Ca^{2+}-independent interaction of annexin I with phospholipid monolayers

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Abstract At pH 6.0, the interaction of annexin I, a proteolytic fragment of annexin I and annexin V, was studied with monolayers composed of dipalmitoylphosphatidylserine (DPPS), dipalmitoylphosphatidylcholine (DPPC) or DPPS/DPPC mixtures (molar ratio 1:4). The measurements reveal that only annexin I shows a significant increase in the surface pressure at constant surface area in the absence of Ca^{2+} ions. We interpret these pressure changes as reflecting penetration of the protein. Kinetic analyses of the annexin I/monolayer interaction at pH 6.0 in the presence and absence of Ca^{2+} ions show differences between the interaction mechanisms that support the occurrence of a pH-regulated process. At pH 7.4, Ca^{2+} ions are required for the interaction.

Key words: Annexin I; Calcium; Phospholipid monolayer; Surface pressure/area isotherm; Protein penetration

1. Introduction

The annexins are a class of Ca^{2+} regulated, phospholipid and membrane binding proteins, which are assumed to participate in various membrane related events such as exocytosis, endocytosis and membrane organization (for review see [1]). The typical annexin structure is characterized by a conserved core domain and a variable N-terminal domain. The core domain consists of four or eight repeats of a 70 amino acids sequence forming five α-helices. These α-helices are arranged in two helix-turn-helix motifs and a connecting helix lying perpendicular to the others. The overall shape of the annexin core domain is a curved disc, with the Ca^{2+} binding sites located at the convex side and the N-terminal region on the concave side [2–4]. Binding of annexins to membranes is generally assumed to be Ca^{2+}-dependent, peripheral in nature and to function via a 'Ca-bridge', in which the Ca^{2+}-ion is coordinated by polar headgroups of the phospholipid molecules and by ligands from the protein [5]. Despite this general principle and despite the structural homology of the annexins which has been revealed by X-ray crystallography [6,2,7–9], different annexins show also specific interactions with calcium ions, phospholipid vesicles and biological membranes.

Annexin I is a member of the annexin family capable of aggregating and even fusing membrane vesicles [10–12]. Within the annexin family this activity is only shared by annexin II provided that it resides in the heterotetrameric complex with its S100A10 (p11) protein ligand [13]. In view of the structural organization of the annexins such specificity is likely to be associated with the unique N-terminal domain. Support for this hypothesis comes from vesicle aggregation/fusion experiments employing chimeric annexins. A derivative containing the N-terminal domain of annexin I (residues 1–45) and the core domain of annexin V (residues 19–320), named I_NV_C, is capable of mediating vesicle aggregation whereas authentic annexin V is not [14,15]. A mechanistic basis for the fusogenic activity of annexin I could be the penetration and/or perturbation of the membrane by the protein. To test this hypothesis we analyzed the interaction of annexin I with phospholipid monolayers which have been well established as model systems for biological membranes (for review see [16]). Information on specific interactions between proteins and lipids can be derived from measurements of the surface pressure of the phospholipid monolayers. Generally, a protein induced increase in surface pressure at constant surface area has been interpreted generally as indication of a penetration of the monolayer by the protein [17,18]. Using this approach, the annexin V interaction was shown to be peripheral in nature and to depend on the presence of Ca^{2+} [19].

The monolayer measurements performed here reveal a Ca^{2+} independent interaction of annexin I with the membrane which results in a distinct surface pressure increase. This activity is pH dependent and not observed for an annexin I core or annexin V. We also recorded the penetration kinetics of annexin I at pH 6.0 and pH 7.4 both in the absence and in the presence of Ca^{2+} ions. In contrast to what is observed at pH 6.0, Ca^{2+} ions are absolutely required at pH 7.4 to obtain the same increase in surface pressure as at the lower pH value. Our results reveal the importance of pH changes in regulating annexin-membrane interactions and point to a role of annexin I in the organization of membrane domains.

2. Materials and methods

2.1. Lipids

Dipalmitoylphosphatidylserine (DPPS) and dipalmitoylphosphatidylcholine (DPPC) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). The lipids were more than 99% pure and were used without further purification.

2.2. Proteins

Recombinant porcine annexin I was purified according to Rosengarth et al. [20]. The protein concentration was determined by UV absorption using an absorption coefficient ε_{280nm} of 0.6 cm^2 mg^{-1} [21]. Purity, integrity and secondary structure of the protein were analyzed by SDS-PAGE and CD-spectroscopy. Recombinant human annexin V purified according to Burger et al. [22] was kindly provided by C.

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Abbreviations: CD, circular dichroism; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; ε_{280nm}, molar extinction coefficient at 280 nm; DPPS, dipalmitoylphosphatidylserine; DPPC, dipalmitoylphosphatidylcholine

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Jatzke (Institut für Physikalische Chemie, WWU Münster, Germany). Lung porcine annexin I and a proteolytic fragment of annexin I truncated in the N-terminal domain were kindly provided by J. Seemann (ICRF, London, UK; [23]).

2.3. Monolayer measurements

Penetration kinetics and surface pressure/area isotherms (Π-A-isotherms) were measured at 20°C with a PC-controlled Riegler and Kirstein Langmuir film balance. The apparatus is equipped with a Wilhelmy plate surface tension measuring system [24]. The teflon trough of the film balance (42 cm^2) has a small hole allowing the addition of protein solution into the subphase. The subphase buffers (V = 20 ml) contained 50 mM MES/NaOH, 100 mM NaCl, pH 6.0, or 10 mM imidazole/HCl, 100 mM NaCl, pH 7.4, with or without 1 mM $CaCl_2$. For optimal mixing, the subphase was stirred by a magnetic stirrer during the experiments. Lipid films were prepared by spreading with a Hamilton syringe onto the respective buffer, respectively, 10 μl of a solution of DPPC in chloroform (0.44 mM), 10 μl of a solution of DPPS in chloroform/methanol (molar ratio 1:3) (0.38 mM) or 10 μl of a 0.4 mM mixture of DPPS/DPPC (molar ratio 1:4) in chloroform/methanol (molar ratio 1:3). After 30 min, the monolayer was compressed to an initial surface pressure of 10 mN/m. The stability of the film was then controlled for 5 min before the experiments were started. The protein was then injected into the subphase to final concentrations of 22–30 nM, and the increase in the surface pressure at constant area was recorded as a function of time until no further increase could be detected. Subsequently, the film was expanded and two cycles of compression to 35 mN/m and expansion were performed to elucidate whether the final pressure observed in the kinetic run reflected an equilibrium situation. A third compression up to 50–55 mN/m was recorded to prove if annexin I is squeezed out of the monolayer under these high surface pressures.

The surface activity of annexin I was analyzed by injecting the protein into the subphase (50 mM MES/NaOH, pH 6.0) to a final concentration of 24 nM. When no further increase in surface pressure of the annexin I monolayer could be detected, the compression and expansion isotherms of the protein film were recorded.

3. Results

3.1. Penetration and interaction kinetics of various annexin species at pH 6.0 in the absence of Ca^{2+} ions

Monolayers of DPPS, DPPC or DPPS/DPPC (molar ratio 1:4) were adjusted to an initial surface pressure of 10 mN/m. Subsequently, the increases in surface pressure (ΔΠ) that resulted from the injection of annexin I purified from porcine lung or transformed bacteria (A), a proteolytic fragment of annexin I (B), and annexin V (C) were recorded as a function of time (Fig. 1). As the penetration kinetics of bacterially expressed annexin I proved to be identical to those of annexin I from porcine lung for all monolayers tested the latter data are not included in the figures shown. The choice of pH = 6.0 in the subphase buffer resulted from previous stability studies. They had revealed that annexin I exhibits the highest thermodynamic stability between pH 5.0 and 6.0 [20]. As a remarkable result we observed that annexin I induces an increase in the surface pressure of all different phospholipid monolayers studied despite the absence of Ca^{2+} (Fig. 1A). The increase in Π is the same for the three different monolayer compositions, although for DPPC monolayers the velocity of the reaction is clearly reduced as compared to the DPPS and the DPPS/DPPC monolayers. In contrast to what is observed for full length annexin I, the proteolytic fragment lacking the N-terminus shows no interaction with DPPC and only a reduced interaction with the DPPS and DPPS/DPPC monolayers (Fig. 1B). Rather than representing an incorporation into the monolayer this residual effect could be due to electrostatic interactions, because the isoelectric point (p*I*) of this truncated

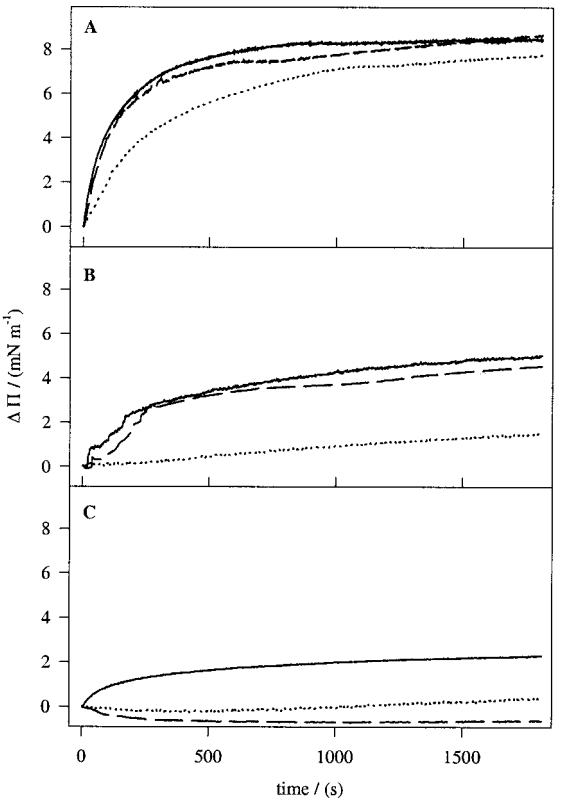

Fig. 1. Annexin penetration kinetics in the absence of Ca^{2+} ions into DPPS (solid lines), DPPC (dotted lines) and DPPS/DPPC (molar ratio 1:4) monolayers (dashed lines). A: Annexin I; B: the proteolytic fragment of annexin I; C: annexin V. The subphase buffer was 50 mM MES/NaOH, 100 mM NaCl, pH 6.0. The initial surface pressure (Π_i) was 10 mN/m and the protein concentrations were 22 nM in all measurements.

annexin derivative is approximately 8.0. Full length annexin I, on the other hand, has a p*I* of 6.4 and the interaction with DPPC was relatively fast and resulted in the same final pressure as observed with the negatively charged lipids. This suggests a specific interaction between annexin I and phospholipids which is not only of electrostatic nature but also based on hydrophobic interactions. Only negligible Ca^{2+} independent interactions with phospholipids are observed with annexin V. This annexin causes only a slight increase in Π of the DPPS monolayers and shows no interaction with pure DPPC or the mixed DPPS/DPPC monolayers (Fig. 1C). The comparison of the kinetics of all three annexin species shows that the observed interaction of annexin I with phospholipid monolayers is complex. However, it is evident that the N-terminal domain of annexin I plays a prominent role in the overall penetration reaction.

3.2. Penetration kinetics of annexin I at pH 6.0 and 7.4 in the presence of Ca^{2+} ions

To study the influence of Ca^{2+} on the interaction of annexin I with DPPS/DPPC (molar ratio 1:4) monolayers we recorded penetration kinetics of annexin I at pH 6.0 and pH 7.4 in the presence and absence of Ca^{2+} ions using an initial surface pressure of 15 mN/m (Fig. 2A). While a significant increase in Π is observed at pH 6.0 in the absence of Ca^{2+} a shift to pH 7.4 basically abolishes this interaction. However, injection of a $CaCl_2$ solution into the buffer to yield a sub-

phase Ca^{2+} concentration of 1.5 mM results in a rapid surface pressure increase to the value that was obtained at pH 6.0 in the absence of Ca^{2+}. In a control it was shown that the injection of $CaCl_2$ alone has no effect on the surface pressure of the monolayer (data not shown). These results demonstrate that at pH 7.4 Ca^{2+} ions are essential for the interaction of annexin I with the membrane.

However, at pH 6.0 the presence of Ca^{2+} ions has an inhibitory effect on the velocity of the interaction of annexin I with DPPS/DPPC (molar ratio 1:4) monolayers. This is shown in Fig. 2B. The interaction is significantly slower in the presence of Ca^{2+} than without Ca^{2+}. Such reduction in the initial velocity could be due to Ca^{2+} binding to the annexin I molecule associated with decreased affinity for the membrane. This effect could result from conformational changes or some steric hindrance. The penetration kinetics of annexin I has been evaluated according to different models and is discussed in Section 3.3. The results support the hypothesis that different mechanisms govern the lipid interaction in the presence and absence of Ca^{2+} ions.

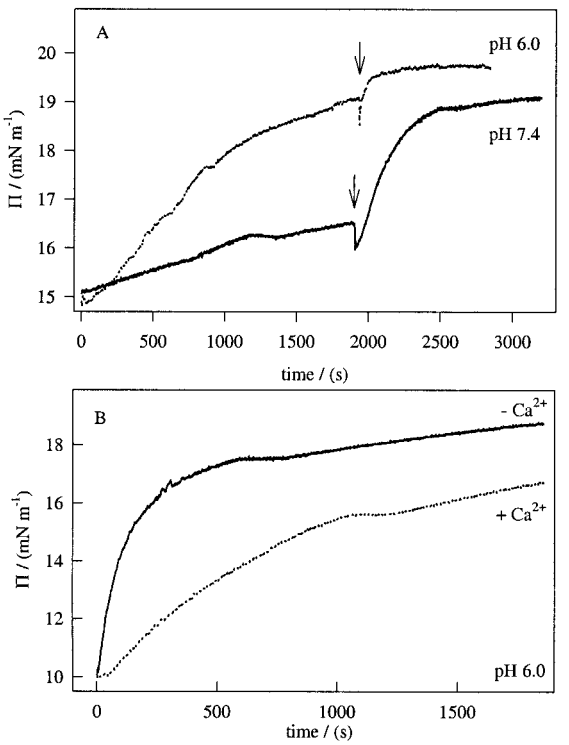

Fig. 2. A: Penetration kinetics of annexin I using DPPS/DPPC (molar ratio 1:4) monolayers at pH 6.0 and pH 7.4. The initial surface pressure was 15 mN/m. The arrows indicate the time of injection of a $CaCl_2$ solution into the subphase. The final Ca^{2+} concentration was 1.5 mM. The subphase buffers were 50 mM MES/NaOH, 100 mM NaCl, pH 6.0 and 10 mM imidazole/HCl, 100 mM NaCl, pH 7.4 and the protein concentrations were 30 nM. Due to the higher initial surface pressure this increase is somewhat slower than that shown in Fig. 1A. B: Penetration kinetics of annexin I into DPPS/DPPC (molar ratio 1:4) monolayers at pH 6.0 in the presence and absence of Ca^{2+} ions. The initial surface pressure was 10 mN/m and the protein concentration was 22 nM. The subphase buffers were 50 mM MES/NaOH, 100 mM NaCl, pH 6.0 ($-Ca^{2+}$) and 50 mM MES/NaOH, 100 mM NaCl, 1 mM $CaCl_2$, pH 6.0 ($+Ca^{2+}$).

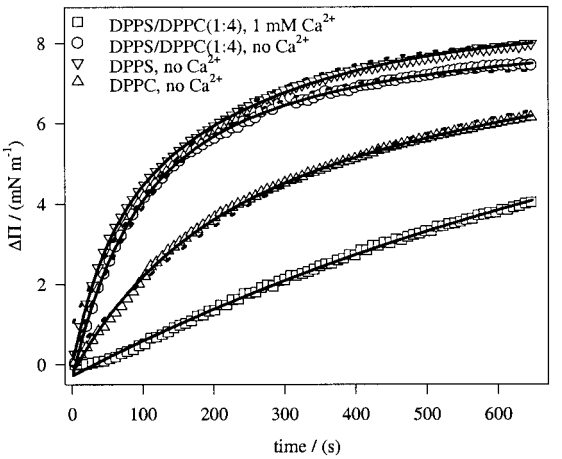

Fig. 3. Evaluation of the penetration kinetics of annexin I into different phospholipid monolayers at pH 6.0 in the presence and absence of Ca^{2+} ions. The symbols refer to the measurements shown in Fig. 1A up to 10 min. The solid lines refer to the sequential fits (Eq. 1) and the dotted lines to the fits according to a first-order reaction (Eq. 2). The fits of the kinetics in the presence of Ca^{2+} ions are superimposed.

3.3. Kinetic analyses of the annexin I/monolayer interaction at pH 6.0 in the presence and absence of Ca^{2+} ions

The penetration kinetics of annexin I into DPPS, DPPC and the DPPS/DPPC (molar ratio 1:4) monolayers at pH 6.0 in the presence and absence of Ca^{2+} ions (Fig. 3) were analyzed according to the model of a sequential reaction:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C.$$

The resulting fit equation is:

$$\Pi = \left(a + \frac{(b \cdot k_1 - c \cdot k_2)}{(k_2 - k_1)} \cdot \exp(-k_1 \cdot t)\right) + \frac{k_1}{(k_2 - k_1)} \cdot (c - b) \cdot \exp(-k_2 \cdot t) + c. \quad (1)$$

The constants k_1, k_2, a, b and c are fit parameters. k_1 and k_2 are apparent first-order rate constants for the individual reactions, and a, b and c are parameters describing the initial, the intermediate and the final states of the overall reaction. For comparison the data were also fitted to a simple first-order reaction mechanism:

$$A \xrightarrow{k_1} B.$$

In this case we used the following fit equation:

$$\Pi = c + (a - c) \cdot \exp(-k_1 \cdot t) \quad (2)$$

with a, c and k_1 as fit parameters. a and c are the initial and the final surface pressure of the monolayer and k_1 the apparent first-order rate constant. The results are shown in Fig. 3 and the parameters resulting from the fits are summarized in Table 1. The solid lines in Fig. 3 refer to fits according to the sequential mechanism and the dotted lines refer to fits according to the simple first-order reaction. The agreement of the experimental and the fitted curves shows that the sequential model represents the best fit for the interaction in the absence of Ca^{2+} ions. When Ca^{2+} ions are present the kinetics of the

penetration reaction is adequately described by one first-order reaction.

In the model of the sequential reaction the first reaction from *A* to *B* could be due to the 'docking' of annexin molecules to the surface of the phospholipid monolayers. This 'docking' results in a perturbation of the monolayer and therefore to a small increase in the surface pressure. The second reaction from *B* to *C* could reflect the incorporation of the protein yielding an additional surface pressure increase. The very small magnitude of k_2 in the presence of Ca^{2+} ions would then indicate that in this case the incorporation of annexin I into the monolayer does not occur or is significantly slower.

3.4. Compression and expansion isotherms of various phospholipid monolayers on subphases containing annexin I

Compression/expansion isotherms of DPPS, DPPC and DPPS/DPPC (molar ratio 1:4) monolayers on a subphase containing 50 mM MES/NaOH, 100 mM NaCl, pH 6.0 plus or minus annexin I are shown in Fig. 4. Despite the absence of Ca^{2+} ions which had been considered to be essential for the interaction between annexins and phospholipid membranes [5] we observed a strong influence of annexin I on the phase behavior of all monolayers tested. When comparing the form of the Π/area isotherms in the presence and in the absence of annexin I, one difference is particularly evident. This is the large increase in the area per lipid molecule at a given surface pressure Π when annexin I is present in the subphase. The effect is particularly pronounced for the DPPS monolayer. It can be interpreted as reflecting a strong electrostatic component of the interaction. We also observed an increase in the area per lipid molecule in DPPS/DPPC (molar ratio 1:4) monolayer isotherms at pH 6.0, when instead of full length annexin I its proteolytic fragment or annexin V were present (data not shown). However, the interaction kinetics of these annexin species showed only little, if any, tendency of penetration. Generally, the compression isotherms of all annexin species tested show the following pattern. Annexin is squeezed out of the lipid monolayer during compression up to 35 mN/m or even higher surface pressure values but it is inserted again during the expansion cycle, once the surface pressure falls below 20 mN/m.

As discussed below the apparent discrepancy between the penetration kinetics and the isotherms of the different annexin species could be due to the intrinsic surface activity of annexin I.

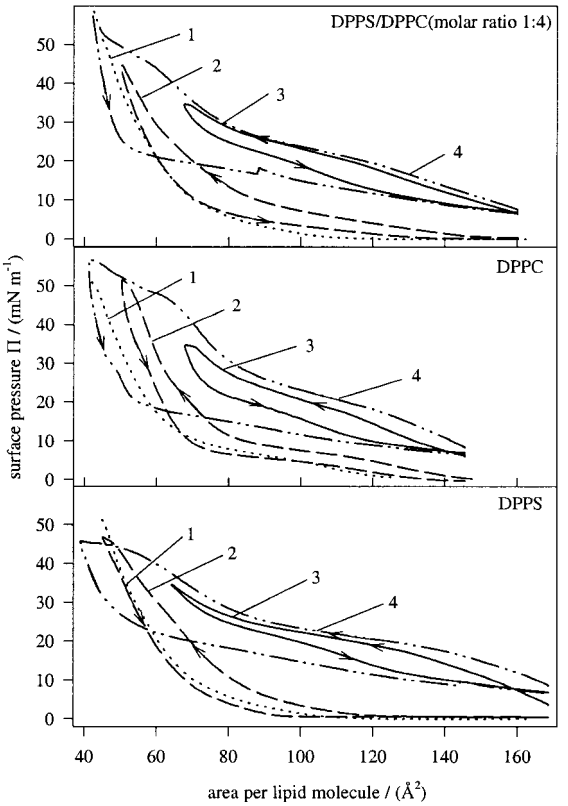

Fig. 4. Surface pressure (Π)/area isotherms of DPPS/DPPC (molar ratio 1:4), DPPC and DPPS monolayers on various subphases at 20°C. The dotted lines (1) show the isotherms of the pure lipid films on water, the dashed lines (2) show the pure lipid films on the buffer (50 mM MES, 100 mM NaCl, pH 6.0), the solid lines (3) refer to the first and the dashed/dotted lines (4) to the third compression and expansion cycles of the monolayers on buffer subphases containing annexin I. The arrows indicate the compression (↑) and the expansion (↓) of the monolayers. Note that annexin I is squeezed out of the monolayer when high pressure values are reached (above 45 mN/m) which is evident from the expansion of the third cycle where the isotherm looks like the one of the pure lipid.

Table 1
Evaluation of the penetration kinetics of annexin I into DPPS, DPPC and DPPS/DPPC (molar ratio 1:4) monolayers in the absence and presence of Ca^{2+} ions

	Monolayer	k_1 (s^{-1})	k_2 (s^{-1})	*a*	*b*	*c*
Seq. fit	DPPS	$2.2\times10^{-2}\pm1.7\%$	$4.0\times10^{-3}\pm0.8\%$	$0.0\pm17\%$	$4.0\pm1\%$	$8.4\pm0.1\%$
First order fit	DPPS	$5.8\times10^{-3}\pm1\%$	–	$1.0\pm3.8\%$	–	$8.2\pm0.2\%$
Seq. fit	DPPC	$7.0\times10^{-3}\pm2.6\%$	$1.0\times10^{-3}\pm6.8\%$	$0.001\pm7.7\%$	$4.3\pm2\%$	$9.1\pm1.8\%$
First order fit	DPPC	$3.0\times10^{-3}\pm1\%$	–	$0.3\pm8.7\%$	–	$7.4\pm0.3\%$
Seq. fit	DPPS/DPPC (1:4)	$1.4\times10^{-2}\pm3.7\%$	$3.5\times10^{-3}\pm6.3\%$	$-0.3\pm7\%$	$4.9\pm2.9\%$	$7.9\pm0.7\%$
First order fit	DPPS/DPPC (1:4)	$7.4\times10^{-3}\pm1\%$	–	$0.2\pm21\%$	–	$7.4\pm0.2\%$
Seq. fit+Ca^{2+}	DPPS/DPPC (1:4)	$1.0\times10^{-3}\pm12\%$	$7.1\times10^{-9}\pm5\times10^{9}\%$	$0.0003\pm7849\%$	$8.7\pm12\%$	$437\pm5\times10^{9}\%$
First order fit+Ca^{2+}	DPPS/DPPC (1:4)	$1.0\times10^{-3}\pm1\%$	–	$-0.3\pm2.7\%$	–	$8.8\pm0.7\%$

Seq. fit refers to the fits according to Eq. 1, first order fit refers to the fits according to Eq. 2. The fitted measurements are those shown in Fig. 1A, but reduced to 10 min. Fits of the kinetics in the presence of Ca^{2+} ions are indicated by $+Ca^{2+}$.

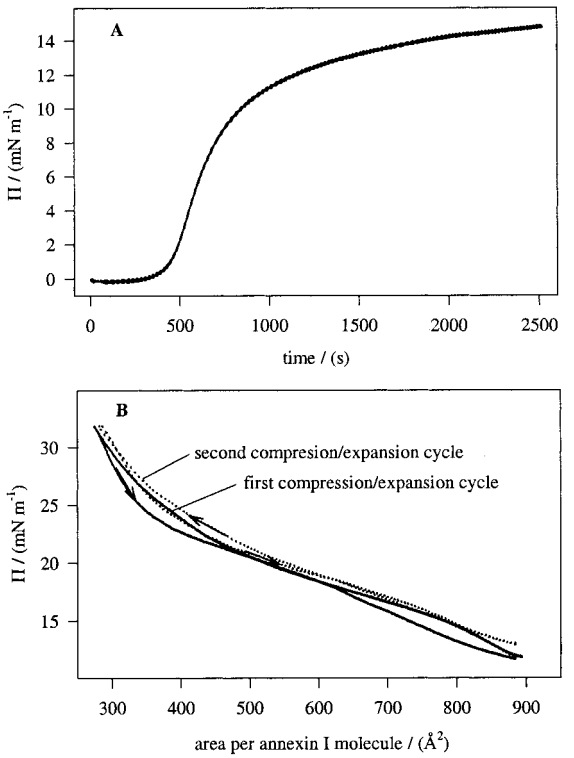

Fig. 5. Kinetics of the formation of an annexin I monolayer (A). Annexin I was injected to a final concentration of 24 nM into the subphase of the Langmuir film balance containing 50 mM MES/NaOH, pH 6.0. B: Compression (↑) and expansion (↓) isotherms of the annexin I monolayer. The solid lines refer to the first compression/expansion cycle, the dotted lines to the second cycle.

3.5. Surface activity of annexin I

Annexin I itself as well as the other annexin proteins employed in the present study (data not shown) exhibit surface activity. Fig. 5A shows the surface pressure of annexin I as a function of time after injecting the protein into the subphase to a final concentration of 24 nM (the protein concentration in the film is undefined). After a lag time of approximately 5 min an increase in the surface pressure is detectable, and after 30 min the annexin I monolayer exhibits a surface pressure of 14 mN/m. As revealed by compression and expansion isotherms this protein monolayer is stable up to a surface pressure of 30 mN/m (Fig. 5B). Thus when the complete surface of the subphase in the trough is available, annexin I (as well as the other annexin derivatives tested) forms a monolayer at the air/water interface. When only limited area is available, i.e. monolayers in the liquid-expanded phase, the annexins will occupy that space and form protein domains within the lipid monolayer. This is, however, not observed when the lipid film is in the non-expanded phase, i.e. at initial surface pressures of the monolayer of more than 15 mN/m. Under these conditions only full length annexin I induces a significant increase in the surface pressure indicative of penetration of the protein into the monolayer.

4. Discussion

We have shown that of the annexin derivatives tested only full length annexin I exhibits a significant interaction with DPPS, DPPC and DPPS/DPPC (molar ratio 1:4) monolayers at pH 6.0 in the absence of Ca^{2+} ions. The interaction of full length annexin I with the lipid films is complex and appears to involve electrostatic and hydrophobic contributions. The speed and magnitude of the interaction are high for native annexin I having an intact N-terminal domain. At the same pH but in the presence of Ca^{2+} ions the mechanism of interaction of annexin I with the DPPS/DPPC (molar ratio 1:4) monolayer is somewhat different from the Ca^{2+}-independent interaction as revealed by the quantitative analyses of the penetration kinetics under the different conditions. While at pH 6.0 in the absence of Ca^{2+} ions the reaction of annexin I with the monolayers appears to follow a sequential reaction scheme that could indicate a docking and an incorporation step, the reaction in the presence of Ca^{2+} ions is satisfactorily described by a first-order reaction.

Ca^{2+}-independent interactions regulated by pH have been reported for other members of the annexin family. Reutelingsperger and co-workers observed that at pH 5.0 both the chimeric annexin $I_N V_C$ and annexin V are able to induce the leakage of PS vesicle content even in the absence of calcium ions [15]. This indicates that the binding of these annexin molecules to the PS bilayer at pH 5.0 results in membrane perturbation. A considerable Ca^{2+}-independent penetration of annexin V into PS vesicles at pH 4.0 was postulated also by Köhler et al. [25] who observed a 4-fold reduction in the lateral diffusion coefficient of the lipids induced by the annexin under these conditions. It is worth noting in this context that the p*I* value of annexin V is 4.8. The annexin V-membrane interaction between pH 5.0 and 4.0 could therefore – at least in part – be due to electrostatic effects but could also be explained by increasing the hydrophobicity of annexin V by protonating the acidic residues in the protein leading to a penetration into the bilayer.

A prerequisite for the Ca^{2+}-independent interaction of annexin I with phospholipid structures is a pH value of about 6. It is possible that such pH values occur transiently within cells as the result of certain physiological stimuli for example during the generation of multivesicular endosomes. It is known that annexin I is present on endosomal structures whose luminal pH is more acidic than that of the cytoplasm [26,23]. Annexin I has been implicated in the inward vesiculation process which generates internal vesicles in the multivesicular endosome probably by inward budding/fusion of the limiting membrane [26]. In case lower pH values would occur at the cytosolic face of the limiting membrane under these conditions, it remains possible that annexin I could penetrate into the lipid bilayer and thereby support the budding/fusion process.

At pH 7.4, on the other hand, Ca^{2+} ions are absolutely required to obtain a significant surface-pressure-increase in DPPS/DPPC monolayers upon addition of annexin I. A Ca^{2+}-independent penetration into anionic phosphatidylglycerol monolayers at pH 7.4, however, has been recently observed for annexin I [27] although these results were obtained using significant lower values for the initial surface pressure of the monolayers ($\Pi_i = 5$ mN/m), lower protein concentrations (4 nM) and another temperature (25°C). The authors observed an increase in the surface pressure of 2–3 mN/m at an initial surface pressure of 5 mN/m of dipalmitoylphosphatidylglycerol (DPPG) and DPPC monolayers in the absence of Ca^{2+} ions. In the presence of 0.5 μM Ca^{2+}, Π reaches a value of 9 mN/m, reflecting an enhancement of the interaction by

Ca^{2+} ions at this pH value. This observation is in line with the penetration kinetics of annexin I with DPPS/DPPC (molar ratio 1:4) at pH 7.4 presented in this work.

The differences in the interaction mechanism between annexin I and the monolayers at pH 6.0 and pH 7.4 suggest that annexin I may exist in different conformational states at different pH values. Interestingly, such pH-dependent conformations have been observed recently for annexin V [28]. If, in analogy, this applies also to annexin I, a functional control of annexin I within the cell by both pH and Ca^{2+} could be envisaged.

An important result of our studies is the finding that annexin I has a strong tendency to penetrate into the monolayer. This is concluded from the observation that upon protein addition the initial surface pressure of 15 mN/m increased to a final pressure of 20 mN/m. Since at 15 mN/m the monolayer is in the liquid condensed or even crystalline phase, any pressure increase can only result from penetration of the protein into the lipid film. Under these conditions mere adsorption of proteins to the surface of a lipid monolayer causes no significant increase in the surface pressure [17,18] as, for example, observed in the case of annexin V (Fig. 1C). Interestingly, the final Π-value of 20 mN/m after annexin I incorporation into the monolayer appears to be independent of the initial pressure of the monolayer. This phenomenon will be addressed in future analyses.

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