Developments in Data Analysis
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A Simple Probabilistic Model for Intergranule Fusion of Rat Melanotroph Secretory Granules

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Abstract

A simple probabilistic model is introduced to explain the possible intergranule fusion of rat melanotroph secretory granules. It is assumed that larger granules are formed by fusion of two or more spherical granules of fixed size and that the surface of the newly formed granule is equal to the sum of the fused granule membranes. The model also takes into account the characteristics of the data collection techniques. The results show a multimodal empirical distribution of profile diameters. The fusion theory can partly describe the shape of this distribution.

1. Introduction

Rat melanotrophs from *pars intermedia* secrete numerous peptides, stored in secretory granules. The formation of secretory granules begins with the condensation of secretory products within the lumen of the trans-Golgi network. During the maturation process the size of secretory granules is increasing due to intergranular fusion. Larger granules may result from fusion between smaller granules of unitary size (Alvarez de Toledo and Fernandez, 1990).

Large variability of the secretory granule diameters in rat melanotrophs has already been reported (Zupančič et al., 1994). The aim of our work is to find out whether intergranular fusion theory adequately explains a part of this variability. For this purpose we develop a simple probabilistic model assuming that larger granules are formed by fusion of two or more spherical granules of fixed size and that the surface of a newly formed granule is the sum of fused granule membranes (Alvarez del Toledo and Fernandez, 1990). The proposed model allows for the characteristics of the data collection techniques.

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2. Material and methods

2.1 Measurements of granule diameters

After ether anaesthesia the animal is killed by decapitation. *Pars intermedia* from one animal is carefully dissected from the pituitary. Ultrathin sections (70 nm) are cut on a ultramicrotom and examined with electron microscope. Diameters of secretory granule profiles are measured in mm from electron micrographs, enlarged to the scale of 126 000. The smallest detectable profile diameter is 8 mm. The accuracy of the data readings is ±1 mm. Very often the contours of the granule profiles are not clear. Some of the profiles appear spheroid and for them the maximal diameter is measured.

2.2 Theoretical model

2.2.1 Sectioning process

Let us assume a population of sphere granules all having equal diameter D. Granules are cut by parallel random planes. For each granule, parallel cuts are uniformly distributed on the granule diameter, as presented on Figure 1.a. Diameters of the obtained sphere profiles are measured.

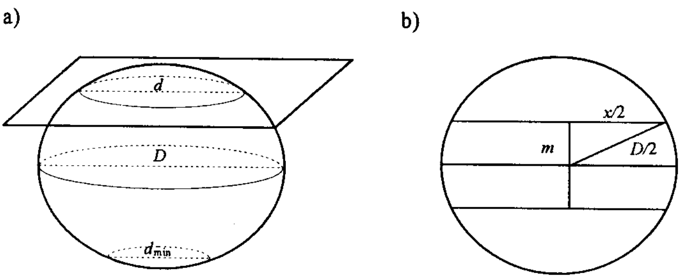

Figure 1: a) Sectioning of a granule with diameter D. On each sphere profile its diameter d is measured. The smallest detectable profile diameter is d_{min}. b) Distribution function $F(x)$ is obtained on the basis of this plot.

Profile diameter d can be regarded as a random variable with values ranging from 0 do D. Its distribution function $F(x)$, $0 < x < D$, is obtained geometrically (see Figure 1.b), and is

$$F(x) = 1 - \frac{2m}{D} = 1 - \frac{\sqrt{D^2 - x^2}}{D}$$

In experimental work small profile diameters can not be recognised reliably. Let us denote the smallest detectable diameter $d_{\min}$, its value is usually known from the experimental work. Then the distribution function is

$$F(x) = 1 - \sqrt{\frac{D^2 - x^2}{D^2 - d_{\min}^2}}, \quad d_{\min} < x < D \tag{1}$$

Derivation of (1) gives the probability density function $p(x)$

$$p(x) = \frac{x}{\sqrt{(D^2 - d_{\min}^2)(D^2 - x^2)}}, \quad d_{\min} < x < D \tag{2}$$

A pole at $x = D$ should be noted. For illustration we present $F(x)$ and $p(x)$ on Figure 2. It turns out that the expected value of d is

$$\mathrm{E}(d) = \int_{d_{\min}}^{D} x p(x) dx = \frac{d_{\min}}{2} + \frac{D^2}{2\sqrt{D^2 - d_{\min}^2}} \arccos\frac{d_{\min}}{D} \geq \frac{\pi}{4} D \tag{3}$$

Thus, the average value of profile diameters is approximately $\pi/4$ of D for small $d_{\min}$. Our analytical results are consistent with commonly used graphical methods presented in Weibel (1979).

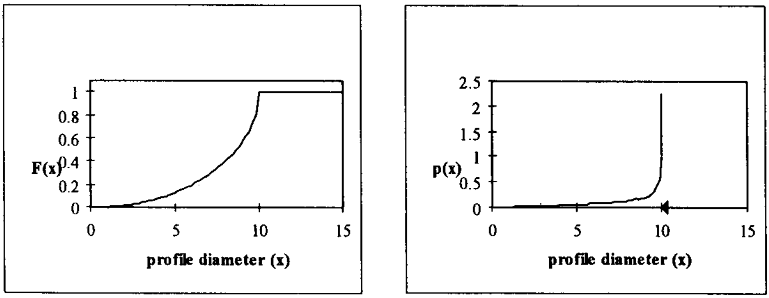

Figure 2. Distribution function $F(x)$ (left) and probability density function $p(x)$ (right) for profile diameters. The granule diameter is $D = 10$. The smallest detectable profile diameter is $d_{\min}=0$.

2.2.2 Fusion theory

The smallest granules, i.e., unit granules, fuse into double-, triple-, ... granules, where the maximal granule size type K is unknown. We assume that the unit granules are spherical and their diameter D_1 fixed but unknown. Following the intergranule fusion theory, the surface of the granule size type k, say S_k, is $S_k = kS_1 (k = 1,\dots,K)$, and consequently its diameter $D_k = \sqrt{k}D_1$. Assume further that the probability of detecting the type k granule follows the Poisson-like distribution

$$p_k = \alpha_K \frac{\lambda^{k-1}}{(k-1)!}, \quad k = 1, 2,\dots K \tag{4}$$

where λ is the unknown parameter representing the expected granular size type in the following way $\mathrm{E}_K(k) \approx 1+\lambda$, and α_K is the normalizing constant

$$\alpha_K = \left(1 + \frac{\lambda}{1} + \frac{\lambda^2}{2} + \dots + \frac{\lambda^{K-1}}{(K-1)!}\right)^{-1}.$$

These granules undergo the sectioning process described previously. Following (1) the distribution function for any granular size type k is

$$F_k(x) = 1 - \sqrt{\frac{kD_1^2 - x^2}{kD_1^2 - d_{\min}^2}}, \quad d_{\min} < x < \sqrt{k}D_1 \tag{5}$$

Pooling together all granular size types with the corresponding probabilities p_k (4), the global distribution function $G(x)$ is as follows

$$G(x) = \sum_{k=1}^{K} p_k F_k(x) \tag{6}$$

For illustration we present $G(x)$ on Figure 3. Relative cumulative function estimates the distribution function $G(x)$ which is very nonlinear. A number of numerical techniques may be applied to find the least squares estimates commonly used in regression models (Everitt, 1987). The parameters to be estimated are D_1 and λ. Their starting values can be estimated from the empirical distribution of profile diameters: starting value for D_1 corresponds to the abscissa of the first observable peak, λ to the granular size type with the highest frequency. The third parameter to be estimated is K, which is an integer value. Fitting procedure for D_1 and λ is repeated for K in a reasonable range.

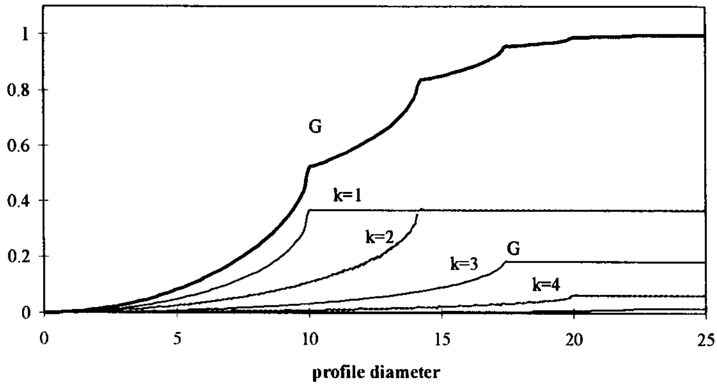

Figure 3: Distribution function G and its components.The following values of parameters are taken into account: D_1=10, λ=1, K=5. The smallest detectable profile diameter is d_{min}=0.

3. Results

Here we present the results for one dataset consisting of 1298 granules. As mentioned before, the smallest detectable profile diameter is d_{min} = 8 mm. Measured profile diameters are in the range from 11 to 139 mm. For 17 granules (1.3%) their profile diameter is greater than 84 and they are not included in further analysis. The mean profile diameter for the remaining 1281 granules is 31.3 mm and the standard deviation 13.9 mm.

Figure 4 presents the data. The plot displays a multimodal distribution. The abscissa of the first observable peak is somewhere inbetween 19 to 27 mm. We started the fitting procedure with the following starting values D_1=19 mm, λ= 1, for each $K = 2,3,\ldots,11$. Afterwards the starting value for D_1 was increased by one and the procedure repeated. The best results obtained are presented in Table 1. We applied the modified Gauss-Newton algorithm to obtain the estimates for D_1 and λ. Module AR in BMDP Dynamic program was used.

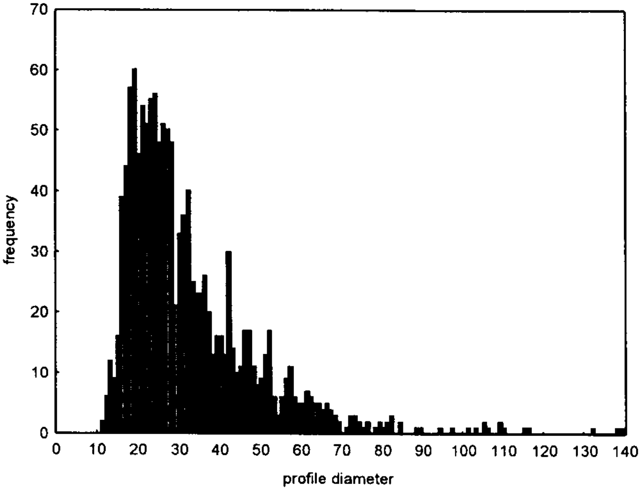

Figure 4: stribution of 1298 profile granule diameters.

Table 1: Residual sum of squares (RSS) and the estimates for D1 and * for maximal granular size type K=2,3...,11. The starting values are D1=26 mm, *= 1.

K	RSS	D_1	λ
2	0.422290	28.76	1.150
3	0.255214	28.45	0.854
4	0.205776	27.03	0.986
5	0.188185	27.01	0.978
6	0.181061	25.64	1.170
7	0.179237	25.63	1.172
8	0.178914	25.64	1.167
9	0.178832	25.62	1.173
10	0.178824	25.62	1.173
11	0.178823	25.62	1.173

Table 2 presents the positions of the peaks and the corresponding probabilities for the model with K=11.

The probabilities for K greater than 7 are less than 0.1%. The corresponding residual sum of squares do not differ considerably. Therefore the estimates accepted are D_1 =25.6 mm, λ=1.17, K=7. Double granules have the greatest probability of appearance (0.36), the probability for unit granules is 0.31 and for all other types 0.22.

Table 2: Granule diameters D_k (mm) and its probability p_k, for each granule size type $k = 1,2,...,11$. The results are based on the estimates, $D_1 = 25.6$ mm, $\lambda = 1.17, K = 11$.

k	1	2	3	4	5	6	7	8	9	10	11
D_k	26	36	44	51	57	63	68	72	77	81	85
p_k	0.3094	0.3630	0.2129	0.0832	0.0244	0.0057	0.0011	0.0002	0.0000	0.0000	0.0000

Comparison of the relative cumulative function with its fit G (see Figure 5) shows that the model describes the empirical distribution reasonably up to the second peak. Deviations increase from the second to the fourth peak and diminish afterwards.

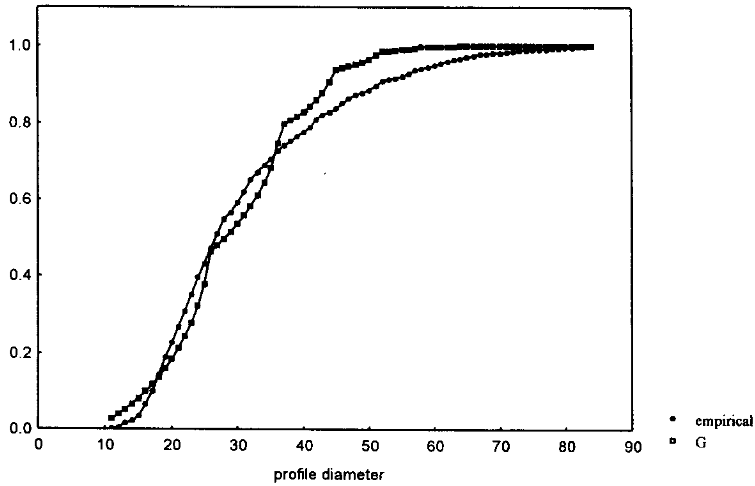

Figure 5: Relative cumulative frequency (empirical) and its fit G. The following values of estimates were obtained: $K=7$, $D_1=25.6$ mm, $\lambda=1.17$.

4. Discussion

A simple probabilistic model is introduced to explain a possible fusion process of rat melanotroph granules. The formation of larger granules may be due to intergranule fusion where the surface of a newly formed granule is equal to the sum of fused granule membranes. Additional three assumptions are taken into account: granules are spherical, unit granule diameters are of *one fixed* size, the probability of detecting a granule of particular type k follows a Poisson-like distribution.

The 'Poisson assumption' comes from the fact that the intergranule fusion is a very rarely observed event. The other two assumptions are taken into account for the sake of simplicity only. Non-spherical shapes were often observed in our experiments indicating that some of the granules are of 'irregular' shapes (see Rusakov, 1993). There is no argument whatsoever the unit granules to be of one fixed size only. The normal distribution of these values could be a reasonable assumption.

As described previously the data collection has several steps. The electron microscopy is a very robust data acquisition technique when the intergranule fusion theory is studied. It may generate many possible types of bias. Therefore one can not expect the peaks to be in the fixed sequence $D, D\sqrt{2}, D\sqrt{3}, \ldots$, even if the fusion theory is correct.

The results show a multimodal empirical distribution of profile diameters (Figure 4). We may conclude that the intergranule fusion theory can partly describe the shape of this distribution. The unit granule diameter is 26 mm. The probability of detecting unit granules is 0.31, for double and triple granules 0.36 and 0.22, respectively. Granules of higher size type are very unlikely to be detected.

It would be much easier to evaluate the results visually if instead of the distribution function the probability density function was fitted. Unfortunately, the probability density function has poles at $D, D\sqrt{2}, D\sqrt{3}, \ldots$ which hinder this approach.

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References

[1] Alvarez del Toledo G. and Fernandez J.M. (1990): Patch-Clamp Measurements Reveal Multimodal Distribution of Granule Sizes in Rat Mast Cells. *J. Cell Biol.* **110**, 1033-1039.

[2] Evans M., Hastings N., and Peacock B. (1993): *Statistical Distributions*. John Wiley.

[3] Everitt B.S. (1987): *Introduction to Optimization Methods and Their Application in Statistics*. Chapman and Hall.

[4] Rusakov D.A. (1993): Estimation of the Size of Closed Cell Elements from Analysis of Their Random Plane Sections. *Biometrika*, **49**, 141-149.

[5] Weibel E.R. (1979): *Stereological Methods for biological morphometry*. Acad. Press.

[6] Zupančič G., Kocmur L., Veranič P., Grilc S., Kordaš M., and Zorec, R. (1994): The Separation of Exocytosis in Rat Melanotroph Membrane Capitance Records. *J. Physiol.* **480**, 539-552.

[7] *BMDP Statistical Software Manual* (1988), University of California Press, Berkley.