Hypothesis

ON THE POSSIBLE PARTICIPATION OF ACID PHOSPHOLIPIDS IN THE TRANSLOCATION OF SECRETED PROTEINS THROUGH THE BACTERIAL CYTOPLASMIC MEMBRANE

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1. Introduction

Significant progress has been made in understanding the molecular basis of bacterial extracellular protein secretion [1,2]. Several pathways for protein translocation have been proposed [1,3–7]. The 'signal' hypothesis emphasizes translocation through a passive protein channel, formed by the specific receptor proteins of membranes, which interact with the signal peptides of the newly synthesized protein [3].

Other models [4–7] assume protein translocation to be a spontaneous process that does not require specific membrane receptors or transport proteins.

The 'membrane-triggered folding' hypothesis emphasizes self-assembly and the role of protein conformational changes during transfer from an aqueous compartment into a membrane [4]. The helical hairpin hypothesis proposes that the secretion of a protein through a membrane is initiated by formation of a helical hairpin structure [7]. This model, in its theoretical treatment of secretion and topological aspects, overlaps with the 'direct transport' model [5] and the 'loop' model [6]. The idea that a crucial role is played by the structure of the secreted protein is central to all these models [1,3–7].

Unfortunately, these models do not take into account the dynamic feature of membrane structure and a possible active role of the membranes in protein translocation. Most recent observations in bacteria, however, point to a link between formation and secretion of proteins and lipid exchange and the lipid structure of the membranes [8–19]. This allows us to suggest that the lipid component of the membrane plays an active role in protein translocation. We propose a new model of protein secretion, which takes into account both the role of protein structure and an active role for membrane in protein translocation. The model proposed suggests the interaction of the signal peptide of the newly synthesized protein with acid phospholipids of the membranes. This results in the initiation of the transmembrane movement of phospholipids and the coupled translocation of secretory proteins and phospholipids across the membrane. Thus phospholipids and the secreted protein promote the movement of each other (fig.1).

2. Coupled translocation of secretory proteins and acid phospholipids through the cytoplasmic membrane of bacteria

2.1. *The interaction of the signal peptide of the nascent protein with acid phospholipids of the membranes*

Most exported proteins in procaryotes are synthesized as larger precursors with an additional peptide extension (signal peptide) near the N-terminal end. This amino-terminal peptide contains two different sites; the N-distal section of the extension is basic and positively charged at neutral pH, because it contains lysine but no acidic amino acid residues; the next region is hydrophobic [1]. However, the bacterial membrane surface is negatively charged at neutral pH because of acid phospholipids (phosphatidylglycerol and cardiolipin in *Escherichia coli*). The structure of both the signal peptide and the membrane provides for the possibility of both ionic and hydrophobic interaction between them.

According to the model proposed, the basic, positively charged site of the N-terminal extension of the nascent peptide is involved in the initial attachment of the protein, and consequently the polysomes, to

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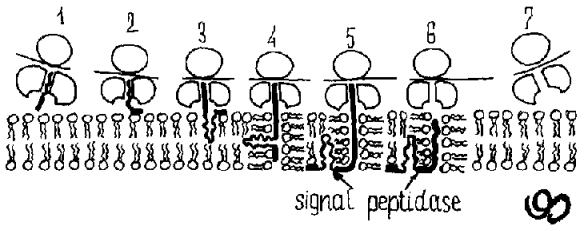

Fig.1. Schematic illustration of the secretion of protein across the cytoplasmic membrane coupled with phospholipid translocation: (1) nascent peptide, containing N-distal charged site of the signal sequence, begins to emerge from a ribosome; (2) nascent peptide binds to negatively-charged phospholipids; (3) hydrophobic site of signal peptide is produced and inserts into the membrane interior; (4,5) acid phospholipid losing its charge starts transbilayer movement, inducing the formation of a hydrophilic channel (hexogonal configuration), and also pulls the hydrophilic part of the secreted protein across the channel; (6) polypeptide elongation completed, the signal sequence is removed by peptidase and protein released from the membrane.

the negatively charged acid phospholipids (probably to cardiolipin) of the inner surface of the cytoplasmic membrane through ionic interaction. The next, hydrophobic region of the nascent peptide is then synthesized and progressively inserted into the membrane interior in U-shaped fashion by hydrophobic interaction with the fatty acid site of phospholipids. This step of our scheme overlaps with the 'loop' model of lipoprotein translocation in [1,6]. This final step results in the anchoring of the newly synthesized peptide in the membrane. The screening of the charged part of the phospholipids and the high mobility of the fatty acid tails are necessary for this insertion.

Some recent observations strongly suggest that the interaction of proteins with acid phospholipids is a widespread phenomenon and plays a definite role in the biogenesis of membranes and secreted proteins. The interaction of integral membrane proteins primarily with acid phospholipids in both native [20,21] and artificial membranes [22] has been demonstrated. In addition, the biosynthesis and assembly of a number of secretory proteins: procoat protein of f_1 phage [9] and alkaline phosphatase in *E. coli* [8], and the electron transport system in *S. aureus* [10] was correlated with the increase in the content and the metabolism of acid phospholipids. The biosynthesis of alkaline phosphatase in *E. coli* correlates, in particular, with the increase in the relative content and metabolism of phosphatidylglycerol (PG) in these cells [8]. The treatment of membranes by phospholipases A_2 and C also revealed a correlation between the extent of PG hydrolysis and the level of biosynthesis of alkaline phosphatase in *E. coli* cells as well as enzymatic activity of membrane-bound form of the enzyme. It was shown that this phospholipid was less available to the action of phospholipases in cells synthesizing the enzyme intensively (6% of PG were hydrolyzed) as compared to cells in which the synthesis and secretion of the enzyme are absent (45% PG were hydrolyzed in these cells) [11]. The results obtained allow us to suggest an interaction between PG and the enzyme during the biogenesis of the latter. This was confirmed by experiments with cells treated before the de-repression of alkaline phosphatase, with the lipotrophic antibiotic polymixine B which interacts with acid phospholipids cardiolipine (CL) and PG [23]. The level of the enzyme in such cells is reduced by half.

This change in the level of enzyme synthesis correlates with a change in the ratio between acid phospholipids, which is probably due to a disturbance in their interconversion, or/and translocation in these cells.

We have also observed a dependence of the biosynthesis of secreted proteins on the fluidity of membranes [12] and the content of unsaturated fatty acids [13,14]. In particular, a correlation was detected between the biosynthesis and secretion of alkaline phosphatase and the content of *cis*-vaccenic acid, which was changed by a temperature shift-down or shift-up [13], as well as by lipotrophic agents (alcohols) [14]. The biosynthesis of the enzyme did not correlate with the degree of unsaturation of total lipids and the level of unsaturation of phospholipids is important probably only in the specific part of the membrane participating directly in the biosynthesis and translocation of the enzyme. *cis*-Vaccenic acid is peculiar to acid phospholipids and the content of this acid increases during temperature shift-down [24].

Probably, increased unsaturation of fatty acids appearing in phospholipids in a translocation site of a membrane increases their mobility and promotes the insertion of hydrophobic sequences into membrane.

2.2. *The initiation of the transmembrane translocation of phospholipids and coupled translocation of secretory proteins and phospholipids*

The most important consequence of the interaction of a signal peptide with acid phospholipids might be the initiation of trans-membrane movements of these

phospholipids. Due to this interaction the latter, losing their charge and, consequently, their hydrophilic properties, would begin to move to the hydrophobic region of the membrane, carrying the bound peptide with them.

In addition, both peptide and phospholipid would promote the movement of each other: the signal peptide during its elongation on the ribosomes is envisaged to push the phospholipid, which in its turn, pulls the signal peptide.

Transbilayer movement of phospholipids is known to be a dynamic feature of a membrane structure [25,26] and has been revealed both in Gram-positive [27,28] and Gram-negative bacteria [29,30]. Molecular mechanisms determining the rate of the transbilayer movement of phospholipids are not completely understood. However, the interaction of proteins with lipids is an important factor. Thus the introduction of peptides [32] or membrane proteins [22,31] into the lipid bilayer causes a change in the surface pressure between the two monolayers. The alteration of a phospholipid charge can also modulate and facilitate transmembrane movement of phospholipids. The introduction of a double bond into phospholipids can also increase the flip–flop of phospholipids [33]. In summary, comparison of conditions necessary for the biosynthesis and translocation of secreted proteins and those inducing transbilayer movements of phospholipids shows many similarities (table 1).

Table 1
Similarity of conditions affecting the synthesis and secretion of secretory proteins and the transbilayer movement of phospholipids

Biosynthesis and translocation of secreted proteins	Phospholipid translocation
1. Are accompanied by the interaction with acid phospholipids [8–11]	Is promoted by interaction with polypeptides and proteins [22,31–32]
2. Are promoted by increases in unsaturation of phospholipids [12–14]	Is promoted by the introduction of double bonds into phospholipids [33]
3. Correlate with the net synthesis of phospholipids [15–19,49][a]	Can be promoted by synthesis and exchange of phospholipids [37]
4. Depend on the membrane potential [34–36]	Depends on the membrane potential [30]

[a] This correlation relates on the whole to soluble, secreted proteins [15,16,18,19] and some specific membrane proteins, e.g., OmpF and OmpC [19]. The bulk synthesis of membrane proteins was shown, however, to be unaffected by the cessation of the phospholipid synthesis [18,38]; synthesis of lipids was shown to be coordinated with protein synthesis [38]

All of the above supports the possibility of a coupling of two important membrane processes: translocation of proteins and phospholipids through membranes. Of particular importance for the comprehension of the dynamic rôle of membranes and transbilayer movements of phospholipids of membranes are recent ideas of the metamorphic, mosaic structure of biological membranes and the demonstration of the capability of lipids to adopt a non-bilayer configuration under certain conditions [39,40]. It was suggested that such a non-bilayer configuration may be involved in multi-functional abilities of biological membranes, including different membrane transport processes [40], and transbilayer movements of phospholipids. Structures, such as intrabilayer 'inverted micelles' or 'inverted cylinders' (H_{II} phase), could serve as intermediaries in flip–flop processes.

One may suggest that the interaction of the signal peptide with acid phospholipids modulates the adoption of a non-bilayer configuration of phospholipids. Thus hydrophilic lipid channels (inverted short cylinders) may form as an intermediary in flip–flop of phospholipids, through which the major hydrophilic part of a secreted protein is linearly translocated during its synthesis, pulled by moving phospholipids with the signal peptide anchored to them. The coupled translocation of proteins and phospholipids is completed with the processing and maturation of the secreted protein, releasing it from the membrane.

This hypothesis is further supported by the observation that acid phospholipids of bacteria have a very high rate of exchange [41] and transbilayer movement [30]. Moreover, cardiolipine plays an important rôle in the adoption of the non-bilayer configuration and formation of the hexogonal structure [40]. It was also observed that there is a slight preference for PG localization in the outer layer of Gram-negative envelopes [42,44] while CL was found to be localized preferentially in the inner layer of membranes [43] and as suggested, the latter transforms to PG during the transbilayer movement [45]. There may be a relationship between this transformation and energy metabolism, because changes in proportions of PG and CL correlate with the cellular phosphorylating ability

[46]. During the transformation of CL to PG, energy could be liberated [47].

If CL transforms to PG during its translocation with the liberation of energy, this energy may be used for translocation of the protein in addition to the energy of polypeptide elongation on ribosome.

3. Conclusion

The model proposed fully agrees with data on the secretion of protein in bacteria and, unlike the previous models, it takes into account both the role of the structure of secreted proteins, and the active role of the membranes in this process. The model is also in agreement with recent data on the interrelation between the formation of secreted proteins and phospholipid exchange, and the dynamic feature of lipid structure of membranes. The signal peptide is presumed to be responsible for the interaction with membrane phospholipids and for the initiation of transmembrane movement of phospholipids. The peptide therefore retains a certain signal function, but does not participate itself in the translocation of the protein. This conclusion for the passive role of the signal peptide, is based on the analysis of the role of hydrophobicity of peptides in protein translocation [48]. Unlike the earlier models, the model proposed suggests a leading role for phospholipids in protein translocation.

References

[1] Inouye, M. and Halegoua, S. (1980) Crit. Rev. Biochem. 7, 339–371.
[2] Hall, M. H., Emer, S. D. and Silhavy, T. J. (1980) J. Supramol. Struct. 13, 147–163.
[3] Blobel, G. and Dobberstein, B. (1975) J. Cell Biol. 7, 835–851.
[4] Wickner, W. (1980) Science 210, 861–863.
[5] Von Heijne, G. and Blomberg, G. (1979) Eur. J. Biochem. 97, 175–181.
[6] Di Rienzo, M., Nakamura, K. and Inouye, M. (1978) Annu. Rev. Biochem. 47, 481–532.
[7] Engelman, D. M. and Steitz, T. A. (1981) Cell 23, 411–422.
[8] Evdokimova, O. A. and Nesmeyanova, M. A. (1977) Biochimia 42, 1791–1799.
[9] Chamberlain, B. K. and Webster, R. E. (1976) J. Biol. Chem. 251, 7739–7745.
[10] Frerman, F. E. and White, D. C. (1967) J. Bacteriol. 94, 1868–1874.
[11] Nesmeyanova, M. A. and Evdokimova, O. A. (1979) Biokhimia 44, 1512–1519.
[12] Kimura, K. and Izui, K. (1976) Biochem. Biophys. Res. Commun. 70, 900–906.
[13] Evdokimova, O. A., Nesmeyanova, M. A. and Kulaev, I. S. (1978) Biokhimia 43, 1680–1687.
[14] Zemljanukhina, O. A., Kolycheva, V. V. and Nesmeyanova, M. A. (1981) Biokhimia 46, 92–98.
[15] Izui, K. (1971) Biochem. Biophys. Res. Commun. 45, 1506–1511.
[16] Beacham, I. R., Taylor, N. S. and Ionell, M. (1976) J. Bacteriol. 128, 522–527.
[17] Cashman, I. S. and Webster, R. E. (1977) J. Bacteriol. 129, 1245–1249.
[18] Paton, I. C., May, B. R. and Elliott, W. (1980) J. Gen. Microbiol. 118, 179–187.
[19] Boequet-Pages, C., Lazdunski, C. and Lazdunski, A. (1981) Eur. J. Biochem. 118, 105–111.
[20] Bevers, E. M., Wang, H. H., Op den Kamp, J. A. F. and Van Deenen, L. L. M. (1979) Arch. Biochem. Biophys. 193, 502–508.
[21] Höchli, M. and Hockenbrock, C. R. (1979) Proc. Natl. Acad. Sci. USA 76, 1236.
[22] De Kruijff, B. and Cullis, P. R. (1980) Biochim. Biophys. Acta 602, 477–490.
[23] Nesmeyanova, M. A., Bogdanov, M. V., Kolot, M. H., Zemlianukhina, O. A. and Kulaev, I. S. (1982) Biokhimia, in press.
[24] Kito, M., Ishinaga, M., Nishihara, M., Kato, M., Iawada, S. and Hata, T. (1975) Eur. J. Biochem. 54, 55–60.
[25] Op den Kamp, J. A. F. (1979) Annu. Rev. Biochem. 48, 47–57.
[26] Van Deenen, L. L. M. (1981) FEBS Lett. 123, 1–16.
[27] Bergelson, L. D. and Barsukov, L. I. (1977) Science 197, 224–226.
[28] Rothman, J. E. and Kennedy, R. P. (1977) J. Mol. Biol. 100, 603–618.
[29] Jones, N. C. and Osborn, M. J. (1977) J. Biol. Chem. 252, 7405–7412.
[30] Donohue-Rolfe, A. M. and Schaechter, M. (1980) Proc. Natl. Acad. Sci. USA 77, 1867–1871.
[31] Van Zoelen, E. J. J., De Kruijff, B. and Van Deenen, L. L. M. (1978) Biochim. Biophys. Acta 508, 97–108.
[32] De Kruijff, B. and Cullis, P. R. (1980) Biochim. Biophys. Acta 601, 235–240.
[33] De Kruijff, B., Cullis, P. R. and Radda, G. K. (1975) Biochim. Biophys. Acta 406, 6–20.
[34] Date, T., Goodman, J. M. and Wickner, W. T. (1980) Proc. Natl. Acad. Sci. USA 77, 1669–1679.
[35] Pugsley, A., Conrard, D. J., Schnaitman, C. A. and Gregg, T. J. (1980) Biochim. Biophys. Acta 599, 1–12.
[36] Enequist, M. G., Hirst, T. R., Harayama, S., Hardy, S. J. S. and Randall, L. L. (1981) Eur. J. Biochem. 116, 227–233.
[37] De Kruijff, B. and Baken, P. (1978) Biochim. Biophys. Acta 507, 38–47.
[38] Mindish, L. (1975) Membrane Biogenesis (Tzageoloff, A. ed) pp. 429–454, Plenum, New York.
[39] Cullis, P. R. and De Kruijff, B. (1979) Biochim. Biophys. Acta 559, 399–420.

[40] Cullis, P. R., De Kruijff, B., Hope, M. J., Nayar, R. and Schmid, S. L. (1980) Can. J. Biochem. 58, 1091–1100.
[41] Ballesta, J. P. G. and Schaechter, M. (1971) J. Bacteriol. 107, 251–256.
[42] Michaelson, D. M., Horwitz, A. F. and Klein, M. P. (1973) Biochemistry 12, 2637–2645.
[43] Berder, J. A., Barker, R. W. and Radda, G. K. (1975) Biochim. Biophys. Acta 375, 186–208.
[44] Barsukov, L. I., Kulikov, V. I. and Bergelson, L. D. (1976) Biochem. Biophys. Res. Commun. 71, 704–711.
[45] Barsukov, L. I., Kulikov, V. I. and Bergelson, G. K. (1977) Biokhimia 42, 1539–1545.
[46] Peterson, R. H. F. and Buller, C. S. (1968) J. Virol. 3, 463–468.
[47] Andreev, L. V. (1978) All-Union Conference of evolutional Biochemistry and Origin of Life, Tbilisi, Proc. pp. 43–44.
[48] Von Heijne (1981) Eur. J. Biochem. 116, 419–422.
[49] Pages, C., Lazdunski, C. and Lazdunski, A. (1982) Eur. J. Biochem. 122, 381–386.