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The effect of hydrostatic pressure on membrane-bound proteins

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Abstract

Many cellular proteins are bound to the surfaces of membranes and participate in various cell signaling responses. Interactions between this group of proteins are in part controlled by the membrane surface to which the proteins are bound. This review focuses on the effects of pressure on membrane-associated proteins. Initially, the effect of pressure on membrane surfaces and how pressure may perturb the membrane binding of proteins is discussed. Next, the effect of pressure on the activity and lateral association of proteins is considered. We then discuss how pressure can be used to gain insight into these types of proteins.

Key words
- High pressure
- Membrane proteins
- Volume changes
- Protein-membrane associations
- Protein-protein association
- Lateral interactions

Introduction

High pressure has been used extensively to measure the volume change that accompanies the association between proteins in solution (for an overview, see Ref. 1). Many studies have reported on the contribution of different types of molecular interactions such as electrostriction, hydrogen bonding and packing voids to the volume change of protein-protein associations. In contrast, the effects of pressure on the interactions between membrane-bound proteins have not yet been considered. While these proteins should be subject to the same molecular interactions that regulate the pressure dependence of aqueous soluble proteins, other considerations must be made. The most prominent feature of membrane-bound proteins is the surface on which the protein resides and pressure affects membranes very differently than it affects the aqueous solvent.

The purpose of this review is to describe the few pressure studies of membrane surfaces and membrane-bound proteins that have been reported and to predict how pressure may affect membrane-bound proteins. We will first discuss the effects of pressure on the basic biophysical interactions that promote membrane binding such as membrane surface charge and structure before discussing its effects on membrane-bound proteins. Pressure effects on membrane proteins are summarized in Figure 1.

The effect of pressure on interactions responsible for protein-membrane binding

Surface charge

Biological membranes are negatively charged and many proteins bind to membranes with a strong electrostatic component. Explicit and theoretical treatment of the role of electrostatic interactions in pro-

tein-membrane interactions can be found elsewhere (e.g., Ref. 2). The binding energy from electrostatic interactions is highly dependent on the local ionic strength and will weaken considerably as the salt concentration is raised.

The ionization state of the lipid head groups may be susceptible to changes under pressure. In non-bilayer, detergent micelles, it has been found that pressure promotes ionization of the head groups due to the large volume change that accompanies electrostriction (3). However, this effect is not observed in bilayers where the effect of electrostriction is counteracted by the energy needed to expand and hydrate the surface (3). A lack of significant change in surface charge appears to be general since it was established for an array of biological head groups including electrically neutral phosphatidylcholine, and negatively charged phosphatidylglycerol, phosphatidylserine and phosphatidic acid (3). Note that this latter head group has the greatest potential to become further ionized under pressure. Thus, the charge of membrane surfaces is not expected to be sensitive to pressure.

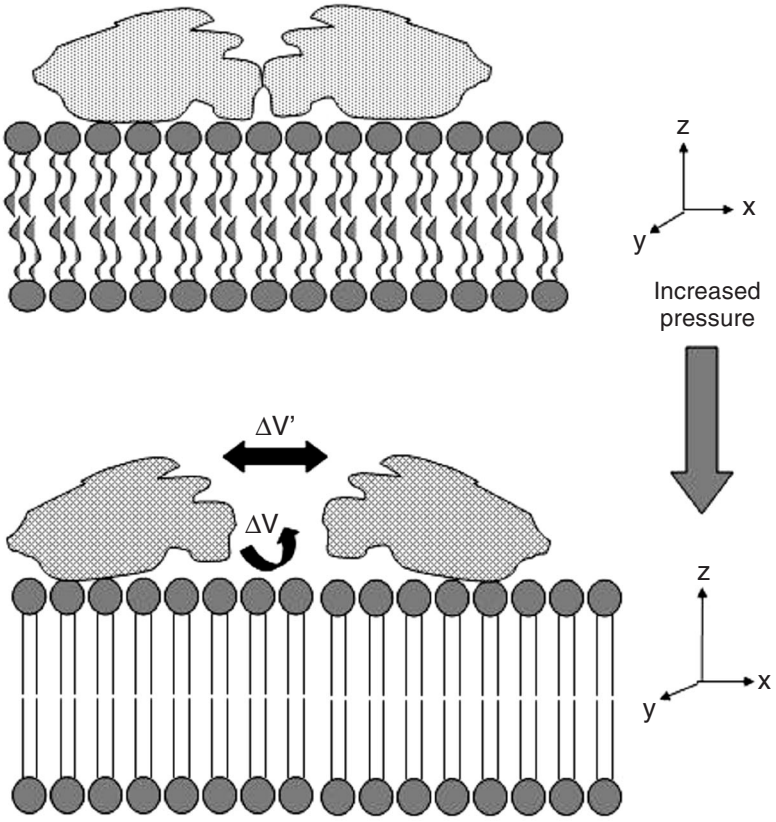

Figure 1. Summary of pressure effects on a membrane-bound protein dimer. The application of pressure on a membrane-bound protein dimer may result in dimer dissociation due to the permeation of solvent into the packing voids in the dimer interface. This dissociation, given by ΔV', should be governed by the same constraints as water-soluble proteins. Increased pressure also compresses the lipid membrane preferentially in the planes perpendicular to the head groups resulting in a lengthening of the bilayer under pressure. However, compression is not expected to greatly perturb the properties of the membrane surface as detailed in the text. Pressure is also expected to promote dissociation of proteins from membranes depending on the size and extent of packing voids between the protein-membrane interface, giving a negative ΔV.

Since many proteins bind to membranes through electrostatic interactions, it is possible that high pressure should reverse binding because of the large volume decrease that accompanies the electrostriction of water around newly formed ions (4). However, this does not appear to be the case. Membrane association of peptides that bind electrostatically is stable under pressure, even when the electrostatic interactions are weakened by elevated salt (5,6). A simple interpretation of this lack of pressure dependence is that the charged groups of both the protein and the membrane do not significantly dehydrate upon binding, thus eliminating the possibility of large volume changes due to electrostriction.

Head group packing

Lipid bilayers have varying compressibilities which depend on their precise localization within the bilayer (7). In general, the head group region is incompressible as compared to the hydrocarbon interior. The application of pressure significantly increases the packing of lipid chains which increases the length of the membrane in the direction parallel to the lipid chains without substantially decreasing the area of the membrane planes perpendicular to the lipid chains (7; see Figure 1). A result of this asymmetric change in membrane dimensions is that the packing of the relatively incompressible lipid head groups is small in the 1-2000 bar pressure range.

When a membrane is highly curved, as in a small unilamellar vesicle, the less polar groups beneath the ionic head groups become exposed, permitting interactions with less charged groups in the protein and may permit partial penetration of these groups (8). This curvature is thought to direct the binding of proteins during the fusion events that occur during endocytosis and the routing trafficking of vesicles through the cell. The geometric factors that contribute to curvature have been well described and therefore we would only like to note that, in many cases, curvature will promote protein binding. It is also important to note that phosphatidylethanolamine lipids, whose head group width is less than their hydrocarbon chains under most physiological conditions, promote bilayer curvature and tend to promote non-specific binding (9).

Since the hydrocarbon interior of a bilayer is more compressible than the head group region, increased pressure is expected to decrease membrane curvature. However, studies comparing the binding of proteins that were pre-bound to small unilamellar, multilamellar and large, unilamellar vesicles showed identical behavior (5,10). More studies that directly compare the rates of association and dissociation of proteins whose binding is curvature-sensitive are needed to fully address the role of curvature in the pressure dependence of protein-membrane interactions.

Surface structure

The surfaces of biological membranes are far from uniform. Irregularities of the membrane surface are due to integral membrane proteins that protrude from the surface, and membrane head groups vary in size (11). Also, membrane lipids have varying length which can cause lipids to either protrude from or sink into the membrane surface. Natural membranes may also contain lipid rafts, i.e., aggregates of lipids in the liquid-ordered (L_o) phase dispersed in liquid-disordered (L_d) phase lipids (for a review, see Ref. 12). The L_o phase is characterized by tight chain packing, reduced fluidity and extended lipid chains, although the lipid mobility is still high. Lipid rafts can be formed in model membranes by mixing high concentrations of cholesterol (i.e., 30 mol%) with sphingomyelin, or with lipids that contain at least one saturated acyl chain. Phase diagrams of these domains have been characterized (e.g., Ref. 13).

Since high pressure promotes chain order, the application of pressure would be expected to reduce the tendency of the L_o phase lipids to phase separate since the physical properties of the phases become increasingly similar with pressure. However, if the lipid rafts consist primarily of cholesterol, this may not be the case since the hydrocarbon rings of cholesterol are not expected to pack well against *cis*-unsaturated lipids. Thus, pressure may reduce the presence of L_o raft phases simply causing the surrounding fluid phase lipids to become ordered, but phase separation may still remain.

We also note that the presence of lipid rafts would be expected to cause irregularities on the membrane surface due to differences in length between the L_o and L_d phase lipids and proteins may also concentrate at these interfaces. As described above, for raft-containing membranes, increasing the hydrostatic pressure would order the fluid phase lipids and thereby decrease mismatches in lipid length.

Post-synthetic modifications

It has been suggested that surface-associating proteins will preferentially partition onto or away from raft phases depending on their post-synthetic modifications (14). Many membrane-associated proteins are modified by one or more hydrocarbon chains, the most common of them being palmitoyl, myristyl, geranylgeranyl, and farnesyl (15). Palmitoyl modi-

fication is distinguished by being a reversible modification, and this reversibility has been proposed to regulate the surface association of some membrane proteins. Isoprenylation, which comprises geranylgeranyl and farnesyl groups, involves *cis*-unsaturated chains. Theoretical predictions and experimental data based on model membrane studies show that if these chains insert into the lipid bilayer they will contribute to the binding energy, and since many surface-associated proteins have more than one *cis*-unsaturated chain, then their role in membrane binding is expected to be significant.

It has been suggested that post-synthetic hydrocarbon modifications play a role in stabilizing their host proteins to lipid rafts (e.g., Ref. 16). This idea is based on the observation that proteins which have a saturated hydrocarbon modification, in particular a palmitoyl group, tend to localize to lipid rafts (14). Localization by palmitoylation is thought to be due to an increase in retention time because of the gel-like nature of the microdomains. In contrast, hydrocarbon modifications that have *cis*-unsaturated bonds, such as geranylgeranyl and farnesylation, would destabilize the tight hydrocarbon packing in lipid rafts, and are thus thought to partition in the fluid domains.

Since hydrocarbon chain modifications are thought to insert into the bilayer, they should stabilize the membrane binding of their host protein. However, this stabilization, if it does occur, is not translated to pressure stability since no changes in the pressure dissociation behavior of a dually palmitoylated protein were observed upon removal of the palmitoyl chains (17). Thus, the idea that the chains are inserted into the lipid bilayer may not be valid in all circumstances.

Behavior of membrane-associated proteins under pressure

In order to study the effect of pressure on membrane-bound proteins, it is important to determine the pressure range that will cause the proteins to remain on the membrane surface. Given that the properties of the membrane surface are not expected to change greatly under pressure, then the membrane-binding stability of a particular protein is governed primarily by its packing efficiency with the membrane surface and its ability to deform under pressure (5). If the protein is not well packed on the membrane surface, then it would dissociate under pressure allowing water to fill in the void volume. In certain specific cases, a protein region close to the membrane surface may be able to deform under pressure and fill in the voids. Since proteins are fairly incompressible, the latter mechanism is less likely.

Example of pressure-induced changes in the activity of a membrane-associated protein

To our knowledge, there is only one example in the literature of changes in the activity of a surface-associating protein while subjected to pressure (18). The enzyme phospholipase Cδ_1 (PLCδ) catalyzes the hydrolysis of a minor lipid component in membranes, phosphoinositol 4,5 bisphosphate, to release the soluble head group inositol 1,4,5 trisphosphate. This reaction also generates a proton allowing the reaction to be monitored spectroscopically through the use of a fluorescent pH indicator. Preliminary control studies using fluorescence resonance energy transfer from PLCδ tryptophan residues to membranes doped with dansyl-phosphatidylethanolamine showed that pressure does not affect the binding of PLCδ to substrate-containing membranes. Also, since the studies monitored lipid hydrolysis it was important to ascertain whether pressure affected the hydration of the membrane surface. This was carried out using a membrane surface probe whose emission is sensitive to water content. It was found that pressure does not significantly affect the surface hydration.

While it was initially predicted that increased pressure would inhibit lipid hydrolysis due to the increase in lipid chain packing, the opposite was observed. The application of pressure was found to significantly increase the rate of hydrolysis. This increase was understood to occur due to two key factors. First, it was found that the enzyme does not significantly penetrate the membrane surface and so the effect of pressure on chain packing does not play a role. Second, it was noted that one of the products generated in the reaction has an additional ionic group permitting a volume reduction due to electrostriction. Since many membrane-bound enzymes are involved in head group hydrolysis and modification of head groups by phosphorylation, their rates under pressure are expected to be governed by electrostriction effects and the number of ions of reactants and products should be considered.

Lateral association of membrane proteins under pressure

Association of proteins on membrane surfaces occurs with higher apparent affinity compared to those in solution for two reasons. First, their effective concentrations are higher due to their confinement to the membrane surface. Second, the number of orientations is reduced on the membrane surface, promoting favorable associations. The increase in the affinity between membrane-bound proteins has been previously treated by assuming that the proteins interact within a reduced volume that is governed by the membrane area in which the proteins are bound multiplied by the length from the membrane surface into the solvent that the proteins associate (19). Usually, this latter parameter is taken from the crystallographic size of the protein.

Even though association is promoted, the volume change of protein associations on membrane surfaces should be identical to the volume change that would be measured if the proteins were freely diffusing in solution. Unfortunately, direct comparison of the volume change of the interaction between membrane-bound versus freely diffusing proteins is quite difficult due to the tendency of membrane proteins to aggregate in the absence of lipids. Inclusion of detergents reduces aggregation but may also perturb protein-protein interfaces. An example of this problem is the study of the pressure behavior of PLCß-Gßγ on membrane surfaces (17). The ΔV for this association was found to be 34 ml/mol. However, when the proteins were dispersed in buffer or in a mild detergent a ΔV of 78 ml/mol was obtained, suggesting that in solution the proteins form higher order aggregates. Clearly, more studies that compare membrane-bound and freely diffusing volume changes are required before general statements can be made.

Discussion

The study of membrane-bound proteins using high pressure clearly has the potential to provide novel information regarding molecular interactions that cause a protein to bind to membranes and their associations with other proteins on the membrane surface. The lack of significant pressure effects on bilayer surfaces greatly simplifies these studies. Since membrane dissociation is specific for an individual protein, pressure may be used to selectively dissociate proteins from the membrane surface (10). Pressure may also be useful for generating reagents due to its ability to change the rate of lipid reactions (18). To date, the effect of pressure on membrane protein associations has not been well characterized. While we propose that pressure effects should be similar to their aqueous counterparts, more examples and direct comparisons must be made.

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