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c-Fos Is Surface Active and Interacts Differentially with Phospholipid Monolayers

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The transcription factor c-Fos forms stable Gibbs and Langmuir monolayers at the air-buffer interface. Its marked surface activity is enhanced by penetration into phospholipid films above the protein's own maximum adsorption surface pressure to a lipid-free interface. The protein-phospholipid stabilizing interactions at the interface depend on the lipid polar head group and the increases of lateral surface pressure generated are comparable to those of membrane-active proteins. The surface activity of c-Fos is strong enough to thermodynamically drive and retain c-Fos at the membrane interface where it may exert direct or indirect effects. © 2001 Academic Press

Key Words: **c-Fos; phospholipids; surface activity; phosphatidylinositol; lipid-protein monolayers.**

The membrane phospholipid composition can vary both globally and locally in practically all cellular processes related to growth, differentiation, division and in the response to internal and external signals, thus changing the membrane phase state and domain microheterogeneity. This affects concomitantly the ability of proteins to associate/interact with the membrane whereby their function can be structurally or functionally regulated (1). Although scarcely studied regarding their association with lipids, some proteins involved in DNA replication and transcription were reported to interact with phospholipids at the membrane level: topoisomerase I (2, 3), DNA polimerase (4, 5), histones (6, 7), and the DnaA protein, which in *Escherichia coli* initiates chromosome replication (8–10). This suggests that, at least in prokaryotes, the membrane interface itself may be an important regulator or transducer of protein-mediated DNA transcription.

The transcription factor c-Fos, a key component of the response to stimuli in eukaryotic cells with a well established role as regulator of transcription, has recently been associated to the endoplasmic reticulum and linked to phospholipid synthesis (11). Monomolecular layers at the air-buffer interface provide a simple, unambiguous method for studying interfacial properties of proteins and lipid-protein interactions under well controlled thermodynamic conditions and intermolecular organization (12). With this technique, we studied the surface activity of Fos and disclosed surface-active properties that may enable this protein to act at the membrane level in a novel way by interacting with defined phospholipids.

MATERIALS AND METHODS

Protein purification. Recombinant Fos was purified by affinity chromatography from induced liquid cultures of *E. coli* transformed with the plasmid p6hisFos containing the rat *c-fos* cDNA linked to six histidine residues (13). Typically, 1 liter culture was grown for 3 h at 37°C, then induced with 1 mM IPTG (Promega) for 2–4 h. The cells were collected by centrifugation, resuspended in lysis buffer containing 8 M urea, sonicated and processed according to Maniatis' procedure for inclusion bodies.

The cell homogenate was run through an HisBind resin (Novagen) column and the affinity purified protein eluted in 60 mM imidazole/8 M urea. The yield of purified protein was ~5 mg per liter of culture. Fos was identified by Western blot using anti c-Fos polyclonal antibody (Ab-1 antibody, Santa Cruz, data not shown), and its concentration (1.1 $\mu g/\mu l$) was determined by the Bradford method.

Membrane lipid extraction. NIH 3T3 fibroblasts were grown to confluence in 10 ml Dulbecco's medium with 10% Fetal Calf Serum (GIBCO BRL) for three days. Cells were harvested, resuspended in 1/20 vol distilled water, sonicated and centrifuged for 2 h at 100,000*g*. The pellet was resuspended in 1/50 vol distilled water. Protein content (2.8 $\mu g/\mu l$) of this fraction was determined by the Bradford method and lipids were extracted by the Folch method.

Lipid monolayers. Spread phospholipid (Avanti Polar-Lipids, Inc., Alabaster, AL) monolayers and protein films formed by adsorption (in a compartment with 18 ml of buffer, 18 cm^2 surface) or by

Abbreviations used: PA, dilauroylphosphatidic acid; doPC, dioleoylphosphatidylcholine; doPS, dioleoylphosphatidylserine; dlPG, dilauroylphosphatidylglycerol; dlPE, dilauroylphosphatidylethanolamine; PIP, phosphatidylinositol-4-phosphate; PI, phosphatidylinositol phosphate; PIP_2, phosphatidylinositol-4,5-diphosphate; π_i, initial lateral surface pressure; ΔV, surface (dipole) potential; HEPES, 4-(2-hydroxylethyl)-1piperazineethanesulfonic acid; IPTG, isopropyl β-D-thiogalactopyranoside.

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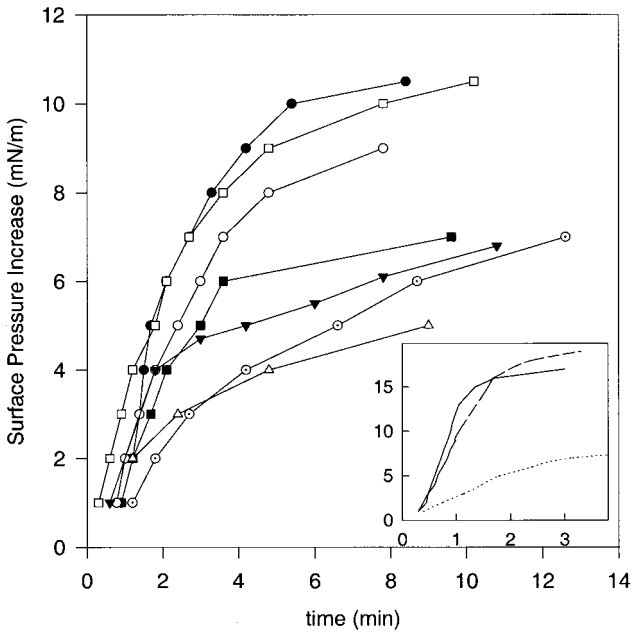

FIG. 1. Penetration of Fos in different phospholipid films. The initial surface pressure of the phospholipid monolayer (before subphase injection of protein) was set at 18 mN/m. The protein concentration in the subphase was 50 nM (△ PE, □ PG, ⊙ PA, ○ PS, ■ PI, ▼ PIP, and ● PIP_2). Inset: adsorption of Fos as a Gibbs monolayer at different protein concentrations in the subphase; black line, 100 nM; dashed line, 50 nM; dotted line, 25 nM.

spreading (in a compartment with 76 ml of buffer, 80 cm^2 surface) were prepared as previously described (14, 15). The buffer contained 64 mM HEPES sodium salt/140 mM NaCl/4.5 mM KCl/0.5 mM $MgCl_2$/5.6 mM glucose, at pH 7. Surface pressure- and surface potential-molecular area compression isotherms or increases of surface pressure at constant surface area were automatically recorded. In the lipid monolayer penetration experiments the amount of protein injected in the subphase was 51 μg (in 46 μl). The molecular weight taken for Fos was 57 000.

RESULTS AND DISCUSSION

Fos forms Gibbs and Langmuir monolayers at the air-buffer interface. The adsorption of c-Fos protein from the subphase as a Gibbs monolayer was monitored by the increase of surface pressure as a function of time (see inset in Fig. 1). At a subphase concentration of 50 nM (a similar increase was obtained at 100 nM) the protein induced a rapid increase in surface pressure to 18 mN/m in 2 min and a concomitant maximum increase of surface potential (ΔV) of 40 mV (discussed below, see also Fig. 4) while the surface pressure increased only to about 8 mN/m after 5 min at 25 nM. These changes are comparable to those found for interfacial adsorption of extrinsic or integral membrane proteins and highly amphipathic peptides (14, 16, 17).

Fos can also spread as a stable Langmuir monolayer directly from aqueous solution or after its prior dissolution in C:M (1:1) giving essentially the same compression isotherms. Thus, the molecular organization of Fos at the interface is similar and independent of the initial conformation adopted in solvents of different polarity, following the behavior of other proteins in monolayers (16–18). The high stability of the Fos monolayer is reflected in the fact that surface pressure- and surface potential-molecular area isotherms are fully reproducible after expansion and recompression cycles (Fig. 2). These isotherms show a hysteresis, again common for membrane lipids and proteins (18, 19), that is equivalent to a rather high compression free energy gap of about 5 Kcal/mole. The monolayer collapse pressure is also very high, occurring at about 45 mN/m, a limiting cross-sectional molecular area of 1.52 nm^2, and a surface potential of about 230 mV. Based on the surface molecular density at 18 mN/m, the overall free energy of adsorption (17) for the protein transfer from the subphase to the interface ($\Delta G_{ads} = -RT \ln C_{int}/C_{sub}$) is −8.1 Kcal/mol. This value is again comparable to that of highly membrane-active and integral proteins (16), reflecting the remarkable surface activity of c-Fos.

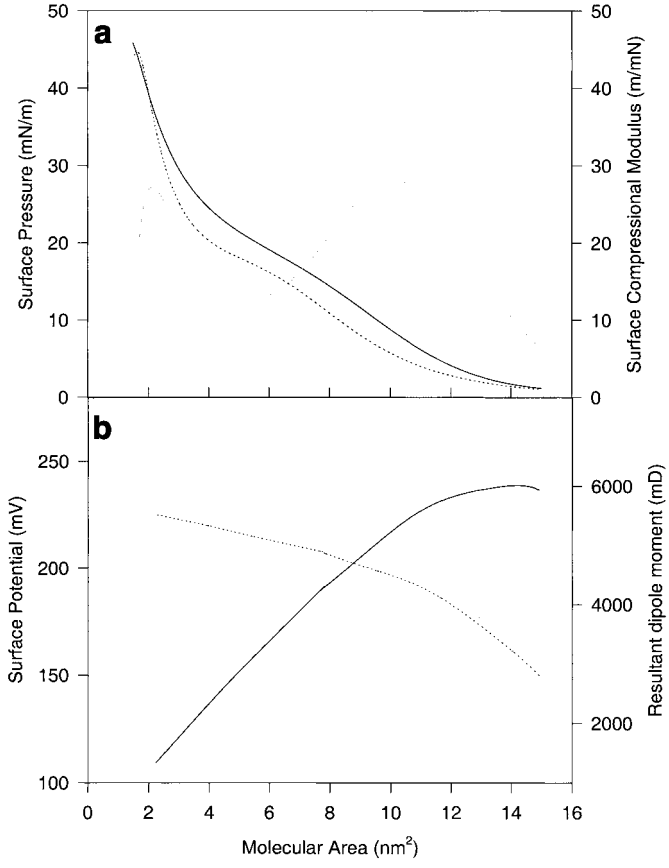

FIG. 2. (a) Compression isotherm (black line) with the corresponding surface compressional modulus (gray line), and decompression isotherm (dotted line) of (a) Langmuir monolayer of Fos. (b) Surface potential (dotted line) and the resultant dipole moment perpendicular to the interface (black line) of the compression isotherm shown in (a).

The closely packed cross-sectional molecular area above 35 mN/m corresponds to a diameter of 1.39 nm which is about the average diameter (1.3–1.5 nm depending on aminoacid side chains) of a protein α-helix with the helical axis perpendicular to the surface (16, 20). A marked rearrangement of the protein intermolecular organization occurs reversibly between about 10–20 mN/m and molecular packing areas between 7–10 nm^2, marked by the slope change in the isotherms and clearly indicated by the variation of surface compressional modulus (Fig. 2a). The change of surface organization at this point is also evidenced by the change in slope of the resultant dipole moment at the same molecular packing areas, due to defined reorientation (16, 21) of the protein resultant dipole in the direction perpendicular to the interface (Fig. 2b). This is also similar to intermolecular reorganizations of membrane proteins in complex monolayer interfaces (18). The electrostatic changes found as the molecular packing increases indicate that the protein resultant molecular dipoles change at a critical pressure of about 10 mN/m from a considerable dipole contributing a positive end pointing toward the hydrophobic (air) side of the monolayer (21), to resultant dipoles pointing progressively away from the interface and into the aqueous subphase. The molecular packing areas at which interfacial reorganization occurs would correspond to about 4–6 α-helical structures perpendicular to the interface. This suggests that at relatively low lateral surface pressures Fos may associate as α-helix bundles, joined by hydrophilic loops in the subphase and less polar sequences on the hydrophobic side of the interface, with resultant dipole moment vectors pointing toward the latter. At higher lateral pressures, or because of pressure fluctuations (about $\pm$15 mN/m) depending on surface compressibility (22, 23), the surface organization of the protein undergoes marked changes and progressively adopts an average packing that corresponds to that of a single α-helix approximately perpendicular to the interface; the overall dipole moment is logically decreased in this case because the reoriented helices previously located at the surface are forced out of it (probably into the aqueous subphase) by the lateral pressure. This may represent an interesting molecular feature of Fos that could enable it to dynamically transduce membrane packing (lateral pressure) and electrostatic (dipole changes) information along the lateral and transverse membrane plane and that would be specifically sensed locally by surrounding lipid dipoles (24). Similar changes have been shown to modulate active transport by membrane pumps and the activity of phosphohydrolytic enzymes related to lipid-mediated membrane signal transduction events (25, 26).

Fos penetrates into phospholipid monolayers. In order to study the penetration of Fos into lipid interfaces we used liquid-expanded phospholipid monolayers. Figure 1 shows increases in lateral pressure after injecting Fos (subphase concentration 50 nM) beneath phospholipid monolayers set at an initial surface pressure (π_i) of 18 mN/m. The latter corresponds to the maximal surface pressure attained by Fos adsorption to lipid-free interfaces (Fig. 1, inset) and further increases beyond this value clearly indicate that Fos is markedly stabilized by interaction with phospholipids. Between about 10–20 min after injecting the protein in the subphase, the increases in surface pressure stabilize at maximal values between 6–10 mN/m, depending on the phospholipid type, which brings the total surface pressure to the rather high value of 24–28 mN/m (14, 16) for the lipid-protein interface. Fos can also interact with, and readily penetrate, a complex lipid interface formed by a mixed monolayer from total lipids extracted from fibroblast membranes, set at a π_i of 18 mN/m, causing surface pressure increases comparable to those found for PI, PIP, or PC films (data not shown). It should be interesting to assess the penetration of Fos into mixed lipid interfaces of defined composition, and whole natural membrane monolayers (18) obtained from different phases of the cell cycle to see if naturally changing physiological conditions at this level can determine differences in Fos-lipid interactions.

It was reported that basic residues in DnaA protein are important for the interaction with defined lipids (27) and that the interfacial insertion of proteins may depend on the membrane phase state (1). In our work, the polar head group of the phospholipid clearly confers different capabilities of Fos to penetrate into monolayers that are in a similar physical state (all liquid-expanded). The interaction appears quite selective: the greater penetration is into monolayers of PIP_2, followed by PG and PS, and the least interaction occurs with PI and PA (and the neutral zwitterionic PC and PE). This points to an interesting possibility regarding the way in which the protein molecules can recognize and associate to the lipid polar head group region and suggests that at least two net negative charges may facilitate the establishment of stabilizing interactions that lead to penetration.

The interfacial penetration of Fos depends on the phospholipid type and the initial surface pressure of the monolayer. The interaction of Fos with each phospholipid species was examined at different initial surface pressures, lower and higher than that of Fos' own maximal adsorption pressure (18 mN/m) (Fig. 3). According to the Gibbs adsorption equation, increases in surface pressure are correlated to increases in the interfacial concentration of molecules (12). In accordance with other membrane and soluble proteins (14, 16), for a given amount of Fos injected into the subphase, protein penetration is inversely

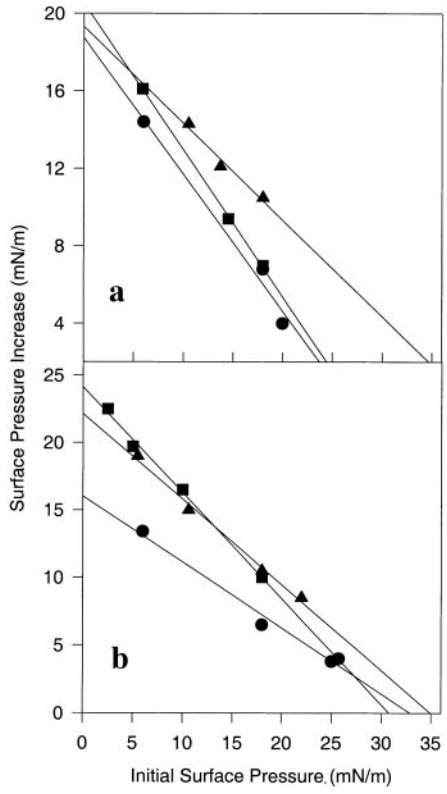

FIG. 3. Dependence of the surface pressure increase with the initial surface pressure (cut-off plot) induced by injection of 50 nM Fos beneath (a) phosphatidylinositol-derivative monolayers, ▲ PIP_2, ● PIP, and ■ PI, and (b) other phospholipid monolayers, ▲ PS, ● PC, and ■ PG.

dependent on π_i. This dependence on π_i is different for different phospholipids; Fos penetrates more into a PIP_2 than into a PIP monolayer at all π_is (Figs. 1 and 3) indicating that phospholipid identity can be distinguished. Moreover, even if doPC does not take protein most avidly, it allows greater monolayer penetration at higher π_i (higher value of the pressure "cut-off" point). These tendencies are shown in Fig. 3: PG and the major membrane phospholipids PC and PS show cut-off values between 30 and 35 mN/m (Fig. 3b), and minor phospholipid components like PIP show cut-off values lower than 25 mN/m, while PIP_2 allows protein penetration to about 35 mN/m. It should be emphasized that the surface activity of Fos is such that it penetrates phospholipid interfaces at the very high average lateral pressures that are likely to occur in biomembranes (22, 23). It seems possible that the amount of Fos associated to the lipid interface can be linked preferentially to local concentrations of PIP_2, permanent or transient, relative to the other phospholipids and be coordinated in this manner with cell cycle or membrane-mediated transduction signals.

The changes in surface potential (ΔV) induced by Fos penetration are positive, and correlate inversely with the initial surface pressure (Fig. 4). Since the surface potential is directly proportional to the resultant dipole moment in a direction perpendicular to the interface (21, 25), its variation suggests that the overall protein dipole contribution in the monolayer is in a direction similar to that of the phospholipids studied so far. At high π_i, the resultant dipole moment contributed by Fos appears reduced by the phospholipid. Both observations—inverse correlation with π_i and dipolar matching or compensation—suggest that the more protein penetrates the film (at low π_i) the less its resultant dipole moment is buffered by the phospholipid dipoles, and vice versa. This is likely pointing out the protein interfacial rearrangement at high surface pressures, in accordance with the changes of intermolecular organization evidenced by the compression isotherm (Fig. 1). Thus, Fos can readily penetrate phospholipid monolayers without substantial variations in its dipolar properties when π_i is below its own maximal adsorption pressure. On the other hand, when π_i of the lipid monolayer is at or above this critical point, the protein penetration involves a surface stabilization by non-ideal interactions with the phospholipid causing a change in the resultant dipole contributions at the interface. Interestingly, PIP_2 is able to keep the maximal change of ΔV at the same level either at high or low π_i (regardless of the variation in Fos penetration) suggesting again that the interaction with this polyphosphoinositide shows particular characteristics whose elucidation requires further studies.

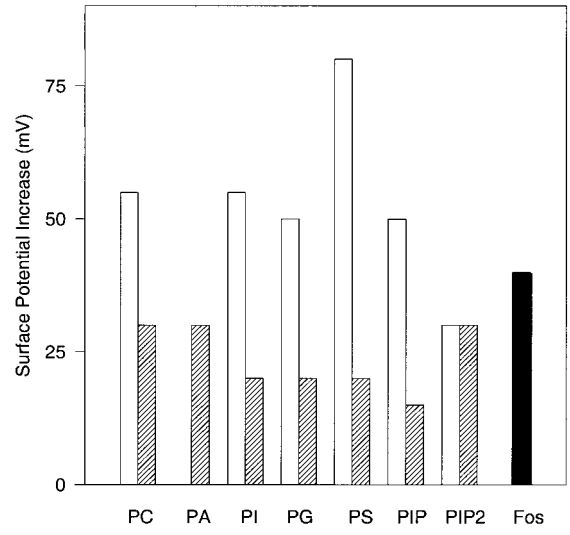

FIG. 4. Net surface potential increases induced by penetration of Fos (50 nM in subphase) into different phospholipid monolayers set at low (5–6 mN/m, white bars) or high (18–19 mN/m, striped bars) initial surface pressures, and adsorption of Fos as a Gibbs monolayer to a lipid-free interface (black bar).

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