Specific Interaction of the Intermediate Filament Protein Vimentin and Its Isolated N-Terminus with Negatively Charged Phospholipids As Determined by Vesicle Aggregation, Fusion, and Leakage Measurements

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ABSTRACT: The interaction of the intermediate filament protein vimentin and its non-α-helical N-terminus with phosphatidylserine and phosphatidylinositol small unilamellar vesicles was investigated by measuring vesicle aggregation, fusion, and leakage. While the N-terminus suppressed Ca^{2+}-induced fusion of phosphatidylserine vesicles, it caused their rapid aggregation in the absence of Ca^{2+}; at a molar ratio of lipid to polypeptide of 25:3, the polypeptide/lipid complexes precipitated from the reaction mixture. This aggregation was efficiently diminished by NaCl. The phosphatidylinositol vesicles, on the other hand, became leaky when interacting with the N-terminus of vimentin, even at a molar ratio of lipid to polypeptide of 500:1. The leakage of phosphatidylinositol vesicles was suppressed by the addition of Ca^{2+} or NaCl to the reaction mixture. Intact vimentin also caused leakage of phosphatidylinositol vesicles, at low and high salt concentration. The results indicate specific and differential interactions of the N-terminus of vimentin with various negatively charged lipid species, although there is an electrostatic component common to these interactions.

Intermediate filaments (IFs), in general, are associated with cellular membrane systems, such as the plasma membrane, the nuclear membrane, the outer mitochondrial membrane, and the endo- and sarcoplasmic reticulum [for a review, see Traub (1985)]. These associations are thought to be involved in the formation and maintenance of cell shape and in the structural organization of the cytoplasm (Lazarides, 1980).

The IFs are bound to these membranes through protein–protein interactions, as in the case of the ankyrin–vimentin (Georgatos & Marchesi, 1985; Georgatos et al., 1985), spectrin–vimentin (Mangeat & Burridge, 1984), spectrin–desmin (Langley & Cohen, 1986), and desmocalmin–cytokeratin (Tsukita & Tsukita, 1985) systems, and/or by their direct interaction with the lipid bilayer. The latter possibility seems to be verified in the binding of newly synthesized vimentin to lens plasma membranes added to a reticulocyte cell-free protein-synthesizing system (Ramaekers et al., 1982). Moreover, vimentin isolated and purified from a number of mammalian cell lines was shown to be highly contaminated with phospholipids and neutral lipids (Traub et al., 1985), suggesting the existence of direct vimentin–lipid interactions in vivo. In support of this view, a series of studies has demonstrated a high affinity of cellular lipids for vimentin filaments (Traub et al., 1985, 1986, 1987; Perides et al., 1986b). Vesicles obtained from negatively charged phospholipids not only bind to vimentin filaments with high efficiency but also inhibit filament formation and even cause dissociation of preformed filaments at higher concentrations (Perides et al., 1986a). Furthermore, circular dichroism studies performed on the isolated N-terminus of vimentin revealed a significant increase in the α-helical content of the polypeptide upon its interaction with vesicles containing negatively charged phospholipids (Perides et al., 1987). Vimentin and its N-terminus were also photoaffinity labeled with the hydrophobic phosphatidylcholine analogue [^{3}H]-1-palmitoyl-2-[11-[4-(trifluoromethyl)diazirinyl]undecanoyl]-*sn*-glycero-3-phosphocholine or with 1-azidopyrene, demonstrating an insertion of both protein molecules into the hydrophobic region of the lipid bilayer (Perides et al., 1986a, 1987).

In the present study, we have further characterized the interaction of vimentin and its N-terminus with a series of negatively charged phospholipids. This was done by investigating the influence of both proteins on the aggregation, fusion, and leakage of small unilamellar vesicles composed of various phospholipid species. We have concentrated our efforts mainly on the study of phosphatidylserine and phosphatidylinositol vesicles because, in previous investigations, these have shown the weakest and strongest effects, respectively, on the formation and stability of vimentin filaments (Perides et al., 1986a).

Materials and Methods

Materials

Egg yolk phosphatidylcholine (PC),[1] soybean phosphatidylinositol (PI), egg yolk phosphatidylglycerol (PG), bovine brain phosphatidylserine (PS), egg yolk phosphatidylethanolamine (PE), bovine heart cardiolipin (CL), and carboxyfluorescein (CF) were purchased from Sigma (Deisenhofen, FRG) and used without any further purification. $TbCl_3$ and 2,6-pyridinedicarboxylic acid (DPA) were obtained from Janssen Chimica (Bruggen, FRG). Vimentin from Ehrlich ascites tumor cells (Nelson & Traub, 1982; Nelson et al., 1982) and its N-terminus (Traub et al., 1986) were prepared as described previously. The amino acid sequences of both polypeptides were determined by Geisler et al. (1983) and Fischer et al. (1986). All other chemicals were obtained from either Merck (Darmstadt, FRG), Roth (Karlsruhe, FRG), or Serva (Heidelberg, FRG) and were of analytical grade.

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[1] Abbreviations: PC, L-α-phosphatidylcholine; PI, L-α-phosphatidylinositol; PG, L-α-phosphatidyl-DL-glycerol; PS, L-α-phosphatidyl-L-serine; PE, L-α-phosphatidylethanolamine; CL, cardiolipin; CF, 5(6)-carboxyfluorescein; DPA, 2,6-pyridinedicarboxylic acid; TES, 2-[[tris(hydroxymethyl)methyl]amino]ethanesulfonic acid.

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Methods

Vesicle Preparation. Small unilamellar vesicles (hereafter termed vesicles) were prepared in the presence of either (a) 15 mM $TbCl_3$ and 150 mM sodium citrate, (b) 150 mM DPA, or (c) 100 mM CF (sodium salt) as described by Wilschut et al. (1980). All solutions contained 5 mM TES adjusted to a pH of 7.4 with NaOH. The lipids or lipid mixtures were freed of chloroform in a gentle stream of N_2 and in vacuo, dispersed in aqueous medium at a concentration of 10 μmol/mL, and sonicated at room temperature for 6 min with a Model B-12 Sonifier (Branson Ultrasonic S.A., Carouge-Geneve, Switzerland) at an output power of 10 W. Subsequently, the preparations were centrifuged for 15 min at 110000*g* and 20 °C in a Beckman TLA 100.1 rotor to remove large vesicles and/or aggregates.

Vesicles were separated from nonencapsulated CF, $TbCl_3$, or DPA at room temperature by gel filtration on a PD-10 column containing Sephadex G-25M (Pharmacia-LKB, Freiburg, FRG) by use of 5 mM TES, pH 7.4, and 100 mM NaCl for column equilibration and elution. EDTA was included at 1 mM in the elution buffer for the preparation of $TbCl_3$- and DPA-loaded vesicles. Aliquots of $TbCl_3$-loaded vesicle preparations were rechromatographed without EDTA for determining the 100% vesicle fusion level (Wilschut et al., 1980). Lipid concentrations were determined as described by Bartlett (1959).

Dialysis of Vimentin. Vimentin dissolved in 10 mM Tris-HCl, pH 7.6, 6 mM 2-mercaptoethanol, and 6 M urea was dialyzed twice at 2 °C against 800 volumes of low ionic strength buffer (5 mM TES, pH 7.4) and was stored on ice until used. The protein content was determined according to Bradford (1976) with bovine serum albumin as a standard.

Fluorescence Measurements. The measurements were performed with an Aminco Bowman spectrophotofluorometer (Silver Spring, MD). The temperature of the cuvette holder was maintained at 25 °C, and the solution in the cuvette was continuously stirred. The measurements were carried out in a final volume of 2 mL of 5 mM TES, pH 7.4, with NaCl or EDTA as indicated below. Aggregation, fusion, and leakage of vesicles were measured as described by Wilschut et al. (1980). Briefly, aggregation of vesicles was determined by measuring the intensity of light (350 nm) scattered at an angle of 90° to the incident light beam. Fusion of vesicles was determined by measuring the fluorescence of the $TbCl_3$/DPA complex, which is formed only through the fusion of vesicles containing $TbCl_3$ and DPA. The $TbCl_3$/DPA complex was excited at 276 nm, and fluorescence was measured at 545 nm through a Scott 06515 cutoff filter. Leakage of vesicles was monitored by measuring an increase in fluorescence due to release of CF, which was loaded into vesicles at a self-quenching concentration of 100 mM (Blumenthal et al., 1977). CF was excited at 493 nm, and the fluorescence was measured at 520 nm through a Scott 06515 cutoff filter to eliminate the contribution to the signal from light scattering.

Results

Phosphatidylserine Vesicles. The time courses of vesicle aggregation, fusion, and leakage obtained when different amounts of Ca^{2+} (0.5–5 mM) were added to samples containing 50 μM empty vesicles, 50 μM concentration of a 1:1 mixture of vesicles loaded with $TbCl_3$ or DPA, and 50 μM vesicles loaded with CF, respectively, are given in Figure 1A–D. Vesicle fusion and leakage were detectable at and above 1 mM Ca^{2+} (Figure 1B–D). Upon increasing the Ca^{2+} concentration to 2.5 mM, a rapid aggregation of vesicles was accompanied by an increase in vesicle fusion (Figure 1C). The

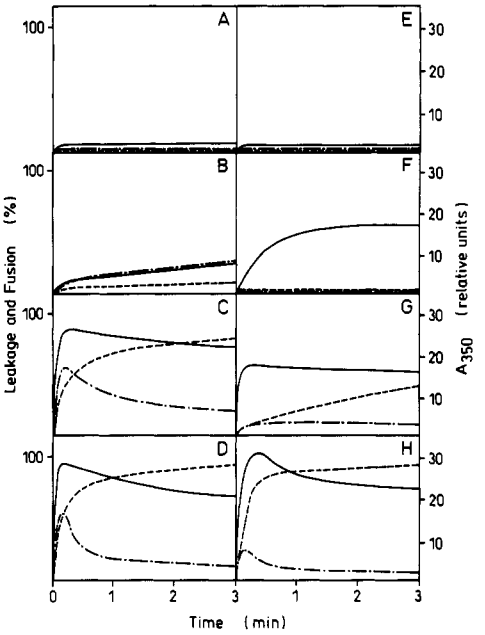

FIGURE 1: Ca^{2+}-induced aggregation, fusion, and leakage of phosphatidylserine vesicles in the absence (A–D) and in the presence (E–H) of the N-terminus of vimentin. The aggregation (—) of vesicles was measured in 5 mM TES, pH 7.4, and 100 mM NaCl. It is expressed in relative units, where the intensity of 350-nm light scattered on 50 μM vesicles, in the absence of Ca^{2+}, was taken as unity. 25 μM $TbCl_3$- and 25 μM DPA-loaded vesicles were used for fusion measurements (–·–) in a buffer containing 5 mM TES, pH 7.4, 100 mM NaCl, and 0.1 mM EDTA. Leakage measurements (– –) were performed in 5 mM TES, pH 7.4, and 100 mM NaCl. The 100% level of vesicle leakage was determined by the addition of Triton X-100 [0.1% (v/v) final concentration] to a sample containing 50 μM CF-loaded vesicles. At time 0, 20-μL portions of $CaCl_2$ stock solutions were added to the various samples. The final concentrations of Ca^{2+} in the samples were as follows: (A and E) 0.5 mM; (B and F) 1 mM; (C and G) 2.5 mM; (D and H) 5 mM. To samples E–H, the N-terminus of vimentin (2 μM final concentration) was added at time 0.

fluorescence intensity of the $TbCl_3$/DPA complex decreased after 10 s as a consequence of the instability of fused vesicles and the presence of EDTA and Ca^{2+} in the external medium (Wilschut et al., 1980). Raising the Ca^{2+} concentration to 5 mM caused a more rapid decrease in the fluorescence intensity of the $TbCl_3$/DPA complex due to an enhanced rate of vesicle fusion (Figure 1D).

By use of the same system, the influence of the N-terminus of vimentin on Ca^{2+}-induced vesicle aggregation, fusion, and leakage was examined (Figure 1E–H). In the presence of 0.5 mM Ca^{2+}, 2 μM N-terminus had no effect, as was observed for 0.5 mM Ca^{2+} alone (compare panels A and E of Figure 1). At 1 mM Ca^{2+}, however, the same concentration of N-terminus effectuated a stimulation of vesicle aggregation but a suppression of both vesicle fusion and leakage (compare panels B and F of Figure 1), whereas at 2.5 mM Ca^{2+} it induced a suppression of all three events (compare panels C and G of Figure 1). Finally, at 5 mM Ca^{2+}, 2 μM N-terminus brought about only a suppression of vesicle fusion in comparison to the effect of 5 mM Ca^{2+} alone (compare panels D and H of Figure 1).

The addition of N-terminus at concentrations of 1–3 μM to a 25 μM solution of vesicles in the absence of Ca^{2+} effected an enhancement of vesicle aggregation probably due to cross-linking of the liposomes (Figure 2A). At an N-terminus concentration of 3 μM, the vesicles aggregated very rapidly, and after 3 min, the intensity of scattered light fluctuated greatly. This instability was caused by the precipitation of the lipid/polypeptide complexes from the reaction mixture.

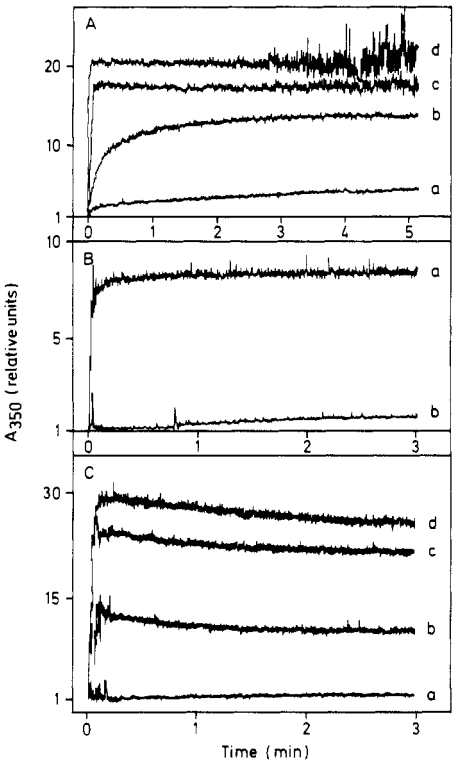

FIGURE 2: (A) N-Terminus-induced aggregation of phosphatidylserine vesicles. A solution of the N-terminus of vimentin was added to samples containing 25 μM PS vesicles in 5 mM TES, pH 7.4, and 100 mM NaCl to yield the following final protein concentrations: (a) 1, (b) 1.5, (c) 2, and (d) 3 μM. (B) Effect of the ionic strength on N-terminus-induced aggregation of phosphatidylserine vesicles. A 2 μM concentration of the N-terminus of vimentin was added to a sample containing 50 μM PS vesicles in 5 mM TES, pH 7.4. The buffer contained (a) 8 mM NaCl or (b) 200 mM NaCl. (C) Ca^{2+}-induced aggregation of phosphatidylinositol vesicles. 20-μL portions of $CaCl_2$ stock solutions were added to samples containing 50 μM PI vesicles in 5 mM TES, pH 7.4, and 100 mM NaCl. The final Ca^{2+} concentrations of the samples were (a) 5, (b) 10, (c) 15, and (d) 20 mM. Light scattering at 350 nm was measured as described in the legend to Figure 1.

At this N-terminus concentration, the vesicle leakage remained below 5% (data not shown).

To investigate the electrostatic nature of the interaction of the N-terminus with PS vesicles, vesicle aggregation was monitored in solutions of increasing NaCl concentration. Raising of the ionic strength clearly suppressed PS vesicle aggregation; it was nearly abolished at a NaCl concentration of 200 mM (Figure 2B).

Phosphatidylinositol Vesicles. To compare different negatively charged phospholipids with respect to their reaction with the N-terminus of vimentin, we repeated the experiments described above with PI vesicles. Ca^{2+} alone only induced rapid aggregation of the vesicles, without causing vesicle fusion or leakage (Figure 2C). Even the highest Ca^{2+} concentration (20 mM) did not cause a significant extent of PI vesicle leakage (data not shown). A concentration of 5 mM Ca^{2+} did not induce a measurable aggregation, whereas this was distinctly enhanced when the Ca^{2+} concentration was raised to the range of 10–20 mM. By contrast, N-terminus alone brought about a rapid and extensive leakage of PI vesicles at very low polypeptide concentrations. The dependence of PI vesicle leakage on the N-terminus concentration is shown in Figure 3A. Total leakage was observed at a molar ratio of polypeptide to lipid of 1:65, and a marked effect was detected even at a molar ratio of 1:500, without any observable vesicle aggregation.

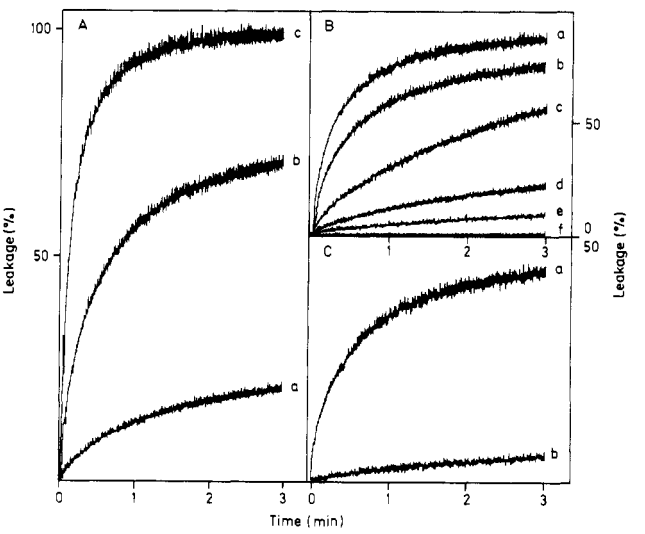

FIGURE 3: (A) N-Terminus-induced leakage of phosphatidylinositol vesicles. The N-terminus of vimentin was added to samples containing 25 μM PI vesicles in 5 mM TES, pH 7.4, and 100 mM NaCl to yield molar ratios of lipid to polypeptide equal to (a) 500:1, (b) 170:1, and (c) 65:1. (B) Influence of Ca^{2+} on N-terminus-induced phosphatidylinositol vesicle leakage. The N-terminus of vimentin and 20-μL portions of $CaCl_2$ stock solutions were added to samples containing 25 μM PI vesicles in 5 mM TES, pH 7.4, and 100 mM NaCl at time 0. The final concentration of polypeptide was 0.125 μM and that of Ca^{2+} was (a) 0, (b) 0.1, (c) 1, (d) 3, (e) 5, and (f) 20 mM. (C) Influence of the ionic strength on N-terminus-induced phosphatidylinositol vesicle leakage. The N-terminus of vimentin was added to a concentration of 125 nM to a sample containing 50 μM PI vesicles in 5 mM TES, pH 7.4. The buffer contained (a) 100 mM NaCl or (b) 220 mM NaCl. Vesicle leakage was determined as described under Materials and Methods.

Ca^{2+} suppressed the N-terminus-induced vesicle leakage very effectively, as shown in Figure 3B. Vesicle leakage decreased markedly at a Ca^{2+} concentration of 0.1 mM, and a concentration of 20 mM Ca^{2+} totally inhibited leakage.

As shown in Figure 3C, N-terminus-induced vesicle leakage was suppressed at higher NaCl concentrations, which suggests that the first step of the interaction of the N-terminus with PI vesicles is of an electrostatic nature.

We examined the influence of various other phospholipid species on the leakage phenomenon to investigate whether the vesicle leakage caused by the N-terminus of vimentin is an event fairly specific for PI vesicles. These results are summarized in Figure 4A. N-Terminus was added to a concentration of 1.125 μM to samples containing 25 μM lipid or lipid mixtures, and the vesicle leakage was measured over a time period of 3 min. At this molar ratio of polypeptide to lipid (1:200), pure PI vesicles showed a marked leakage. Vesicles prepared from a PI:PC mixture of 9:1 (mol/mol) showed a leakage of only about 50% of that of vesicles consisting of PI alone. The presence of PE in the PI vesicles suppressed the leakage even more efficiently; the presence of 5% PE within PI vesicles diminished their N-terminus-induced leakage to 15%. The vesicles of other negatively charged phospholipids such as PG and CL showed a vesicle leakage of about 2% of that of pure PI vesicles at this molar ratio of polypeptide to lipid.

Finally, we examined the influence of intact vimentin on the behavior of PI vesicles. At 100 mM NaCl, vimentin caused a concentration-dependent leakage of PI vesicles similar to the effect exerted by its isolated N-terminus (Figure 4B). As depicted in Figure 4C, this reaction had a distinct ionic strength optimum. The best effect was observed at a salt concentration close to physiological.

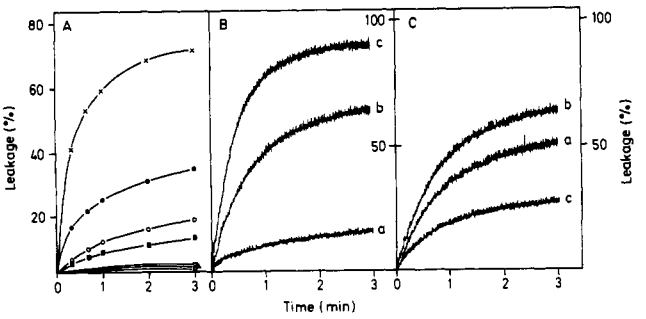

FIGURE 4: (A) Influence of lipid composition on N-terminus-induced vesicle leakage. The N-terminus of vimentin was added to a concentration of 0.125 μM to samples containing 25 μM lipid vesicles in 5 mM TES, pH 7.4, and 100 mM NaCl. The vesicles were obtained from lipid mixtures of the following compositions (molar ratios): (×) 100% PI; (●) PI:PC = 9:1; (○) PI:PC = 8:2; (■) PI:PE = 95:5; (□) PI:PE = 8:2; (▽) 100% PG; (△) 100% CL. (B) Vimentin-induced leakage of phosphatidylinositol vesicles at different vimentin concentrations. Vimentin was added to a sample containing 25 μM PI vesicles in 5 mM TES, pH 7.4, and 100 mM NaCl. The final protein concentration was (a) 80, (b) 330, and (c) 840 nM. (C) Influence of the salt concentration on vimentin-induced leakage of phosphatidylinositol vesicles. Vimentin was added to a concentration of 330 nM to a sample containing 25 μM PI vesicles in 5 mM TES buffer, pH 7.4. The buffer contained (a) 0, (b) 100, and (c) 225 mM NaCl. The vesicle leakage was determined as described under Materials and Methods.

Discussion

We have further characterized the interaction of the N-terminus of vimentin and of the intact protein itself with phospholipids (Traub et al., 1986, 1987; Perides et al., 1986b, 1987) by monitoring the aggregation, fusion, and leakage of vesicles prepared from negatively charged phospholipids. For better comprehension of the points made in this discussion, the amino acid sequence of the N-terminal polypeptide spanning amino acid residues 1–96 is presented (Fischer et al., 1986):

$$\text{STRSVSSSSY}_{10}\text{RRMFGGSGTS}_{20}\text{SRPSSNRSYV}_{30}\text{-}$$
$$\text{TTSTRTYSLG}_{40}\text{SALRPSTSRS}_{50}\text{LYSSSPGGAY}_{60}\text{-}$$
$$\text{VTRSSAVRLR}_{70}\text{SSVPGVRLLQ}_{80}\text{DSVDFSLADA}_{90}\text{-}$$
$$\text{INTEFK}$$

The results depicted in Figures 2B and 3C show that the first stage of the association of the positively charged N-terminus with the negatively charged bilayer of PS and PI vesicles is very likely an electrostatic interaction. Such interactions have also been found for poly(L-lysine), for cytochrome *c* (Papahadjopoulos et al., 1975; De Kruijff et al., 1985), and for other extrinsic membrane-bound proteins (Jain & Zakim, 1987; Yung & Green, 1986). The different interactions of cytochrome *c* with CL and PS are also reflected in its interaction with lipid extracts of natural membrane preparations containing these anionic lipids (Demel et al., 1989). However, electrostatic interactions must not always result in nonspecific associations of proteins with lipid bilayers, since, for example, spectrin (Mombers et al., 1977, 1980), myelin basic protein (Boggs et al., 1981), and the antibiotic polymyxin (Sixl & Galla, 1979) cannot be desorbed by cations from anionic phospholipid bilayers.

After having contacted the lipid bilayer, the N-terminus of vimentin seems to undergo rather specific secondary reactions with different negatively charged phospholipids. PS vesicles, for instance, aggregated very rapidly after the addition of the polypeptide, and at a molar ratio of lipid to protein of 25:3, the complexes precipitated from the reaction mixture after 3 min (Figure 2A). Similar precipitations of protein/lipid complexes have been described for the reaction of poly(L-lysine) with a CL dispersion where CL remained as a stable bilayer (De Kruijff & Cullis, 1980), of melittin with a dioleoylphosphatidic acid dispersion with the concomitant appearance of a hexagonal H_{II} phase (Batenburg et al., 1987), and also of melittin with dioleoyl-PS and egg PG dispersions (Batenburg et al., 1987).

However, the N-terminal polypeptide caused no fusion and leakage of PS vesicles as did Ca^{2+} (Wilschut et al., 1980), suggesting that it does not disrupt the PS bilayer. Since the N-terminus can protect the vesicles from Ca^{2+}-induced fusion, it probably coats the PS vesicles and exerts an effect antagonistic to that of Ca^{2+} (Figure 1).

The interaction of the N-terminus with PI vesicles seems to be based on a mechanism quite different from that of its interaction with PS vesicles. It did not cause an aggregation of PI vesicles but interacted more intimately with the bilayer, resulting in a rapid leakage of vesicles even at a molar ratio of protein to lipid as low as 1:500. As shown previously, during the association of the N-terminus with PC vesicles containing 50% PI, the polypeptide undergoes a conformational change with an increase in its α-helical content from 10 to 30% (Perides et al., 1987). This is similar to protein conformational changes observed for the interaction of apocytochrome *c* with negatively charged phospholipids (Rietveld et al., 1985; Walter et al., 1987).

The cause of the increased permeability of PI vesicles after their interaction with the N-terminus is unknown, but it seems to be a highly specific event with respect to the lipid moiety, as suggested by the marked decrease of the leakage of PI vesicles containing a small amount of various other phospholipids (see Figure 4A). In this context, it is difficult to visualize how the incorporation of, for instance, 5% PE into the PI vesicles can effect a reduction of vesicle leakage by a certain amount of N-terminal polypeptide from 70 to 10% within a reaction time of 3 min (Figure 4A); the charge interaction postulated to initiate the association of the reactants cannot be diminished to that extent by the added PE. The ability of the N-terminus to cause PI vesicle leakage was also very efficiently inhibited by Ca^{2+} (Figure 3B). Concerning the mechanism governing the leakage phenomenon, the polypeptide might either insert into the PI bilayer causing a defect in lipid packing or even create a transmembrane channel, as does the α-toxin of *Staphylococcus aureus* (Batenburg et al., 1987) and melittin (Talbot et al., 1987). The N-terminus also might trigger an isothermal phase transition of the lipid membrane to a nonlamellar phase after interaction with the polar head groups of the lipid molecules, as is the case in the gramicidin–dielaidoyl-PE interaction (De Kruijff et al., 1985; Van Echteld et al., 1981). Any of these events would be expected to be accompanied by a concomitant release of CF from the vesicles. In this context, it is interesting to note that a perturbance of the lipid bilayer is also caused by signal peptides of mitochondrial precursor proteins (Nagaraj et al., 1987; Pilon et al., 1987) which are similar in structure to the N-terminal polypeptide of vimentin with regard to their amphiphilic character, their high positive charge (due to a wealth of basic amino acids, particularly arginine residues), and the total absence of acidic amino acids [Geisler et al., 1983; Horwich et al., 1985; Roise et al., 1986; Tamm, 1986; for a brief review, see Hurt and van Loon (1986)]. Electron microscopy and ^{31}P NMR studies could shed light on the morphology of the lipid/polypeptide complexes.

It is a well-established fact that the ability of vimentin to form filamentous structures is dependent on a high salt concentration of the medium (Traub & Vorgias, 1983, 1984), and

it has been demonstrated that filament structure and assembly are severely disturbed by negatively charged phospholipid vesicles (Perides et al., 1986a). It also has been shown that, in addition to the α-helical core domain, the non-α-helical N-terminal polypeptide of vimentin plays an important role in filament formation and stability (Traub & Vorgias, 1983). Posttranslational modification of the N-terminus such as limited proteolysis (Traub & Vorgias, 1983), phosphorylation (Evans, 1988; Inagaki et al., 1988), or deimination of arginine residues (Inagaki et al., 1989) prevents filament assembly from protofilaments or, if intact intermediate filaments are the substrates of such modifications, cause disassembly of the filaments. It is thus conceivable that the interaction of critical regions of the N-terminal polypeptide with negatively charged PI (vesicles) provokes similar effects (Perides et al., 1986a).

On the basis of these observations and considerations, it is somewhat surprising that the leakage of PI vesicles caused by protofilamentous vimentin is significantly increased at 100 mM NaCl, a salt concentration that favors filament formation (Figure 4C). There might be two explanations for this effect. The enhanced leakage could be due to a local concentration of N-termini as a result of their dense and regular arrangement on the surface of filament fragments which form and interact with the surface of the vesicles. Alternatively, there may be salt-induced promotion of hydrophobic interactions, for instance, between the α-helical core domain of vimentin and the hydrophobic interior of the PI bilayer. In the first case, the dissociating effect of high salt on the interaction of the N-termini with the vesicle surface would be overcome by the local increase in the density of positive charges on the protein aggregate, and in the second case, it would be compensated for by the formation of nonionic bonds between protein and lipid molecules; conformational changes in the protein molecule or protein aggregates might facilitate the formation of such nonionic interactions. At the higher ionic strength of 225 mM KCl, a more efficient shielding of ionic groups might occur on the N-terminal polypeptide chains as well as on the PI vesicle surface, thus diminishing the initial electrostatic attraction of the reactants and causing a reduction of vesicle leakage (Figure 4C). It should be considered, however, that intact vimentin filaments are totally disintegrated, probably with the formation of tetrameric protofilaments or perhaps dimers or monomers by an excess of PI vesicles at 150 mM KCl (Perides et al., 1986a). This observation would argue against the local concentration of positively charged N-terminal polypeptide chains as being responsible for the enhancement of vimentin-induced PI vesicle leakage at higher ionic strength. Therefore, the second explanation invoking the additional involvement of the α-helical core domain of vimentin in the perturbation of the PI bilayer at higher salt concentration seems more likely. A theoretical treatment of this problem has been submitted for publication elsewhere.

No definite conclusions can be drawn from the observations described here and in our previous reports (Perides et al., 1986a,b; 1987) as to the physiological significance of the interaction of vimentin and its filaments preferentially with PI vesicles. However, the ability of vimentin to cause PI vesicle leakage together with the fact that even small amounts of other phospholipids are very efficient in suppressing N-terminus-induced PI vesicle leakage (Figure 4A) suggests, in agreement with Burn's (1988) work, a possible regulatory role of PI-rich domains of biological membranes in the interaction of vimentin and its filaments with membranes. These lipid domains may provide a signal for lipid–protein interactions to occur and may therefore represent a driving force for the dynamic association of cytoskeletal proteins with membranes (Burn, 1988). Conversely, through its high affinity for PI, and particularly for phosphatidylinositol 4-monophosphate and 4,5-diphosphate (Perides et al., 1986a), vimentin filaments might cause local accumulations of these biologically important phospholipids in membranes and by that contribute to an intensification of signal transduction. These mechanisms, and thereby the kinetics of the interactions of cytoskeletal proteins with membranes, may be sensitively controlled by variations of the intracellular Ca^{2+} concentration (Horkovics-Kovats and Traub, submitted for publication).

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