Mechanism of interaction between actin and membrane lipids: a pressure-tuning infrared spectroscopy study

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Using pressure-tuning Fourier transform infrared spectroscopy to study an *in vitro* system consisting of actin and distearoylphosphatidylcholine (DSPC) liposomes, we have determined the mechanism of interaction between actin and membrane lipids. This interaction results in a significant conformational change in actin molecules. Analysis of the amide I band of actin shows an increase in the β -sheets to α -helix ratio, in random turns, and in interactions between actin monomers. In the absence of lipids, the actin molecules are denatured by pressures of 8×10^8 Pa and more, which give rise to a random organization of the peptide

chain. However, in the presence of DSPC liposomes, pressure greater than 2×10^8 Pa induces a change in actin conformation, which is dominated by strongly interacting β -sheets. As the spectra of the lipid molecules are not changed by the presence of actin, the organization of the lipid molecules in the bilayer is not affected by the protein. It is concluded from these results that this interaction of actin with membrane lipids involves very few lipid molecules. These lipid molecules may interact with actin at a few specific sites on the protein.

INTRODUCTION

Actin, a ubiquitous cytoskeletal protein, is involved in fundamental cellular processes including motility, division, phagocytosis, organelle movement and cell adhesion. Actin filaments, in association with microtubules and intermediate filaments, also contribute to the establishment and maintenance of cell morphology. These functions require that actin filaments be attached to the plasma membrane. Indeed, membrane-anchorage of actin filaments was first observed by electron microscopy and subsequently confirmed by biophysical analysis.

Because of their involvement in many different cell functions, cytoskeleton–membrane interactions are the object of intensive research which tries to identify the molecular mechanism of attachment of these filaments to the membrane. Although there are probably as many systems of attachment as there are different cell types, general organization themes are emerging. The basic concept is that actin filaments are attached to membranes by complexes of membrane proteins. Several membrane proteins which participate in the anchorage of actin filaments have been identified: ponticulin [1], the laminin receptor [2], the glycoprotein II/IIIa complex in platelets [3], hisactophilin [4], myosin I [5–7], the glycoprotein complex in tumour-cell microvilli [8], vinculin [9], talin [10] and spectrin/band 4.1 in erythrocytes. Some working models exist in the case of erythrocyte membranes [11,12], focal adhesions [13], striated muscle [14], brush border [15], and microvilli [8]. Several excellent reviews have covered recent developments in this field [16–20].

The possibility that actin may also directly interact with membrane lipids has rarely been considered [21], and is absent from recent reviews concerning cytoskeleton–plasma membrane interactions [22]. However, by using an *in vitro* system composed of pure lipid vesicles, i.e. liposomes, and purified actin, we have found that actin may also interact with membrane lipids without the need of any intermediate protein [23]. The mechanism of this interaction seems to involve electrostatic interactions. Electron

microscopy and differential scanning calorimetry (DSC) analysis have shown changes in actin conformation when it interacts with membrane lipids [24,25].

In the present study, we used pressure-tuning i.r. spectroscopy to investigate the mechanism of interaction between actin and membrane lipids further. This technique makes it possible to determine the relative contributions of α -helices, β -sheets, turns and coils in proteins [26–29], as well as the strength of hydrogen bonds in these secondary structures [30], and the intermolecular interactions with lipids [31,32]. We found that the interaction of actin with lipids induces an important change in the conformation of the protein but does not affect the molecular packing of the lipids.

MATERIALS AND METHODS

Preparation of actin

Actin was prepared from rabbit striated muscle by the technique of Spudich and Watt [33] as modified by Nonomura et al. [34]. Actin was obtained as monomers and dissolved in the following buffer called G buffer (2 mM Tris/HCl, 0.2 mM ATP, 0.2 mM CaCl₂, 0.01 %, sodium azide, 0.5 mM 2-mercaptoethanol, pH 8.0).

Preparation of liposomes

Liposomes were prepared by the freezing–thawing technique [35]. Briefly, 40 mg of distearoylphosphatidylcholine (DSPC) from Sigma Chemical Co. (St. Louis, MO, U.S.A.) was dissolved in 10 ml of chloroform and evaporated under vacuum in a rotary evaporator to obtain a thin film of lipid in the flask. The lipids were resuspended in G buffer by vortexing, transferred in a cryogenic tube and rapidly frozen in liquid nitrogen. Thereafter, they were thawed and heated at 70 °C, above the phase transition of DSPC. The freezing–thawing procedure was repeated three times.

Actin-liposomes interaction

G actin (10 mg) was mixed with 10 mg of DSPC liposomes in 10 ml of G buffer and incubated for 30 min at room temperature. Polymerization of actin was induced by the addition of MgCl_2 at 2 mM (final concentration). After 1 h of incubation, to complete actin polymerization, the mixture was centrifuged at 170 000 g for 60 min, and the mixture of actin-liposomes collected as a gelatinous pellet.

For Fourier transform infrared (f.t.i.r.) analysis of the amide I band and several bands of lipids, the pellet was washed twice in G buffer prepared with $^2\text{H}_2\text{O}$.

F.t.i.r. spectroscopy

The pellet of actin-liposomes was placed at room temperature, together with powdered α -quartz, on a 0.37 mm diam. hole in a 0.23-mm-thick stainless steel gasket mounted on a diamond anvil cell. The α -quartz was used as an internal pressure calibrant by following the frequency shifts of the 695 cm^{-1} phonon band [36]. I.r. spectra were collected with a Bomem Model Michelson 110 Fourier transform spectrophotometer with a liquid nitrogen-cooled mercury cadmium telluride detector. The spectrometer set-up for recording f.t.i.r. spectra under pressure and deconvolution of the spectra to quantify the contributions to the overall spectrum made by individual peaks was done using computer programs developed in our Ottawa laboratory [37].

Electron microscopy

The actin and liposome mixtures were diluted in G buffer to a concentration of 0.1 mg/ml. One drop was deposited on a formvar-carbon-coated grid previously treated with a plasma glow discharge and negatively stained with 1% (w/v) uranyl acetate in water.

RESULTS

Electron microscopy

When actin is mixed with liposomes composed of DSPC, a precipitate immediately forms, indicating an interaction between both constituents. Examination of the precipitate by electron microscopy (Figure 1) shows liposomes covered by a sheet of linear, parallel actin filaments, denoting a paracrystalline organization. Filaments which are not in direct contact with the liposomes are typically wavy and randomly dispersed. This suggests the occurrence of a conformational change in the actin when it comes into contact with the lipid surface, producing a regular, highly ordered packing of the actin filaments on the liposome surface.

Centrifugation in $^2\text{H}_2\text{O}$ buffer

When actin alone is centrifuged at 170 000 g for 30 min in $^2\text{H}_2\text{O}$ buffer, the protein forms a clear gelatinous pellet at the bottom of the tube. When only DSPC liposomes are centrifuged under the same conditions, these form a creamy layer on the surface. However, when actin and DSPC liposomes are mixed, both are sedimented as a white pellet. Therefore, the actin filaments are able to draw the liposomes to the bottom of the tube. This simple experiment is clearly indicative of an interaction between actin and the DSPC liposomes.

Amide I band i.r. spectrum analysis

Figure 2 shows the normal and deconvoluted amide I band i.r. spectra of actin alone. This band is due to the in-plane C=O

stretching weakly coupled with the C-N stretching, and the in-plane N-H bending of the amide groups in proteins [28]. The maximum peak of the amide I band is sensitive to the secondary structure of the protein because specific substructures have different types of H bonding. This is used to analyse the secondary structure of proteins [26–28,30]. The spectra become clearer after band narrowing using Fourier self-deconvolution with an enhancement factor of 1.8 and band-width of 25 cm^{-1} .

The band of actin has two principal maxima. The predominant one at 1653 cm^{-1} is due to the α -helix conformation, and the second one at 1631 cm^{-1} to the β -sheets. This is in good agreement with the known actin secondary structure determined recently by X-ray diffraction [38,39]. It shows that α -helices are the predominant secondary structures. Moreover, the amide I band of actin has two shoulders, the first one at 1673 cm^{-1} and the second one at 1613 cm^{-1} . The first band has been attributed previously to the antiparallel β sheet, but it may also be partially due to unordered turns as it has also been observed in the i.r. spectrum of myoglobin in which there is no β sheet substructure [37]. The second shoulder is due to the amide I mode of the amide groups arising from the intermolecular interactions between neighbouring monomers [40,41].

The i.r. spectra in the amide I mode region of actin in the presence and absence of DSPC are shown in Figure 3. In the presence of DSPC liposomes, the secondary structure of actin is significantly modified. These modifications indicate that a con-

Figure 1 Electron micrograph of negatively stained mixture of actin and DSPC liposomes

Actin filaments are deposited on the surface of the liposome, and organized in highly ordered parallel arrays. Bar = 0.5 μm .

Figure 2 Infrared absorption of the amide I band of actin

---, Original spectrum; —, spectrum after narrowing using Fourier self-deconvolution.

Figure 3 Deconvoluted infrared spectra in the amide I region of actin alone (—) and actin in the presence of DSPC liposomes (---)

formational change occurs in the actin molecule. The main change observed is an increase of the contribution of the 1631 cm^{-1} frequency. This frequency is too high to be interpreted as a normal vibrational mode of the intermolecular amide group. Therefore, it must correspond to an increase in β -sheet structure. The intensity ratio of β -sheets to α -helices increases from 0.84 to 0.90 in the presence of lipids. The shoulder at 1673 cm^{-1} increases and becomes a well-defined peak. These changes in conformation in the actin molecule also result in increased intermolecular interactions between the actin monomers, as indicated by the increase in the band at 1613 cm^{-1} . All of these results are consistent with our observations by electron microscopy which show that the actin filaments at the surface of liposomes have a different morphology and organization compared with free filaments (Figure 1). In particular, the actin filaments at the surface of liposomes have a paracrystalline organization, producing additional interactions between the monomers which may explain the increase of the 1613 cm^{-1} band. In the presence of

Figure 4 Comparison of the deconvoluted spectra of the amide I band of actin paracrystalline sheets at the surface of liposomes (---) and actin paracrystals made by 20 mM MgCl_2 (—).

DSPC, a new peak appears at 1585 cm^{-1} . Judging from the frequency region, this band is most likely due to the vibrational mode of the functional groups in the actin side chains. Indeed, this band was not only observed in the presence of DSPC, but was also observed in pure actin at high pressure (see Figure 5a).

As actin filaments form paracrystalline sheets at the surface of liposomes, one may ask whether the spectral changes reflect actin-lipid interactions, or the fact that actin forms paracrystals. To answer this question, we have determined spectra of actin paracrystals made by 20 mM MgCl_2 . Figure 4 shows that the spectrum of actin paracrystals is very different from the actin paracrystalline sheets at the surface of liposomes, having much lower intermolecular interactions at 1613 cm^{-1} , and less β -sheets at 1631 cm^{-1} . Therefore, the conformation of actin at the surface of liposomes is unique and results from its interaction with lipids.

Pressure effects on the amide I band

Figure 5 shows the effects of high pressure on amide I band of actin alone (Figure 5a), and actin in the presence of DSPC liposomes (Figure 5b). Pressure strongly affects the whole spectrum of the amide I band. For actin alone, the whole spectrum is reduced to a single peak at 1643 cm^{-1} , corresponding to a random organization of the protein at pressures above $1 \times 10^9\text{ Pa}$. This dramatic change in the i.r. spectra of the amide I band is irreversible and remains after the sample is turned to atmospheric pressure. This corresponds to the denaturation of the protein under high pressure.

A more detailed examination of the i.r. spectra shows that pressure does not affect equally the different secondary structures in the protein. The contribution of the β -sheets slightly increases with pressure, up to $8 \times 10^8\text{ Pa}$, while contributions due to the α -helix, random turns, and intermolecular bondings remain constant. At $8 \times 10^8\text{ Pa}$, the protein denatures, giving rise to a sudden burst of random coiling at 1643 cm^{-1} . A shoulder remains at 1673 cm^{-1} .

In the presence of DSPC liposomes, increasing pressure changes the actin conformation differently. From $2 \times 10^8\text{ Pa}$ up, the proportion of β -sheets increases and exceeds that of α -helices. At the same time, the peak at 1614 cm^{-1} due to intermolecular bonds shifts to higher frequencies and finally overlaps with the peak of β -sheets, fusing in a single peak at 1630 cm^{-1} . α -Helix and random turns are stable up to $5.5 \times 10^8\text{ Pa}$ and suddenly vanish at higher pressures.

Figure 5 Effect of pressure on the spectra of the amide I region for actin alone (a) and actin in the presence of liposomes (b)

Pressures are indicated in kbar in the margin (1 kbar = 10^8 Pa).

It appears that, in the absence of DSPC liposomes, pressure denatures the actin molecule into a random organization. By contrast, in the presence of DSPC liposomes, the pressure-induced changes in the conformation of the actin molecule are dominated by strongly interacting β -sheets.

Lipid bands i.r. spectra

Figure 6 shows the effects of increased pressure on the vibration frequency of the ester-stretching mode at 1740 cm^{-1} and 1725 cm^{-1} . The presence of two bands in the C=O-stretching mode of DSPC may reflect the non-equivalence of the two carbonyl groups of the *sn*-1 and *sn*-2 chains. This is typical in many phospholipids. Figure 6 also indicates that actin does not change the frequencies of the C=O-stretching mode at any

Figure 6 Pressure dependencies of the C=O-stretching frequencies of DSPC liposomes alone (●), and DSPC liposomes in the presence of actin (+)

Figure 7 Pressure dependencies of the CH_2 -bending frequencies of DSPC liposomes alone (●), and DSPC liposome in the presence of actin (+)

pressure, and therefore, that actin does not modify the behaviour of this C=O functional group.

Figure 7 represents the effect of pressure on the frequencies due to the CH_2 bending of methylene chains in DSPC in the presence and in the absence of actin. Figure 8 is the same experiment looking to the anti-symmetric vibration of the phosphate group in DSPC. We have also examined other frequencies of lipids, in particular the CH stretching at 2850 cm^{-1} , the CH_3 end group at 1370 cm^{-1} , and the symmetric vibration of phosphate located at 970 cm^{-1} . None of these frequencies is affected by the presence of actin. Moreover, there was no broadening or change in band shape in the i.r. bands of the lipid in the presence of actin (results not shown).

In summary, our results show that actin interacts with lipids. This interaction greatly modifies the conformation and stability of the actin molecule. However, the organization of the bilayer lipids is not disturbed during this interaction.

Figure 8 Pressure dependencies of the anti-symmetric vibration of the phosphate group of DSPC liposome alone (●), and DSPC liposome in the presence of actin (+)

DISCUSSION

Previous results obtained by electron microscopy, centrifugation analysis, and fluorescence have established that an interaction between actin and membrane lipids may occur [23,25]. This interaction requires millimolar concentrations of bivalent cations. Therefore, this interaction occurs only with F actin since G actin polymerizes in the presence of millimolar concentrations of bivalent cations. On the other hand, high concentrations (> 200 mM) of monovalent cations inhibit the interaction, a clear indication of an electrostatic phenomenon [23,24]. Interaction of actin with membrane lipids induces an important change in actin conformation that produces a complete disappearance of actin phase transition in DSC. Our f.t.i.r. results demonstrate that this change in conformation of actin consists of an increase of β -sheets, intermolecular interactions and turns. Moreover, actin filaments attached on the liposomes surface cannot be decorated with heavy meromyosin. The binding site for myosin is therefore modified or buried on the lipid surface [42].

These results raise the possibility that change in actin con-

formation might result from local denaturation. It does not seem to be the case because actin still contains a bound nucleotide, mainly ADP [43], and inhibition of actin interaction with a high concentration of KCl detaches actin filaments, showing the same DSC phase transition as native F actin [24]. Therefore, the phenomenon appears to be reversible because actin at the surface of liposomes is not denatured.

By using high-pressure f.t.i.r., our aim was to determine whether actin molecules penetrate into the lipid bilayer matrix, or whether they are adsorbed at the surface via the polar head groups of phospholipids. Our results show that no lipid vibration frequencies are modified during the interaction with the protein. However, important conformational changes in the actin molecules take place in the presence of DSPC. Therefore, we suggest that actin molecules do not penetrate into the lipid bilayer matrix and that lipids on the bilayer surface interact only with a specific site on the actin molecule (Figure 9). It has been previously shown by Herbet et al. [44] that calcium binds preferentially to the phosphate moiety of the phospholipid head groups of dipalmitoylphosphatidylcholine (DPPC) bilayers, neutralizing the negative charge of the phosphate. Therefore, in the presence of bivalent cations, membranes made with zwitterionic lipids become positively charged, and thus may interact with the negative charges of actin through electrostatic interactions. As the surface of one actin monomer is much larger than that of a lipid molecule, only very few lipid molecules are expected to interact directly with actin. Changes in the i.r. spectra of the lipids would then be too weak to be detected. On the other hand, attachment of these lipid molecules to the specific site of the actin molecule will change the conformation of the protein.

Hence, our finding and characterization of a direct interaction between actin and membrane lipids raises the question: does this phenomenon exist *in vivo* or is it an *in vitro* artifact? One may argue that in our experimental conditions (actin, approx. 0.025 mM; phospholipids, 1.3 mM; bivalent cations, 2.2 mM) many bivalent cations are expected to be bound tightly to the lipid bilayer [44–46], giving rise to a charged surface of the sort that has been previously shown to polymerize actin and bind actin filaments [42,43] and paracrystals [47,48]. Although membrane phospholipids may become charged *in vitro* in the presence of bivalent cations, it should be noted that the concentration of bivalent cations used in the present experiments is compatible with that existing in living cells [49]. Thus, one may expect that the same 'artifact' will also occur *in vivo*.

The present experiments have been done at low concentrations of monovalent cations because actin-lipid interaction is greater at such concentrations. However, we have established by DSC, that 50 % of the interaction still exists in the presence of 100 mM KCl [24], a concentration compatible with those existing *in vivo*.

Several experiments using the hydrophobic photolabel INA (iodonaphthylazide) incorporated into chromaffin-granule membranes, erythrocyte membranes, or brush-border membranes show that this probe does not react with actin [50–52]. This leads to the conclusion that the actin molecule is not in direct contact with the lipids, and has been presented as an argument that actin does not interact with lipids *in vivo*. However, if, as we suggest, actin interacts with only a few lipid molecules, then the labelling of actin with INA should be scarce, and the conclusion that there is no direct contact between actin and the lipid molecules will have to be questioned.

Many studies have established that actin filaments are attached to the membrane by membrane actin-binding-proteins. Using our system, we found that actin may also interact directly with the membrane lipids *in vitro*. We did not prove that the phenomenon exists *in vivo*, but our results strongly suggest that

Figure 9 Proposed model of interaction between actin and membrane lipids

Polar heads of the lipids bind a bivalent cation which results in a positively charged surface attracting actin molecules.

it might be so. Our results are not incompatible with those of others, and therefore suggest that two mechanisms of attachment of actin to the membrane may exist: direct interaction with lipids and attachment through other membrane proteins.

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