New insight into the interaction of TRAF2 C-terminal domain with lipid rafts microdomains

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In this study we provide the first evidence of the interaction of a truncated-TRAF2 with lipid rafts microdomains. We have analyzed this interaction by measuring the diffusion coefficient of the protein in large and giant unilamellar vesicles (LUVs and GUVs, respectively) obtained both from synthetic lipid mixtures and from natural extracts. Steady-state fluorescence measurements performed with synthetic vesicles indicate that this truncated form of TRAF2 displays a tighter binding to raft-like LUVs with respect to the control (POPC-containing LUVs), and that this process depends on the protein oligomeric state. Generalized Polarization measurements and Spectral Phasor Analysis revealed that truncated-TRAF2 affects the membrane fluidity, especially when vesicles are heated up at physiological temperature. The addition of nanomolar concentration of TRAF2 in GUVs also seems to exert a mechanical action, as demonstrated by the formation of intraluminal vesicles, a process in which ganglioside GM1 plays a crucial role.

Figure 1 – Panel a: binding of alexa-labeled TRAF2 to a POPC-GUV (diameter 20 µm); panel b: visualization of the GUV through the membrane marker CellMask Orange.

Figure 2 – Diffusion coefficients of labeled TRAF2 bound to GUVs. Vesicles were obtained from 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), raft-like mixture (i.e. 1,2-dioleoyl-sn-glycero-3-phosphocholine/sphingomyelin/cholesterol (DOPC/SM/Cholesterol), BBM samples (Kidney Juxtamedullary Cortex (JMC), Kidney Superficial Cortex (SC), Intestinal Duodenum (ID), and Intestinal Jejunum (IJ)) and POPC + 1 % GM1. Data are expressed as mean ± SD values of three independent experiments; * and ** denote p < 0.05 and p < 0.01, respectively, versus TRAF2 bound to POPC - GUVs.
Figure 3 – Laurdan GP values calculated at room temperature (blue and green) and body temperature (red and yellow) for all samples, before (blue and red) and after the addition of non-labeled TRAF2 (green and yellow). Data are expressed as mean ± SD values calculated from at least 15 – 20 GUVs of two different preparations. * and ** denote p < 0.05 and p < 0.01, of green and yellow samples versus the blue and red ones, respectively.

Figure 4 – Panel A: GUVs diameter changes observed after TRAF2 addition (red). Control experiments have been conducted adding KB (black horizontal line) and BSA (green). Points have been connected only to guide the eye. Panel B: Percentage of intraluminal vesicles occurring in GUVs in the absence of TRAF2 (blue, green, cyan), after TRAF2 addition (red) and in control experiments in which KB (purple) or BSA (orange) have been added.