Changes in the size of isolated chromaffin granules in ATP-evoked catecholamine release

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The average size of chromaffin granules isolated from bovine adrenal medullae was analyzed by a quasi-elastic laser light scattering method. The granule diameter increased by a factor of 1.3 by addition of Mg-ATP in the medium. The ATP effect was completely suppressed in the presence of an anion transport blocker (SITS), and partly depressed by a proton transport blocker (DCCD).

Adrenomedullary chromaffin granule *Quasi-elastic light scattering* *Proton pump*

1. INTRODUCTION

It is known that isolated chromaffin granules rapidly release catecholamines when stimulated by Mg-ATP added in the external medium. A mechanism of the ATP-evoked release has been proposed by Pazoles and Pollard [1]. In their model Mg-ATP activates inwardly directed proton-pumping across the vesicle membrane, so that the granule interior becomes relatively positively charged. This promotes a chloride influx and solubilizes the contents of the granule, which leads to its osmotic lysis.

Here, a quasi-elastic laser light scattering technique is used to examine the validity of the above model since the method provides a direct measure of granule size in various solutions.

2. MATERIALS AND METHODS

Chromaffin granules were prepared from bovine adrenal medulla homogenate by differential centrifugation as reported by Hoffman et al. [2]. The granule suspension in 0.3 M sucrose was stored at 0°C until use. The suspension was diluted so as to make appropriate compositions of the medium and to have protein concentrations of 1–2 mg/ml. Then, it was filtered with a 0.8-μm membrane filter shortly before each measurement. Experiments were carried out within 36 h of preparation.

Quasi-elastic light scattering measurements using a 5-mW He-Ne laser (GLG 2034, NEC, Tokyo) were performed under an optical microscope frame (FM-200 A, Tiyoda, Tokyo) as in [3,4]. A space (0.5 mm) made between a glass slide and a cover glass was filled with 40 μl sample solution for measurement. Correlation functions were measured with a signal averager (ATAC 250, Nihon Kohoden, Tokyo) by the method of Kam et al. [5]. The measurements were done at 18–20°C.

Abbreviations: SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid; DCCD, dicyclohexylcarbodiimide

3. RESULTS

3.1. *Average size of chromaffin granules*

The photocurrent noise of scattered light from chromaffin granules in 0.3 M sucrose at different scattering angles (θ = 23° and 43°) gave autocorrelation functions, $C(\tau)$, with distinct time constants as shown in fig.1A. Although the semi-logarithmic plots of $C(\tau)$ in fig.1B show slight deviations from single exponentials due to a size distribution of the granules, the correlation time, τ_D, determined conventionally from the time when $C(\tau)$ was attenuated to $1/e$ of its initial value,

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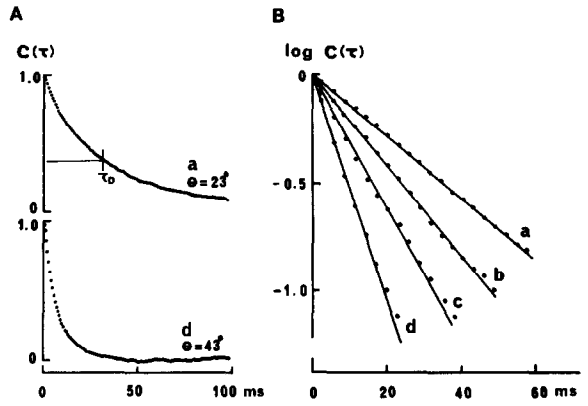

Fig.1. (A) Photocurrent autocorrelation functions, $C(\tau)$, for the light scattered from chromaffin granules in 0.3 M sucrose buffered with 10 mM Hepes at pH 7.0. The scattering angles are (a) 23° and (b) 43°, respectively. (B) Semi-logarithmic plots of the autocorrelation functions measured at scattering angles: (a) 23°; (b) 28°; (c) 35°; (d) 43°.

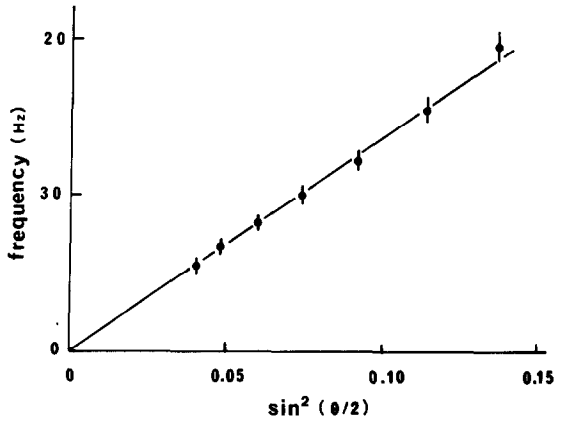

Fig.2. The inverse of correlation time, $(2\pi\tau_D)^{-1}$, was plotted against $\sin^2(\theta/2)$. The data points with standard deviations are means of 4 independent determinations.

obeyed well the following theoretical relation [6]

$$\tau_D = \frac{3\eta r\,(\lambda/n)^2}{16\pi kT \sin^2(\theta/2)} \quad (1)$$

where θ is the scattering angle, r the radius of the granule, η and n the viscosity and refractive index of the medium, λ the wavelength of the laser light used (6328 A). In fig.2, the experimental data are represented in terms of eq.(1) by plotting the inverse of correlation time $(2\pi\tau_D)^{-1}$ against $\sin^2(\theta/2)$. Thus, the average diameter of chromaffin granules was evaluated to be 4.9 μm from the slope of the regression line.

3.2. *Effect of ATP on the granule size*

The effect of 0.6 mM ATP on the average size of chromaffin granules in various media B–E (for compositions, see table 1) was evaluated by measuring the correlation times after 10 min incubation of granules at 37°C in those media. The obtained values, τ_D^i (i denotes B–E) were compared, by taking ratios, with τ_D^A that was obtained in ATP-free medium A. By the addition of ATP (in medium E), τ_D increased by a factor of 1.36. Longer incubations beyond 10 min did not further increase it. Under the circumstances described above, the increase in the correlation time may indicate either an enlargement of individual granules or a formation of aggregates in the granule solution. If granule aggregates occur, it can be detected by an increase in the deviation of $C(\tau)$ from a single exponential as shown with pancreatic zymogen granules [4]. In the present case, however, the extent of deviation after ATP application was not appreciably altered from the level shown in fig.1 (not shown). Thus, ATP most likely

Table 1

Effect of medium compositions on correlation time for scattered light from chromaffin granules

	Medium	(τ_D^i/τ_D^A) mean ± SD (n = 5)
A	Control medium (150 mM sucrose, 75 mM KCl, 50 mM Hepes, 1 mM $MgSO_4$)	1.00
B	+ 0.5 mM SITS + 0.6 mM ATP	1.01 ± 0.02
C	− 1 mM $MgSO_4$ + 0.6 mM ATP	1.03 ± 0.04
D	+ 50 μM DCCD + 0.6 mM ATP	1.20 ± 0.05
E	+ 0.6 mM ATP	1.36 ± 0.10

τ_D^A and τ_D^i represent the correlation times measured in the control medium (A) and the test media (B–E), respectively. The measurements were done at a scattering angle of 35°

causes an increase in the size of individual granules.

It was confirmed that mitochondrial and microsomal contaminations did not account for the observed change in the correlation time by addition of ATP because a suspension of 'loose layer' in the centrifuge sediments, which mainly consisted of mitochondria and microsomes [1], did not show the ATP effect under conditions similar to those with the chromaffin granule suspension.

Without magnesium (medium C), ATP caused little increase in the granule size as shown in table 1. An anion transport inhibitor, SITS [7], in medium B also suppressed the ATP effect completely. On the other hand, a proton pump blocker, DCCD [8], in medium D only moderately suppressed the increase of τ_D by ATP.

3.3. *Effects of chemicals on ATP-evoked catecholamine release*

Since the ATP-evoked increase in chromaffin granule size is thought to lead to catecholamine leakage from the vesicles [1], the turbidity of the granule solution is expected to decrease due to the loss of granule contents. In fact, the absorbance (A) of the granule solution was decreased after an addition of 0.6 mM ATP as shown by curve E in fig.3. When the same compositions of media (A–E) as those used for the experiments of table 1 were employed, a good correlation between the increase of τ_D and the decrease of A is exhibited in a qualitative sense.

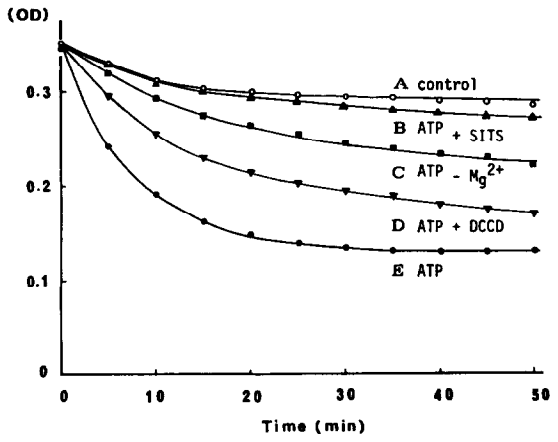

Fig.3. Changes in absorbance with 500 nm light during incubation of chromaffin granules in various media A–E, of which compositions are the same as labelled in table 1. The granules were suspended in a 1-mm cuvette at a protein concentration of 2.5 mg/ml. Temperature was kept at 37°C. OD, absorbance.

4. DISCUSSION

The correlation time of photocurrent noise generated by the light scattered by bovine chromaffin granules increased by a factor of 1.36 when stimulated by the addition of ATP in the external medium. This increase is most likely associated with osmotic expansion and lysis of the vesicle membrane, and is consistent with the mechanism proposed by Pazoles and Pollard [1]. The present observation that the ATP effect was suppressed in the absence of Mg^{2+} or in the presence of DCCD probably reflects the involvement of Mg-ATP-driven proton transport in the mechanism. The presence of chloride transport that is in some way coupled with the proton transport is also suggested from an inhibitory effect of SITS on the ATP-evoked lysis. Thus, the results obtained here are as a whole consistent with those expected from Pazoles-Pollard's model [1].

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