Journal of Electroanalytical Chemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Electrochemical measurement of antibody-antigen recognition biophysics: Thermodynamics and kinetics of human chorionic gonadotropin (hCG) binding to redox-tagged antibodies

Lee I. Partington[a], Stephen L. Atkin[a,b,c], Eric S. Kilpatrick[b,d], Samuel H. Morris[e], Mark Piper[e], Nathan S. Lawrence[f], Jay D. Wadhawan[e,f,*]

[a] Hull-York Medical School, The University of Hull, Cottingham Road, Kingston-upon-Hull HU6 7RX, United Kingdom
[b] Department of Clinical Biochemistry, Hull Royal Infirmary, Anlaby Road, Kingston-upon-Hull HU3 2JZ, United Kingdom
[c] Weill Cornell Medical College Qatar, Education City, P. O. Box 24144, Doha, Qatar
[d] Department of Clinical Chemistry, Sidra Medical and Research Centre, P. O. Box 26999, Doha, Qatar
[e] Department of Chemistry, The University of Hull, Cottingham Road, Kingston-upon-Hull HU6 7RX, United Kingdom
[f] School of Engineering, The University of Hull, Cottingham Road, Kingston-upon-Hull HU6 7RX, United Kingdom

ARTICLE INFO

Keywords:
Electroimmunoassay
Frumkin isotherm
Partially blocked electrodes
Molecular wire
Chemoreception biophysics

ABSTRACT

The thermodynamics and kinetics of antigen binding under diffusive conditions to an electrode surface modified with ferrocene-tagged antibodies is studied within this work, and realised experimentally for the case of human chorionic gonadotropin (hGC) as the antigen with monoclonal anti-hCG antibodies immobilised on an electrode surface *via* a molecular wire platform. The formation of the antigen-antibody complex is monitored through the blocking of the ferrocene voltammetry, thereby enabling the fractional coverage of the electrode binding sites to be unravelled as a function of time. It is found that, at low antigen concentrations, a Frumkin adsorption isotherm fits the data, with repulsive interactions between bound antigens playing a significant rôle, with an affinity constant that is an order of magnitude larger than in the case of an untagged antibody, suggesting that the chemical hydrophobicity of the redox tag may encourage stronger binding. Comparison of the experimental temporal data with relevant diffusion-adsorption models under activation control allows for the extraction of the kinetic parameters; at zero coverage, the rate constants for adsorption and desorption are, respectively, larger and smaller than the untagged antibody. The kinetic study enables the confirmation that this type of platform may be utilised for rapid (15 min) and quantitative electroimmunoassay. This is validated through proof-of-concept analytical measurements, yielding a limit of detection around 25 mIU mL^{-1} (corresponding to 2.7 ng mL^{-1}) – a value used clinically for urine hCG measurements corresponding to around four weeks of gestational age.

1. Introduction

The hallmark of the specific adaptive immune response is the recognition of antigens (often proteins or carbohydrates) through binding to immunoglobulins (antibodies) present on the surface of *B* lymphocytes (*B* cells) [1,2]. Such protein-protein binding, mediated by a mixture of electrostatic, hydrophobic, hydrogen bonding or van der Waals' forces, occurs with a strong affinity constant ($10^{8\pm3}\ M^{-1}$), and, at least for the case of monoclonal antibodies, is highly specific, so that the binding may discriminate between closely related compounds [3,4].

The formation of such antibody-antigen complexes has been exploited extensively for the *in vitro* bioassay of a huge diversity of chemical structures and organisms [1–4], which has, *inter alia*, enabled a growth in analytical point-of-care sensors detecting antigenic disease biomarkers [5]. In general [6], two classes of measurement are used by such sensors; in a first approach, the binding assay uses an excess of the binding protein (antibody) that is conjugated with a suitable chemical tag, affording a *stoichiometric assay*. Such systems require separation of labelled and unlabelled antibody, typically through the use of a solid support. In a second approach, the binding protein is kept in limited amount, so that only a proportion of the antigen forms the antibody-antigen complex at equilibrium. In this scenario, initially bound, labelled, antigen may be displaced from the binding equilibrium by free, unlabelled antigen, enabling detection through the *substoichiometric*

* Corresponding author at: Department of Chemistry, The University of Hull, Cottingham Road, Kingston-upon-Hull HU6 7RX, United Kingdom.
E-mail address: j.wadhawan@hull.ac.uk (J.D. Wadhawan).

https://doi.org/10.1016/j.jelechem.2018.02.062
Received 31 July 2017; Received in revised form 19 February 2018; Accepted 27 February 2018

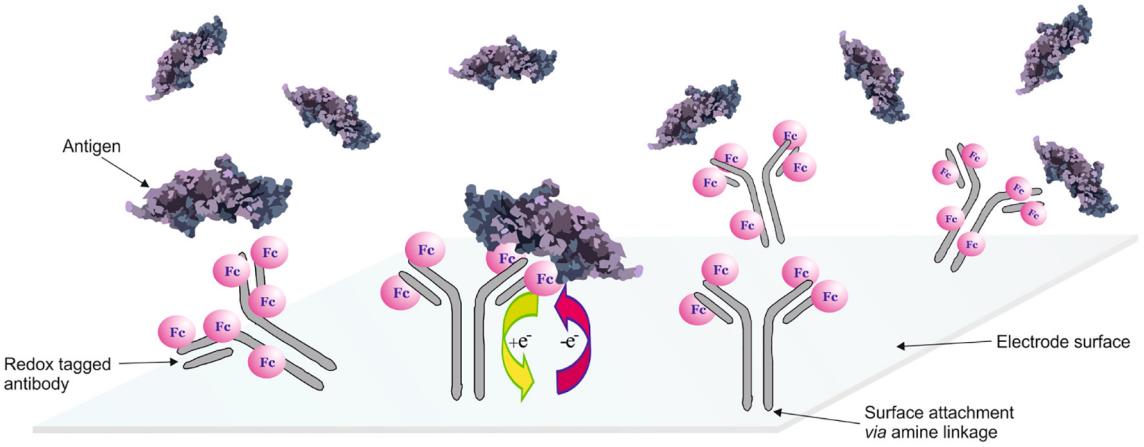

Fig. 1. Cartoon illustrating the nature of the electrochemical responses considered within this manuscript. Although the electrode surface is shown as a monolayer in this illustration, it is recognised that this is likely not the case (see text).

dilution principle [7].

Immunoassay using electrochemical methods is highly popular, owing to the ease through which developed sensing chemistry may be adapted to afford portable, low cost and reliable "dipstick" sensors of high sensitivity [8], especially for the case where the electrode surface itself is modified with antibody; rapid quantitative assay then relies on the antigen concentration in solution, the rate of antigen transport to the electrode surface and the kinetics of the interaction. In a previous work [9], we employed ferrocene-tagged antibodies attached to an electrode surface through a molecular wire platform to monitor the presence of biomarkers for cardiovascular disease in a physiological Krebs-Henseleit solution, where we demonstrated successful quantitative analysis using an antigen-incubation time of 20 min. In this work, we are interested in unravelling the physicochemical whys and wherefores underpinning the chemoreception of that platform, to determine how low an antigen incubation time is possible, whilst reliably providing quantitative analysis. We use human chorionic gonadotropin (hCG) as the antigen, as the clinical interest is of increasing hCG concentration [6], so that we may work with known minimum biomarker levels (*vide infra*). This hormone is a 37 kDa glycoprotein secreted by specialist syncytiotrophoblast cells of the developing placenta following implantation of a corpus leteum [10,11]. The presence of hCG can be detected in serum and urine, in some cases as early as three to four days following implantation, and therefore plays an important rôle in early pregnancy diagnosis [12]. However, the presence of hCG may indicate other health issues, including ectopic pregnancy [13], choriocarcinoma and hydatidiform moles [14–16], thereby offering further clinical utility. The hormone comprises two dissimilar, non-covalently bound structures: the α and β subunits [11]. The α-subunit is identical to those in the glycoprotein family, such as luteinizing hormone, whereas the β-subunit is unique to hCG and determines its immunological properties, providing an ideal target for specific detection *via* immunoassay techniques [10].

The majority of hCG immunoassays most commonly use enzyme-linked immunosorbent assay (ELISA) and other sandwich assay techniques [17], with recent advancements reported using chemiluminescence [18,19], fluorescence [20], and electrochemical immunosensor approaches [21–23], in addition to on-disc advancements [24]. The diagnostic cut-off for a positive reading is generally 25 milliinternational units per millilitre (mIU mL^{-1}), corresponding to 2.7 ng mL^{-1}, although this varies significantly from test-to-test [25]. An hCG level of < 5 mIU mL^{-1} generally indicates a negative result [26].

Previous work [27] has illustrated that for electrodes modified with anti-hCG immunoglobulins, the antibody orientation, in terms of binding site position, is extremely important for maximising the sensitivity of an hCG sensor. Moreover, neutron reflection studies have illustrated that for the ionic strengths employed in this work (typically ~200 mM), hCG binding to IgG1 mouse monoclonal anti-β-hCG antibodies immobilised on silicon oxide wafers, is influenced by both steric hindrance and electrostatic interaction between the antigen and the wafer surface [28], and that decreasing the packing density of adsorbed antibody increases the antigen binding capacity [29]. It is clear that such steric crowding and steric hindrance effects on binding arising from the adsorbed antibody may be complemented by effects hitherto unreported pertaining to the mutual repulsion that results from the binding of the antigen to the antibody. In this paper, we examine the thesis that such antigen-antigen repulsions on binding to an antibody-modified surface may be manifested through alterations in the binding isotherm, which may be measured electrochemically, and which may additionally affect the rate constants for hCG binding, and thence the capability of this system in responding reliably for rapid immunoassay.

2. Theory

2.1. Development of a global antigen coverage parameter

Our previous work on the development of a versatile platform for electroimmunoassay [9], was exploited as the foundation for the method described herein. In short, a redox-labelled, monoclonal antibody was attached to an electrode surface through a covalently bound molecular wire, as schematically illustrated in Fig. 1. The use of electrode-grafting by reducing diazonium functionalities means that a multilayer molecular wire platform is present [30]. In this work, we use the electrochemical reduction of a nitrobenzene diazaonium salt. Accordingly, in order to form an amide link with the antibody, the nitro-moieties need to be completely reduced to the amine. Owing to the complexity of this reduction [31], there is no easy way in which to coulomb count this; we therefore assume the resulting surface is sprinkled with nitro-, nitroso-, hydroxylamine and amine functionalities. This gives rise to a platform that is chemically non-uniform, and with a unique nanostructure. The antibody is likewise randomly modified with the redox tag – typically this is achieved through the formation of an amide linkage between the free primary amine moieties on the antibody and an aldehyde or acid functional group on the redox compound (such as ferrocenecarboxaldehyde or *cis*-bis(isothiocyanato) bis(2,2′-bipyridyl-4,4′-dicarboxylato)ruthenium(II)). Whilst there is an abundance of free primary amines at the paratopes of the antibody, there is also the possibility of immobilising the redox functionality all over the antibody. Furthermore, as indicated in Fig. 1, other than a statistical likelihood of the free carboxylic acid on the labelled antibody combining with the free amine groups on the modified electrode, there is a degree of uncertainty within our antibody-modified electrode