Anal Bioanal Chem (2010) 397:1217–1224
DOI 10.1007/s00216-010-3641-8

ORIGINAL PAPER

Competitive capacitive biosensing technique (CCBT): A novel technique for monitoring low molecular mass analytes using glucose assay as a model study

Mahmoud Labib · Martin Hedström · Magdy Amin · Bo Mattiasson

Received: 8 December 2009 / Revised: 4 March 2010 / Accepted: 7 March 2010 / Published online: 18 April 2010

Abstract A novel technique for monitoring of low molecular mass analytes using a flow-injection capacitive biosensor is presented. The method is based on the ability of a small molecular mass analyte to displace a large analyte–carrier conjugate from the binding sites of an immobilized biorecognition element with weak affinity to both compounds. A model study was performed on glucose as the small molecular mass analyte. In the absence of glucose, binding of a glucose polymer or a glycoconjugate to concanavalin A results in a capacitance decrease. Upon introduction of glucose, it displaces a part of the bound glucose polymer or glycoconjugate leading to a partial restoration of capacitance. Experimental results show that the change in capacitance depends linearly on glucose concentration within the range from 1.0×10^{-5} to 1.0×10^{-1} M, corresponding to 1.8 μg ml^{-1} to 18 mg ml^{-1} in a logarithmic plot, with a detection limit of 1.0×10^{-6} (0.18 μg ml^{-1}) under optimized conditions. In addition, by modifying the molecular mass of the glucose polymer, amount of biorecognition element, and buffer composition, we were able to tune the analyte-sensing range. The developed technique has the benefits of expanded dynamic range, high sensitivity, and excellent reusability.

Keywords Biosensor · Competitive capacitive · Concanavalin A · Flow-injection · Glucose · Glycoconjugate

M. Labib · M. Hedström · B. Mattiasson (✉)
Department of Biotechnology, Lund University,
Box 124, 221 00 Lund, Sweden
e-mail: Bo.Mattiasson@biotek.lu.se

M. Labib · M. Amin
Microbial Biotechnology Center, Faculty of Pharmacy,
Cairo University,
Kasr El-Aini, Box 115 62, Cairo 11451, Egypt

Introduction

Glucose is the major carbon and energy source in cellular metabolism. Its deficiency or overproduction could have a deleterious effect on cellular functions. Therefore, 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 adopted by many groups due to their high sensitivity and superior specificity [7, 8]. However, the sensor performance suffers from nonspecific changes in fluorescence which could compromise the sensor's 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 electroactive 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 focused on Con A, since it had been first employed for glucose sensing by Schultz et al. in 1982 [19], 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 have attracted wide-spread attention in clinical, environmental, and biotechnological applications. They are based on the theory of the electrical double layer which exists at the surface of a metal electrode in contact with an electrolyte solution. Binding of an analyte to a biorecognition element immobilized on the electrode surface increases the thickness of the dielectric layer above the electrode surface and hence, causes a parallel decrease in capacitance that is proportional to the concentration of the target analyte. Unfortunately, capacitive biosensing is restricted to analytes capable of increasing the thickness of dielectric layer either directly by virtue of their large sizes, such as proteins [24, 25], glycoproteins [26], polysaccharides [27, 28], and nucleic acids [29], or indirectly by inducing a conformational change in the biorecognition element upon binding to it, such as heavy metals [30]. On the other hand, low molecular mass analytes lacking the previous properties cannot be detected by capacitive biosensors. This paper demonstrates a new technique to enable capacitive biosensors to measure low molecular mass analytes, using glucose as a model. In brief, the sensing mechanism is based on the ability of glucose to displace a glucose polymer or glycoconjugate from the glycoligand binding sites of Con A. To demonstrate proof-of-concept of the developed technique, factors affecting the sensor response were elucidated, including the size of the glucose polymer, amount of immobilized Con A, and buffer composition.

Materials and methods

Chemicals

Concanavalin A from *Canavalia ensiformis*, dextran (average molecular weight: 10, 39, 71, 298, 500 kDa), D-fructose, D-maltose, methyl α-D-glucopyranoside (Me-α-Glu), methyl α-D-mannopyranoside (Me-α-Man), α-lipoic acid, and acetonitrile 99.8%, were all from Sigma-Aldrich Chemie (Steinheim, Germany). D-glucose and *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride (EDC), were purchased from Fluka, Sigma-Aldrich Gmbh, Germany. 1-Dodecanethiol was obtained from Aldrich (Deisenhofen, Germany). Immunoglobulin G (IgG) was the product of Pharmacia (Uppsala, Sweden). All buffers were prepared with water treated with a Milli-Q system, preceded by a reverse osmosis step, both from Millipore (Bedford, MA, USA). All dextran solutions were dialyzed against distilled water (molecular weight cut off, 8 kDa).

Concept of competitive capacitive biosensing technique

The technique is based on the competition between a small molecular mass analyte and a large analyte–carrier conjugate for binding to an immobilized biorecognition element with weak affinity to both competitors. Binding of a large glucose polymer such as dextran or a glycoconjugate like IgG to the biorecognition element (Con A) immobilized on the electrode surface results in a capacitance decrease. This is reversed by introduction of the low molecular mass analyte (glucose) which will displace either the large dextran or IgG at the glycoligand binding sites of Con A. Consequently, the glucose concentration can be interrogated by measuring the shift-back in capacitance. The electrode can be regenerated with the glucose polymer or glycoconjugate since the nature of binding between glucose polymer- or glycoconjugate-Con A and glucose is well known to afford reversible glucose sensing [31]. The mechanism of analysis is schematically depicted in Fig. 1. As earlier mentioned, the direct binding between glucose and Con A cannot 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. It is noteworthy to mention that we have successfully employed this technique in a reversed manner to measure IgG and detect its aggregation, using the same analytical platform with immobilized Con A [26]. The decrease in capacitance caused by injecting a glyconjugate (IgG) was used to determine its concentration. The electrode surface was successfully regenerated with concentrated glucose solution. However, in the present study, the increase in capacitance caused by injecting glucose is employed to determine its concentration. Conversely, the electrode surface is regenerated with either a glucose polymer or a glycoconjugate solution.

Preparation of the Con A-modified gold electrode

The sensing platform is based on self-assembled monolayers (SAMs) of α-lipoic acid with the carboxylic group covalently coupled to Con A. Prior to SAM deposition, the gold electrode (Ø 3 mm, 99.99% purity) was sequentially polished using alumina suspension with particle diameters of 0.1 and 0.05 μm for 3 and 2 min, respectively. Next, the electrode was thoroughly washed with distilled water to

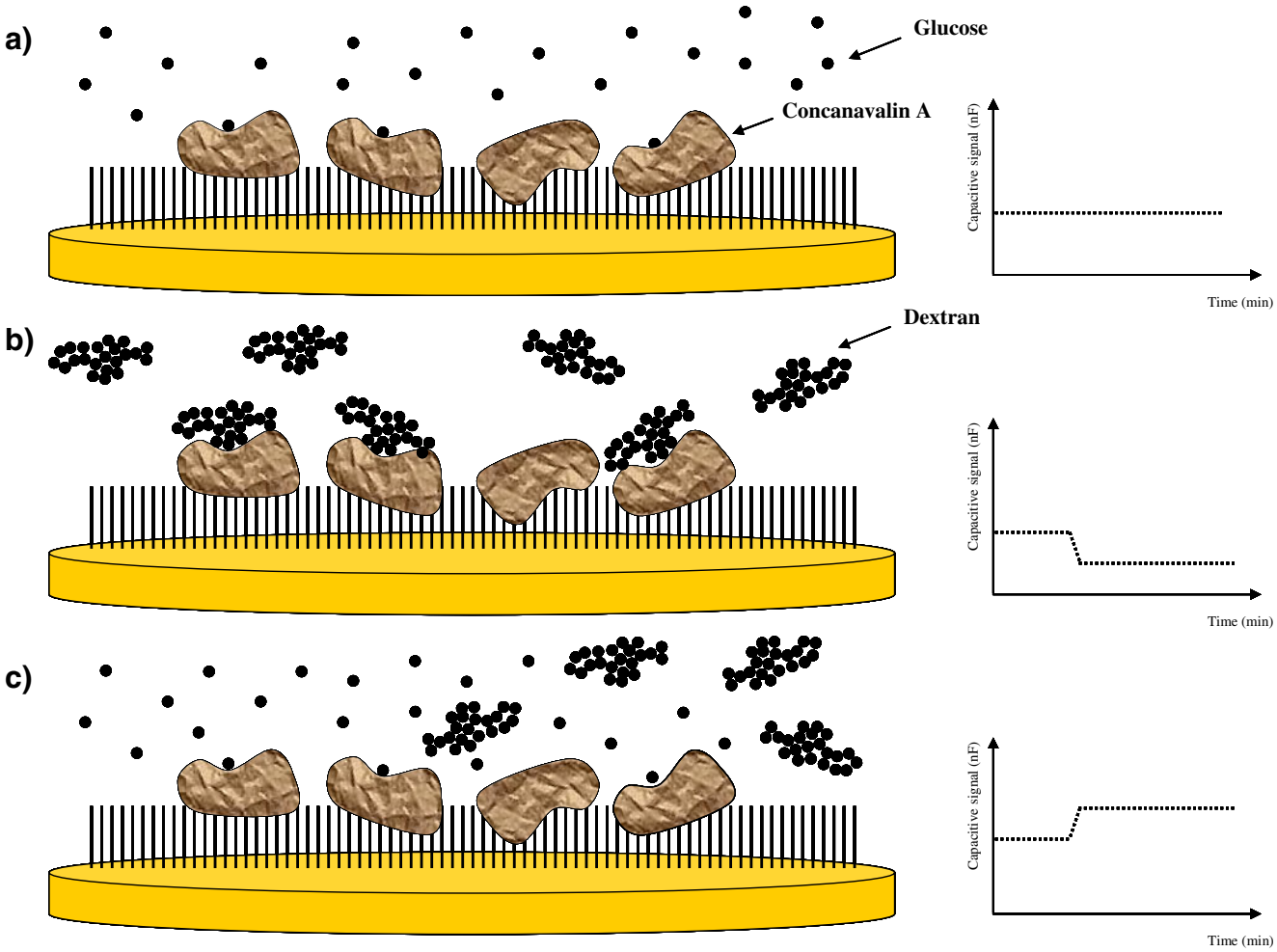

Fig. 1 Schematic representation of the mechanism of CCBT for glucose assay. In **a** glucose is infused to the transducer with surface bound concanavalin A. The binding will not result in a capacitive change (*graph on the right*) due to the small size of the target molecule. In **b**, the glucose polymer dextran is infused and binding to Con A results in a registered decreased capacitance. **c** Shows the displacement of dextran by glucose where the capacitance is restored to the original baseline level

remove the alumina. Consequently, the electrode surface was checked for any alumina remnants using an optical microscope. Thereafter, the electrode was placed in a Teflon holder and plasma-cleaned for 15 min (Model PDC-3XG, Harrich, NY). The modified electrode was prepared according to a four-step procedure, starting with (1) construction of SAM by immersing the electrode in a solution of 1% α lipoic acid in absolute ethanol overnight. This was followed by (2) activation of the carboxylic acid group by immersing the electrode in a solution of 1% EDC in dry acetonitrile for 5 h then drying with high-purity nitrogen. (3) Protein coupling step was achieved by incubating the electrode with 40 μL of 1.0 mg mL^{-1} Con A in 5.0 mM Tris–HCl buffer containing 1.0 mM of $CaCl_2$, $MgCl_2$, and $MnCl_2$, pH 7.4 overnight at 4 °C. The same buffer was further employed as a mobile phase for capacitance measurements. (4) Finally, a surface blocking step was performed, using 10 mM 1-dodecanethiol in ethanol, to assure a complete electrochemical insulation of the gold electrode.

Capacitance detection system

The capacitive biosensor system consists of a four-electrode flow-injection manifold cell with a volume of 10 μL. The Con-A-modified gold electrode serves as the working electrode. A platinum foil and a platinum wire serve as the auxiliary and the pseudo-reference electrode, respectively. An external reference (Ag/AgCl) electrode was placed in the outlet stream to correct for the potential drift of the platinum reference. The electrodes are connected to a potentiostat, which is further 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 diagrammatic presentation of the system is provided elsewhere [26]. The platinum pseudo-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 mobile phase was pumped with a peristaltic pump (M-312 Gilson, France) at a flow rate of 50 μL min^{-1}. All injections were performed via a 250-μL loop. Prior to loading the glucose sample, the glucose polymer or 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. The principle of detection involves capacitance measurements made by applying a potential pulse of +50 mV to the working electrode at every minute during the binding event between Con A and either the glucose polymer or glycoconjugate as well as glucose. Consequently, the current decay evoked by the potential step was sampled with a frequency of 50 kHz. The total capacitance at the working electrode/solution interface was continuously monitored using an in-house built computer software.

Results and discussion

Capacitance measurements

The Con-A-modified gold electrode was placed as a working electrode in the four electrodes flow-injection manifold cell. To evaluate the capacitance resulting from the potential step, a model describing the electrode/solution interface with the equivalent circuits must be applied. The simplest model to describe this interface is a resistor–capacitor in series (RC-model). According to this model, the current evoked by the potential pulse will follow Eq. 1:

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

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 [32].

$$\ln i(t) = \ln(u/R_s) - (t/R_s \times C) \quad (2)$$

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.

As shown in Fig. 2a,b, binding of dextran to Con A brought about a consequent decrease in capacitance, ΔCd. The average value of ΔCd can be determined from the following formula: $\Delta Cd = C_0 - C_1$, where C_0 and C_1 represent the average of five consecutive baseline values before and after loading the dextran, respectively. However, upon injection of glucose, it displaces dextran from the glycoligands binding sites of Con A with a consequent increase in capacitance ΔCc. The average value of ΔCc can be calculated as follows: $\Delta Cc = C_2 - C_1$, where C_1 and C_2 represent the average of five consecutive baseline values before and after the glucose injection, respectively. The necessity to evaluate an average of five capacitance values was previously proved by calculations [33], and the last five cycles of capacitance related responses were evaluated as a reliable analytical signal, as described elsewhere [34]. It is worth noticing that the binding between glucose and the bare Con A surface could not produce any change in capacitance, and hence it is necessary to measure glucose using a displacement assay.

Tuning of sensing range and sensor response

To demonstrate proof-of-concept of the competitive capacitive biosensing technique (CCBT) and optimize the sensor performance, we investigated the effects of the glucose polymer size and Con A and metal salts concentrations on the sensor response.

Effect of glucose polymer size

It was hypothesized that the displaced glucose polymer or glycoconjugate, specifically, its size and binding affinity to Con A, can cause a significant effect on the glucose sensing range and the signal detection sensitivity [35, 36]. In this study, the glycoligand binding sites of Con A were saturated with 1.0×10^{-4}M of dextran (500, 298, 71 kDa), and 1.0×10^{-3}M of dextran (39, 10 kDa) and IgG, each at a time. Saturation was evidenced by the lack of capacitance change upon further glycoconjugate injection. A series of glucose concentrations, ranging from 1.0×10^{-8} to 1.0 M,

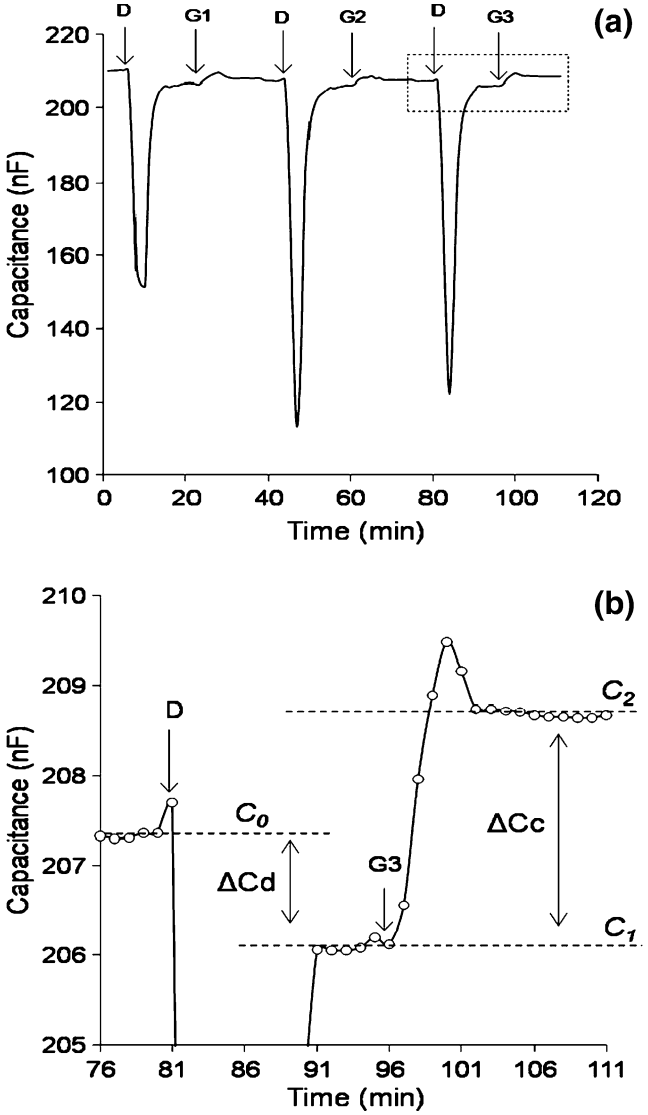

Fig. 2 **a**,**b** Time course of capacitance changes obtained upon loading the glucose polymer followed by its dissociation by the injected sugar. **a** Capacitive measurements of several glucose concentrations, including 1×10^{-3}M (*G1*), 1×10^{-2}M (*G2*), and 1×10^{-1}M (*G3*), whereas the biosensor was subsequently regenerated with 1.0×10^{-3}M of the 39 kDa dextran (*D*) after each glucose injection. **b** Magnification of the capacitance response obtained after loading 1×10^{-3}M of the 39 kDa dextran followed by an injection of 1×10^{-1}M glucose. ΔCd is the decrease in capacitance caused by binding of the glucose polymer to Con A (C_0-C_1) and ΔCc is the increase in capacitance due to the glucose polymer dissociation (C_2-C_1)

were injected and ΔCc for each aliquot was calculated, where ΔCc is the capacitance increase due to the dissociation of the glycoconjugate by the injected glucose. The biosensor was subsequently regenerated with an injection of the tested glucose polymer or glycoconjugate after each glucose injection. As shown in Fig. 3, the largest change in capacitance in response to glucose (ΔCc) was observed with the 39 kDa dextran. When the molecular weight of dextran was increased, smaller changes in capacitance were typically observed. This is because the equilibrium constant of the interaction was higher and requires higher glucose concentrations to displace the dextran bound to the glycoligand binding sites of Con A [37, 38]. Furthermore, the glucose sensing range itself was reduced when the 500 kDa dextran was used. We also noticed that using IgG, instead of dextran, resulted in a smaller change in capacitance in comparison to those obtained with dextran. Again, this could be ascribed to the high molecular weight of IgG (150 kDa), compared to the 39 kDa dextran. However, this observation clearly illustrates that the signal obtained is not solely dependent on the molecular mass of the displaced molecule, but also depends on other factors such as charge density, mode of interaction, binding strength, etc. [39]. On the other hand, a concomitant narrowing of the sensing range was associated with dextran (10 kDa), presumably due to its small size which was not sufficient to produce major changes in capacitance upon dissociation from the surface of Con A.

Effect of Con A and metal salts concentrations

A preliminary determination of the linear range concentrations of glucose was performed and four glucose concentrations (1.0×10^{-4}–1.0×10^{-1}M) were utilized in a one-factor-at-a-time optimization approach. As shown in

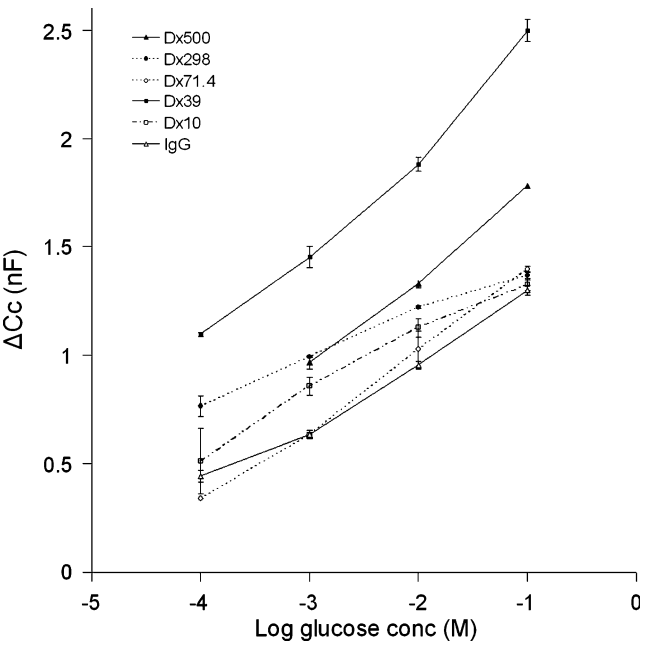

Fig. 3 Glucose calibration curves by CCBT using five glucose polymers, including 1×10^{-4}M dextran 500 kDa (*Dx500*), 298 kDa (*Dx298*), 71 kDa (*Dx71*), and 1×10^{-3}M dextran 39 kDa (*Dx39*), 10 kDa (*Dx10*), and one glycoconjugate, namely 1×10^{-3}M IgG. *Symbols* denote mean values of triplicate measurements of capacitance. The capacitance is represented by mean values and standard deviations of all measurements

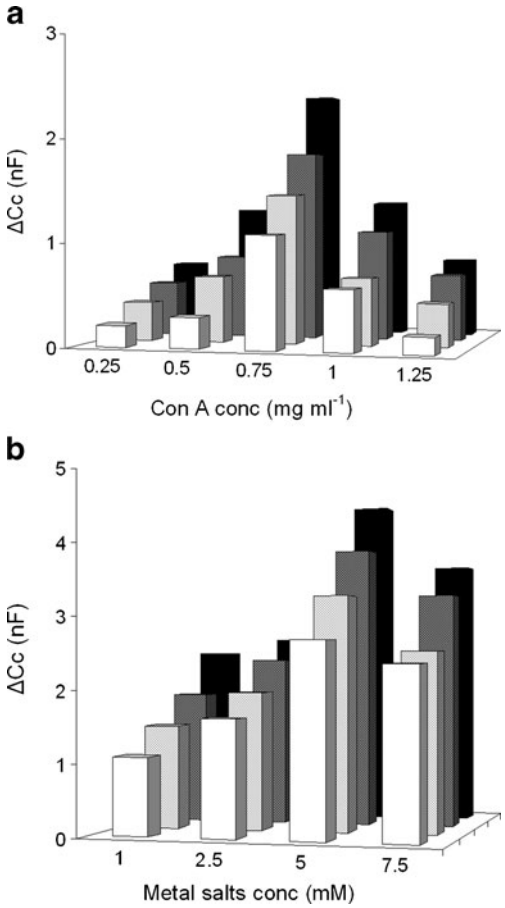

Fig. 4 **a**,**b** Modulation of sensor response by Con A and metal salts concentrations, at four glucose concentrations, including 10^{-4}M (□), 10^{-3}M (▒), 10^{-2}M (▓), and 10^{-1}M (■). **a** Effect of Con A concentration on the sensitivity of biosensor, using 5 mM Tris–HCl buffer containing 1 mM of the metal salts ($CaCl_2$, $MgCl_2$, and $MnCl_2$), pH 7.4 at a flow rate of 50 µL min^{-1}. **b** Effect of metal salts concentration, using 0.75 mg mL^{-1} Con A and 5.0 mM Tris–HCl buffer (pH 7.4), containing different molar concentrations of the metal salts at a flow rate of 50 µL min^{-1}

Fig. 4a, the sensor response increased with increasing the Con A concentration up to 0.75 mg $mL,^{-1}$ which is equivalent to 37.5 µg of Con A on the electrode surface. Further increase in the Con A concentration was accompanied by a reduced sensor response, presumably due to increasing the electrode insulation as a result of increasing the thickness of the protein layer immobilized on the electrode surface [40]. The effect of the metal salts concentration on the sensor response was elucidated, as shown in Fig. 4b. The largest sensor response was observed with a metal salt concentration of 5 mM, probably due to enhancing the Con A–carbohydrate interaction. Further increase in the concentration was accompanied by an increase in capacitance and instability of the base line, which impair capacitance measurements. This could be attributed to the fact that solutions of high ionic strengths may produce pinholes on the electrode surface due to the removal of the insulating SAM layer [28]. Furthermore, increasing the electrolyte concentration causes a compression of the diffuse ion cloud above the protein layer, resulting in an increased capacitance [41].

Post-optimization glucose sensor response

A validation experiment was performed to assess the sensor performance and to verify the optimum conditions for the factors investigated in this study. We studied the sensor response toward various glucose concentrations ranging from 1.0×10^{-8} to 1.0 M in 5 mM Tris–HCl buffer containing 1 mM of $CaCl_2$, $MgCl_2$, and $MnCl_2$, pH 7.4. The biosensor was subsequently regenerated with 10^{-3}M dextran (39 kDa) after each glucose injection. It was observed that the detection of glucose is linear from 1.0×10^{-5} to 1.0×10^{-1}M, corresponding to 1.8 µg mL^{-1} to 18 mg mL^{-1}, with the regression equation of $y=0.694x+5.428$ ($R^2=0.997$), 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 (R.S.D.) values were between 1.5 and 3.7% at the tested glucose concentrations. The reversibility and response time of the sensor were investigated using the same experimental setup. Reversibility of the sensor response was demonstrated by prompt return to baseline after introduction of 1.0×10^{-3}M dextran (39 kDa) subsequent to challenge with 1.0×10^{-5}–1.0×10^{-1}M glucose. The response time was about 15 min. Such relatively long response time makes it suitable only for offline analyses. After optimization, the sensor exhibited an average of 150.5%, 126.8%, 115.7%, and 89.2% increase in response at glucose concentrations of 1.0×10^{-4}, 1.0×10^{-3}, 1.0×10^{-2}, 1.0×10^{-1}M, respectively.

Potential applications of CCBT

Capacitive monitoring of the molecular weight of dextran

The effect of the molecular weight of dextran on the change in capacitance obtained upon dextran–Con A binding was investigated. Dextran polymers with different molecular weights (71, 298, 500 kDa), and 1:1 combination of 71 and 298 kDa, with a number-average molecular weight (Mn) of 185 kDa, and a similar combination of 298 and 500 Da, with Mn of 399 kDa, were tested. Consequently, the capacitance change (ΔCd) after each sample injection was registered, where ΔCd is the capacitance decrease due to the binding between Con A and dextran. The system was subsequently regenerated with 1.0 M glucose after each sample injection. As shown in Fig. 5, it was observed that increasing the molecular weight of dextran, within the

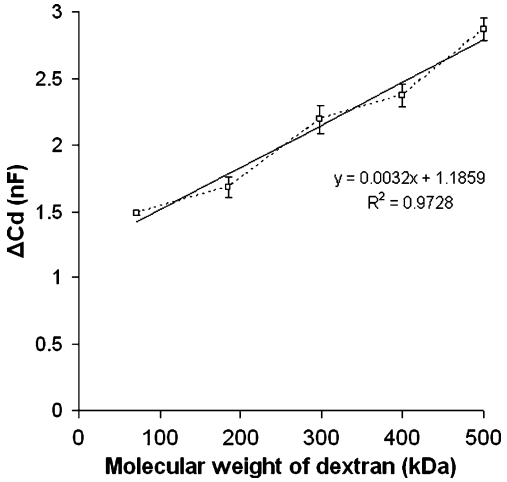

Fig. 5 Linear plot of capacitance change as a function of the molecular weight of dextran using CCBT. Reference points: 1×10^{-4}M of dextran (71, 185, 298, 399, 500 kDa). *Symbols* denote mean values of triplicate measurements of capacitance. ΔCd is represented by mean values and standard deviations of all measurements

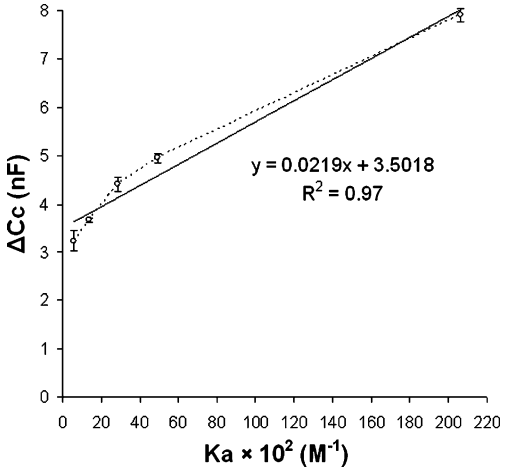

Fig. 6 Linear plot of capacitance changes versus the affinity constant (Ka) of several sugars to Con A. Reference sugars are: 1.0×10^{-3}M of glucose ($Ka=5.9\times10^{2}M^{-1}$), fructose ($Ka=13.7\times10^{2}M^{-1}$), maltose ($Ka=28.8\times10^{2}M^{-1}$), Me-α-Glu ($Ka=49.4\times10^{2}M^{-1}$), and Me-α-Man ($Ka=206.0\times10^{2}M^{-1}$). *Symbols* denote mean values of triplicate measurements of capacitance. ΔCc is represented by mean values and standard deviations of all measurements

tested range, was accompanied by a proportional increase in the ΔCd value. The linear regression equation was ΔCd (nF)=0.0032×molecular weight of dextran (kDa)+1.1859, with a correlation coefficient of 0.973. The R.S.D. values were 0.9, 4.5, 4.6, 3.6, and 3.0% for triplicate measurements of dextran molecular weights of 71.4, 185, 298, 399, and 500 kDa, respectively. The response time was about 20 min. The results ensure that the newly developed technique can be utilized for the estimation of the molecular mass of glycoconjugates, providing simplicity and high sensitivity.

Capacitive monitoring of carbohydrates binding affinity to Con A

In the carbohydrates–Con A study, several small sugars (glucose, fructose, maltose, Me-α-Glu, and Me-α-Man) were allowed to displace dextran at the glycoligand binding sites of Con A in a dynamic situation and the course of interaction was quantitatively monitored. The glycoligand binding sites of Con A were saturated with a solution of 1.0×10^{-3}M dextran (39 kDa) in the optimized mobile phase. After establishing a stable baseline, a solution of 1.0×10^{-3}M of the tested sugar was subsequently injected and ΔCc for each sugar was registered. The biosensor was subsequently regenerated with the dextran solution after each sugar injection. As shown in Fig. 6, it was observed that there is a linear agreement between the ability of a certain sugar to displace dextran at the Con A surface, represented by its ΔCc value, and its binding affinity to Con A (Ka). The linear regression equation was $y=0.022x+3.502$ ($R^2=0.97$), where y is ΔCc value in nanofarad and x is $Ka\times10^{2}$ (M^{-1}). The R.S.D. values were between 1.2 and 6.5% for the tested sugars. The response time was about 15 min. The ΔCc values were found to obey the following order: Me-α-Man>Me-α-Glu>maltose>fructose>glucose. The association constants of these carbohydrates to Con A were previously determined using equilibrium dialysis in 0.025 M cacodylate buffer containing 1 M NaCl, pH 6.2 at 2 °C. A good agreement was achieved between our results and the reported Ka values for these sugars: 206×10^{2}, 49×10^{2}, 29×10^{2}, 14×10^{2}, and $5.9\times10^{2}M^{-1}$, respectively [42]. The substantial increase in capacitance obtained with Me-α-Man and Me-α-Glu could be ascribed to the high binding affinity of methylated sugars to Con A compared to the non-methylated counterparts [43]. Our results strongly suggest the potential of CCBT for monitoring carbohydrates–Con A interactions with simplicity and high sensitivity.

Conclusions

This work represents the first trial to develop a capacitive biosensor for monitoring of a low molecular mass analyte in a competitive fashion. The technique employed in this study is based on employing a weak binder as a biorecognition element immobilized on the electrode surface followed by charging it with a polymer comprising several units of the analyte of interest. Upon loading the tested analyte, it displaces the polymer allowing a sensitive interrogation of the tested analyte. Combining the ability of this technique to detect small molecules together with the inherent sensitivity and broad detection range of capacitive biosensors offers a promising tool for monitoring of small biomolecules. This technique also offers the possibility to tailor the dynamic sensing range by modifying and/or

changing the polymer. In other words, 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. The possibility of tailoring biorecognition elements with controlled affinity to their target analytes employing combinatorial chemistry [44], computational chemistry [45, 46], molecular imprinting [47], rational design [48], or combinations of one or more of these will undoubtedly open new avenues for the CCBT. The versatility of this technique lies in the fact that it can be utilized in a reversible manner. For instance, it can be utilized for estimating the molecular mass of glucose polymers, whereas the system can be regenerated by a concentrated glucose solution. On the other hand, the technique can be employed for glucose analysis and monitoring of the binding affinities between different oligosaccharides and Con A. In this case, the system can be regenerated by a concentrated glucose polymer solution. This is acceptable since the binding between either the glucose polymer- or glycoconjugate-Con A and a small sugar is well known to afford reversibility [31]. Future work will be devoted to expand the model study described here into some relevant analytical challenges using a competition between a hapten and a macromolecular structure conjugated with several hapten molecules for binding to an immobilized bioelement with weak affinity for both competitors.

Acknowledgment Ministry of Higher Education, Egypt is gratefully acknowledged for financial support to Mahmoud Labib during his study at the Department of Biotechnology, Lund University.

References

1. Choy V, Patel N, Thibault J (2007) Biotechnol Lett 29:1075–1080
2. Lieu Y-C, Wang FS, Lee W-C (2001) Biochem Eng J 7:17–25
3. Ballerstadt R, Kholodnykh A, Evans C, Boretsky A, Motamedi M, Gowda A, McNichols R (2007) Anal Chem 79:6965–6974
4. Diem P, Kalt L, Haueter U, Krinelke L, Fajfr R, Reihl B, Beyer U (2004) Diab Technol Ther 6:790–799
5. Malitesta C, Losito I, Zambonin PG (1999) Anal Chem 71:1366–1370
6. Gallego RG, Haseley SR, van Miegem VFL, Vliegenthart JFG, Kamerling JP (2004) Glycobiology 14:373–386
7. Rounds RM, Ibey BL, Beier HT, Pishko MV, Coté GL (2007) J Fluoresc 17:57–63
8. Chinnayelka S, McShane MJ (2004) Biomacromolecules 5:1657–1661
9. Khalil OS (2004) Diab Technol Ther 6:660–697
10. Pickup JC, Hussain F, Evans ND, Rolinski OJ, Birch DJS (2005) Biosens Bioelectron 20:2555–2565
11. D'Auria S, Di Cesare N, Gryczynski Z, Rossi M, Lakowicz JR (2000) Biochem Biophys Res Commun 274:727–731
12. D'Auria S, Di Cesare N, Taiano MS, Gryczynski Z, Rossi M, Lakowicz JR (2002) Anal Biochem 303:138–144
13. Wenholt I, Hoekstra J, DeVries J (2006) Diabet Care 29:1805–1811
14. Ivanov AE, Thammakhet C, Kuzimenkova MV, Thavarungkul P, Kanatharana P, Mikhalovska LI, Mikhalovsky SV, Galaev IY, Mattiasson B (2008) J Mol Recognit 21:89–95
15. Ye K, Schultz JS (2003) Anal Chem 75:3451–3459
16. Engström HA, Johansson R, Koch-Schmidt R, Gregorius K, Ohlson S, Bergström M (2008) Biomed Chromatogr 22:272–277
17. Struve C, Kristensen JS, Gregorius K, Yu Y, Denmark, Patent: WO2006061208
18. Liang F, Pan T, Sevick-Muraca EM (2005) Photochem Photobiol 81:1386–1394
19. Schultz JS, Mansouri S, Goldstein IJ (1982) Diabet Care 5:245–253
20. McCartney LJ, Pickup JC, Rolinski OJ, Birch DJS (2001) Anal Biochem 292:216–221
21. Cheung KY, Mak WC, Trau D (2008) Anal Chim Acta 607:204–210
22. Sato K, Anzai J-I (2006) Anal Bioanal Chem 384:1297–1301
23. Beyer U, Fleischer A, Kage A, Haueter U, Ehwald R (2003) Biosens Bioelectron 18:1391–1397
24. Labib M, Hedström M, Amin M, Mattiasson B (2009) Anal Chim Acta 634:255–261
25. Labib M, Hedström M, Amin M, Mattiasson B (2009) Anal Bioanal Chem 393:1539–1544
26. Labib M, Hedström M, Amin M, Mattiasson B (2009) Biotechnol Bioeng 104:312–320
27. Labib M, Hedström M, Amin M, Mattiasson B (2010) Anal Chim Acta 659:194–200
28. Jiang DC, Tang J, Liu BH, Yang PY, Shen X, Kong JL (2003) Biosens Bioelectron 18:1183–1191
29. Numnuam A, Kanatharana P, Mattiasson B, Asawatreratanakul P, Wongkittisuksa B, Limsakul C, Thavarungkul P (2009) Biosens Bioelectron 24:2559–2565
30. Bontidean I, Kumar A, Csöregi E, Galaev IY, Mattiasson B (2001) Angew Chem Int Ed 40:2676–2678
31. Gestwicki JE, Cairo CW, Strong LE, Oetjen KA, Kiessling LL (2002) J Am Chem Soc 124:14922–14933
32. Berggren C, Bjarnason B, Johansson G (1999) Instrum Sci Technolog 27:131–139
33. Ramanaviciene A, Ramanavicius A (2004) Anal Bioanal Chem 379:287–293
34. Ramanaviciene A, Ramanavicius A (2004) Biosens Bioelectron 20:1076–1082
35. Lee S, Pérez-Luna VH (2005) Anal Chem 77:7204–7211
36. Liao K-C, Hogen-Esch T, Richmond FJ, Marcu L, Loeb GE (2005) Proc SPIE 5691:129–145
37. Aslan K, Lakowicz JR, Geddes CD (2004) Anal Biochem 330:145–155
38. Aslan K, Zhang J, Lakowicz JR, Geddes CD (2004) J Fluoresc 14:391–400
39. Nesatyy VJ, Suter MJ (2004) J Mass Spectrom 39:93–97
40. Berggren C, Johansson G (1997) Anal Chem 69:3651–3657
41. Berggren C, Bjarnason B, Johansson G (1998) Biosens Bioelectron 13:1061–1068
42. So LL, Goldstein IJ (1968) Biochim Biophys Acta 165:398–404
43. Mandel DK, Kishore N, Brewer CF (1994) Biochemistry 33:1149–1156
44. Chen B, Bestetti G, Day RM, Turner APF (1998) Biosens Bioelectron 13:779–785
45. Piletska EV, Turner NW, Turner APF, Piletsky SA (2005) Biosens Bioelectron 108:132–139
46. Subrahmanyam S, Piletsky SA, Piletska EV, Chen B, Karim K, Turner APF (2001) Biosens Bioelectron 16:631–637
47. Piletsky SA, Subrahmanyam S, Turner APF (2001) Sens Rev 21:292–296
48. Chianella I, Lotierzo M, Piletsky SA, Tothill IE, Chen B, Karim K, Turner APF (2002) Anal Chem 74:1288–1293