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Demonstration of a new biosensing concept for immunodiagnostic applications based on change in surface conductance of antibodies after biomolecular interactions

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Abstract: We report an important observation that the surface conductivity of antibody layer immobilized on polylysine-coated glass substrate decreases upon the formation of complex with their specific antigens. This change in conductivity has been observed for both monoclonal and polyclonal antibodies. The conductance of monoclonal mouse IgG immobilized on polylysine-coated glass substrate changed from 1.02×10^{-8} Ω^{-1} to 1.41×10^{-11} Ω^{-1} at 10 V when complex is formed due to the specific biomolecular interactions with rabbit anti-mouse IgG F(ab′)$_2$. Similar behavior was observed when the same set up was tested in two clinical assays: (1) anti-Leishmania antigen polyclonal antibodies taken from Kala Azar positive patient serum interacting with Leishmania promastigote antigen, and (2) anti-p21 polyclonal antibodies interacting with p21 antigen. The proposed concept can represent a new immunodiagnostic technique and may have wide ranging applications in biosensors and nanobiotechnology too.

Key words: Immunodiagnosis, Antibodies, Immune complex, Conductance
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Electrical conductivity in biomolecules and other large polymeric systems has become an important topic of current research. Schlag *et al*.(2000a; 2000b) demonstrated that charge migration in proteins is highly efficient although the mechanistic origin is still debated and largely unknown even though various models have been proposed by Weinkauf *et al*.(1995; 1996; 1997). According to them, the charge transfer between amino acids takes place by hole hopping between local sites of lowest ionization potential in the amino acid chain i.e. the highest occupied molecular orbital (HOMO) of peptide groups, -CONH- assisted or driven by the torsional motions of the floppy backbones. Charge transfer mechanisms in peptides had been described in detail by Sheu and Schlag (2002), Sheu *et al*.(2002), and Long *et al*.(2005). Our observations demonstrate change in conductance of polylysine-coated glass biochip due to surface immobilization of protein A, mouse IgG and anti-mouse IgG F(ab′)$_2$.

Mouse IgG molecules were immobilized on polylysine-coated glass biochip employing 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and protein A (Fig.1). The amino groups of the polylysine-coated glass slide were crosslinked to the carboxyl groups of protein A employing EDC crosslinker. Protein A was employed as it binds to the constant F_c region of antibodies keeping their antigen binding sites on the variable F_{ab} region free to bind to antigens.

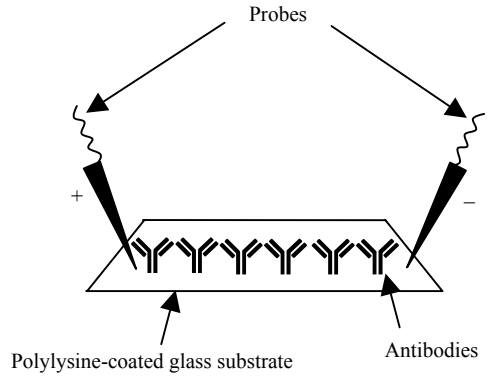

Fig.1 Schematic of the proposed biosensing principle. The antibodies are immobilized employing protein A, which is crosslinked to polylysine-coated glass substrate by EDC crosslinker

Current (I)-voltage (V) measurements (as shown in Table 1 and Fig.2) were taken using a two-point probe Probing Station (Signatone) in the voltage range from −10 to 10 V keeping the distance between the probes fixed at 200 μm. Varying the distance between the probes did not cause significant changes in the conductance of immobilized biomolecules. This may be explained based on the fact that the density of the immobilized biomolecules remains the same. All experiments were conducted in air at room temperature. The conductance values of blank polylysine-coated glass slide and EDC-coated polylysine glass biochip at 10 V were found to be 9.70×10^{-12} Ω^{-1} and 2.20×10^{-11} Ω^{-1} respectively. An increase in conductance from 2.20×10^{-11} Ω^{-1} to 2.07×10^{-8} Ω^{-1} was observed when protein A was immobilized on EDC-coated polylysine glass substrate. The conduction decreased from 2.07×10^{-8} Ω^{-1} to 1.02×10^{-8} Ω^{-1} upon the binding of mouse IgG to protein A. When the substrate immobilized mouse IgG was provided with rabbit anti-mouse IgG $F(ab')_2$, the conductance showed a decrease from 1.02×10^{-8} Ω^{-1} to 1.41×10^{-11} Ω^{-1}. The decrease in conductance may be due to the clogging/inactivation of certain charge conducting groups in the mouse IgG molecules after the formation of complex with rabbit anti-mouse IgG $F(ab')_2$. Detailed theoretical studies are required to gain understanding of the principles of charge transfer in solid substrate immobilized protein molecules and to unravel the mechanisms responsible for change in conductance of antibody functionalized biochip after specific biomolecular interactions.

Table 1 Conductance values at 10 V as determined from the current (I)-voltage (V) measurements at different steps of biomolecular interactions

Steps	Description of steps	Conductance at 10 V (mean±*SD*) (Ω^{-1})
1	Polylysine-coated glass biochip	$(9.70\pm1.62)\times10^{-12}$
2	Step 1+EDC	$(2.20\pm0.36)\times10^{-11}$
3	Step 2+protein A	$(2.07\pm0.45)\times10^{-8}$
4a	Step 3+monoclonal mouse IgG	$(1.02\pm0.29)\times10^{-8}$
5a	Step 4a+rabbit anti-mouse IgG $F(ab')_2$	$(1.41\pm0.26)\times10^{-11}$
4b	Step 3+polyclonal anti-p21	$(4.35\pm1.24)\times10^{-10}$
5b	Step 4b+p21	$(8.19\pm1.73)\times10^{-11}$
4c	Step 3+polyclonal antibodies against Leishmania antigen	$(4.27\pm1.17)\times10^{-10}$
5c	Step 4c+Leishmania antigen	$(2.18\pm0.28)\times10^{-11}$

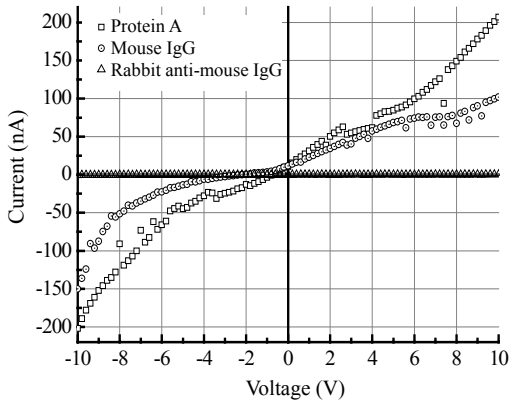

Fig.2 Current-voltage static measurements of biomolecules immobilized on polylysine-coated glass biochip at different stages of biomolecular interactions i.e., after protein A was immobilized, after mouse IgG was bound to protein A, and after mouse IgG formed complex with rabbit anti-mouse IgG $F(ab')_2$

A possible explanation of what we observed is that if charge is introduced at one polypeptide chain of the Y-shaped antibody, it moves along the polypeptide chain through amino acid hopping and then goes to the other polypeptide chain through disulfide bond and reaches the free site of the antibody where due to spatial hopping this charge jumps to the next antibody molecule (Fig.3). When the mouse IgG functionalized polylysine-coated glass substrate was provided with anti mouse IgG $F(ab')_2$, which selectively binds to the mouse IgG, certain sites on anti-

bodies get clogged or inactivated resulting in the measured decrease in surface conductivity.

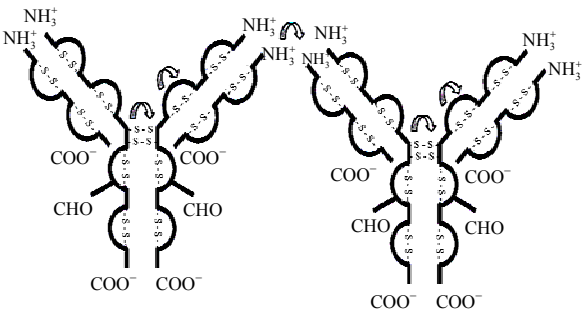

Fig.3 Proposed charge transfer mechanism in antibodies immobilized on polylysine-coated glass biochip

The proposed concept was practically tested in two clinical situations. In one case, polyclonal antibodies from Kala Azar positive patient were immobilized on the polylysine-coated glass biochip. The measured conductance at 10 V decreased from 4.27×10^{-10} Ω^{-1} to 2.18×10^{-11} Ω^{-1} when the antibody functionalized polylysine-coated glass biochip was provided with Leishmania promastigote specific antigen. In another case, antibodies against p21 were immobilized on the polylysine-coated glass biochip and the change in conductance was measured after interactions with p21 antigen. The same tendency of conductance to decrease from 4.35×10^{-10} Ω^{-1} to 8.19×10^{-11} Ω^{-1} was observed. But the amount of decrease in these cases was not as much as observed in the case of biomolecular interactions with monoclonal antibodies i.e., mouse IgG.

Charge transport in biomolecules is of recent interest and the demonstrated concept can have potential applications in various fields ranging from healthcare to biomolecular electronics. The development of simple, efficient, easy to use, cost effective and rapid immunodiagnostic kit for a number of diseases is one such application that is of paramount importance. Conventional diagnostic assays such as enzyme linked immunosorbent assay (ELISA), radio immunoassay (RIA) and enzyme immunoassay (EIA) require enzyme labelling or radio tagging, complex and time-consuming procedure and sophisticated instruments. On the other hand, the biosensing principle based on change in surface conductance of biomolecules immobilized on biochip surface after specific biomolecular interactions is less time consuming, cost effective and hold great promise for application in the field of biosensors.

The studies required highly interdisciplinary approach with persons from biology, medicine, physics, chemistry, computer science and engineering. The demonstrated concept is of extreme importance for fast and reliable clinical diagnosis and is a direct application of the much debated charge transfer in proteins in the field of medicine.

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