Determination of the affinity of talin and vinculin to charged lipid vesicles: a light scatter study

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Abstract Recent experimental findings have demonstrated that both talin and vinculin bind to phospholipids and insert into the hydrophobic region of lipid membranes. Here, we show that the light scatter method can be used for measuring the affinity of proteins to phospholipid membranes. Large unilamellar DMPC/DMPG vesicles were produced by the extrusion technique (LUVETs). We have used repeated heating/cooling scans between 15°C and 35°C to ensure protein–lipid interaction/insertion. A molar affinity of talin, $K = 2.9 \times 10^6$ M^{-1} and of vinculin, $K = 3.3 \times 10^5$ M^{-1} to lipid vesicles, respectively, was determined from the plot; light scatter signal at 380 nm against protein concentrations by fitting the term, ln $(I_0/I - 1) = A - K \times c$ to the data.

Key words: Lipid binding affinity; Talin; Vinculin; Light scattering

1. Introduction

Talin and vinculin are major protein components of focal adhesions and cell–cell attachments. Both proteins are believed to form with each other and with other components complexes which serve to link filamentous actin to the membrane [1].

Talin in vitro binds vinculin [2], α-actinin [3], interacts with actin [4], promotes actin nucleation [5] and induces an increase in internal F-actin chain stiffness [6]. Further, talin is capable of interacting with phospholipid membranes. The lipid-binding activity of the talin molecule has been demonstrated in vitro by various techniques on lipid mono- and bilayers: differential scanning calorimetry in combination with Fourier transformed infrared spectroscopy [7], hydrophobic photolabeling [8,9] and the film balance technique [10].

Vinculin in vitro binds talin (see above), α-actinin [11], paxillin [12] and filamentous actin [13]. Furthermore, the vinculin molecule inserts into the hydrophobic core of mono- and bilayers as shown in vitro by the film balance technique [14] and in lipid photolabeling experiments [8,15]. The incorporation of talin and vinculin into zwitterionic phospholipid bilayers (PC) is low, however, greatly enhanced in the presence of negatively charged phospholipids (PG, PS).

In the present work, we demonstrate that the light scatter method allows to measure directly the affinity of proteins, here talin and vinculin, to charged lipid vesicles.

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Abbreviations: PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; DMPC, dimyristoyl-L-α-phosphatidylcholine; DMPG, dimyristoyl-L-α-phosphatidylglycerol; LS, light scattering; cps, counts per second.

2. Materials and methods

2.1. Protein preparations

Buffer reagents were purchased from Sigma. Water purified by Millipore Mili-Q-System was used for all buffers. Buffer A: 20 mM HEPES/NaOH, 1 mM EGTA, 1 mM EDTA, 0.2 mM DTT, 40 mM NaCl, 0.005% NaN_3, pH 7.4.

Platelet talin and vinculin were isolated from outdated (not older than 10 days) human thrombozytes as described by Collier and Wang [16]. Talin was further purified by passing it through a gel filtration column as described by Kaufmann et al. [5]. After the first ionic exchange column according to this protocol vinculin was purified by an additional hydroxylapatite column and eluted by a linear gradient from 0.02 M to 0.4 M KH_2PO_4. The purity of the proteins was analyzed on SDS mini slab gels. Protein concentrations were determined according to Bradford [17]. Prior to light scatter measurements talin and vinculin were extensively dialyzed against the vesicle buffer A.

2.2. Lipid vesicle preparation

The phospholipids dimyristoyl-L-α-phosphatidylcholine (DMPC) and dimyristoyl-L-α-phosphatidylglycerol (DMPG) were purchased from Avanti Polar Lipids (Birmingham, USA) and used without further purification.

Lipid stock solutions were prepared by dissolving mixtures (50:50 w/w) of crystalline phospholipids (DMPC, DMPG) in chloroform/methanol, 2:1 (v/v). From aliquots of these solutions a dry lipid film was formed on the walls of a thoroughly rinsed glass beaker by evaporating the solvent with a stream of nitrogen, followed by a vacuum desiccation for at least 2 h. Large unilamellar vesicles by extrusion techniques (LUVETs) with diameters ≤200 nm were generated by pressing the lipid dispersion through the two (stacked) 200 nm Nucleopore polycarbonate filters of an Extruder (Lipex, Canada) 10 times. All extrusion procedures were conducted at least 10°C above the *gel–liquid–crystal* transition temperature. The vesicle solutions formed were slightly turbid.

2.3. Light scatter experiments

Light scattering (LS) was carried out in a Spex Fluorolog 1680 0.22 double spectrophotometer. The cell-holder was thermostatically temperature-controlled by an external water bath (± 0.1°C), and the temperature was measured electronically by a sensor in the cell. A 1 ml four-sided quartz cuvette of 1 cm path length was used in these measurements. Proteins and lipid vesicle solutions were added and gently mixed by hand to prevent the formation of air bubbles. The light scatter signal was recorded at 380 nm at a 90° angle and at a band pass width of 1 nm. This wavelength was chosen in order to avoid interference from phospholipid and protein absorption at 225 nm and 280 nm, respectively. Data were collected on a Spex computer (program DM3000) and later transferred to an Apple Macintosh LC computer and analysed on a commerically available program (IGOR).

Prior to measurements samples were equilibrated at 4°C for 30 min. Talin and vinculin were reconstituted into preformed LUVETs by repeated cycles of heating/cooling between 15°C and 35°C in the Spex Fluorolog. This procedure prevented thermal denaturation of talin and vinculin which starts at ~40°C [8]. The heating/cooling cycles were carried out at a scan rate of 2.4°C/min (Fig. 1).This method allowed direct observation of the influence of talin and vinculin on the lipid

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phase transition at ~23.8°C. The samples were rescanned 5 times. In contrast to measurements with pure lipids, lipids in the presence of talin or vinculin clearly showed visible difference between the first and subsequent scans. After the second to fifth heating scan, the light scattering signals were similar, indicating that the reconstituted lipid/protein system was at equilibrium.

3. Results and discussion

In order to examine the effects of proteins on the melting behaviour and on the order of phosholipid membranes, the phase transition of LUVETs containing different concentrations of talin or vinculin was examined by heating/cooling cycles between 15°C and 35°C in a spectrophotometer. Fig. 2 shows the LS amplitude changes which reflect the main-phase *gel-to-liquid-crystalline* transition of DMPC/DMPG (50:50; w/w) LUVETs at different protein concentrations for vinculin and talin. The averaged traces with increasing protein concentrations reveal a gradual reduction in LS signal amplitude at 380 nm. At a talin/lipid molar ratio of 1:2150 (Fig. 2, bottom trace) and at a vinculin/lipid molar ratio of 1:350 (data not shown) the amplitude change around 23.8°C could hardly be observed above the baseline noise. These results — with respect to the LS signal amplitude reduction — can be interpreted in terms of conformationally inhibited lipid molecules adjacent to bound talin or vinculin which therefere are unable to undergo a phase transition (cf. [18]). In the experiments presented here, we have determined the molar affinity of talin and vinculin to charged phospholipid vesicles by light scattering. We have measured the light scatter signal at 380 nm of the solutions before, I_o and after, I the main phase transition at ~23.8°C and plotted the data against various protein concentrations, using the following linear equation:

$$\ln (I_o/I - 1) = A - K \times c$$

where A is the ordinate intercept, K is the slope, and c is the protein concentration of the solutions. From the best linear fit

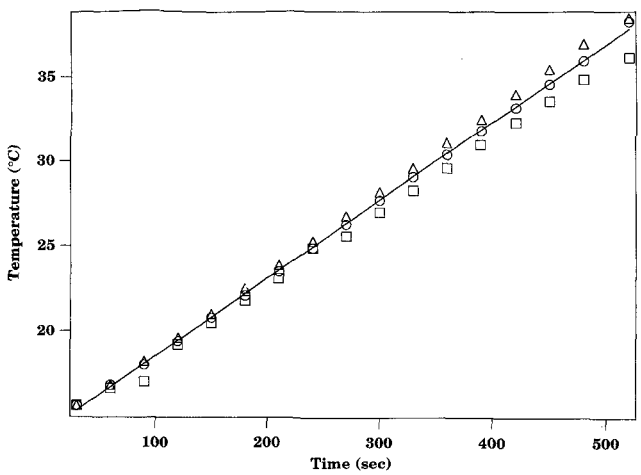

Fig. 1. Determination of the heating rate of a 1 ml Buffer A solution in a quartz cuvette. The temperature is maintained (± 0.1°C) by an external heater and electronically controlled by a sensor in the cell. Buffer A: 20 mM HEPES/NaOH, 1 mM EGTA, 1 mM EDTA, 0.2 mM DTT, 40 mM NaCl, 0.005% NaN_3, pH 7.4. Slope of the computer fitted line = 2.4°C/60 s. The symbols represent data points for three different experiments.

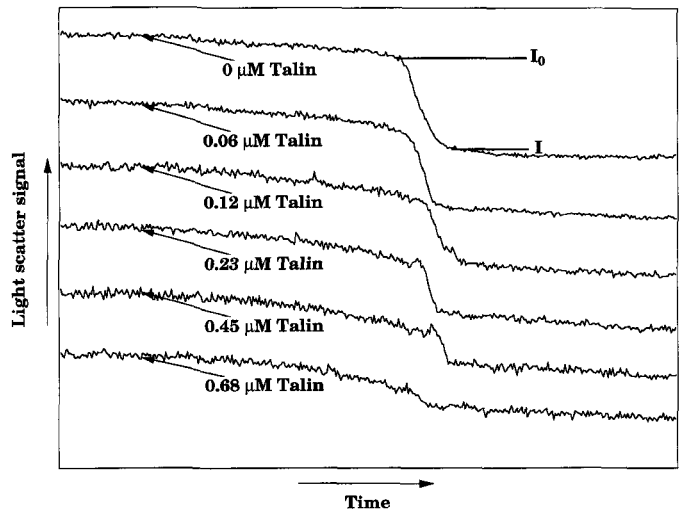

Fig. 2. Temperature-induced changes in light scatter signal between 15°C and 35°C at a rate of 2.4°C/60 s of a 1 ml solutions containing: Buffer A, 1.46 mM DMPC/DMPG (50:50; w/w) and 0–0.68 μM talin. I_o, and I, indicate the start and end of the signal at 380 nm of the lipid (= main phase transition of DMPC/DMPG) at ~23.8°C. The traces present an average of 4 heating scans. The signal unit: (cps) = counts per second.

to the data a molar affinity to charged lipid vesicles, $K = 2.9 \times 10^6$ (S.D. $\pm 0.21 \times 10^6$) M^{-1} for talin (Fig. 3) and $K = 3.3 \times 10^5$ (S.D. $\pm 0.63 \times 10^5$) M^{-1} for vinculin (Fig. 4) was calculated.

The difference in affinity observed between talin/lipid vesicles and vinculin/lipid vesicles compares well with data achieved by the hydrophobic lipid photolabeling technique [8]: here, lipid membrane insertion of talin and vinculin was probed by a photoactivatable lipid analogue. Talin and vinculin incorporated 0.1–0.3.% and 0.1–0.2% of total label, respectively, upon incubation and photolysis with charged phospholipids containing trace amounts of the photoactivatable phospholipid [^{3}H]PTPC/11.

In control experiments, protein interaction with and/or insertion into lipid vesicles was tested, using 1–4μM ovalbumin. We observed a ± 10% change in LS signal (ln (I_o/I−1)) compared to pure lipid vesicles (Figs. 3,4, dotted line), indicating that ovalbumin incorporates insignificantly into lipid vesicles. Interestingly, in lipid photolabeling control experiments isolated rat IgG, carbonic anhydrase and ovalbumin also incorporated only ~1/10th of the total label compared to talin (cf. [8,9]).

Using differential scanning calorimetric (DSC), it was previously reported [19] that one possible effect of proteins on the thermotropic properties of lipid vesicles is reflected by a shift of the chain melting onset temperature, T_s, i.e. the solidus line to lower temperatures. In general, a low temperature shift of the solidus line, T_s, is indicative of a weak hydrophobic interaction of proteins with DMPC/DMPG LUVETs. A possible explanation for the origin of this phenomenon in membranes is phase separation. This corresponds to the segregation of membrane-bound talin or vinculin into protein-rich domains in the membranes, with the concomitant formation of protein-poor domains. In protein-rich domains the *trapped* lipid is considered as part of an eutectic protein–lipid complex, existing below the phospholipid phase transition.

As shown in Fig. 2 talin most likely interferes with the lipid

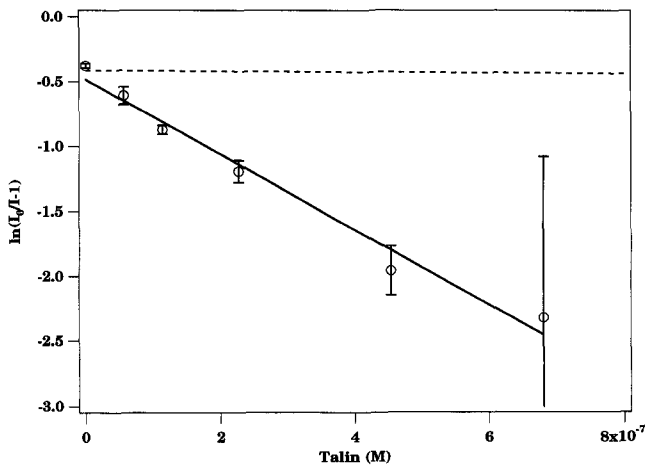

Fig. 3. Plot of observed light scatter signals ln $(I_0/I - 1)$ at ~23.8°C and 380 nm against buffer A containing various talin and 1.46 mM DMPC/DMPG (50:50; w/w) concentrations. The line represents the best fit to the data and gives a slope, $K = 2.9 \times 10^6$ M^{-1} (S.D. $\pm 0.21 \times 10^6$ M^{-1}; $n = 4$). In control experiments (performed under identical conditions, using various ovalbumin and 1.46 mM DMPC/DMPG (50:50; w/w) concentrations)) the light scatter signals are indicated by the dotted line. Note: the linear equation ln $(I_0/I - 1)$ was used because of the relation.

vesicle packing and order. This suggests a partial incorporation of the protein into the lipid layer. The use of charged phospholipid vesicles gives further evidence that the disturbing effect of talin on the chain melting behavior is enhanced which is probably due to additional forces of either pure electrostatic or electrostatic/hydrophobic character (cf. [10]). Heise et al. [7] specifically demonstrated in their DSC study that the presence of talin dramatically changed (at protein/lipid molar ratios between 1:6700 and 1:1400) the solidus, liquidus lines and transition amplitudes, indicating partial penetration into the hydrophobic region of the lipid membrane. At talin/lipid molar ratios >1:1400 changes in ΔH could not be deconvoluted. Similarly,

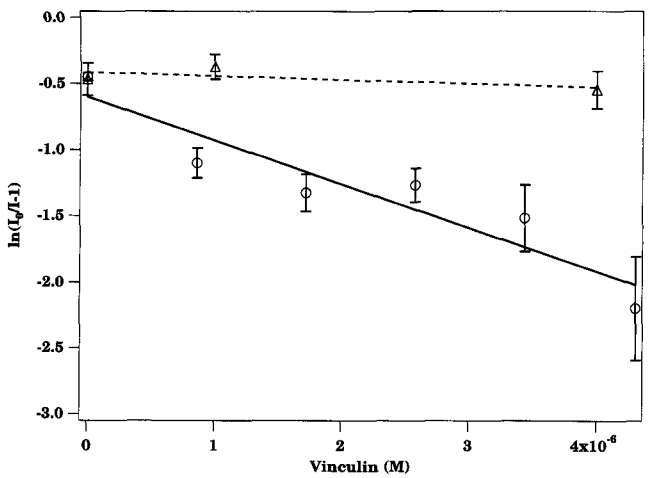

Fig. 4. Plot of observed light scatter signals ln $(I_0/I - 1)$ at ~23.8°C and 380 nm against buffer A containing various vinculin and 1.46 mM DMPC/DMPG (50:50; w/w) concentrations. The line represents the best fit to the data and gives a slope, $K = 3.3 \times 10^5$ M^{-1} (S.D. $\pm 0.63 \times 10^5$ M^{-1}; $n = 4$). Control experiments are indicated by the dotted line; for conditions see Fig. 3.

analyses of the light scatter signal changes failed at talin/lipid molar ratios of >1:2150.

In this work we have demonstrated that the light scatter technique can be used as an effective tool for the study of temperature-dependent protein–lipid interactions/insertions. Using this method we confirm previous observations by DSC and lipid photolabeling [7,8,9] that talin and vinculin insert into the hydrophobic core of lipid membranes and provide for the first time values of the molar affinity of talin to acidic phospholipid vesicles. Thus, for the interaction of vinculin with acidic phospholipids a higher dissociation constant, between 5–10 μM, has previously been reported, using the gel filtration assay [20].

Meanwhile, we have also determined in a computer calculation study the hydrophobic stretches of these proteins. These results are published in detail elsewhere [21]. In short: specific interactions of amino acid regions (a) 21–39, 287–342 and 385–406 of mouse talin and 348–364 of *Dictyostelium* talin (NH_2-terminal region), and (b) 935–978 and 1020–1040 (COOH-terminal region) of chicken embryo vinculin and nematode vinculin are suggested with acidic phospholipids.

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