RB376 and RB377 antibodies recognize the Dictyostelium AlyA protein by Western blot

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Abstract

The recombinant antibodies RB376 and RB377 detect by Western blot the full-length AlyA protein from *Dictyostelium discoideum*. RB343, RB344 and RB378 antibodies do not.

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 ability of two recombinant antibodies (RB376 and RB377) to detect the full-length AlyA protein by Western blot.

Materials & Methods

Antibodies: ABCD_RB343, ABCD_RB344, ABCD_RB376, ABCD_RB377 and ABCD_RB378 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 mouse Fc (MRB343, MRB344, MRB376, MRB377 and MRB378). 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: RB343 and RB344 were raised against a N-biotinylated synthetic peptide corresponding to the residues 141 to 173 (DSRPLGPFNVTESEMAQLFIDHEIAMAQ CEAEK). RB376, RB377 and RB378 were raised against a N-biotinylated synthetic peptide corresponding to the residues 67 to 102 (DSQRFGCGKYLNLCRSGKCVKAQI YDAGPAMWVEQD). *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-mouse IgG (Biorad #170-6516, 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 MRB376 and MRB377 specifically recognize the AlyA protein in *D. discoideum* cells overexpressing a 6xHis-tagged AlyA protein (Fig. 1). The tagged protein was also detected with an anti-6xHis antibody (AD946). MRB343, MRB344 and MRB378 antibodies did not detect the same band (Fig. 1 and data not shown).

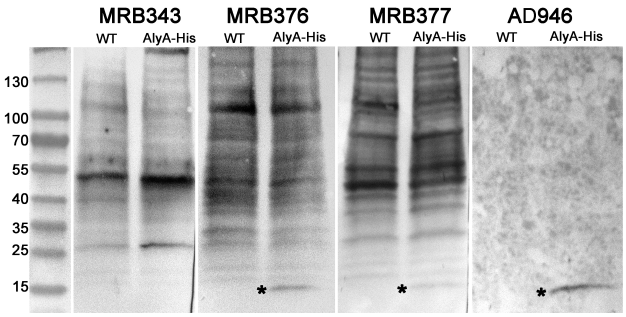

Fig. 1. Specific binding of MRB antibodies to cells overexpressing AlyA-His. AlyA-His was successfully detected by RB376 and RB377 and by the anti-6xHis AD946 antibody (positions indicated by an asterisk), but not by MRB343, MRB344 or MRB378 (data not shown for the last two). The endogenous AlyA protein was not detected in WT cells.

References

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Conflict of interest

The authors declare no conflict of interest.

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