RB818 and RB819 antibodies recognize a low-molecular weight highly sulfated marine exopolysaccharide derivative by ELISA

[1]Philippe Hammel, [2]Corinne Sinquin, [3]Noemi Veraldi

[1]*Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland*
[2] *Ifremer, MASAE Microbiologie Aliment Santé Environnement, F-44000 Nantes, France*
[3]*Department of Paediatrics,Gynaecology and Obstetrics, University of Geneva, Geneva, Switzerland*

Abstract

The recombinant antibodies RB818 and RB819 detect by ELISA a low-molecular weight highly sulfated marine exopolysaccharide derivative.

Introduction

The HE800 exopolysaccharide (HE800 EPS) is a biopolymer secreted by a deep-sea hydrothermal bacterium *Vibrio diabolicus* HE800 strain (Rougeaux *et al.*, 1999). The native high-molecular weight HE800 EPS, named diabolican, consists of a linear non-sulfated tetrasaccharidic repeating unit. The diabolican repeating unit is a combination of both hyaluronan and non-sulfated chondroitin disaccharide repeating units (Fig. 1). A diabolican derivative characterized by a low molecular weight and a high sulfate content was previously described for its heparin-sulfate mimetic behaviour (Senni *et al.*, 2013) and its neuroprotective properties (Veraldi *et al.*, 2023). Here we describe the ability of two recombinant antibodies (RB818 and RB819) to detect by ELISA this diabolican derivative.

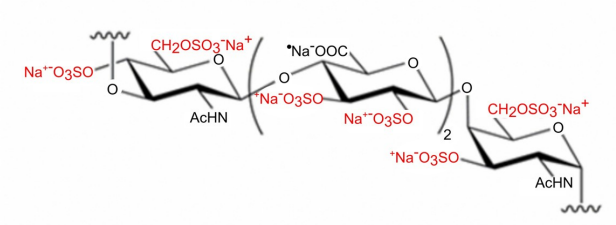

Fig. 1. Tetrasaccharide repeating unit of the diabolican derivative from HE800 exopolysaccharide (Rougeaux *et al.*, 1999).

Materials & Methods

Antibodies: ABCD_RB818 and ABCD_RB819, antibodies (ABCD nomenclature, http://web.expasy.org/abcd/) were discovered by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) and produced as minibodies with the antigen-binding VHH portion fused to a rabbit IgG Fc. HEK293 suspension cells growing in HEK TF medium (Xell #861-0001, Sartorius), supplemented with 0.1% Pluronic F68 (Sigma #P1300), were transiently transfected with the vector coding for the VHH-Fc of each antibody. Supernatants (~50-80 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against a biotinylated diabolican derivative (Seffouh *et al*, 2019). This antigen was also used in the ELISA experiment. Heparin-biotin (Sigma #B9806) was used as control.

Protocol: The whole procedure was carried out at room temperature. Biotinylated diabolican derivative and heparin at saturating concentration (~10 pmol/well) were immobilized on streptavidin-coated ELISA plates (Pierce #15124) for 30 min. Each well was rinsed three times with 100 µl of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 µl of RB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma #A8275, dilution 1:1000, 50 µl per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 µl per well). The reaction was stopped by the addition of 25 µl of 2 M H_2SO_4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies RB818 and RB819 bound in a concentration-dependent manner to the biotinylated diabolican derivative against which they were raised, but not to heparin (Fig. 2).

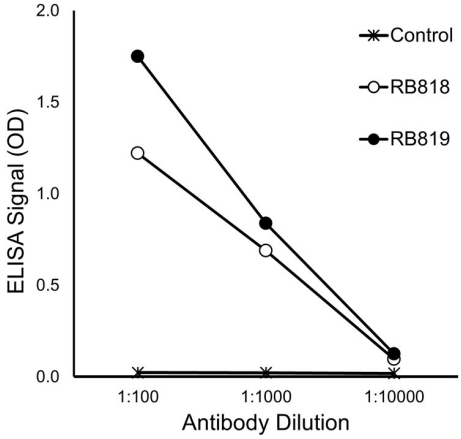

Fig. 2. Specific binding of RB antibodies to the biotinylated diabolican derivative, as detected by ELISA. 'Control' indicates the binding of RB818 to biotinylated heparin (RB819 control curve was superimposed).

References

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Conflict of interest

Philippe Hammel is a cofounder and a shareholder of ABCD Antibodies SA.