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Research paper

Immobilized carboxymethylated dextran coatings for enhanced ELISA

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ABSTRACT

We here report the development of a new generation of enzyme-linked immunosorbent assay (ELISA) that takes advantage of a low-fouling carboxymethylated dextran (CMD) layer chemically grafted on ELISA wells. In our approach, the overnight capture antibody adsorption step found in classical ELISA was replaced by a covalent attachment step to the CMD layer completed in 15 min. As a model, the potential of our approach was highlighted using commercially available anti-human epidermal growth factor (EGF) antibodies to quantify EGF present in various samples. Of interest, the grafted CMD layer was found to be as efficient as the commonly used bovine serum albumine (BSA) to reduce non-specific adsorption, thus eliminating the need of a time-consuming BSA blocking step normally required in classical ELISA. Our results demonstrated similar specificity, affinity, and intra- and inter-assay variations regardless of the diluent used in the assay (BSA-based diluent or protein-free buffer solution) when compared to standard ELISA. Finally, accuracy and precision of the CMD-based ELISA were verified by a spike and recovery test. Dilutions of recombinant human EGF in serum from healthy human volunteers showed almost-perfect linearity and mean recovery rates ranging between 90 and 110%.

1. Introduction

Enzyme-linked immunosorbent assay (ELISA) is the most commonly used tool for protein detection and quantification in complex solutions. In its standard 'sandwich' setup, multi-well polystyrene plates are first coated with capture antibody by physical adsorption overnight. The plates are then blocked by incubation with a blocking agent, such as bovine serum albumin (BSA) or low-fat milk or gelatin (Steinitz, 2000; Sentandreu et al., 2007). In this original setup, the weak attachment between the plate and the biomolecules (i.e. capture antibody and blocking agent) could however be disrupted during the washing steps involving detergent molecules (Prusaksochaczewski and Luong, 1989) or under shaking (Steiner et al., 2003). Inclusion of a blocking agent in the assay buffer is therefore usually required to fill open spaces that may be created due to the detachment of biomolecules (Sentandreu et al., 2007). Incubations with solutions containing the ligand to be detected (standard or unknown samples), enzyme-conjugated secondary antibodies as well as substrate solutions are then performed stepwise and are all separated by additional washing steps to remove unbound species. Altogether, the entire process is time-consuming (up to 20 h, including the overnight adsorption of capture antibody) and tedious due to the multiple incubation and washing steps, when manually performed.

Several improvements to the classical setup have been proposed in the literature to overcome some ELISA weaknesses. ELISA sensitivity and specificity can be enhanced by precoating a polymeric layer on the polystyrene well surfaces. It was indeed proposed to physically adsorb a poly-L-lysine layer or a dextran layer on polystyrene microplates in order to increase the surface

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amount of capture antibodies (Peterfi and Kocsis, 2000; Steinitz, 2000) or reduce the non-specific adsorption of ELISA reagents (Bocher et al., 1997). The weak antibody/surface interaction as well as the time-consuming passive adsorption step could be avoided by capturing the antibody via a strong physical attachment or a covalent link on chemically activated microplates, e.g. streptavidin-exposing (Douglas and Monteith, 1994) or aldehyde-exposing surfaces (Prusaksochaczewski and Luong, 1989). In a previous work, we have also demonstrated the benefits of an ELISA approach based on the direct capture of tagged-antigens via coiled-coil interactions (Liberelle et al., 2010a), eliminating the use of capture antibodies. Finally, the impact of blocking agents on ELISA specificity and sensitivity has been the focus of numerous studies, in which exhaustive lists of proteins (Vogt et al., 1987; Sentandreu et al., 2007; Jeyachandran et al., 2010) or neutral polymers, such as dextran, polyethylene glycol or polyvinyl alcohol (Prusaksochaczewski and Luong, 1989; Huber et al., 2009) have been experimentally tested. The ultimate goal was to limit undesired interactions between ELISA reagents and blocking agents or plate surface for specific antigen/antibody systems.

Based on the previously addressed limitations of classical ELISA setup, we here report the development of a new generation of polymer-coated ELISA plates allowing for faster, thus more convenient ELISA. More specifically, we here propose to replace both steps corresponding to the overnight adsorption of capture antibody and BSA-mediated blocking of classical ELISA wells by a unique step corresponding to the covalent grafting of the capture antibody onto carboxymethylated dextran (CMD)-coated wells (Fig. 1). Due to its low-fouling properties and the availability of free carboxylic groups homogeneously distributed on the polymer, CMD coating may efficiently allow for the chemical grafting of capture antibodies while acting as a protein adsorption blocking layer per se (Monchaux and Vermette, 2007; Liberelle et al., 2010b). In this endeavor, the chemical grafting of CMD and capture antibody layer was first optimized to minimize the non-specific adsorption of ELISA reagents while maximizing the amplitude of standard dose–response curve, respectively. The potential of our ELISA approach was then highlighted by comparing it to a commercially available classical ELISA setup for the detection of the human epidermal growth factor (EGF), in terms of time-consumption, ability to decrease the reagent concentration and versatility regarding the blocking solution formulation. Finally, the reliability of our CMD-coated ELISA as a detection tool was evaluated using biological samples, standard addition and recovery test.

2. Materials and methods

2.1. Material and reagents

Aminated microplates (96 Well Clear Polystyrene Amine Surface Stripwell Microplate, Corning catalog #2388) were purchased from Fisher Scientific Co. (Ottawa, ON). Bare (standard) microplates (96 Well Clear Polystyrene Stripwell Microplates, Costar catalog #2592) and recombinant human epidermal growth factor (rhEGF, catalog #236-EG) were obtained from R&D Systems (Minneapolis, MN). Dulbecco's Phosphate Buffered Saline (modified PBS, without calcium chloride and magnesium chloride), Tween 20, sodium acetate (99 + % purity), N-hydroxysuccinimide (NHS, 98% purity), ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 99 + % purity) and monobromoacetic acid (99 + % purity) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON). Sulfuric acid (98% purity), acetic acid (99.7 + % purity), sodium acetate (99 + % purity), chlorhydric acid (37.7% v/v), and sodium hydroxide (98.7% purity) were purchased from VWR International Inc (Mont-Royal, QC). MilliQ quality water (18.2 MΩ·cm; total organic compounds (TOC) = 4 ppb) was generated with a Millipore Gradient A 10 purification system. Commercially available DuoSet ELISA kit containing mouse anti-human EGF antibody (capture antibody), biotinylated goat anti-human EGF antibody (detection antibody), streptavidin-horseradish peroxidase (streptavidin-HRP), bovine serum albumin (BSA), and substrate solution (hydrogen peroxide/tetramethylbenzidine) were purchased from R&D Systems (Minneapolis, MN). Dextran (Mw = 500, 70 and 10 kDa) was obtained from Pharmacosmos A/S (Holbaek, Denmark).

2.2. Dextran carboxymethylation and characterization

Dextran carboxymethylation was performed by adapting previously reported protocols (Liberelle et al., 2010b). Briefly,

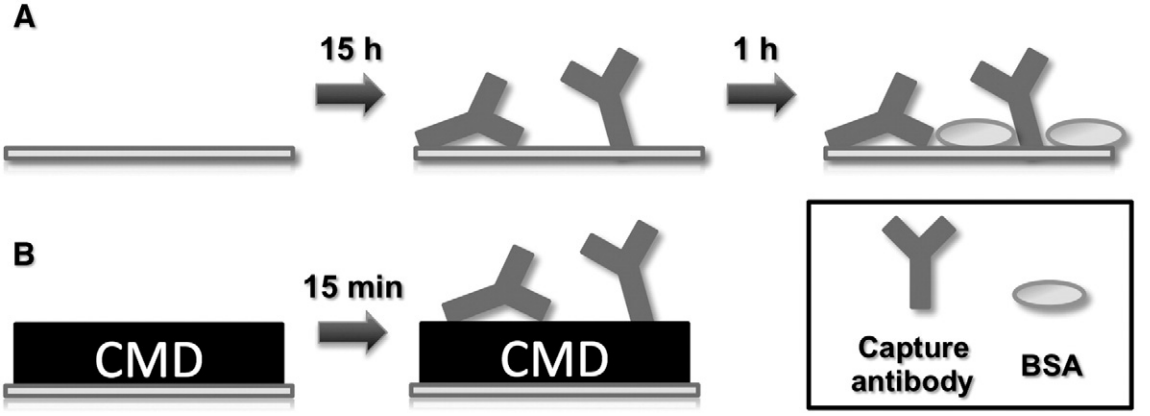

Fig. 1. Schematic illustration of capture antibody immobilization. (A) Standard ELISA procedure based on capture antibodies adsorption followed by BSA adsorption on polystyrene wells, and (B) modified ELISA procedure involving the covalent attachment of capture antibody onto chemically grafted carboxymethylated dextran (CMD) layer on aminated wells.