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Coupling non invasive and fast sampling of proteins from work of art surfaces to surface plasmon resonance biosensing: Differential and simultaneous detection of egg components for cultural heritage diagnosis and conservation

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ABSTRACT

Despite the wide application of surface plasmon resonance (SPR) to a broad area of interests, from environment to food analysis, from drug discovery to diagnostics, its exploitation in cultural heritage conservation is still unexplored. Water-based highly viscous polymeric dispersions (HVPD) composed by partially hydrolyzed polyvinyl acetate (PVA), borax, and water, were recently developed and successfully applied for the selective removal of surface degradation patinas (i.e. protein materials, natural resins etc.) from paintings of historical and artistic interest. This approach is here coupled for the first time to a SPR biosensor to simultaneously recognize albumen, yolk, or their mixtures in HVPD extracts. Ovalbumin and immunoglobulin Y are selected as analytes for egg white and yolk recognition, respectively. The biosensor was first characterized on standard analytes within the range 0–400 mg L^{-1} and then on fresh and dried egg albumen and yolk down to $2 \cdot 10^4$ and $1 \cdot 10^5$ dilution factors, respectively. Once optimized, the biosensor was combined to the HVPD application on simulated and real art samples for the evaluation of hen egg presence in the extract, i.e. albumen, yolk, or their co-presence in the matrix. For a contemporary 'sacred icon', realized by the traditional egg tempera procedure described by Cennino Cennini, the biosensor successfully distinguished different uses of egg components for the realization of painted and gilded areas, i.e. yolk and albumen, respectively. Finally, a XVIII century italian painting whose the realization technique is unknown, was tested confirming its egg tempera-based realization technique.

1. Introduction

Biosensors based on Surface plasmon resonance (SPR) are applied to a wide panorama of analytical problems with excellent results in label free and real time molecular diagnostics in medicine, environmental and food analysis, drug discovery and anti doping (Homola, 2006; Mayer and Hafner, 2011; Minunni et al., 2008; Scarano et al., 2010; Shankaran et al., 2007; Willets and Van Duyne, 2007). However, despite their large diffusion in many fields of chemistry and biochemistry, the literature on the use of these devices in the field of cultural heritage conservation is still surprisingly scarce. In fact, the (bio)chemical characterization of artworks is nowadays an important information to set up effective and low invasive restoration interventions and authentication studies helped by protein fingerprinting (Marijnissen, 1985; Ramírez et al., 2005). Therefore, considering that SPR technique has been extensively and successfully applied to proteins and peptides identification (Nguyen et al., 2015), its application to art diagnosis, conservation, and restoration could be very promising. In this framework, we developed an immuno-based SPR biosensor able to simultaneously identify and distinguish different egg proteins sampled on artwork surfaces, addressing the need in distinguishing pure components from whole egg, i.e. pure albumen or yolk from their mixture. In fact, traditional egg-based tempera is usually prepared by using only yolk as binding medium for pigments, but other preparations are based on whole homogenized egg or pure albumen. Pure albumen is also applied as "glue" for the realization of gildings layers (Mayer, 1985; Mounier et al., 2011). Moreover albumen, extensively used as surface protective in past restorations, is prone to spontaneous degradation causing alteration of the color surface. This requires the selective removal of albumen layers as one of the first steps to be taken in account in the restoration of paintings. Up to now, a limited number of

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attempts in exploiting affinity-based biosensors for such as investigations on artworks have been reported in literature (Micheli et al., 2012; Bottari et al., 2014). Only recently, Ugo and coworkers reported an electrochemical biosensor based on gold microelectrodes with application to art samples for the detection of immunoglobulin Y (IgY) (Bottari et al., 2014), as indicative of egg use in paintings (Osticioli et al., 2008). However, if singly detected, IgY are not informative of different uses of egg in artworks. Furthermore, labeled techniques suffer from possible enzyme activity depletion or catalysis interference by matrix components (mainly pigments), as also demonstrated in ELISA-based immunoassays for egg detection in simulated temperas (Cartechini et al., 2010), even if sandwich-based strategies can successfully eliminate matrix effects thanks to the specificity of the secondary antibody used to reveal the target analyte. In this framework, the use of label free techniques is thus very attractive, and SPR-based biosensing represents a promising platform for analysis of proteins dedicated to cultural heritage. Other analytical approaches than biosensors are based on expensive and/or labeled techniques (Cartechini et al., 2010; Gambino et al., 2013; Mazurek et al., 2014), that have limited perspectives for a widespread diffusion. On the contrary, recent innovations in the field of SPR platforms (mainly based on Localized SPR) have already gained encouraging results as cheap and portable analytical platforms in this sense. Working in this direction, this paper reports the successful coupling of an innovative, low invasive, and highly versatile sampling method based on a Highly Viscous Polymeric Dispersion (HVPD) to the simultaneous identification of egg albumen and yolk by SPR. These HVPD have been recently optimized for the selective removal of surface degradation patinas from painted surfaces of historical and artistic interest (Angelova et al., 2011; Baglioni et al., 2015). These systems are composed by partially hydrolyzed polyvinyl acetate (PVA) covalently crosslinked with borax. Its intrinsic shear elastic modulus allows its complete removal after the cleaning action simply by peeling it off from the artwork surface, without leaving any instrumentally detectable residue onto the cleaned area (Natali et al., 2011). PVA-based HVPDs are thus a very interesting material to be coupled to optical biosensing to both investigate surface material from artworks and/or monitor the progress of a cleaning action. This approach allows to evaluate the composition of the extracted material and to provide information on the progression of the cleaning process in fast and label free mode, compared to traditional immuno-based assays such as ELISA. The biosensor was first developed and tested on standard analytes (ovalbumin and IgY) and on dried egg films. Then its detection ability was checked on HVPD extracts after its application on simulated and real art samples. The direct detection through primary antibodies (a-OVA and a-IgY) was reinforced by a secondary sandwich-based detection tailored for each analyte, exploited to solve uncertain direct responses on yolk. As proof of concept, the method was finally applied to a contemporary 'Sacred icon', first, and then to a XVIII century italian painting. SPR data confirmed the ability of the whole procedure in both detecting the egg presence and in distinguishing its different uses for the realization of painted and gilded areas. The method results non-invasive, fast and reproducible, giving specific information within 1 h from HVPD application to SPR results.

2. Materials and methods

2.1. Reagents and buffers

N-Hydroxysuccinimide (NHS) was from Fluka (Milan, Italy); 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDAC) was from Merck-Calbiochem (Darmstadt, Germany); 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), sodium hydroxide, hydrochloric acid, Tween 20, ethylenediaminetetraacetic acid (EDTA), ethanolamine hydrochloride (EA), chicken ovalbumin (OVA) and sodium tetraborate decahydrate (borax, $>99.5\%$) were all from Sigma Aldrich (Milan, Italy). Skim milk powder (analytical grade) was purchased from Oxoid Ltd. (Hampshire, UK) and used without any further purification. 80PVAc was supplied by the Kuraray Co. Europe, Ltd. (Hattersheim am Main, Germany) as a random copolymer, PVA-424 h (MW=47 300, 80% hydrolyzed), and was used as received. Water was purified by a Millipore Elix3 ($R \geq 15\ M\Omega \times cm$). Other chemicals were purchased from standard commercial sources at analytical grade. Running buffer used in Biacore X™ experiments was the HBS-EP buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% Tween 20, pH 7.4), both for protein dilution and binding. All salts used for buffers were analytical grade and were purchased from Merck (Darmstadt, Germany). Deionized water (Millipore) was used throughout the preparations and buffer solutions were finally filtered (0.22 μm) before use. Purified chicken IgY, whole molecule, and the relative polyclonal antibody ((anti-IgY), goat) were from GenScript (Twin Helix Srl, Milan, Italy). Purified polyclonal (anti-OVA)lbumin (rabbit, 1.0 mg mL^{-1}) were from Rockland Inc. (Tebu-bio, Milan, Italy). Secondary antibodies used for the sandwich strategy were rabbit (anti-OVA) from Sigma Aldrich (Milan, Italy), goat (anti-IgY) from Abcam (Cambridge, UK).

2.2. SPR instrumentation and biochip preparation

SPR measurements were performed on Biacore X™, by using carboxymethylated dextran CM5 biochips (General Electric Healthcare Bio-Sciences AB; Uppsala, Sweden). Antibodies were immobilized on CM5 biochips by amino-coupling reaction as follows: at a constant flow rate of 5 μL min^{-1}, the dextran layer was activated with a water solution of NHS/EDAC (50 mM/200 mM) for 7 min Afterwards, 200 mg L^{-1} of each antibody in 10 mM sodium acetate solution at proper pH was flowed separately on the active surface of a channel, and the binding monitored in real time for 15 min The biochip surface was finally saturated by 1 M EA pH 8.5 for 20 min and the biochip was let under flow until equilibration of the baseline. All experiments were performed at least three times at a temperature of 25.00 ± 0.01 °C to determine the reproducibility of the biosensor. After each measuring cycle, the biosensor surface was regenerated by an injection of 100 mM NaOH aqueous solution for 30 s Dual channel biochips worked with the two measuring channels opened in series, each one carrying one immobilized antibody ((anti-OVA) or (anti-IgY)) for simultaneous detection. For this reason, preliminary experiments aimed to check the absence of unspecific binding of analytes on CM5 biochip treated to work as negative reference were carried out (see Supporting information and Fig. S1).

2.3. Sandwich on standard analytes solutions

Secondary (anti-OVA) and (anti-IgY) antibodies (different from the primary ones) were used to bind the analytes by a different epitope after their binding with the primary antibodies. The secondary antibodies were serially injected following the binding process in real time, and a specific amplification of each signal was obtained. The concentration of the secondary antibodies was optimized and consisted in 10 mg L^{-1} for both. It resulted enough to amplify the direct signals of standard analytes in a dynamic fashion, up to the highest concentration tested (400 mg L^{-1}). Also the effect of the injection order (aOVA/aIgY or aIgY/aOVA) on the overall amplified signals was evaluated, showing that the injection of secondary (anti-IgY) at first allows to avoid some unspecific binding of the secondary (anti-IgY) on the secondary (anti-OVA),