated that gold nanoparticles can strongly adsorb proteins, particularly antibodies, due to their large surface area. Therefore, they represent a potential platform to immobilize biospecies and efficiently retain their biological activity [36–38]. In this work, colloidal gold was assembled onto a polytyramine-modified gold electrode based on the high affinity of the amino group of polytyramine for nanogold and this could result in larger electrode surface area and easier attachment of the biorecognition molecules. Thereafter, Con A was adsorbed onto the nanogold layer and the electrochemical characteristics of the sensor surface were assessed by cyclic voltammetry. Finally, the analytical characteristics of the prepared biosensor were discussed, including the detection range, reproducibility, reusability, and stability.

2. Principles

2.1. Capacitance measurement

Capacitance measurement is based on the theory of the electrical double layer. A metal electrode, modified with the biorecognition element resembles a capacitor in its ability to store charge. The capacitive biosensor system consists of a four-electrode flow-injection manifold cell with a volume of 10 μL. A sputtered gold electrode covered with the biorecognition element serves as the working electrode. A platinum foil and a platinum wire serve as the counter and the pseudo-reference electrode, respectively. An external (Ag/AgCl) reference electrode is placed in the outlet stream to correct for any potential drift. The electrodes are connected to a potentiostat, which further is linked to a personal computer via a fast data acquisition unit (Keithley Instruments, Cleveland, OH, USA). In order to reduce the noise in the system, the potentiostat is driven by the data acquisition unit, which in turn, is powered by the computer through a galvanically insulated power supply. This is to isolate the noisy computer power from the sensitive analogue parts. A schematic drawing of the system setup is provided in Fig. 1. The platinum reference electrode controls the potential applied on the working electrode though it does not have a well-defined potential. However, such electrode is necessary to improve the potentiostatic control of the working electrode, leading to a sharp response in a small flow cell. Therefore, the potentials of platinum and Ag/AgCl reference electrodes are compared prior to pulse application and the computer calculates the potential, which has to be applied to the platinum electrode in order to make it behave as an Ag/AgCl reference. The capacitance is measured by discharge of the chemical capacitor. This can be achieved by applying a potential pulse of +50 mV every minute and recording the current transients following the potential step, using an in-house built computer software. To evaluate the capacitance resulting from

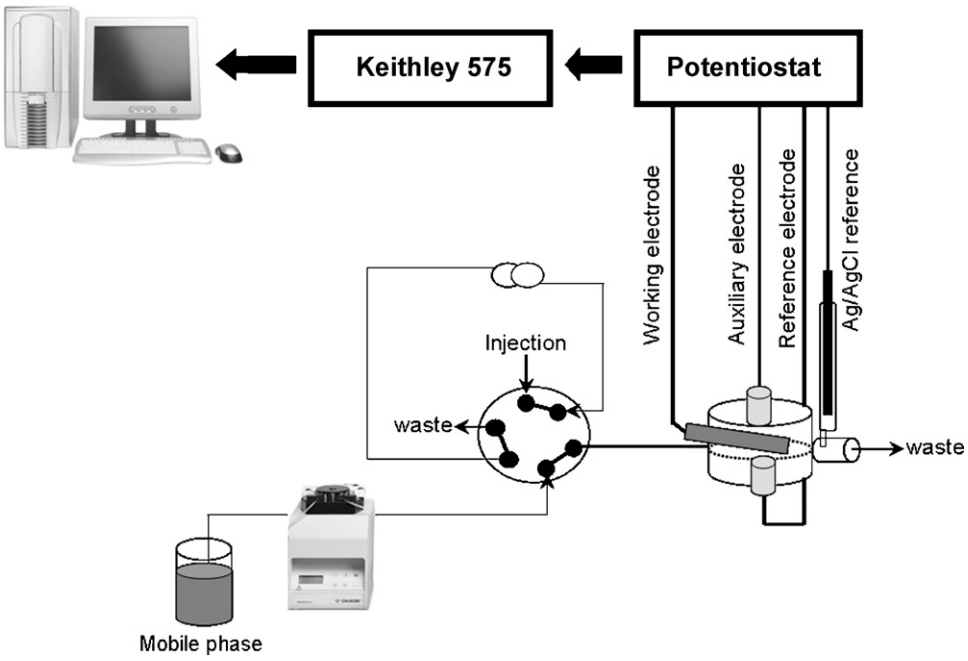

Fig. 1. Schematic diagram of the flow-injection capacitive biosensor setup.

the potential step, a model describing the electrode/solution interface with the equivalent circuits must be applied [39]. The simplest model to describe this interface is a resistor–capacitor in series (RC-model) [40]. According to this model, the current evoked by the potential pulse will follow Eq. (1):

$$i(t) = \frac{u}{R_s} e^{(-t/R_s \cdot C)} \quad (1)$$

where $i(t)$ is the current at time t, u is the applied pulse potential, R_s is the resistance of the recognition layer, t is the time elapsed after the potential pulse was applied, and C is the total capacitance measured at the working electrode/solution interface. The values of R_s and C were determined by the computer software, using the linear relationship between ln(i) and t as shown in Eq. (2) [41].

$$\ln i(t) = \ln\left(\frac{u}{R_s}\right) - \left(\frac{t}{R_s \cdot C}\right) \quad (2)$$

The analytical signal appears when the analyte binds to unoccupied binding sites of the immobilized biorecognition element and consequently increase the thickness of the electrode sensing layer. The displacement of the electric double layer created during the potentiostatic pulse will cause a decrease in capacitance, which is strictly proportional to the analyte concentration.

2.2. Principle of glucose assay

The assay principle is based on the competition between a small glucose molecule and a large glucose polymer or a glycoconjugate for binding to an immobilized Con A with weak affinity to both competitors. Binding of a glucose polymer or a glycoconjugate to Con A immobilized on the electrode surface results in a capacitance decrease. Upon introduction of glucose, which competitively will occupy the glycoligand binding sites of Con A, displacement of the bound glucose polymer or glycoconjugate occurs, followed by restoration of the measured capacitance. This shift-back in capacitance will hence be proportional to the applied glucose concentration. The electrode can be regenerated with the glucose polymer or the glycoconjugate solution since the nature of competitive binding between glucose polymer- or glycoconjugate-Con A and glucose is well known to afford reversible glucose sensing [42]. As earlier mentioned, the direct binding between glucose and Con A in a non-competitive assay fashion can not produce any change in capacitance. This is due to the small size of glucose which is not sufficient to increase the thickness of the dielectric layer above the electrode surface. In addition, binding of glucose to Con A does not induce any conformational changes in the tertiary structure of Con A.

3. Experimental

3.1. Materials

Con A from *Canavalia ensiformis*, β-cyclodextrin, β-cyclodextrin polymer, dextran (average molecular weight: 10, 39, 71 and 298 kDa, respectively), and glycogen type II from oyster, were all from Sigma–Aldrich Chemie (Steinheim, Germany). D-Glucose, gold(III) chloride hydrate, and tyramine were purchased from Fluka, Sigma–Aldrich Gmbh, Germany. 1-Dodecanethiol was obtained from Aldrich (Deisenhofen, Germany). Bovine serum albumin (BSA) was purchased from ICN Biomedicals Inc (Malmö, Sweden). Sputtered gold electrodes were a generous gift from the Biophysics research unit, Prince of Songkla University, Thailand. All other chemicals used were of analytical grade. All buffers were prepared with water treated with a Milli-Q system from Millipore (Bedford, MA, USA).

3.2. Procedures

3.2.1. Electropolymerization and preparation of the glucose biosensor

Electropolymerization and cyclic voltammetry (CV) were performed at ambient temperature in a conventional three-electrode electrochemical cell using a potentiostat/galvanostat, Autolab Model PGSTAT 12 (Utrecht, The Netherlands), controlled with GPES 4.8 software. The electrodes were a sputtered gold transducer, a platinum wire counter electrode and Ag/AgCl (saturated KCl) reference electrode. All potentials were registered versus the reference electrode. Before electrochemical polymerization, the gold electrode was rinsed with ethanol and deionized water in order to remove organic and inorganic contaminants prior to drying with a stream of high-purity nitrogen. Electropolymerization of tyramine was performed from a solution of 50 mM tyramine in a degassed 1:3 mixture of ethanol/sodium phosphate buffer saline (PBS, 2.0 mM phosphate buffer, pH 7.0 + 100 mM NaCl) by cycling the potential between 0 and 0.8 V at a scan rate of 0.05 V s^{-1}, as described elsewhere [43]. The mixed solvent was a necessity due to the poor solubility of tyramine in water. The obtained electrode was thoroughly washed with deionized water to remove the physically adsorbed tyramine, and then dried with nitrogen. Thereafter, the electrodes were immersed in gold colloid, prepared according to the method described by Wang et al., at 4 °C overnight [44]. Finally, the electrodes were washed thoroughly with deionized water and then incubated with 0.75 mg mL^{-1} Con A in 5.0 mM Tris–HCl buffer containing 5.0 mM of $CaCl_2$, $MgCl_2$, and $MnCl_2$ (pH 7.4) at 4 °C overnight. The same buffer was further employed as a mobile phase for capacitance measurements. Prior to use, any defects in the prepared layer were filled by immersing the electrodes in a solution of 10 mM 1-dodecanethiol in ethanol for 20 min. The electrochemical characteristics of the modified electrodes were studied by CV after each step during the fabrication process. Electrochemical experiments were performed using the same cell in a solution of 100 mM $K_3[FeCN_6]$ in 100 mM KCl by sweeping the potential between −0.3 and 0.8 V at a sweep rate of 0.1 V s^{-1}.

3.2.2. Capacitive monitoring of glucose

The Con A-modified gold electrode was placed as a working electrode in the four-electrode flow-injection manifold cell (see Fig. 1). The same cell was previously employed in a capacitive biosensor system for detection of cholera toxin [45] and staphylococcal enterotoxin B [46], with slight modifications. The mobile phase, filtered through a 0.22 μm filter and degassed before use, was pumped with a peristaltic pump at a flow rate of 50 μL min^{-1}. All samples were injected via a 250-μL loop. Prior to loading the sample glucose solution, the glucose polymer or the glycoconjugate was injected into the flow cell and a stable base line was achieved after 20 min. Subsequently, the glucose sample was loaded to the flow cell and the capacitance was monitored for 15 min. A constant flow rate of 50 μL min^{-1} was maintained during either the glucose polymer or the glycoconjugate injection as well as the glucose injection. Finally, the data were collected and manually processed.

4. Results and discussion

4.1. Electrochemical characterization of the fabricated biosensor

Proper insulation of the electrode surface is an essential feature in capacitive biosensors. The degree of electrode insulation can be probed with CV using an electro-active species in the contacting aqueous solution. The reason is that the electron transfer between this species and the electrode must occur either by tunneling through the layer covering the electrode surface or by approaching the electrode at a defect “pinhole” in this layer [47].

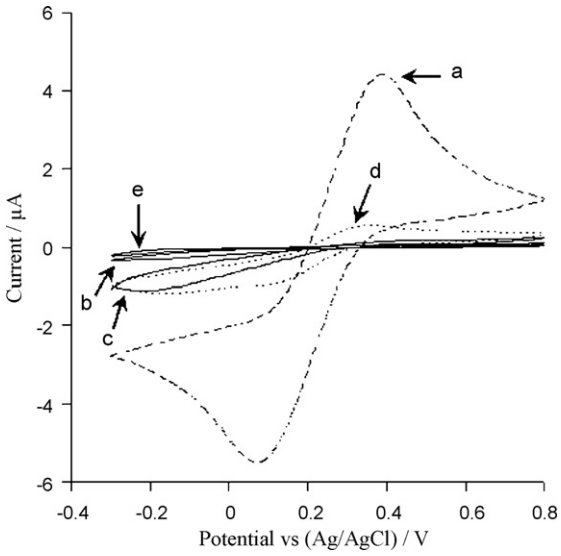

Fig. 2. Cyclic voltammograms recorded in a solution of 100 mM KCl containing 100 mM $K_3[Fe(CN)_6]$ using: (a) bare gold electrode; (b) gold electrode coated with a layer of electropolymerized tyramine; (c) the same as (b) but after nanogold treatment; (d) as in (c) with subsequent immobilization of Con A; (e) as in (d) after treatment with 1-dodecanethiol. The scan range was from −0.3 to +0.8 V (vs. Ag/AgCl), at a scan rate of 0.1 V s^{-1}.

The electrochemical characteristics of the modified electrode were investigated by CV using the redox component $K_3[Fe(CN)_6]$ at different stages of the fabrication process, as shown in Fig. 2. A cyclic voltammogram for the bare gold electrode demonstrated clear redox peaks (*curve a*). The successful formation of a polytyramine film on the electrode surface seems to effectively prevent the penetration of the conducting ions. This was evidenced by a significant reduction in the voltammetric response of the electrode, as shown in *curve b*. As can be seen in *curve c*, the interfacial resistance decreased again after the gold nanoparticles were assembled on the polytyramine layer, which implies that they can act as tiny conduction centers to facilitate the electron transfer at the electrode surface [48–50]. The mechanism of coupling between the polytyramine film and gold nanoparticles remains as unsolved matter. However, one opinion is that it mainly occurs by an electrostatic attraction between the protonated amino groups of polytyramine and the citrate anions bound to the surface of the gold nanoparticles during preparation [51]. Another opinion postulates strong covalent bonds between gold and amines [52,53]. As can be seen in *curve d*, the immobilization of Con A on the modified electrode surface caused a slight increase in the peak current, indicating that the Con A molecules are not closely packed on the surface [54,55]. It is noteworthy to mention that the sensing interface provided by the gold nanoparticles, which possess large surface area and good biocompatibility, can allow for a large amount of Con A to be loaded. Furthermore, the three-dimensional structure of the particles can allow a free-orientation of the immobilized molecules to retain their active configuration. Therefore, it is reasonable to assume that the Con A immobilized on the nanogold layer could maintain its high biological activity [56]. As mentioned above, the electrode surface should be completely insulated. For this reason, a long chain 1-dodecanethiol was employed to fill the defects in the formed layers. Under this treatment, the current peak disappeared completely (*curve e*), which indicates a highly capacitive (blocked) interface.

4.2. Capacitive measurement of glucose

The total capacitance was continuously monitored at the working electrode/solution interface using the capacitive biosensor

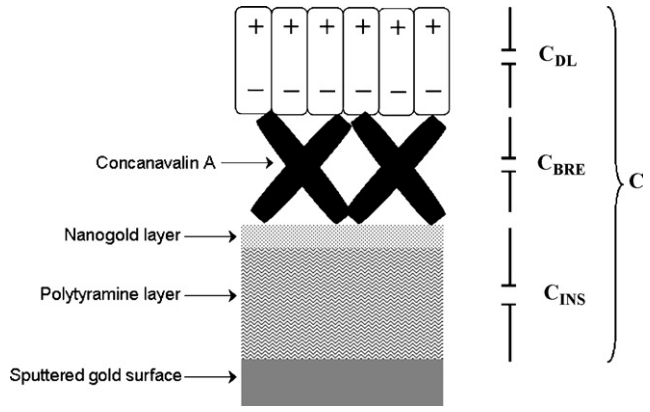

Fig. 3. Schematic drawing of the different layers covering the electrode surface which contributes to the registered total capacitance.

software. The total capacitance (C) described by Eq. (3) is composed of a series of three capacitances, namely the capacitance of the insulating layer (C_{INS}), which comprises the capacitance of the polytyramine layer and the assembled nanogold layer. The second capacitance involves the capacitance of the biorecognition element (C_{BRE}), represented by the immobilized Con A. The capacitance of the electrical double layer (C_{DL}) represents the third capacitance in this series. Such capacitance is caused by the solvated ions of the liquid forming a space-charge, as schematically depicted in Fig. 3.

$$\frac{1}{C} = \frac{1}{C_{INS}} + \frac{1}{C_{BRE}} + \frac{1}{C_{DL}} \quad (3)$$

The smallest capacitance in this series dominates the value of the total capacitance. For this reason, it is necessary to make each capacitance as large as possible so that the change caused by the recognition dominates. In other words, a highly sensitive capacitive biosensor could be obtained by reducing the thickness of the insulating film covering the electrode surface as well as thickness of the biorecognition element. The former can be achieved by using the non-conducting polymer film employed in this study whereas; the latter is achieved via optimizing the concentration of the biorecognition component immobilized on the insulating film. As shown in Fig. 4a, the binding of the glucose polymer (dextran) to the immobilized Con A molecules was accompanied by a consequent decrease in capacitance. However, upon introduction of glucose, it occupied competitively the glycoligand binding sites of Con A with a partial shift-back in capacitance (Fig. 4b). The average value of the capacitance change (ΔC) can be calculated from the following formula: $\Delta C = C_2 - C_1$, where C_1 and C_2 represent the average of five consecutive values before and after the glucose injection, respectively. The necessity to evaluate an average of five capacitance values was previously proved mathematically [57] and the last five cycles of capacitance related responses were evaluated as a reliable analytical signal, as described elsewhere [58]. The response time was about 15 min. Such relatively long response time makes it suitable only for offline analyses. This process has been shown to be reversible, meaning that the system was successfully regenerated by loading the glucose polymer solution after each glucose injection and the capacitance was again reduced. The metal salts concentration in the buffer (5.0 mM of $CaCl_2$, $MgCl_2$, and $MnCl_2$) was selected after an optimization study where the developed biosensor exhibited the largest response at this concentration. Further increase in the metal salts concentration brought about a significant increase in the capacitance baseline accompanied by signal instability, which interfered with the accurate determination of capacitance changes (*data not shown*). One possible reason for this is that the increase in the electrolyte concentration causes the diffuse ion cloud outside the protein layer to be compressed resulting in a parallel increase

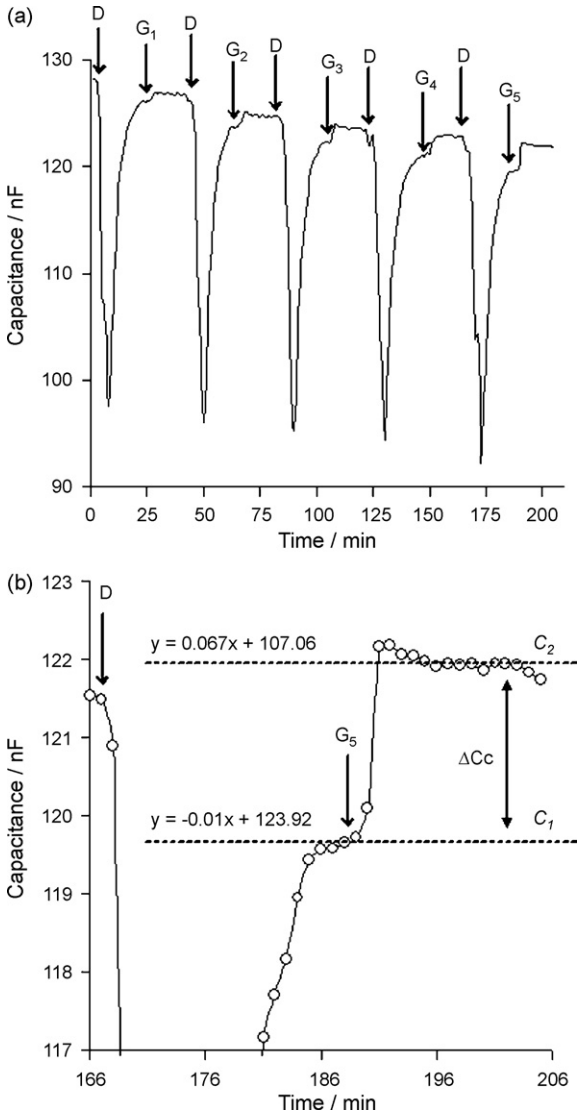

Fig. 4. Time course of capacitance changes upon loading the glucose polymer followed by its dissociation by the injected glucose. (a) Capacitive measurements of several glucose concentrations, including 1.0×10^{-6} M (G_1), 1.0×10^{-5} M (G_2), 1.0×10^{-4} M (G_3), 1.0×10^{-3} M (G_4), 1.0×10^{-2} M (G_5), whereas the biosensor was subsequently regenerated with 1.0×10^{-3} M of the 39 kDa dextran (Dx) after each glucose injection. (b) Magnification of the capacitance response obtained after loading 1.0×10^{-3} M dextran (39 kDa) followed by an injection of 1.0×10^{-2} M glucose. $\Delta C(C_2 - C_1)$ represents the capacitance change due to the dissociation of the glucose polymer by the added glucose.

in capacitance [55]. Noteworthy is that the direct binding between glucose and the bare Con A in a non-competitive mode did not produce any changes in capacitance and hence, it is necessary to measure the glucose in a competitive mode.

4.3. Comparison between macromolecular glycoconjugates

It was previously demonstrated that the molecular mass of the displaced glucose polymer or glycoconjugate as well as its affinity to Con A, can cause a significant effect on the glucose sensing range and the signal detection sensitivity [59]. In this context, one synthesized glycoconjugate, namely 5% BSA-grafted dextran and several glucose polymers, including 1.0×10^{-4} M dextran (298 and 71 kDa), 1.0×10^{-3} M dextran (39 and 10 kDa), β-cyclodextrin, glycogen, and β-cyclodextrin polymer, were employed individually to saturate the biosensor surface until a constant capacitance baseline

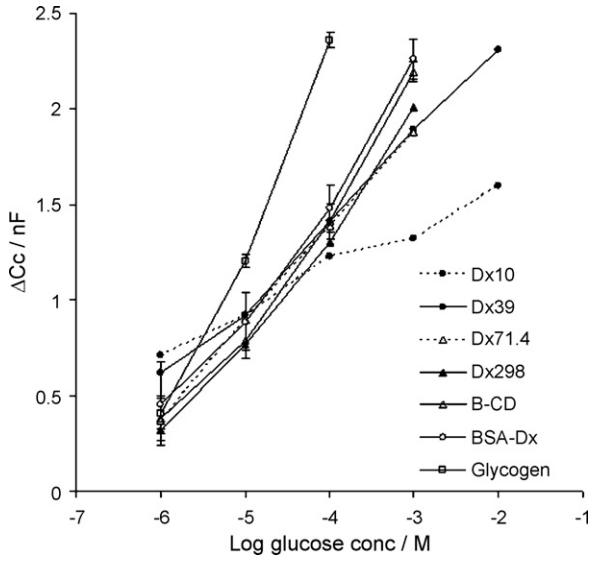

Fig. 5. Glucose calibration curves by CCBT using one glycoconjugate, namely 5% BSA-grafted dextran and six glucose polymers, including 1.0×10^{-4} M dextran 298 (Dx298), 71 (Dx71) and 1.0×10^{-3} M dextran 39 (Dx39), 10 (Dx10), 5% β-cyclodextrin polymer, and 5% glycogen. Symbols denote mean values of triplicate measurements of the capacitance change. The capacitance value is represented by mean values and standard deviations of all measurements.

was accomplished. Consequently, a series of glucose concentrations ranging from 1.0×10^{-8} to 1.0 M, were injected and ΔC for each concentration was calculated, where ΔC is the capacitance increase due to the dissociation of the glucose polymer or the glycoconjugate from the Con A surface by the tested glucose solution. The biosensor surface was subsequently regenerated with either the glucose polymer or the glycoconjugate, previously employed to saturate the biosensor surface, after each glucose injection. A calibration curve was plotted and the glucose concentrations, showing a linear agreement with the registered signals, were determined.

As shown in Fig. 5, using the 39 kDa dextran was accompanied by a broad glucose sensing range extending from 1.0×10^{-6} to 1.0×10^{-2} M, corresponding to 0.18 μg mL^{-1} to 1.8 mg mL^{-1} of glucose with the regression equation of $y = 0.43x + 3.16$ ($R^2 = 0.985$), where y is the change in capacitance in nanofarad and x is the logarithm of glucose concentration in moles. The detection limit was 1.0×10^{-6} M, corresponding to 0.18 μg mL^{-1}, at a signal-to-noise ratio of 3. The relative standard deviation (RSD) values were between 1.5% and 5.7% at the tested glucose concentrations. When the molecular weight of dextran was increased (71 and 298 kDa), a narrower sensing range was typically observed. The same feature was exhibited when β-cyclodextrin polymer and a BSA-dextran conjugate, prepared by the periodate method, were utilized because the equilibrium constant of the interaction was higher [60]. Interestingly, a concomitant narrowing of the dynamic range was associated with dextran (10 kDa); presumably due to its small size which was not sufficient to produce major changes in capacitance upon its dissociation from the Con A surface. For the same reason, β-cyclodextrin monomer (1.1 kDa) could not produce any changes in capacitance upon binding to Con A. It was also noticed that using glycogen, instead of dextran, brought about a significant narrowing in the glucose sensing range. The reason is that the glycogen mainly consists of α-1,4 glycosidic linkages whereas, α-1,6 linkages are predominant in dextran, even though they are similarly composed of D-glucose units. Hence, it is likely that glycogen binds to Con A more strongly than dextran does [61]. Therefore, it is reasonable to assume that the dissociation of glycogen from the Con A surface will be more difficult than the dextran counterpart and logically, this will lead to a narrower dynamic range.

4.4. Repeatability, reproducibility, stability and selectivity of the glucose biosensor

The measurement repeatability of the glucose biosensor was studied at a glucose concentration of 10 mM and an RSD value of 7.5% was obtained for 10 successive measurements with the same sensor. The system was subsequently regenerated with 1.0×10^{-3} M dextran (39 kDa) after each glucose injection. The fabrication reproducibility was estimated from the response of four different electrodes, prepared under the same conditions, to a concentration of 10 mM glucose and the RSD was calculated to be 7.9%. The stability of the biosensor was assessed by repeatedly measuring the capacitive response to 10 mM glucose. When not in use, the electrode was stored in 5.0 mM Tris–HCl buffer, pH 7.4 at 4 °C. The biosensor maintained about 70% of the initial response after 1 day of storage then there was a steady decline in response within the first week of storage. It should be stressed that the selectivity of the biosensor is controlled by the selectivity of the immobilized ligand. Con A, employed in this study, has different binding affinities for some small sugar molecules [62]. Some small sugars, including D-fructose, D-mannose, D-maltose, methyl-α-D-glucopyranoside, and methyl-α-D-mannopyranoside were able to partially displace the glucose polymer or the glycoconjugate bound to Con A, thus can interfere with glucose detection (*data not shown*). However, in most cases this will not cause problems when glucose solely exists in the medium or when the measurement is performed under physiological conditions. The same sensing mechanism, using Con A, was frequently employed by many researchers in different analytical techniques, including fluorescence resonance energy transfer (FRET) based assay [63], piezoelectric sensors [64], viscometric affinity sensors [4], and intraocular lens sensors [65]. However, this was the first time to apply this sensing mechanism in capacitive biosensors.

5. Conclusions

The concept illustrated in this study is based on employing a weak binder as biorecognition element immobilized on the gold electrode surface followed by charging it with a polymer comprising of several units of the analyte of interest. Upon loading the tested analyte, it competitively displaces the polymer allowing a sensitive interrogation of the target analyte (glucose). Combining the ability of the aforementioned technique to detect small molecules together with the inherent sensitivity of capacitive biosensor, this strategy offers a promising tool for monitoring of small biomolecules. It also offers the possibility to tailor the dynamic assay range by modifying and/or changing the polymer. Meaning that, a high sensitivity assay can be obtained by utilizing a polymer with low affinity to the biorecognition element and hence, it can be easily displaced with a low concentration of the target analyte, and the converse is true. Future work will be devoted to expand the technique described here into relevant analytical challenges using the competition between a hapten and a macromolecular structure conjugated with several hapten molecules for binding to an immobilized bioelement. Further results will be released in due course.

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