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Molecular diagnostics with electrochemical biosensors and arrays

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Abstract — Biosensors are self-contained analytical devices in which a bioreceptor is integrated with a transducer. The interaction between the bioreceptor and a target analyte generates a signal suitable for analytical purposes. In electrochemical biosensors, a change in the redox state of the biorecognition/analyte system generates a change in an electrochemical quantity which can be monitored by electroanalytical techniques. Electrochemical sensors can be miniaturized using ultramicroelectrodes and nanoelectrodes and their arrays as transducers. These devices are characterized by high specificity and sensitivity and improved detection limits. Biosensors can be used by non-specialist operators at the point of care. For the above reasons, within the frame of the Trans2care project, the Laboratory of Electrochemical Sensors of the University Ca' Foscari of Venice will collaborate with the project partners to develop electrochemical sensors suitable for specific clinical needs.

Index Terms — electrochemistry, biosensors, affinity, proteins, analysis, electrodes, array.

1 INTRODUCTION

Biosensors are analytical devices in which a molecular recognition layer is integrated with a transducer. The immediate environment of the bioreceptor can change as a consequence of the interaction with the target analyte so generating a measurable signal. With a biosensor it is possible to measure the target molecule directly, without using any additional reagent.
In electrochemical biosensors a change in the redox state of the biorecognition layer produces a change in an electrochemical quantity (a Faradaic current or an electrical potential) which can be monitored by electroanalyticall techniques [1]. In this

research the focus will be on amperometric and voltammetric methods of detection. Interestingly, electrochemical systems can be miniaturized from the millimeter down to the sub-micrometer scale; moreover, it is possible to use individual micrometer or nanometer sized working electrodes as well as arrays of them. In particular, the use of arrays of ultramicroelectrodes (UMAs) or nanoelectrodes (NEAs) allows one to overcome problems related to the requirement for high signal amplification and careful shielding from electrical noise. Signals given by UMA and NEAs are indeed the summation (weighed by diffusion effects) of the signals generated at each single micro(nano)electrode [2]. UMAs and NEAs are characterized by very high signal/noise ratios, therefore they allow one to achieve very low detection limits, of the order of nanomolar concentrations or, as absolute quantity, few picomoles or even femtomoles.

In typical schemes used in electrochemical biosensors, a biorecognition layer is directly immobilized on the electrode surface and the signal is produced by exchange of electrons between this layer and the underlying electrode. Analytes which can be detected includes small redox molecules such as enzymatic co-factors, vitamins, oligopetides, enzyme substrates, antigens and antibodies, aptamers, oligo- and polinucleotides. Note that in the case of miniaturized electrodes, such as in the case of nanoelectrodes, the amount of immobilized biomolecules can be very small with obvious advantages in the case of expensive or difficult to purify bioreceptors.

Depending on the nature of the biorecognition layer, electrochemical sensors can be classified into two categories: biocatalytic and bio-affinity sensors.

In biocatalytic sensors, the biorecogniton layer is composed by an enzymatic layer, (typically an oxido-reductase or a dehydrogenase, immobilized on the electrode/array) which exchanges electrons with the metal surface of the electrode via a suitable redox mediator (see Figure 1).

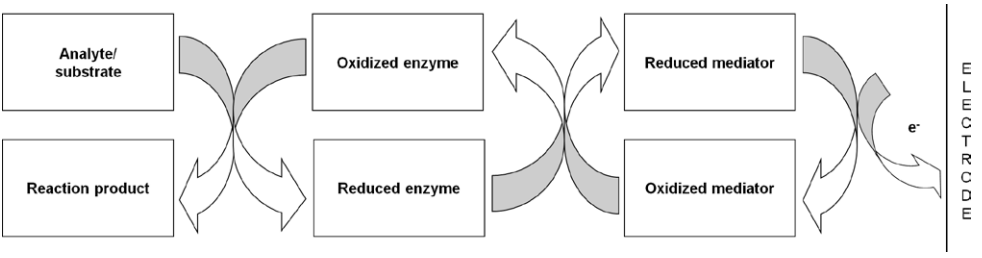

Figure 1. Recognition and signal generation in electrochemical enzymatic biosensor.

In bioaffinity electrochemical biosensors, the recogniton layer is obtained by immobiliation of antigen or antibody molecules; typically, redox enzymes are used as labels. The functioning scheme of some typical electrochemical immunosensors are summarized in Figure 2.

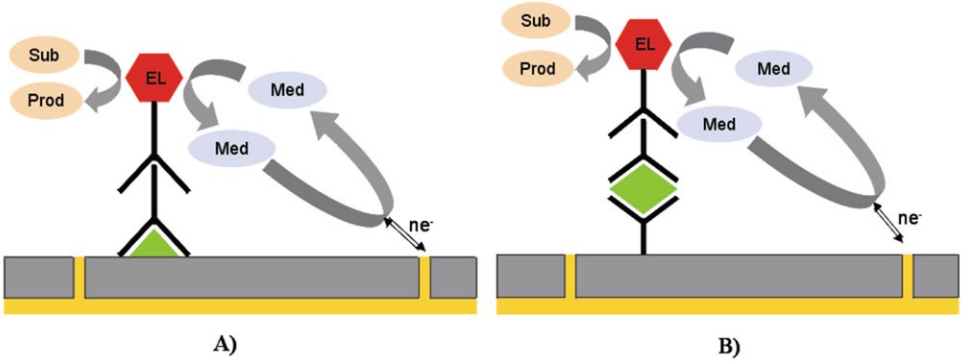

Figure 2: Schematic illustration of the two detection approaches used with electrochemical immunosensors: (A) the target protein is immobilized on the electrode/array, then it binds a primary antibody and secondary antibody with the enzyme label (EL); a soluble mediator (Med) shuttle electrons from the electrode to EL. (B) A primary antibody, specific for the target protein, is immobilized in order to capture the target protein. The other components follow as in scheme A.

The blood glucose sensor is a biocatalytic sensor which represents the most successful commercial biosensor developed so far. It employs amperometric detection and exploits the specificity of the enzyme glucose oxidase and a ferrocene-based redox mediator to produce a measurable current proportional to the blood glucose concentration. About 5-6 % of the population in western countries suffer from diabetes so that the glucose biosensor market growth is continuously growing.. According to a recent report [3], the global market for glucose biosensors and strips will reach 11.5 billion USD by 2012. It is obvious to expect that other kinds of biosensors will be developed to be ready for practical application in the near future. Note that biosensors can be used by non-specialist operators at the point of care and this allows for immediate action to be taken.

2 ACTIVITY AT THE UNIVERSITY CA' FOSCARI OF VENICE

2.1 The University Ca' Foscari of Venice

Established on August 6th 1868 as a Royal Business College, the University Ca' Foscari University of Venice actively participates in the city cultural life, organizing over 400 events every year, collaborating with other prestigious cultural institutions. The activity in Ca' Foscari is summarized by the following data:
4 main scientific-cultural areas: Economics, Humanities, Languages, Sciences;
15 First Cycle Degree Programmes;
29 Second Cycle Degree Programmes;
28 Specialist Master's Programmes;
1 Doctoral School, 16 Research Doctorate Programs;
9 Summer and Autumn Schools;
8 Departments, 6 Interdepartmental Schools;

1 Learning Centre Library,4 subject-related Libraries, 7 Departmental Libraries;
20,000 students, 4,000 new enrolments per year, 3,300 graduates per year
1,700 professors, lecturers, native language teachers and administrative staff.
Scientific research is carried out through Departments, Interdepartmental Schools and Doctoral Schools, which often work together on inter-disciplinary projects.
Ca' Foscari holds relationships with several associations and institutions through agreements for cooperation in the area of scientific information, teaching and research.
The University is a member of various research bodies including the Venice International University - VIU, Consorzio Venezia Ricerche and VEGA Science and Technology Park of Venice.

2.2 The laboratory of electrochemical sensors

In the last sixteen years the group of Electrochemical Sensors at the University Ca' Foscari of Venice, Department of Molecular Sciences and Nanosystems, has developed original know-how in the field of the fabrication and analytical application of ensembles and arrays of nanoelectrodes, nanostrutured electroactive membranes and polymer-based electrodes [2].
Among the other fabrication methods, the preparation of arrays of nanoelectrodes by controlled deposition of metal nanoelements within the pores of ultrafiltration membranes used as template, is an attractive and increasingly used nanofabrication procedure. Electroless deposition of gold in polycarbonate templates for producing NEAs was introduced some years ago by Menon and Martin [4] and was refined more recently in our laboratory [5]. Thanks to their peculiar geometry, NEAs are characterized by improved signal/noise ratios and by detection limits 2-3 orders of magnitude lower than those achievable with regular electrodes.
Among the bioanalytical applications of NEAs and nanostructured membranes developed in Venice it is worth listing:
-an electrochemical biosensor for the detection of nitrate which employs nitrate reductase as the biorecognition element [6];
-the direct electroanalysis of the redox enzymes such as cytochrome c with NEAs and with polymer coated electrodes [7, 8];
-an electrochemical sensor for glucose analyses based on NEAs which employs glucose dehydrogenase and a special redox mediator (a nitrofluorenone), developed by coworkers at the University of Bordeaux 1, which is very efficient in catalyzing NADH oxidation [9];
-the development of NEA-based electrochemical immunosensors applied, in collaboration with ICGB-Trieste (prof. G. Stanta) for the detection both of general model protein such as the single chain fragment variable (ScFV) protein [10], and of clinically relevant proteins such as the HER2 receptor [11]. The HER2 receptor is an important target protein for the identification of cancer that can be treated successfully with Herceptin (Trastuzumab) by the so called personalized therapies.
In recent studies carried out in collaboration with AB-Analitica srl (Padua), with CIVEN (Coordinamento interuniversitario veneto per le nanotecnologie; Porto Marghera-

Venezia) and with the Karlsrue Institute of Technology (group of Dr. Ljljiana Fruk), we developed a new procedure suitable for immobilizing oligonucleotides on NEAs aimed at using the biofuntionalized electrodes for the electrochemical detection of viruses.

3 ACTIVITY WITHIN THE TRANS2CARE PROJECT

The collaboration with other Trans2care partners will be finalized to the development both of biocatalytic and bioaffinty electrochemical biosensors, to detect suitable target analytes relevant for the partners involved in clinical and diagnostic practice.

IAs far as biocatalytic sensors are concerned, the development of an enzymatic electrochemical sensor for bilirubin analysis will be studied. Bilirubin is the breakdown product of the haem moiety of haemoglobin and other haemoproteins. Because of internal hydrogen bonding, bilirubin is water-insoluble and requires enzyme-mediated glucuronidation in the liver for biliary excretion. In normal circumstances, plasma bilirubin is mostly unconjugated and is tightly bound to circulating albumin. In cases of inherited or acquired deficiencies of bilirubin storage or excretion, both conjugated and unconjugated bilirubin accumulate in the plasma [9].The biosensor to be developed will be based on bilirubin specific enzymes such as bilirubin oxidase or biliverdin reductase, focusing on the discrimination between conjugated and unconjugated bilirubin. This work will be developed mainly in collaboration with the leader partner.

In the field of bioaffinty sensors, the focus will be on electrochemical immunosensors with capabilities to detect allergens, gluten and related antibodies, to be developed in collaboration with the leader partner and with project partner 2. The work can be potentially extended to the analysis of other proteins taken as biomarkers of different diseases, so involving also other project partners.

4 CONCLUSION

The know-how in the field of analytical application of electrochemical nano-biosensors acquired by the group at the University Ca' Foscari thanks to the present project can be extended and find practical application to the determination of clinically relevant molecules. Our participation in the Trans2Care project together with the other specialized partners, each bringing different know-how ranging from biochemistry to clinical practice, constitutes an important occasion and a challenge for new developments in the field of molecular diagnostics. Electrochemical biosensors can indeed be very helpful for obtaining quick and reliable analytical information thanks to their sensitivity, relative low cost, possibility of decentralized use and simple applicability.

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