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Quantification of the trichothecene Verrucarin-A in environmental samples using an antibody-based spectroscopic biosensor

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ABSTRACT

Verrucarin A[2] (VerA) is a toxic trichothecene mycotoxin that can be produced indoors at very low level by moulds contaminating dwellings and may be associated with several human health problems. In this study we describe a spectroscopic label-free biosensor for VerA. This sensor is based on the high sensitivity of Fourier transform infrared-attenuated reflection (FTIR-ATR) spectroscopic detection and the use of a new anti-VerA rat monoclonal antibody (mAb). This antibody was directly grafted at the surface of the infrared element. Competitive ELISA and FTIR-ATR techniques were compared for detection of VerA in buffer and in complex dust samples obtained from dwellings. After optimization, the competitive ELISA showed a sensitivity of 7.43 ng/ml of VerA in PBS and a dynamic range below one order of magnitude. The FTIR technique improved the detection of the VerA by three orders of magnitude (2 pg/ml in buffer and 6 pg/ml when spiked in dust samples). The dynamic range for its detection extended over four orders of magnitude. The percentage of recovery of VerA spiked (1000 ng to 0.1 ng) in a complex dust matrix ranged from 99 to 68%. Our results clearly show that this antibody-based spectroscopic biosensor allow a better detection of VerA as compared to classical immunoassays and can be very efficiently used in the field of indoor mycotoxin detection.

1. Introduction

Damp building-related illness is a syndrome with an heterogenous group of respiratory, immunological, and neurological symptoms whose causes and symptoms can be directly attributed to one or more pollutants within a building. As patients experiencing these illnesses and children suffering from idiopathic pulmonary hemorrhage often have histories of living in water-damaged buildings, they have been associated with airborne exposure to fungi [1] particularly moulds that flourish in damp indoor environments as for instance *Aspergillus versicolor*, *Stachybotrys chartarum*, *Cladosporium* spp and *Penicillium* spp. These moulds are well known mycotoxin producers that may play a role in the adverse health effects reported during damp-related illnesses. Mycotoxins are in fact secondary metabolites that are produced to give strategic advantages to moulds over encroaching organisms and very often these mycotoxins are found in spores and mycelial fragments of moulds. These spores and mycelia finally set down in dust and can become airborne and inhaled under certain conditions and then cause a number of adverse effects [2]. Among the mycotoxin plethora, the trichothecenes consist of a family of over 200 structurally related low molecular weight (~200–500 Da) compounds closely related to sesquiterpenoids with a common 12,13-epoxide-trichothecene ring system [3,4]. VerA belongs to the family of macrocyclic trichothecenes containing a cyclic diester or triester ring linking C-4–C-15 [5,6]. This family is considered to include the most toxic trichothecenes ever observed [1]. Macrocyclic trichothecenes such as VerA can be produced indoor by moulds contaminating dwellings [7] and are known to interfere with ribosome function, inhibit protein synthesis [8], induce activation of caspases and apoptosis [9] and also activate inflammasome-associated innate immunity [10]. Although

Abbreviations: ATR, Attenuated total reflection; FTIR, Fourier transform infrared; IR, Infrared; mAb, Monoclonal antibody; Ror A, Roridin A; VerA, Verrucarin A.

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a causal connection has not been unequivocally proven, damp building-related illnesses could be attributed in part to the mycotoxins produced by the indoor growing moulds [11–13]. Because of their high toxicity, the detection of trichothecenes indoors is an important task. However this detection can be hampered by their very low concentrations and their presence on "difficult matrix" (e.g., wallpapers, dust, etc.) that may interfere with the analytical methods used. These drawbacks can therefore prevent the development of preventive strategies for this kind of indoor pollution [1].

Recently a new type of generic biosensor has been developed, based on the high sensitivity of Fourier transform infrared (FTIR) spectroscopy and on an attenuated total internal reflection (ATR) element transparent in the infrared (IR) [14]. This IR configuration allows the spectrometric analysis of compounds in solution when they are brought in contact with the ATR element. The FTIR provides quantitative information as well as spectroscopic signatures allowing the precise determination of the interacting molecules, while standard sensors as a rule only indicate the mass loading on the surface. The chemical signature of the analyte bound is of major importance and allows the detection of potential non-specific interactions. The specificity of this FTIR-ATR label-free configuration is based on specific ligand–receptor interactions. A germanium or silicon crystal can be used as an ATR element onto which an organic layer is directly grafted at the surface. Then bifunctional spacer molecules allow the covalent binding of receptors which can capture and concentrate the target ligands at the surface of the ATR crystal.

We previously used this FTIR-ATR configuration for the competitive detection of the small molecule 2,4-dinitrophenol [15] and for the direct detection in a complex medium of factor VIII molecules using phospholipid membrane fragments grafted to the organic layer [16]. Here we present the development of an FTIR-ATR immunosensor for the direct detection of a macrocyclic trichothecene contaminating dwellings and show that it allows a better detection as compared to a classical detection by immunoassay.

2. Materials and methods

2.1. Reagents

Unless otherwise stated, the purchased chemicals were of analytical grade. VerA, Roridin A, Ovalbumin (OVA), Bovine Serum Albumin (BSA), Methanol, H_2SO_4 and H_2O_2 were purchased from Sigma–Aldrich. Phosphate buffered saline (PBS, 0.010 M pH 7.4) solution was prepared from Sigma–Aldrich dry powder.

2.2. Production of a VerA specific rat mAb

Rat mAbs were produced as initially described [17]. All experiments were performed in accordance to the local ethics committee of the WIV-ISP.

2.3. Preparation of dust extracts

Dust samples were collected during environmental audits. To prepare the dust extracts, 50 mg of dust in a microtube was extracted in 1 ml of PBS by agitation for 16 h at room temperature. Supernatants were then clarified by centrifugation (2000 × *g* for 15 min) and stored at −20 °C until use. To prepare the spiked dust extracts, various concentrations of VerA diluted in 100 μl of PBS were added to 50 mg of dust in a microtube. After drying, the material was extracted as described above.

2.4. ELISA

A quantitative competitive ELISA was developed using a selected VerA specific antibody (F24-1G2). Microplates were coated with 0.5 μg of BSA-VerA (or OVA-VerA) in 100 μl of borate buffer per well. Plates were washed in PBS and saturated with PBS supplemented with 10% of Foetal Calf Serum (300 μl/well) for 2 h at 37 °C. After washing in PBS, serial twofold dilutions of VerA in 50 μl of PBS or dust were applied. Wells devoid of inhibitor (VerA) were used as positive (Max) or negative controls (Blank). Fifty microlitres of F24-1G2 diluted at 1 μg/ml in PBS 0.1% Tween 20 1% BSA were added immediately to each well except for the negative controls. Plates were incubated for 1 h at 37 °C and then washed in PBS 0.1% Tween 20. Peroxidase-labelled mouse anti-rat IgK (1 μg/ml in PBS, 0.1% Tween 20, 1% BSA, 100 μl/well) was then added and the plates were incubated for 2 h at 37 °C. Plates were finally washed and developed by the addition of 100 μl of TMB (Sigmafast, Sigma–Aldrich). The reaction was stopped with 50 μl of 2 N H_2SO_4 and O.D. was read at 450 nm. The percentage of inhibition was calculated as follows: % Inh = $(1 - (OD_{492}\ \text{Sample} - OD_{492}\ \text{Blank})/(OD_{492}\ \text{Max} - OD_{492}\ \text{Blank})) \times 100$. Non-linear least squares (ordinary fit) regression and the best fit parameters were calculated for each data set using the GraphPad Prism software.

2.5. VerA IR reference spectra

To determine the IR fingerprint of the VerA, 10 μl of VerA (1 mg/ml in ethanol) were placed onto the ATR crystal on the sampling area. After complete ethanol evaporation, spectra were recorded. The VerA IR spectra showed an intense band at 1716 cm^{-1} due to an ester C═O bond and two minor bands at 1635 and 1582 cm^{-1} corresponding to C═C functions. A broad absorption band around 3500 cm^{-1} indicated the presence of OH groups and three stretching vibrations of CH_3 and CH_2 between 3000 and 2850 cm^{-1} were also observed. The vibrations involving the stretching of the C—O bonds gave several strong IR bands below 1000 cm^{-1} indicating the presence of different compounds (carboxylate, cyclopentanone and aromatic structures). Several other bands at 1083 cm^{-1} (C—O—C), 2158 cm^{-1} (—C—CH_3), 1269 (C—O), and 1209 cm^{-1} (C—O—H), 1188, 1126, 1083, 1029, 996, 967, 879, 820 cm^{-1} were also observed.

2.6. Functionalization of the biosensors and grafting of the VerA-specific mAb

The IR element used was a triangular-shaped germanium crystal (4.8 mm × 4.8 mm × 45 mm, ACM, France). With this geometry, a single reflection occurs and fifteen consecutive tracks can be used on a single crystal. The cleaning, activation, covalent grafting of 2,5,8,11,14,17,20-heptaoxadocosan-22-yl 3-(triethoxysilyl) propylcarbamate and of the spacer N-Succinimidyl (4-azidophenyl) 1,3′-dithiopropionate (NHS) molecules onto germanium crystals, leading to a multitracking biosensor with the appropriate microfluidic, were performed as already described [18].

The functionalized surfaces were placed in an ATR flow cell (Specac, UK) connected to a Watson-Marlow 403U/VM2 peristaltic pump (Farmount, UK). IR spectra were recorded on an FTIR 6700 IR spectrophotometer (ThermoFischer) equipped with a Mercury-cadmium-telluride (MCT) detector at a resolution of 4 cm^{-1} with a mirror speed of 0.6329 cm/s continuously purged using an air dryer (Parker-Zander, Germany) at a flow rate of 30 standard cubic feet per hour (SCFH).

Each experiment was performed on a single track under a semi-continuous flow. Five-hundred microlitres of the VerA-specific rat mAb (F24-1G2) at a concentration of 0.1 mg/ml was injected at a flow rate of 12 μl/min for 30 min. The track was then washed with