Volume 285, number 2, 220–224 FEBS 09959 July 1991

ADONIS 0014579391006802

Minireview

Biogenesis of secretory granules

Implications arising from the immature secretory granule in the regulated pathway of secretion

Sharon A. Tooze

EMBL, Meyerhofstrasse 1, 6900 Heidelberg, Germany

Received 7 May 1991

In endocrine cells the regulated secretion of hormones, peptides, enzymes and neurotransmitters into the external medium occurs when mature secretory granules fuse with the plasma membrane. Secretory granules form at the trans-Golgi network (TGN) by envelopment of the dense-core aggregate of regulated secretory proteins by a specific membrane. The secretory granules initially formed at the TGN, referred to here as immature secretory granules, are morphologically and biochemically distinct from mature secretory granules. The functional similarities and differences between the immature secretory granule and the mature secretory granule, and the events involved in the maturation of the secretory granules are briefly discussed.

Regulated secretion; Endocrine; Immature secretory granule; Maturation; Clathrin adaptins; GTP-binding proteins

1. INTRODUCTION

All cells secrete newly synthesized molecules into their environment. Most cells secrete proteins constitutively but some specialized cells, such as endocrine and exocrine cells, have two secretory pathways, the ubiquitous constitutive pathway and a second regulated pathway. While the constitutive pathway of secretion functions to release molecules continuously into the environment, the regulated pathway of secretion is under the control of external factors such that the release of biologically active substances including hormones, peptides, enzymes and neurotransmitters, depends on receipt of an external signal. As far as is known, both pathways of secretion utilize the same cellular machinery for the synthesis and transport of the secreted molecules until the latter reach the trans-Golgi network. In the trans-Golgi network (TGN; [1]) the two pathways diverge and acquire the particular characteristics which enables one to differentiate them. The divergence of the two pathways, and thus the formation of two distinct populations of secretory vesicles, must entail the sorting and segregation of both the secreted cargo molecules, and the cellular machinery involved in the regulation of release of the vesicles. The purpose of this review is to discuss the recent data on one specific aspect of regulated secretion in endocrine cells, namely the formation of the immature secretory granule, which has recently been biochemically identified using a cell-free assay [2] and the events involved in the maturation of the immature secretory granule to the mature secretory granule. For another recent review on regulated secretion, the reader is referred to Miller and Moore [3].

Correspondence address: S.A. Tooze, EMBL, Meyerhofstrasse 1, 6900 Heidelberg, Germany. Fax: (49) (6221) 387306.

2. THE SECRETORY PROCESS

2.1. *Formation of secretory granules*

Secretory granules form at the TGN by envelopment of a dense core aggregate of regulated secretory proteins by membrane. The processes involved in the formation of the secretory granule can be considered as three distinct events: (i) the selective condensation or precipitation of the proteins which aggregate to form the dense core followed by (ii) the selection of the membrane which envelops the aggregate, and (iii) fusion of the membrane bilayers to release the nascent secretory granule. The first step is believed to be selective due to the intrinsic properties of the regulated proteins packaged in the cores, which enable them to condense

Published by Elsevier Science Publishers B.V.

and come out of solution, either because of an increased concentration [4] of the proteins in the TGN or a change in milieu [5]. The second step may be mediated by a specific receptor, or receptors, in the TGN membrane that bind aggregated secretory proteins although to date no common motif has been identified on any regulated secretory protein which might function as a receptor binding domain. Virtually no information is available with respect to the third and final step, although it has been recently shown that GTP-hydrolysis is required for the budding of secretory vesicles from the TGN in a cell-free system [6] suggesting that GTP-binding proteins may be involved.

2.2. *Properties of secretory granules*

Biochemical studies have shown that in endocrine cells, mature secretory granules contain processed hormones, peptides, small molecules, catecholamines (in chromaffin cells) (for review see Winkler [7]) and a family of proteins called the granins [8]. Secretory granules also contain enzymes such as (i) the pro-hormone processing enzymes, now referred to as PC2 [9] and PC3 [10], that generate the final processed products from the pro-hormone precursor, (ii) enzymes that further modify the processed products such as carboxypeptidase E [11], and peptidyl-glycine-α-amidating monooxygenase [12], and (iii) enzymes involved in the biosynthesis of catecholamines such as dopamine-β-hydroxylase [7]. Some of these molecules are believed to have both a membrane associated and soluble form [7,13,14]. In addition, the membrane of the secretory granule must also contain a subset of specific proteins such as the H^+-ATPase [15], responsible for the acidification of the secretory granule, and in certain cells (for example chromaffin cells) the catecholamine transporter [16]. Finally, for the fusion of the secretory granule with the plasma membrane, the secretory granule must have associated with it one or more GTP-binding proteins (see below) and the machinery required for the actual fusion event [17]. Recently, secretory granules isolated from adrenal chromaffin cells have been shown to have small GTP-binding proteins [18–20].

2.3. *Exocytosis of secretory granules*

The secretory granule must be translocated through the cytoplasm to the plasma membrane for fusion of the granule membrane with the plasma membrane to occur. In neurons, the translocation of the secretory granule in the cytoplasm is mediated by interaction with microtubules [22], and recent experiments with AtT20 cells demonstrate this is also true for endocrine cells [23,24]. At the plasma membrane, changes occur in the sub-membranous cytoskeleton, in particular the actin filament network, which allow the secretory granule to dock at the plasma membrane [17]. The cascade of events involved in the exocytosis of the granule content is regulated by a complex system of stimulatory and inhibitory external signals, involving specific receptors and second-messengers that are themselves mediated in part by changes in the intracellular Ca^{2+} concentration, and by the oligomeric GTP-binding proteins, the G-proteins (for reviews see [3,17,25]). Studies employing permeabilized chromaffin cells have clearly demonstrated that GTP-binding proteins are involved in the exocytotic event and that at least one of these of these proteins, called G_e, must be present in the granule membrane [21].

3. CHARACTERISTICS OF IMMATURE SECRETORY GRANULES

One central question in the study of regulated secretion is when do the secretory granules acquire the properties listed above? Does the newly budded secretory granule contain any, or all, of the molecules required, and are they active? Can the immature secretory granule undergo regulated exocytosis? Of equal interest is the question does the immature secretory granule contain any molecules that the mature secretory granule lacks? If so, how are these molecules removed from the immature secretory granule, and are they recycled to the TGN or degraded in a separate compartment? Questions such as these can only be addressed by examining the first vesicular product, the immature secretory granule.

3.1. *Morphological data*

Using electron microscopic autoradiography, and conventional morphological techniques, it has been possible to identify immature secretory granules in several cell types. Immature secretory granules, containing a dense-core aggregate have been observed by autoradiography in close proximity to the TGN [26]. It has been further shown, using immunogold labelling, that processing of pro-hormones occurs in the immature secretory granules [27,28], that the immature secretory granules are acidic [29], and finally, that portions of the immature secretory granule membrane have a clathrin coat [30,31].

How similar are the properties of the immature secretory granules and the mature secretory granules? The results of morphological studies suggest that the immature secretory granules contain the pro-hormone processing enzymes and the H^+-ATPase, and that these proteins are active in the immature secretory granule. Morphologists have noted one interesting difference between the immature and mature secretory granules. The immature secretory granule membrane is often partially clathrin-coated, whereas the membrane of mature secretory granules does not have any clathrin coat. This suggests that a member, or members, of the family of adaptins, proteins which bind clathrin to the membrane

[32], are present and functional exclusively in the membrane of the immature secretory granule. It remains to be tested whether the adaptins found on the membrane of the immature secretory granule belong to the family of 'Golgi'-specific adaptins. These proteins must be either retrieved from the membrane of the immature secretory granule, or inactivated, before it becomes a mature secretory granule. Orci and colleagues [33] found that in pancreatic β-cells a non-cleavable, amino acid analog-modified form of pro-insulin remained in clathrin-coated secretory granules. This result implies that proteolytic processing of pro-hormones may be required for the loss of the clathrin coat from the immature secretory granule, and therefore for the removal, or inactivation, of the clathrin binding proteins.

3.2. *Biochemical identification of the immature secretory granule*

To address further questions about the functional properties of molecules present in the immature secretory granule, a biochemical approach is required. Recently, immature secretory granules have been identified using biochemical techniques in PC12 cells and in a cell-free system derived from them [2]. Using these systems, it has been directly shown that sorting of the secreted molecules into either constitutive secretory vesicles or immature secretory granules occurs before the two classes of vesicles bud from the TGN. It was also demonstrated that a resident membrane protein of the TGN, sialyltransferase, was not found in either constitutive secretory vesicles or in immature secretory granules [2]. Furthermore, it is known from morphological data that whereas the immature secretory granule has a clathrin coat (see above), the constitutive secretory vesicle does not. The implication of these results is that at the exit from the TGN, membrane proteins as well as cargo proteins are sorted such that the membrane of the immature secretory granule contains a selected population of specific membrane proteins. The finding that GTP-hydrolysis is required for the budding of both constitutive and regulated secretory vesicles from the TGN [6] offers a proof-reading mechanism [34] for the observed and anticipated selection, and retention, of proteins in the TGN.

It has also been shown that in PC12 cells the core of the immature secretory granule (80 nm) is smaller than the core of the mature secretory granule (114 nm, [2]), and the difference in the size of the two populations of secretory granules reflects the difference in core size (S. Tooze, T. Flatmark, and W.B. Huttner, submitted). Two possible mechanisms could be envisaged for increasing the size of the secretory granule core: first, addition of content and membrane via vesicular traffic from the TGN to the immature secretory granule, and secondly, fusion of one or more immature secretory granules together. The difference between the two mechanisms is that the first is a fusion event between two different vesicles (for example a carrier vesicle and an immature secretory granule), while the second is a fusion between two identical vesicles (an immature secretory granule with an immature secretory granule). However, there is no evidence for the existence of an additional population vesicles budding from the TGN carrying regulated secretory proteins which are not secretory granules, and thus the second mechanism seems more likely. Indeed, fusion of immature secretory granules has been morphologically documented in the pituitary mammotroph cell [26].

3.3. *Role of GTP-binding proteins in regulated secretion*

If immature secretory granules do fuse with each

Table I

Functional properties and location of some components of secretory granules

Secretory granule contents and properties	Implications	Location of protein(s)	
		MSG[1]	ISG[2]
1. Processed hormones and peptides	processing enzyme(s), i.e. pc2 [9] and pc3 [10]	yes	yes
2. Modified hormones and peptides	carboxypeptidase E [11] and PAM [12]	yes	yes
3. Catecholamines	catecholamine transporter [16]	yes	probably[3]
4. Clathrin coats	receptor for 'Golgi'-specific clathrin adaptors [32]	no	yes
5. Fuse with the plasma membrane	exocytotic fusion machinery [17]	yes	yes[4]
6. Acidified interiors	H^+-ATPase [15]	yes	yes

[1] MSG: mature secretory granule
[2] ISG: immature secretory granule
[3] It is not known whether the catecholamine transporter, if present on the immature secretory granule, is active
[4] S. Tooze, T. Flatmark, and W.B. Huttner, submitted

other to form mature secretory granules it is tempting to speculate that the regulation of this fusion event may involve GTP-binding proteins. GTP-binding proteins have been postulated to have a regulatory role in membrane traffic [35] and have been implicated in a wide range of intracellular fusion events (see review by Balch [36]), including a 'like-like' fusion event [37], similar to the proposed (S. Tooze, T. Flatmark, and W.B. Huttner, submitted) immature-immature secretory granule fusion event. If a GTP-binding protein, or proteins, are involved in the fusion of immature secretory granules they would have to be distinct from those involved in the fusion of mature secretory granules with the plasma membrane to impart a specificity and regulation to the fusion events. This raises the question do the GTP-binding proteins, or their specific receptors, which mediate the 'like-like' fusion event, themselves recycle from the maturing secretory granule to the TGN for example, or do they remain in an inactive state in the membrane of the secretory granule?

Likewise, are the GTP-binding proteins, or their specific receptors, that mediate the secretory granule-plasma membrane fusion event present in the immature secretory granule? If so, are they in an inactive conformation, or can they mediate fusion of the immature secretory granule with the plasma membrane? Data from the PC12 cell system indicate that immature secretory granules can indeed fuse with the plasma membrane after the appropriate stimulus (S. Tooze , T. Flatmark, and W.B. Huttner, submitted). This demonstrates that the GTP-binding protein, or receptor for the GTP-binding protein, specific for secretory granule fusion with the plasma membrane is present and active on the immature secretory granule membrane as well as the mature secretory granule membrane. As several GTP-binding proteins have already been detected in secretory granule membrane preparations [18-20], elucidation of their function with respect to the fusion events discussed above is an important future goal.

4. SUMMARY

Table I summarizes the known properties and functions of mature secretory granules, and attempts to predict which are present in immature secretory granules. It is apparent from the Table that most of the known activities present in mature secretory granules are also present in immature secretory granules, with the exception of the adaptins, the clathrin binding proteins. This gives rise to two separate questions: (i) what is then the difference between an immature secretory granule and a mature secretory granule? and (ii) what is the function of the clathrin-coated membrane on the immature secretory granule? The answer to the first may lie in the second, and presents a challenge for future work.

Acknowledgements: I would like to thank Drs. John Tooze and Torgier Flatmark for critical reading of the manuscript and helpful comments. The work cited in this manuscript was carried out in the laboratory of Dr W.B. Huttner, and I thank Dr W.B. Huttner for support, criticism and stimulating discussions.

REFERENCES

[1] Griffiths, G. and Simons, K. (1986) Science 234, 438-443.
[2] Tooze, S.A. and Huttner, W.B. (1990) Cell 60, 837-847.
[3] Miller, S.G. and Moore, H.-P. (1990) Curr. Top. Cell Biol. 2, 642-647.
[4] Tooze, J., Kern, H.F., Fuller, S.D. and Howell, K.E. (1989) J. Cell Biol. 109, 35-50.
[5] Gerdes, H.-H., Rosa, P., Phillips, E., Baeuerle, P.A., Frank, R., Argos, P. and Huttner, W.B. (1989) J. Biol. Chem. 264, 12009-12015.
[6] Tooze, S.A., Weiss, U. and Huttner, W.B. (1990) Nature, 347, 207-208.
[7] Winkler, H., Apps, D.K. and Fischer-Colbrie, R. (1986) Neuroscience 18, 261-290.
[8] Huttner, W.B., Gerdes, H.-H. and Rosa, P. (1991) Trends Biochem. Sci. 16, 27-30.
[9] Smeekens, S.P. and Steiner, D.F. (1990) J. Biol. Chem. 265, 2997-3000.
[10] Smeekens, S.P., Avruch, A.S., LaMendola, J., Chan, S.J. and Steiner, D.F. (1991) Proc. Natl. Acad. Sci. USA 88, 340-344.
[11] Fricker, L.D. (1988) Annu. Rev. Physiol. 50, 309-321.
[12] Eipper, B.A. and Mains, R.E. (1988) Annu. Rev. Physiol. 50, 333-344.
[13] Eipper, B.A., Park, L.P., Dickerson, I.M., Keutmann, H.T., Thiele, E.A., Rodriguez, H., Schofield, P.R. and Mains, R.E. (1987) Mol. Endocrinol. 1, 777-790.
[14] Fricker, L.D., Das, B. and Angeletti, R.H. (1990) J. Biol. Chem. 265, 2476-2482.
[15] Apps, D.K. and Percy, J.M. (1987) New York: NY Acad. Sci. 493, 178-187.
[16] Henry, J.P. and Scherman, D. (1989) Biochem. Pharmacol. 38, 2395-2404.
[17] Burgoyne, R.D. (1990) Annu. Rev. Physiol. 52, 647-659.
[18] Burgoyne, R.D. and Morgan, A. (1989) FEBS Lett. 245, 122-126.
[19] Doucet, J.-P., Fournier, S., Parulekar, M. and Trifaró, J.-M. (1989) FEBS Lett. 247, 127-131.
[20] Darchen, F., Zahraoui, A., Hammel, F., Monteils, M.-P., Tavitian, A. and Scherman, D. (1990) Proc. Natl. Acad. Sci. USA 87, 5692-5696.
[21] Gomperts, B.D. (1986) Trends Biochem. Sci. 11, 290-292.
[22] Grafstein, B. and Forman, D.S. (1980) Phys. Rev. 60, 1167-1283.
[23] Tooze, J. and Burke, B. (1987) J. Cell Biol. 104, 1047-1057.
[24] Kreis, T.E., Matteoni, R., Hollinshead, M. and Tooze, J. (1989) Eur. J. Cell Biol. 49, 128-139.
[25] Gilman, A.G. (1987) Annu. Rev. Biochem. 56, 615-649.
[26] Farquhar, M.G., Reid, J.J. and Daniell, L.W. (1978) Endocrinology 102, 296-311.
[27] Orci, L., Ravazzola, M., Amherdt, M., Madsen, O., Vassalli, J.-D. and Perrelet, A. (1985) Cell 42, 671-681.
[28] Tooze, J., Hollinshead, M., Frank, R. and Burke, B. (1987) J. Cell Biol. 105, 155-162.
[29] Orci, L., Ravazzola, M., Amherdt, M., Madsen, O., Perrelet, A., Vassalli, J.-D. and Anderson, R.G.W. (1986) J. Cell Biol. 103, 2273-2281.
[30] Orci, L., Ravazzola, M., Amherdt, M., Louvard, D. and Perrelet, A. (1985) Proc. Natl. Acad. Sci. USA 82, 5385-5389.
[31] Tooze, J. and Tooze, S.A. (1986) J. Cell Biol. 103, 839-850.

[32] Robinson, M.S. (1990) J. Cell Biol. 111, 2319-2326.
[33] Orci, L., Halban, P., Amherdt, M., Ravazzola, M., Vassalli, D. and Perrelet, A. (1984) J. Cell Biol. 99, 2187-2192.
[34] Bourne, H.R., Sanders, D.A. and McCormick, F. (1990) Nature 348, 125-132.
[35] Bourne, H.R. (1988) Cell 53, 669-671.
[36] Balch, W.E. (1990) Trends Biochem. Sci. 15, 473-477.
[37] Gorvel, J.-P., Chavier, P., Zerial, M. and Gruenberg, J. (1991) Cell 64, 915-925.