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Hypothesis

Electrostatic attraction at the core of membrane fusion

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Abstract SNARE proteins appear to be involved in homotypic and heterotypic membrane fusion events [Söllner et al. (1993) Nature 362, 318–324]. The crystal structure of the synaptic SNARE complex exhibits a parallel four-helical bundle fold with two helices contributed by SNAP-25, a target SNARE (t-SNARE), and the other two by a different t-SNARE, syntaxin, and a donor vesicle SNARE (v-SNARE), synaptobrevin. The carboxy-terminal boundary of the complex, predicted to occur at the closest proximity between the apposed membranes, displays a high density of positively charged residues. This feature combined with the enrichment of negatively charged phospholipids in the cytosolic exposed leaflet of the membrane bilayer suggest that electrostatic attraction between oppositely charged interfaces may be sufficient to induce dynamic and discrete micellar discontinuities of the apposed membranes with the transient breakdown at the junction and subsequent reformation. Thus, the positively charged end of the SNARE complex in concert with Ca^{2+} may be sufficient to generate a transient 'fusion pore'.

Key words: SNARE protein; Electrostatic attraction; Membrane fusion

1. Introduction

Neurons communicate with each other by means of neurotransmitters. Membrane fusion is essential for synaptic transmission, a process by which neurotransmitters are released from excited nerve terminals [1]. Recently, the crystal structure of a SNARE complex, a key entity involved in the specific recognition and ultimately fusion of synaptic vesicles with the neuronal plasma membrane, was described [2]. The complex is formed by the specific interaction between segments of three proteins: synaptobrevin-II, a vesicle associated protein, and syntaxin-1A and SNAP-25B, two distinct proteins anchored to the plasma membrane. The clostridial botulinum and tetanus neurotoxins proteolytically cleave these three proteins consequently preventing vesicle fusion and thereby abrogating transmitter release [3]. The SNARE complex folds into a parallel four-helical bundle with a left handed superhelical twist [2,4]: two helices are contributed by a molecule of the t-SNARE SNAP-25, the other two by synaptobrevin and syntaxin. Such a structure may bring into juxtaposition the surfaces of the apposed vesicle and plasma membrane bilayers to facilitate fusion. How this may happen is not known, however, Ca^{2+} is required and other proteins may catalyze and confer additional specificity to the process [5,6]. Here, we focus on highlighting a number of features of this fascinating structure that may provide clues to understand how it mediates bilayer fusion.

2. Electrostatic attractions between oppositely charged interfaces and fusion

A key finding emerged from electrostatic calculations that showed a conspicuous enrichment of positively charged residues at the carboxy-terminal end of the complex [2]. This boundary is assigned to be at the membrane-anchored end of the complex, and therefore, at the minimum distance between the apposed membranes. It is known that negatively charged lipids are preferentially distributed in the inner leaflet of the bilayer plasma membrane [7]. It is also well recognized that Ca^{2+} and highly basic polypeptides interact with negatively charged lipids to induce a lamellar to hexagonal phase transition with the consequent generation of local micellization foci and, if propagated, the breakdown of the bilayer structure [7–10]. And it is well established that acidic lipids are required for the insertion into and translocation across bilayers of a number of channel-forming proteins, conspicuous among them diphtheria [11] and tetanus [12] toxins, colicin [13] and Bcl-2 family proteins [14]. It appears therefore, that electrostatic interaction energy between oppositely charged interfaces might drive discrete micellizations of the apposed membranes with the transient breakdown of the hydrophobic membrane barrier and the consequent release of the transmitter. Thus, the positively charged end of the SNARE complex in concert with Ca^{2+} may be sufficient to generate a transient 'fusion pore'. This hypothesis could be tested using purified SNARE proteins reconstituted into separate bilayer vesicles of defined phospholipid composition [15]. This model system has demonstrated that SNARE proteins are necessary and sufficient for fusion in the absence of other protein components, albeit at a low rate and efficiency [15].

3. Acidic phospholipids and Ca^{2+} ions as mediators of fusion

Another striking feature of the SNARE complex is the occurrence of four shallow grooves at the surface of the helical bundle [2]. Such grooves, particularly those present at the basic charged end of the complex, may provide specific binding pockets for acidic lipids. There is structural evidence for such lipid binding pockets: the crystal structure of a type IIβ phosphatidylinositol phosphate kinase reveals an extensive flat basic surface well suited for the interfacial binding of phosphoinositides and catalysis [16]. The crystal structure of the annexin XII hexamer displays a prominent concave disc with numerous surface exposed Ca^{2+} ions on the perimeter [17].

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Presumably, these Ca^{2+} ions mediate annexin binding to phosphatidylserine, its insertion into membranes and lead to channel formation and membrane fusion [18].

Specific protein-lipid interactions are considered key events in viral fusion mechanisms and the analogy to the SNARE coil-coiled complex has been drawn [15]. The recent identification of a mitochondrial v-SNARE [19] combined with the abundance of acidic lipids in mitochondrial membranes [7] raise the intriguing possibility that SNAREs could be at the fusion interface between mitochondria and other organelles, as it appears to be between yeast vacuoles [20,21]. It would be interesting to examine in molecular detail if the electrostatic attraction between oppositely charged interfaces is sufficient to induce dynamic and discrete micellar discontinuities of the apposed membranes with the transient breakdown at the junction and subsequent reformation. Attention to Ca^{2+} as a mediator of protein-lipid interactions at membrane fusion interfaces is worth revisiting in view of the new structural information.

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References

[1] Söllner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. and Rothman, J.E. (1993) Nature 362, 318–324.
[2] Sutton, R.B., Fasshauer, D., Jahn, R. and Brunger, A.T. (1998) Nature 395, 347–353.
[3] Montecucco, C. and Schiavo, G. (1995) Q. Rev. Biophys. 28, 423–472.
[4] Poirier, M.A., Xiao, W., Macosko, J.C., Chan, C., Shin, Y.-K. and Bennett, M.K. (1998) Nature Struct. Biol. 5, 765–769.
[5] Shao, X., Li, C., Fernandez, I., Zhang, X., Südhof, T.C. and Rizo, J. (1997) Neuron 18, 133–142.
[6] Rettig, J., Heinemann, C., Ashery, U., Sheng, Z.-H., Yokoyama, C.T., Catterall, W.A. and Neher, E. (1997) J. Neurosci. 17, 6647–6656.
[7] De Kruijff, B. (1997) Nature 386, 129–130.
[8] Sherwood, D. and Montal, M. (1975) Biophys. J. 15, 417–434.
[9] Montal, M. (1972) J. Membr. Biol. 7, 245–266.
[10] Siegel, D.P. and Epand, R.M. (1997) Biophys. J. 73, 3089–3111.
[11] Donovan, J.J., Simon, M.I. and Montal, M. (1982) Nature 298, 669–672.
[12] Gambale, F. and Montal, M. (1988) Biophys. J. 53, 771–783.
[13] Zakharov, S.D., Lindeberg, M., Griko, Y., Salamon, Z., Tollin, G., Prendergast, F.G. and Cramer, W.A. (1998) Proc. Natl. Acad. Sci. USA 95, 4282–4287.
[14] Schendel, S.L., Xie, Z., Oblatt-Montal, M., Matsuyama, S., Montal, M. and Reed, J.C. (1997) Proc. Natl. Acad. Sci. USA 93, 5113–5118.
[15] Weber, T., Zemelman, B.V., McNew, J.A., Westermann, B., Gmachl, M., Parlati, F., Söllner, T.H. and Rothman, J.E. (1998) Cell 92, 759–772.
[16] Rao, V.D., Misra, S., Boronenkov, I.V., Anderson, R.A. and Hurley, J.H. (1998) Cell 94, 829–829.
[17] Luecke, H., Chang, B.T., Maillard, W.S., Schlapfer, D.D. and Haigler, H.T. (1995) Nature 378, 512–515.
[18] Langen, R., Isas, J.M., Hubbell, W.L. and Haigler, H.T. (1998) Proc. Natl. Acad. Sci. USA 95, 14060–14065.
[19] Isenmann, S., Khew-Goodall, Y., Gamble, J., Vadas, M. and Wattenberg, B.W. (1998) Mol. Biol. Cell 9, 1649–1660.
[20] Ungermann, C., Sato, K. and Wickner, W. (1998) Nature 396, 543–548.
[21] Peters, C. and Mayer, A. (1998) Nature 396, 575–580.