RB481, RB482, RB483, RB484, RB485, RB486, RB498 and RB499 antibodies recognize the coatomer COPI complex by ELISA

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Abstract

The recombinant antibodies RB481, RB482, RB483, RB484, RB485, RB486, RB498 and RB499 detect by ELISA a purified COPI complex from COS7 cells.

Introduction

COPI (Coatomer or COat Protein complex I) is a large polypeptide complex (composed of seven different subunits) involved in retrograde traffic from the Golgi apparatus to the endoplasmic reticulum (Cosson and Letourneur, 1994). Here we describe the ability of eight recombinant antibodies (RB481, RB482, RB483, RB484, RB485, RB486, RB498 and RB499) to detect by ELISA a purified COPI complex from COS7 cells.

Materials & Methods

Antibodies: ABCD_RB481, ABCD_RB482, ABCD_RB483, ABCD_RB484, ABCD_RB485, ABCD_RB486, ABCD_RB498 and ABCD_RB499 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 portion fused to a rabbit IgG Fc (RRB481, RRB482, RRB483, RRB484, RRB484, RRB485, RRB486, RRB498 and RRB499). 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.

Antigen: The antibodies were raised against a pulled-down COPI complex from COS7 cells (Fig. 1). Briefly, synthetic N-terminally biotinylated peptides KKLETFKKTN or KKLETFSSTN were immobilized at saturating concentration on Dynabeads® M-280 Streptavidin (Invitrogen #11205D). COS7 cells were lysed in Hepes-Triton buffer [50 mM Hepes (pH 7.3), 90 mM KCl, 0.5% Triton X100 and protease inhibitors] at $4x10^6$ cells/ml. 1 mg of beads (approximately 200 pmoles of peptide) were incubated with 1 ml of COS7 cell lysate. After incubation (2 h at 4 °C), beads were washed 3 times in Hepes-Triton buffer and once in 50 mM Hepes. To analyze the proteins bound to the beads, samples were boiled in 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] and run on a 4-15% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #456-1086). The gel (Fig. 1) was stained using PageBlue Staining Solution (Thermo Scientific #24620). Antibodies were raised against the KKLETFKKTN peptide (the last 10 C-terminal residues from yeast WBP1; Uniprot #P33767); KKLETFSSTN was used as a negative selection during phage display panning and as negative control for ELISA.

Protocol: Biotinylated KKLETFKKTN and KKLETFSSTN peptides 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 2 hour with 50 µl of COS7 cell lysate prepared in the same conditions as for the pull down (every antibody was also tested against a KKLETFKKTN peptide not incubated with COS7 cell lysate). After 3 washes with washing buffer, wells were incubated for 1 hour with 50 µl of RRB antibody-containing supernatant diluted in washing buffer (Fig. 2). 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 RRB481, RRB482, RRB483, RRB484, RRB484, RRB485, RRB486, RRB498 and RRB499 bound in a concentration-dependent manner to the KKLETFKKTN peptide loaded with COS7 cell lysate against which they were raised, but not to the unloaded KKLETFKKTN peptide, nor to the KKLETFSSTN peptide loaded with COS7 cell lysate (Fig. 2). For some of the antibodies the binding was strong (RB481, RB482, RB484, RB486), while it was weaker, but detectable, for all other antibodies tested.

References

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Conflict of interest

The authors declare no conflict of interest.

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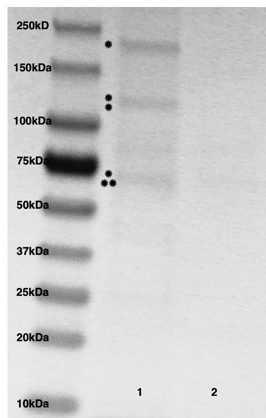

Fig.1. The KKLETFKKTN peptide pulls down the COS7 COPI complex: subunits *alpha 170 kDa, **beta, betaprime and gamma 98-110 kDa, ***delta 61 kDa (lane 1). The KKLETFSSTN peptide pull down is shown as control (lane 2)

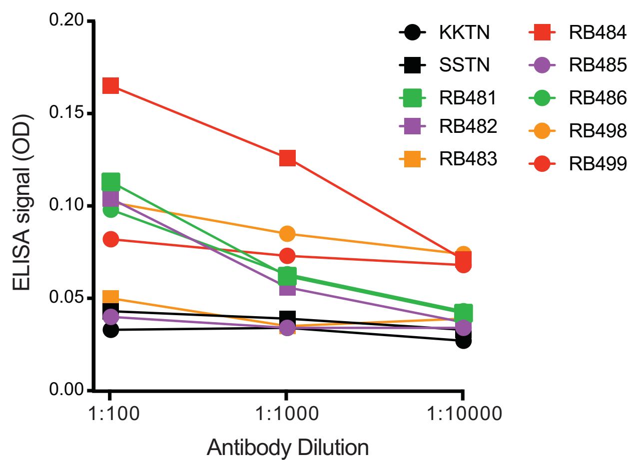

Fig. 2. Specific binding of RRB antibodies to the target KKLETFKKTN peptide loaded with COS7 cell lysate (*i.e.* COPI complex), as detected by ELISA. SSTN indicates the binding of RRB481 to the KKLETFSSTN peptide loaded with COS7 cell lysate and KKTN indicates binding of the same antibody to the unloaded KKLETFKKTN (all other control curves were superimposed).