ACTA STEREOL 1982; 211-217
STEREOL 82 SHEFFIELD

UNFOLDING DETERMINATION OF SPHEROIDAL SIZE-SHAPE DISTRIBUTION FROM SMALL PROFILE SAMPLES

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ABSTRACT

Computer simulation illustrated that a characteristic parameter of spheroidal particles, corresponding to their biological function, could be obtained from their elliptical profiles. By reducing the size-shape distribution of spheroidal particles to a one dimensional problem, it was then possible to analyze melanin granules from small number of profiles.

INTRODUCTION

The size-shape distribution of cell organelles and granules provide a bridge between their structure and function (Weibel, 1969). Such distributions being unfoldingly determined from two dimensional distributions of their profiles (Cruz-Orive, 1978), it might require a very large number of profiles (Weibel, 1980). The difficulty of large sample size has not been eliminated by exchanging the parameter (Miyamoto et al ., 1982).

Some spheroidal particles have morphological characteristic parameter corresponding to their biological function. Then the parameter will be sharply defined. The size-shape distributions of the particles are described by a conjugate parameter. The unfolding determination of such particles being a one dimensional problem, the required number of elliptical profiles for unfolding determination is considerably smaller than the number of the two dimensional problem.

In the present study, examinations, if a characteristic parameter of spheroidal particles could be obtained from their elliptical profiles, were simulated with a computer for spheroidal particles having five kinds of characteristic parameters, and we tried to analyze melanin granules by the unfolding determination.

METHODS

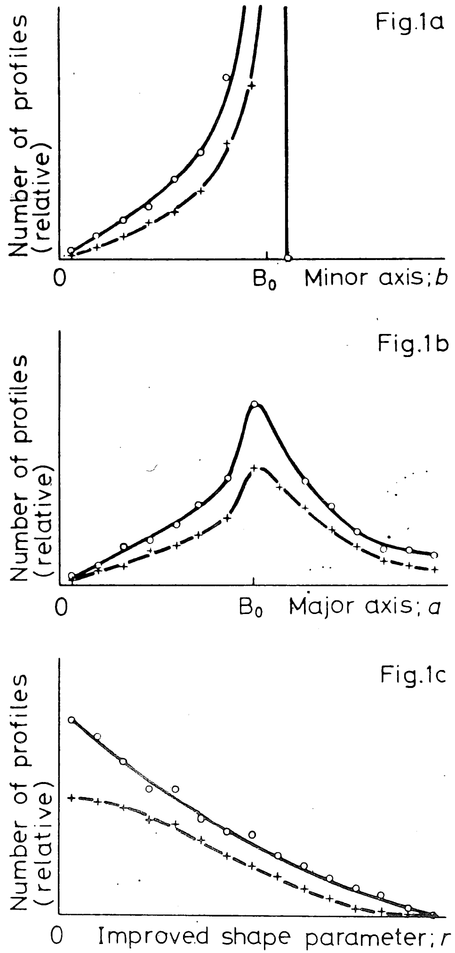
In the first case, the minor axis B, of the prolate spheroid was chosen as a morphological characteristic parameter of the particle. The major axis A conjugate with B. Size-shape of the spheroids distribute over A. Instead of A, improved shape parameter R, was defined by $1-B/A$ (Miyamoto et al ., 1982), since $A=B/(1-R)$, also decided the size-shape distribution of spheroidal particles. Gaussian distribution which was defined by a peak at R=1/2 and by standard deviation σ was used for a model of the size-shape distributions of the particles. The spheroids of Gaussian distribution were randomly sectioned to ellipses. The major axes a and minor axes b of the ellipses were observed. Each ellipse distributed over a, b and improved shape parameter r that was calculated from $1-b/a$. Especially, each distribution was compared with two Gaussian distributions, $\sigma=1/32 \log 2$ and $\sigma \to \infty$. For oblate spheroid, similar computer program was run.

In the other cases, similar experiments were carried out by use of four kinds of morphological characteristic parameters. The parameters are, in the second case, improved shape parameter R (De Hoff, 1962), in the third case, major axis A for prolate spheroid or minor axis B for oblate spheroid (Wicksell, 1926), in the forth case, volume V (Wicksell, 1926) and in the fifth case, area of surface S.

RESULTS AND DISCUSSION

In the first case, results were obtained from prolate spheroids of which minor axes B were sharply defined by B_0. Distributions over b, a and r of the ellipses are shown in Fig.1a, 1b and 1c. In Fig.1a, there are maximum and cut-off peaks of the same position at $b=B_0$. In Fig.1b, there are maximum peaks of the same position at $a=B_0$. These distributions can be elucidated that ellipses with $a=B_0$ occurred, as frequently as ellipses with $b=B_0$ occurred, for many ellipses are circular shape, that is $a=b=B_0$. In Fig.1c, there were no remarks on the distributions over r.

It was found that the distribution over an elliptical parameter corresponding to the characteristic parameter of



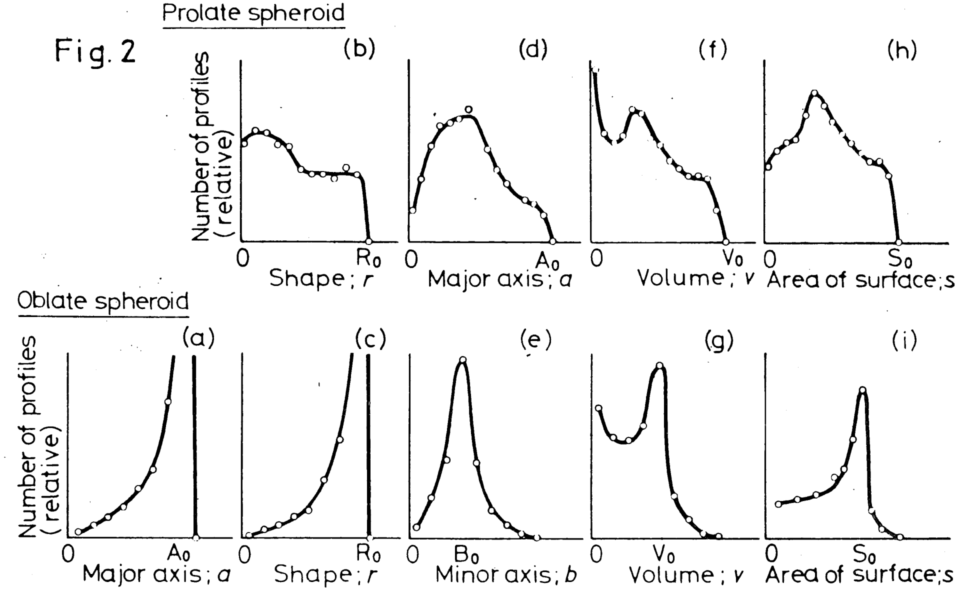


Fig. 1: Distributions over b, a and r of elliptical profiles, which are random sections of spheroidal particles having characteristic parameter $B=B_0$. (o: $B_0=1/2$; $\sigma\to\infty$, +: $B_0=1/2$; $\sigma=1/32 \log 2$).

Fig. 2: Distributions over the elliptical parameter corresponding to a characteristic parameter of spheroids.

spheroids always possessed a remarkable point, for example maximum and cut-off peak at b=B_0. Deducing above, detecting this remarkable point at B_0 on the distribution over b of the ellipses, it will be identified that morphological characteristic parameter of the spheroids is the minor axis B.
For oblate spheroids with characteristic major axis A_0 the experimental result is shown in Fig.2a. There was maximum and cut-off peak at a=A_0. On distributions over the other parameters, there were no remarks.

In the other four cases, just experimental results, distributions over elliptical parameters corresponding to the characteristic parameters of spheroidal particles, are shown in Fig.2 (from b to i). Fig.2b shows a cut-off edge at r=R_0 and distributions over the other parameters have no remarks. Fig.2c shows a maximum and cut-off peak at r=R_0 and distributions over the other parameters have no remarks. Fig.2d shows a shoulder with cut-off edge at a=A_0 and distributions over the other parameters have no remarks. Fig.2e shows a maximum peak at b=B_0 and distributions over the other parameters have no remarks. Fig.2f shows a shoulder with cut-off edge at v=V_0 and distributions over the other parameters have no remarks. Fig.2g shows a maximum peak at v=V_0 and distributions over the other parameters have no remarks. Fig.2h shows a shoulder with cut-off edge at s=S_0 and distributions over the other parameters have no remarks. Fig.2i shows a maximum peak at s=S_0 and distributions over the other parameters have no remarks.

Consequently, sharply defined characteristic parameter of spheroidal particles could be obtained by a remarkable point in the distribution over elliptical parameters.

SIZE-SHAPE DISTRIBUTIONS OF MELANIN GRANULES

Fig.3 shows an example of electron micrograph of melanoma of human lung. The melanin granules are approximated as spheroidal particles for the profiles have elliptical shape. Since there are more small profiles with circular shape than large profiles with elongate elliptical shape, the melanin granules are prolate spheroid (Hennig and Elias, 1963).

On electron micrographs enlarged at x12,410, the major axes and minor axes of 755 profiles in 1,040 $m\mu^2$ sliced tissue area, were measured with a transparent ruler. Fig.4 portrays the distribution of the major axis of the profile, and shows a shoulder with a cut-off edge at about 800 mμ.

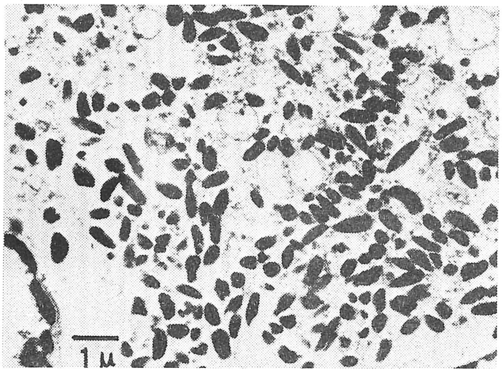


Fig. 3: An example of examined electron micrograph of melanin granules.

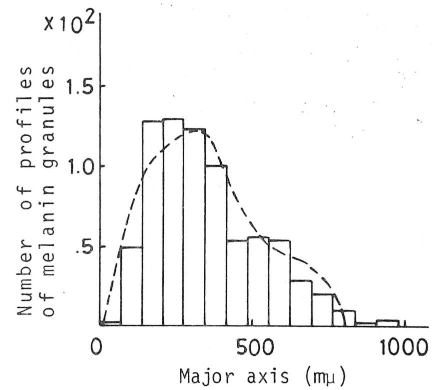


Fig. 4: Distribution over major axis of profiles of melanin granules. Broken line is the distribution of Fig.2d.

The characteristic parameter of melanin granules is sharply defined. Furthermore, the distribution resembles the broken line, which is the distribution of Fig.2d. The difference between the distribution and the broken line is about the variance of the total profile number. Then, we decided that the morphological characteristic parameter of the melanin granules is its major axis.

The relations between $n_\sigma(i,j)$, the numerical density of elliptical profile whose size parameter b lies within $(i-1)\Delta<b\leq i$ (Δ=(maximum minor axis of prolate spheroidal particle)/K) and shape parameter r lies within $(j-1)/L\leq r<j/L$ and $N_\upsilon(I,J)$, the numerical density of prolate spheroidal particle whose size parameter B lies within $(I-1)\Delta<B\leq I\Delta$ (I=1,2,...,K) and shape parameter R lies within $(J-1)/L\leq R<J/L$ (J=1,2,...,L) are described as follows,

$$n_\sigma(i,j) = \Delta \sum_{I=i}^{K} \sum_{J=j}^{L} \Omega(i,I) N_\upsilon(I,J) \Phi(J,j), \qquad (1)$$

where coefficients $\Omega(i,I)$ are size corrector and coefficients $\Phi(J,j)$ are shape corrector (Miyamoto, 1982). Being the major axis sharply defined, the size and shape of spheroidal particle is determined by a size parameter. Equation (1) is changed into the relation between size parameter of elliptical profile and size parameter of spheroidal particle. We obtained such equation by summation from j equals one to L of both side of equation (1).

$$\sum_{j=1}^{L} n_\sigma(i,j) = \Delta \sum_{J=j}^{L} [\{\sum_{I=i}^{K} \Omega(i,I) N_\upsilon(I,J)\} \sum_{j=1}^{L} \Phi(J,j)]. \qquad (2)$$

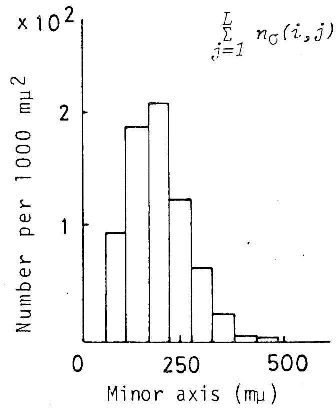


Fig. 5: Distribution over minor sxis of profiles of melanin granules.

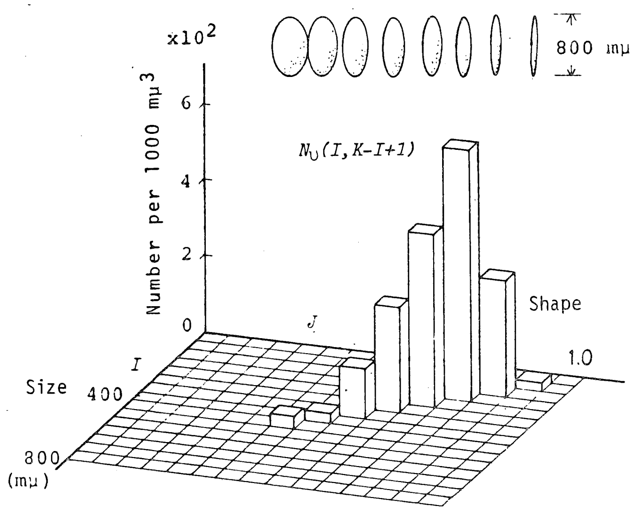


Fig. 6: Resulting size-shape distribution of melanin granules.

In the case of $K=L$ and the range of size parameter lies within $0<B\leq A$, the size-shape of spheroidal particles are described by the admissible combinations of I and J satisfying the condition $I+J=K+1$ (diagonal compornents of KxK matrix). Since $J=K-I+1$, the required values $N_\upsilon(I,K-I+1)$ are K unknowns in K linear equations. The solution of these equations yields as set of K equations of the form,

$$N_\upsilon(I,K-I+1) = \sum_{i=I}^{K}\{\Omega^{-1}(I,i)\sum_{j=1}^{K} n_\sigma(i,j)\}/\Delta\sum_{j=1}^{K}\Phi(K-I+1,j), \quad (3)$$

where $\Omega^{-1}(I,i)$ is Iith element of the inverse of a matrix having $\Omega(i,I)$ as iIth element.

Substituting the observed values $\Sigma_{j=1}^{K} n_\sigma(i,j)$, the size distribution of profiles of melanin granules (Fig.5), the coefficients $\Omega^{-1}(I,i)$ and coefficients $\Phi(J,j)$ for K=15; Δ=800/K mμ into equation (3), the required values $N_\upsilon(I,K-I+1)$ were calculated. Fig.6 shows the resulting size-shape distribution of melanin granules. They have an extremly elongated shape with major axes defined at about 800 mμ and minor axes varied from 50 to 500 mμ with a peak at about 200 mμ.

CONCLUSIONS

The above results can be summarized as follows. The improvement of the shape parameter and the introduction of a morphological characteristic parameter for the spheroidal particles reduced the two dimensional problem to a one dimensional problem; this permitted the unfolding determination of the melanin granules from a small number of profiles.

ACKNOWLEDGEMENT

I thank Dr. K. Baba, School of Medicine, University of Occupational and Environmental Health, Japan, for encouragement of this research and Dr. S. Ishizaka, The University of Tsukuba, for his valuable comments on the manuscript.

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