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Review

When a transmembrane channel isn't, or how biophysics and biochemistry (mis)communicate

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ABSTRACT

Annexins are a family of soluble proteins that bind to acidic phospholipids such as phosphatidylserine in a calcium-dependent manner. The archetypical member of the annexin family is annexin A5. For many years, its function remained unknown despite the availability of a high-resolution structure. This, combined with the observations of specific ion conductance in annexin-bound membranes, fueled speculations about the possible membrane-spanning forms of annexins that functioned as ion channels. The channel hypothesis remained controversial and did not gather sufficient evidence to become accepted. Yet, it continues to draw attention as a framework for interpreting indirect (e.g., biochemical) data. The goal of the mini-review is to examine the data on annexin-lipid interactions from the last ~30 years from the point of view of the controversy between the two lines of inquiry: the well-characterized peripheral assembly of the annexins at membranes vs. their putative transmembrane insertion. In particular, the potential role of lipid rearrangements induced by annexin binding is highlighted.

1. Introduction

Most biomedical scientists today will be familiar with annexin A5 (sometimes still referred to as annexin V) due to its widespread use for labeling activated platelets, apoptotic cells, some cancers, etc., in research and clinical settings [1]. These applications are based on the high affinity this protein exhibits for the phospholipid phosphatidylserine (PS) in the presence of calcium. In healthy, non-activated cells, PS is restricted to the inner leaflet of the cell membranes, but becomes exposed upon platelet activation, as a part of apoptosis, or as a result of malignant transformation [2]. What is perhaps less widely appreciated is the rich history of this protein in the areas of structural biology and biophysics.

Annexin A5 (endonexin II, placenta protein 4 (PP4), vascular anticoagulant protein alpha (VAC-α)), was independently discovered by W. Kraus in Germany and C. Reutelingsperger in Holland (reviewed in ref. [1]). Its currently accepted name is based on the nomenclature described in Gerke and Moss [3]. It is an archetypical member of a family of proteins that share two common features: the Ca-dependent affinity for negatively charged phospholipids, such as PS, and the so-called annexin fold—four or eight repeats ~70 amino acids long that comprise the C-terminal core containing the Ca-binding sites [4,5]. Functionally, different annexins are distinguished mainly by their N-terminal domains [5,6], although there are also differences in the core domains [7,8].

There were several driving forces for the extensive and systematic studies of annexins in general and annexin A5 in particular; chief among them was, I think, that this protein challenged the common dogma of structural biology, which contends that structure determines function, and therefore, that it should be possible to deduce the function from the structure [9]. The structure of annexin A5, published by Huber et al. in 1990 [10], was known some 20 years before firm ideas about its function in cell membrane repair started to take shape [11]. The structure also offered very few clues as to what this protein was there for, and some of these clues turned out to be misleading. Without the understanding of its function, its rich and beautiful behavior on lipid membranes was also perplexing. Scientists are a curious lot, and several puzzles in one protein were impossible to resist. The novelty of the annexin fold, distinct from that found in other Ca-binding protein families, further contributed to the interest.

2. Peripheral Assembly of Annexins on Lipid Membranes

Whatever the driving forces, the annexin A5–lipid interactions were studied by an impressive array of techniques. Most important among them was electron microscopy (EM) on lipid monolayers and liposomes [12–21], but small angle neutron scattering (SANS) [22] and grazing angle X-ray diffraction [23], atomic force microscopy (AFM) [24–29], quartz crystal microbalance (QCM) [28,29], ellipsometry [30,31] and Brewster angle microscopy (BAM) [32–34], also made their

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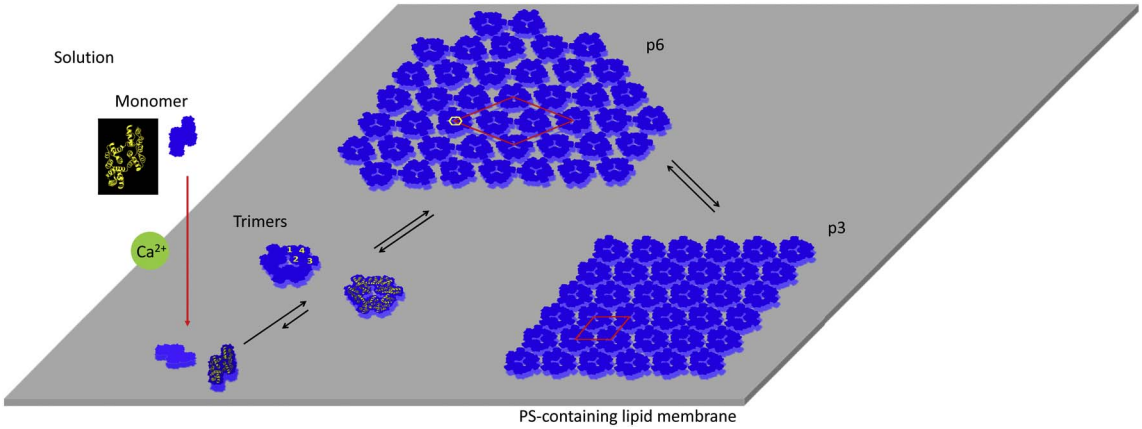

Fig. 1. A schematic illustration of peripheral assembly of annexin A5 on lipid membranes.
The structure of rat annexin A5 (1a8a [42]) was used as the basis for creating this figure. Monomeric protein in solution (blue: surface rendering, yellow: ribbon diagram, slightly enlarged for enhanced visibility) binds to PS in the lipid bilayer (gray) in a calcium-dependent manner and rapidly forms trimers, that serve as the basic motif for the assembly of the classical p6 and p3 crystal forms, as well as other, less common, ordered assemblies described in ref. [20] not indicated in this figure. Trimers are shown in a stylized fashion based on the surface-rendered structure. Domain numbering appearing on one of the trimers is after Huber et al. [10] One of the monomers in the other trimer is overlaid with the ribbon diagram. Lattice constants of the p6 and p3 crystal forms (unit cells are indicated with red lozenges) are a = b = 17.7 nm [19] and a = b = 9.4 nm [19], respectively; $\gamma = 120^{\circ}$ in both cases. Note the presence of the three-fold symmetrical trimers in the six-fold symmetry centers of the p6 lattice; one is highlighted with a yellow hexagon. The transition between the p6 and p3 crystal forms is described in ref. [27] Similar figures appear in refs [6,21,28] and ref. [109]. Further information, including the heights of the annexin A5 molecules above the lipid surface measured with various techniques, can be found in the text. UCSF Chimera [110] was used in the preparation of this figure.

contributions. These techniques will be referred to as “direct”, in the sense that they directly visualize the geometry and topology of the protein-lipid interface (microscopic techniques) or infer its organization via a robust interpretation of scattering profiles (scattering methods).

The results of more than two decades of these efforts can be summarized as follows (see Fig. 1 for a pictorial summary) [6,21,28]: The soluble annexin A5 monomers bind to the PS-containing lipid assemblies in a Ca-dependent manner. On the fluid-phase lipid monolayers, bilayers, or liposomes, the bound protein assembles into trimers that form a variety of ordered arrays, of which the so-called “p6” and “p3” 2D crystal forms are the most common [12,15,16,19,21,35]. Examples of other trimer-based assemblies of annexin A5 can be found in ref. [20]. The assembly process is rapid and occurs at very low amounts of the bound protein [28], as opposed to a similar process, the crystallization of streptavidin on the lipid monolayers and bilayers, which occurs at a surface coverage of ~ 75% [36]. Membrane-bound monomers have not been reported, although in one study we did observe by AFM net-like structures that were probably composed of monomers on lipid bilayers containing sufficient amounts of dipalmitoyl phosphatidylcholine (DPPC) to limit diffusion [26]. On the other hand, there are reports of close-packed (non-crystalline) assemblies of annexin A5 trimers [21]. The requirement for lipid fluidity implies that the trimers form on the membrane rather than in solution [21], where dimers but not trimers have been reported [37,38]. PS content in the lipid phase and calcium concentration in solution govern the assembly process; the higher the calcium concentration, the lower is the PS concentration required for the appearance of the ordered arrays. (A similar effect is observed for the PS/Ca relationship governing the calcium-induced fusion of PS-containing liposomes [39].) Indeed, at sufficiently high (20 mM) Ca concentrations, binding to the fluid-phase PC has been reported [8,21,28,40,41]. Direct physiological relevance of the annexin A5-PC interactions observed at such high calcium concentrations is questionable. Annexin A5 also interacts with phosphatidylethanolamine (PE) [4,8,42,43], but at the physiological PE levels, the interaction does not appear to be relevant either [44]. Other ions, such as Mg, cannot replace calcium in mediating the A5–PS interactions [4,5,30,34]. Here, a parallel with liposome fusion is again evident, because Mg^{2+} and Ca^{2+} behave differently in regards to inducing fusion of PS-containing liposomes; [45] more generally, there is extensive recent literature on the ion-specific effects at interfaces with particular relevance to biology [46–49].

X-ray crystallographic studies and MD simulations resolved the locations of the calcium ions in the protein and the role they play in the interactions between the protein and PS, while correlative X-ray/EM studies established that the conformations of the lipid-bound and soluble protein forms were very similar indeed [13,16,18,19,42,50,51].

The thicknesses of the annexin A5 assemblies relative to the lipid bilayers were determined by various methods. They came very close to the size of the protein in the direction normal to the bilayer expected from X-ray crystallography. Most clear cases include SANS, 3.5 nm [22]; BAM, 3.5 nm [34]; AFM, 2.6 nm (p6 form [24]); QCM, ~ 3 nm [28,52]. In other words, these studies demonstrated unequivocally that annexin A5 behaved as a soluble, membrane-binding protein that assembled on the PS-containing membrane models in a peripheral manner; that insertion of the annexin into the bilayer was neither a prerequisite for its assembly into trimers or higher-order arrays, nor a consequence of such assembly. The assembly of annexin A5 into arrays was recently shown to be important for its cell membrane repair function [11], bringing the biophysical studies of this protein full circle to its biological function [53,54].

Structure, assembly, and interactions of other annexins with lipids have also been studied in considerable detail. Structural data are reviewed in Gerke et al. [6], Gerke and Moss [3], Liemann and Lewit-Bentley [38], and Swairjo and Seaton [5]. Trimer-based assemblies were reported for annexin A4 as well as A6 on lipid monolayers [35,55–57]. On the other hand, several annexins form so-called lipid-lipid junctions where the protein is located between two apposing lipid bilayers, binding them together. Annexin A2-p11 complexes probably represent the most studied example of the junction-forming annexins [3,43,58–62], but lipid junctions are also formed by A1, A4, and A7 annexins. Notably, human annexin A5 does not exhibit this behavior, but chicken annexin A5 has been reported to aggregate lipid vesicles [63]. Human annexin A6 exhibits two types of behavior, depending on the calcium concentration: non-aggregating at low calcium (60–150 μM), aggregating at high calcium (2 mM) [64].

The organization of the junctions formed by the A2, A2-p11, and A1 annexins was analyzed by cryoEM by Lambert et al. [65], and, in the case of annexin A4, by Kaetzel et al. [55] Interestingly, annexin A4 forms both trimer-based lateral membrane arrays and the transverse junctions, apparently simultaneously [55]. The cryoTEM analysis of the annexin junctions involved careful measurements of protein layer thicknesses relative to the lipid bilayers that are clearly visible in the