Psakis, Georgios, Polaczek, Julia and Essen, Lars-Oliver

AcrB et al.: Obstinate contaminants in a picogram scale. One more bottleneck in the membrane protein structure pipeline

Original Citation


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Supplementary figures

S1. MALDI-TOF identification of AcrB (A) and SdhA (B), from the tryptic digestion of the corresponding protein bands in Fig. 1. Colored in blue the peptide sequences which were identified.

S2. Detergent screening of GluP-RGS(His)$_6$ comprising BLR(DE3) E. coli membrane fractions and (NH$_4$)$_2$SO$_4$ solubilization of AcrB. 250-400 µg of inner membranes containing GluP-RGS(His)$_6$, were solubilized in 50 mM Tris-HCl pH 8.5, 10% (v/v) glycerol, 2 mM β-mercaptoethanol in the presence of the selected detergent. Solubilization mixtures were incubated at 4 °C for 3-16 hrs with mixing (1000 rpm). The samples were centrifuged at 30 psi for 15 min using an air-centrifuge (Beckman, ~200,000 g). Solubilized (S) and pelleted (P) fractions were analyzed by SDS-PAGE gel electrophoresis (30 µg - A), Western Blotting (10 µg - B) and Dot-Blots (10 µg - C). (A) Detergent based solubilization of AcrB (open arrow) and of GluP (closed arrow). The following detergents were used: n-dodecyl-β-D-maltoside (DDM) 1.5% (w/v), lauryl-dimethyl-amine oxide (LDAO) 2.0% (v/v), n-octyl-β-D-glucopyranoside (OG) 2.0% (w/v), polyoxyethylene-9-laurylether (C$_{12}$E$_9$) 3.0% (w/v), Triton X-100 (TX-100) 3.35% (v/v), octanoyl-n-methylglucamide (MEGA$_8$) 100 mM, decanoyl-n-methylglucamide (MEGA$_{10}$) 2.4 mM, n-undecyl-β-D-maltoside (NUM) 3.0 mM, n-decyl-D-glucoside (DG) 10 mM, n-decyl-β-D-maltoside (DM) 2.0% (w/v). Only the optimal detergent concentrations are shown. All detergents were purchased from Applichem. CONTR: Control solubilization (buffer without detergent). (B) Western blot of the fractions in A using the anti-RGS(His)$_5$ antibody (Qiagen). (C) Dot blot on selected fractions from A, including n-decyl-β-D-maltoside (DM), and using the anti-
(His)$_5$ antibody (Qiagen). **(D)** Selective solubilization of AcrB (lane 2) from BLR(DE3) *E. coli* membrane fractions harbouring GluP (lane 1), in the presence of 0.2 M (NH$_4$)$_2$SO$_4$. Coomassie brilliant blue staining (lane 3) and silver staining (lane 4) of purified GluP-RGS(His)$_6$ following the 0.2 M (NH$_4$)$_2$SO$_4$ washing.

**S3.** Purification of GluP(His)$_6$ from mixed membrane fractions of *E. coli* K-12 cells, on 12% Coomassie Blue stained SDS-PAGE gels. Lane M, molecular weight markers (Sigma-Aldrich, 2 µg); Lane 1, *E. coli* JM110 membranes containing GluP(His)$_6$ (30 µg); Lane 2, detergent extract of *E. coli* JM110/ GluP(His)$_6$ (30 µg); Lanes 3-4, purified GluP(His)$_6$ (30 µg) from *E. coli* JM110 cells; Lane 5, purified GluP(His)$_6$ (15 µg) from RE707 *E. coli* cells. *E. coli* JM110 cells were grown in minimal media (M9) and RE707 cells in rich media (LB). Open arrows show the position of SdhA (Lanes 3-4) and of AcrB (Lane 5). Closed black arrows show the position of GluP(His)$_6$. Closed red arrows indicate the presence of a small aggregated population of GluP(His)$_6$ migrating at the position of a dimer.