Talin-lipid interaction.

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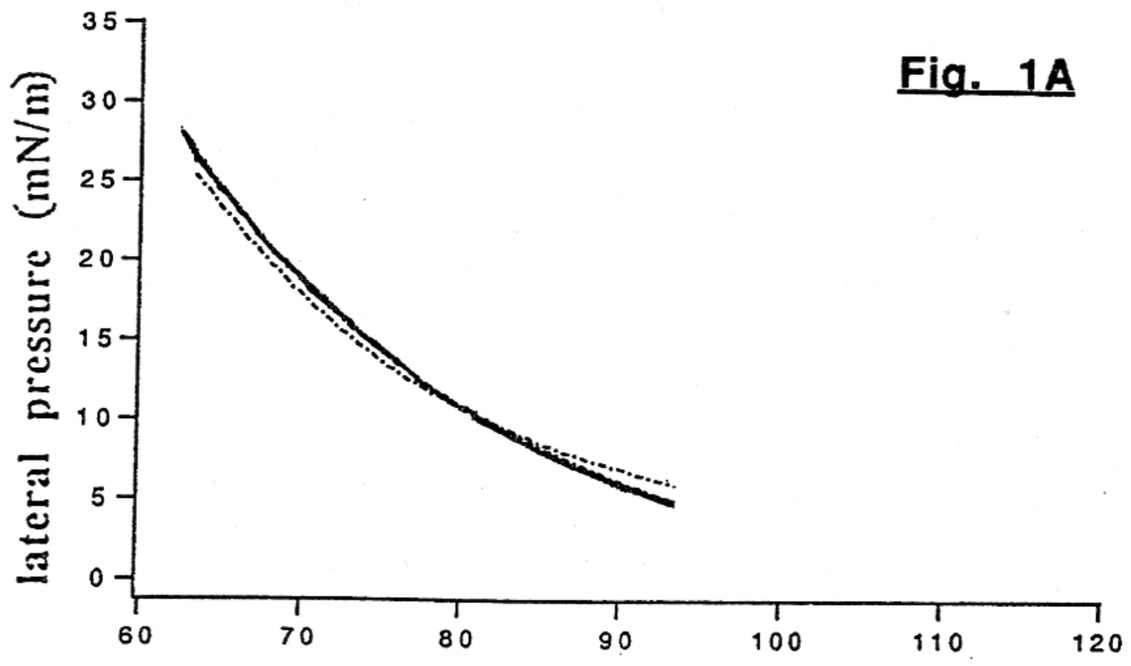
The interaction of many actin binding proteins to cell membrane lipids in vitro has been described. However, the nature of the interaction has only been determined for a few proteins [1]. For talin, Heise et al. using differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) showed that this protein a) interacts electrostatically and hydrophobically with lipids b) is highly selective for negatively charged phospholipids in mixtures and c) binds more strongly to dimyristoylphosphatidylglycol (DMPG) and dimyristoylphosphatidylserine (DMPS) than to dimyristoylphosphatidylcholine (DMPC) [2; 3]. In this study the interaction of DMPG and DMPC to talin is examined by film balance technique.

DMPG and DMPC were purchased from Sigma, Deisenhofen, FRG. Smooth muscle talin was purified as described in [4]. Protein concentration was determined according to Bradford [5] and the purity was analysed on SDS mini slab gels. G-Buffer conditions: 2mM Tris-HCl, pH 7, 0.2mM $CaCl_2$, 0.2mM ATP, 0.2mM DTT, 0.005% NaN_3.

All experiments were performed on a film balance apparatus developed by Gaub et al. [6]. The unit consists essentially of a microscope and a Langmuir trough which is mounted on a motorized x-y translation stage underneath the microscope. The base of the 30ml trough is copper-plated and Peltier elements are installed below for temperature regulation (accuracy ~0.2°C). The top of the trough is covered by a glass slide to protect a spread lipid monolayer from impurities, air convection and fluid condensation. Surface pressure of a solution in the trough is measured by a Wilhelmy system which consists of an inductive displacement transducer. The vertical force detected by the transducer is porportional to the surface pressure at the subphase/air interface. Pressure area diagrams of lipid monolayers (DMPG or DMPC) are obtained by isothermal compression or expansion. Temperature control and scanning speed of the film balance machine and the process of recorded lateral pressure measurements are performed by an IBM-compatible PC-AT, respectively. Hard copies of the data are obtained from an Apple LaserWriter.

First results using this method are shown in Fig. 1A+B. In these experiments both the DMPC and DMPG monolayer have been compressed in the absence and expanded in the presence of talin at 20.5°C. The compression curves (—) for DMPC and DMPG without proteins have been reported elsewhere [7]. The addition of 0.6nM talin to the solutions shows that the curve in each measurement deviates slightly indicating some effect. The expansion traces (····) for DMPC and DMPG in the presence of talin have different points of intersection with the compression curves; for DMPG ~12.6 mN/m and DMPC ~10.5mN/m.

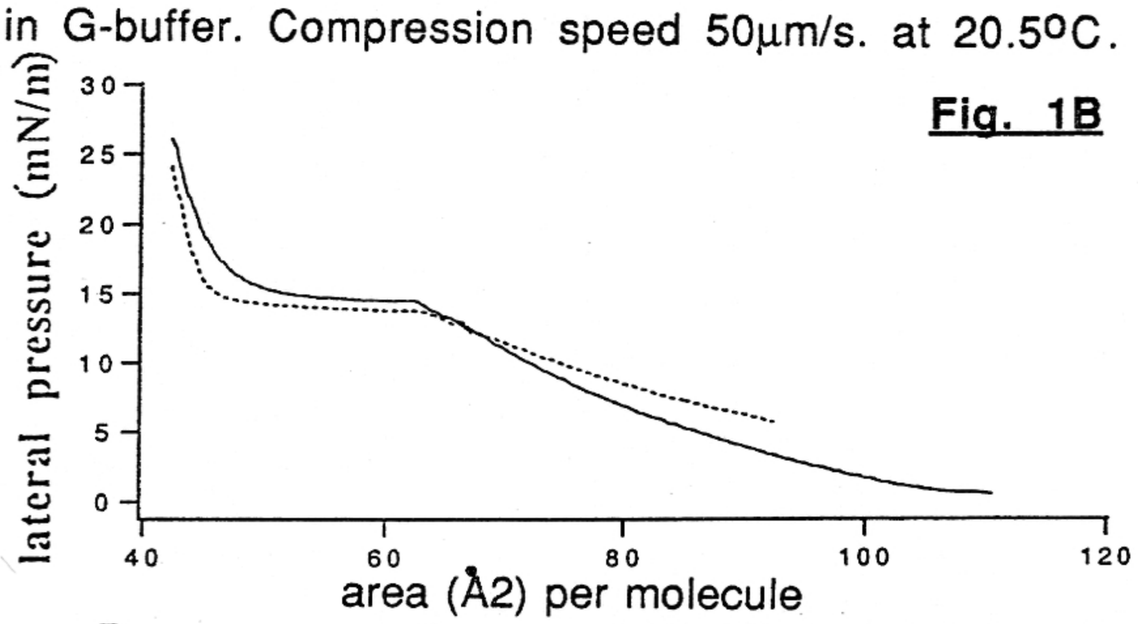
Fig. 1A



area (Å2) per molecule

Pressure area diagram of DMPC and 0.6nM talin in G-buffer. Compression speed 50µm/s. at 20.5°C.

Fig. 1B



area (Å2) per molecule

Pressure area diagram of DMPG and 0.6nM talin in G-buffer. Compression speed 50µm/s at 20.5°C.

These findings are intriguing as these support the assumption made by Heise et al. [2] that interaction forces between DMPG and talin are somewhat stronger than those observed between DMPC and talin. Currently fluorescent markers are being incorporated into DMPG/DMPC and talin. This method would allow binding regions to be idendified by simultaneously using a fluorescent microscope when compressing/expanding the solution [7]. This study was supported the by Sonderforschungsbereich (SFB 266/C5). I thank Ms. H. Kirpal for technical assistance and protein preparations. A special thanks goes to Mr. C. Dietrich for the lipid monolayer preparations and his excellent work on the film balance apparatus.

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