RB464, RB465, RB466 and RB467 antibodies do not recognize the Dictyostelium AlyA protein by Western blot

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Abstract

The recombinant antibodies RB464, RB465, RB466 and RB467 do not detect by Western blot the full-length AlyA protein from *Dictyostelium discoideum*.

Introduction

AlyA (Amoeba LYsozyme, DDB_G0275123, UniProt #Q8T1G4) is a member of the amoeba lysozyme family in the amoeba *D. discoideum* (Muller *et al*., 2005). Here we describe the inability of four recombinant antibodies (RB464, RB465, RB466 and RB467) to detect the full-length AlyA protein by Western blot.

Materials & Methods

Antibodies: ABCD_RB464, ABCD_RB465, ABCD_RB466 and ABCD_RB467 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies; Blanc *et al*., 2014) as mini-antibodies with the antigen-binding scFv fused to a rabbit Fc (RRB464, RRB465, RRB466 and RRB467). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~50 mg/L) were collected after 5 days. As a positive control, the anti-6xHis antibody AD946 (Lamrabet and Jauslin, 2018) was used.

Antigen: RB464, RB465, RB466 and RB467 were raised against a N-biotinylated synthetic peptide corresponding to 43 residues close to the AlyA C-terminus (LTDSRPLGPFNVTESEMAQLFIDHEIAMAQCEAEK TCNGFDLE). *D. discoideum* DH1 (WT) cells expressing a 6xHis-tagged AlyA protein (AlyA-His, 6xHis-tag fused to the C-terminus) were used to detect the full-length AlyA protein.

Protocol: $5x10^6$ *D. discoideum* cells were pelleted and resuspended in 200 µL of reducing sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, 6% (v/v) β-mercaptoethanol). 20 µL of each sample was migrated (200 V, 30 min) in a 4-15% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #456-1086), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked during 1 hour in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk, and washed three times for 15 minutes in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with each of the tested antibodies (dilution 1:2 in PBS-Tween), overnight at 4 °C, then washed three times for 15 minutes. The membranes were then incubated with horseradish peroxidase-coupled goat anti-rabbit or anti-mouse IgG (Biorad #170-6515 and #170-6516, respectively; dilution 1:3000) and washed twice for 15 minutes and once for 5 minutes in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (K-12043, Advansta Corporation) using a PXi-4 gel imaging systems (Syngene).

Results

Antibodies RRB464, RRB465, RRB466 and RRB467 did not specifically recognize the endogenous AlyA protein, nor the overexpressed 6xHis-tagged AlyA protein in WT cells (Fig. 1). The tagged protein was detected in the AlyA-His expressing cells with an anti-6xHis antibody (AD946).

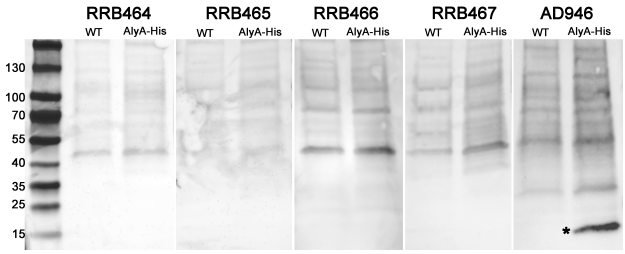

Fig. 1. No specific binding of RRB antibodies to cells overexpressing AlyA-His. AlyA-His was successfully detected by the anti-6xHis AD946 antibody (position indicated by an asterisk), but not by any of the RRB antibodies tested here.

References

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Conflict of interest

The authors declare no conflict of interest.