Direct visualization of lipid vesicle changes on addition of filamin.

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Filamin -a dimeric molecule of ~500.000 MW- is highly flexible, posseses at each end actin binding sites and is distributed in stress fibres and alpha-actinin-rich dense bodies of smooth muscles and in non-muscle cells [1, 2]. Its physiological role and binding to lipid membranes, however, is not yet lucid. Here, we report the interaction of purified filamin with reconstituted lipid vesicles in solution.

Dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglyerol (DMPG) were purchased from Sigma. Lipid vesicle preparation of DMPG/DMPC at a ratio of 1:1 was carried out by the method in [3]. Filamin was isolated from chicken gizzard [4] and further purified as described in [5]. Experimental buffer condition: 2mM Tris-HCl pH 8.0; 0.2mM $CaCl_2$, 0.2mM ATP, 0.2mM DTT, 0.005% NaN_3.

Differential scanning calorimetry (DSC) was performed on an MC-2 apparatus from Microcal (Amerst, MA, USA). Data storage and analysis were carried out on an IBM-AT computer using DA-2 software, also provided by Microcal. Before each experiment, samples were precooled on ice. The heating scan was started after 20 min of temperature equilibration at a rate of 90°C/h with a 15 sec time increment between each data collection.

Lipid vesicles were observed by phase contrast microscopy using a Zeiss Axiovert 10 inverted microscope equipped with a phase contrast air objective of 40 x magnification (Zeiss 40 x Ph 2 nA = 0.75). For documentation the microscope was equipped with a CCD-camera with 604 x 588 pixels and a video recorder [6].

Recording the specifc heat capacity (C_p) of a lipid vesicle solution (DMPG/DMPC of 1 : 1 at 1mg/ml) in the absence of filamin with temperature in the calorimeter, we observed two transitions which peaked at ~23°C and at ~25°C. These phase changes were first believed to be due to separation of the lipid components in solution. Thus, corresponding microscopy of the same solution showed fractions of smaller (densely packed) and bigger (lightly packed) vesicles. Incubating increasing concentrations of filamin (>0.4μM) with DMPC/DMPG vesicles and scanning these solutions in the calorimeter, however, resulted in the dissappearance of the first peak (~23°C) and in a broadening of the base of the second peak (~25°C) with an insignificant temperature shift. This intriguing and very reproducable observation by (DSC) led us assume that filamin when present in solution acts as a possible fusinogen at the lipid surface of smaller (densely packed) vesicles which could be attracted by filamin to form bigger (less densely packed) vesicles. In order to confirm this hypothesis we used phase contrast microscopy of lipid-filamin solutions. Results from these experiments were, however, different to our assumption. Not the packing of vesicles was changed, but surprisingly enough the surface structure of these (from an undulated to a more rounded, smooth surface). (see Fig. 1)

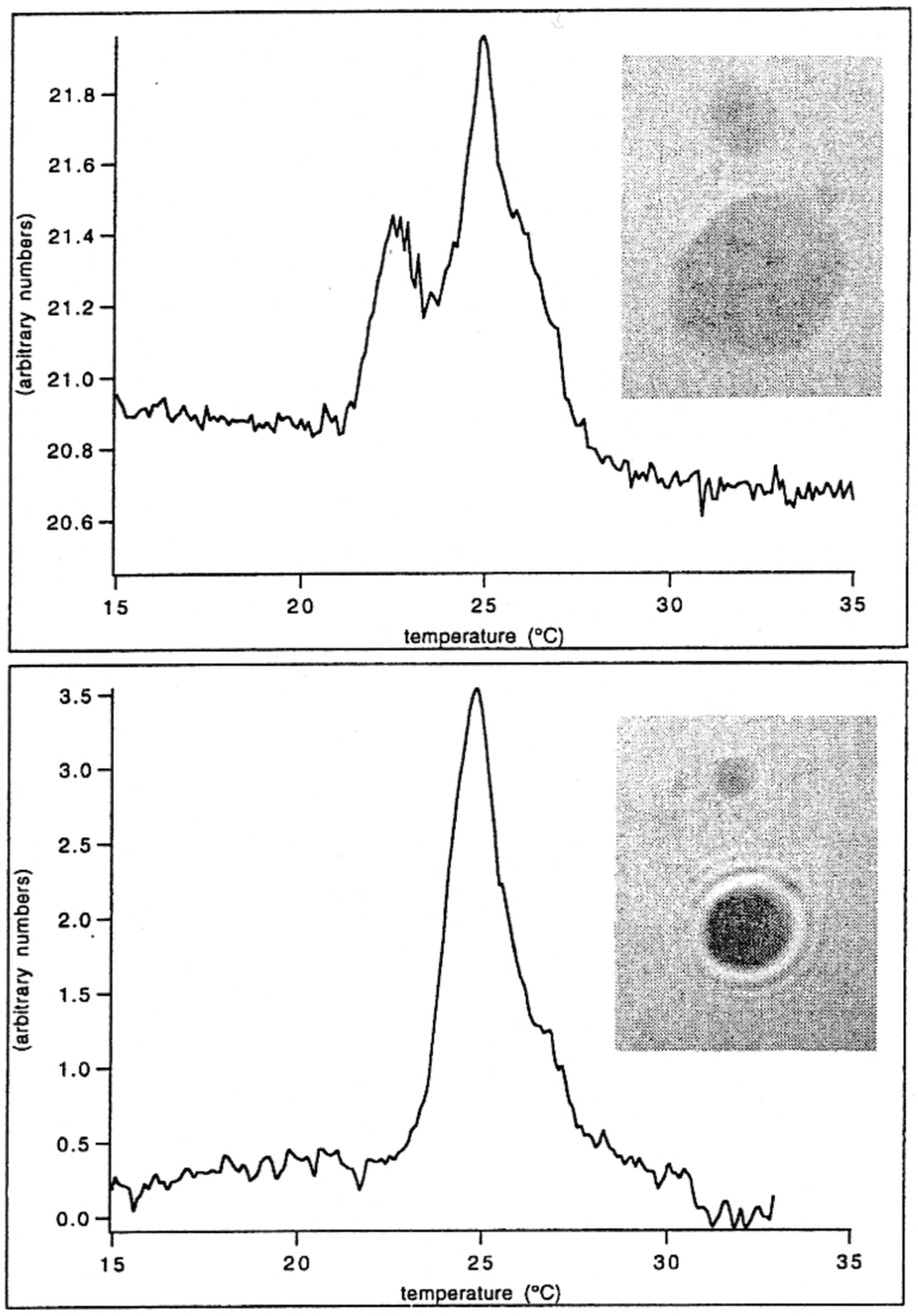

Figure legend: Top trace of 1mg/ml DMPG/DMPC at 1:1 (-) filamin; bottom trace (+) 0.48μM filamin scanned in (DSC) from 15°C - 35°C. (C_p) in arbitrary numbers. Inset: Hard copies from phase microscopy.

These changes could only be observed at filamin concentrations >0.4μM which might be the consequence of a high (commulative) charged effect of the protein with the polar head groups of the phospholipid vesicles. This kind of interaction where various forces (Coulomb, van der Waals and ondulation) act independently and antagonistically, has to be strong in order to remove hydration water from the polar head groups of the vesciles. The binding of filamin to the surface of the vesicle is more likely compared to an incorporation into the lipid membrane from the analysis of (DSC) traces. The observed negligible change in temperature of the second peak and the broadening of its base towards higher temperatures are indicative of (lipid surface)-protein attachment.

Future work will, therefore, involve other techniques (EM, fluorescence labelling) under various buffer conditions in order to substantiate these observations. This work was supported by the Deutsche Forschungsgemeinschaft. We thank Ms. H. Kirpal for protein preparations.

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