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Regulation by Ca^{2+} of membrane elasticity of bovine chromaffin granules

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In a range of $[Ca^{2+}]$ similar to cytosolic transient, a drastic reduction from about 20 dyn/cm to almost zero was observed in the membrane elastic modulus of bovine chromaffin granules, isolated in a solution containing 0.3 M sucrose and 5 mM Hepes at pH 7.0, and measured by combination of osmotic swelling and dynamic light-scattering (DLS) methods. This result suggests that the granule membrane becomes extremely flexible as a prelude to exocytosis.

Chromaffin granule; Membrane elastic modulus; Dynamic light scattering; Osmotic swelling

1. INTRODUCTION

The exocytotic release of catecholamines from adrenal chromaffin cells is normally triggered by nicotinic receptor-activated influx of extracellular Ca^{2+} and subsequent increase in cytosolic $[Ca^{2+}]$ [1]. Although exocytosis was first described using biochemical techniques, the underlying mechanisms triggered by Ca^{2+} have not yet been elucidated. In the process of exocytosis, rearrangement of cytoskeletal actin filaments (F-actin) and other proteins might be activated by a rise of cytosolic $[Ca^{2+}]$ [2–4]. Attention is now being concentrated on a change in physical properties of secretory granules. It has been proposed from experimental and theoretical studies of phospholipid vesicles that the vesicle membrane should become flaccid just before fusion [5–7]. In the resting state of a secretory cell, granules exist as if they were an elastic sphere. However, the granule membrane has to become very flexible enough to flatten when apposed to the cell membrane. We report our measurements on membrane elasticity of isolated chromaffin granules.

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Abbreviation: DLS, dynamic light scattering

2. MATERIALS AND METHODS

Bovine chromaffin granules were prepared according to Bartlett and Smith [8] with slight modifications. Preparation 1 (2) used an unbuffered 0.3 M sucrose (plus 0.5 mg/ml DNase 1) solution in all purification steps of granules. The final pellet was resuspended in 0.3 M sucrose, 5 mM Hepes (pH 7.0) and 1 mM EGTA, and stored at 0°C until use. The stock suspension (~5 mg/ml protein) was observed by use of a differential-interference microscope (Optiphoto; Nikon, Tokyo) equipped with a video-image processing unit (PIP-4000; ADS, Osaka), installed at the National Institute of Physiology.

Granules with an initial diameter d_0 are prepared in an aqueous solution containing membrane-impermeable solute at iso-osmolarity C_0. When osmolarity outside the granule is reduced to C_e, water flows into the granule, and the osmotic stress T_s induces an increase in membrane area $\Delta A = (d_f^2 - d_0^2)/d_0^2$, where d_f is the final diameter. The stress-strain relation $T_s = M\Delta A$ gives

$$d_f - d_0 = (d_0 d_f K_0/8)(1/M)[(d_0/d_f)^3 C_0 - C_e] \quad (1)$$

where K_0 is the osmotic coefficient and M (dyn/cm) the membrane elastic modulus [9,10]. The factor $(d_0/d_f)^3$ in eqn 1 should be corrected for the dead volume occupied by osmotically inactive materials inside the granule. However, this is negligible because the dead volume is at most 10% of the total [11].

The diameter of the granules was determined by a DLS method. The DLS apparatus and method of analysis are detailed elsewhere [10]. The decay rate $\bar{\Gamma}$ of the DLS spectrum for a suspension of spheres is related to the particle diffusion coeffi-

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cient D and diameter d by $D = \bar{\Gamma}/K^2 = k_B T/(3\pi\eta d)$, where $K = (4\pi n/\lambda)\sin(\theta/2)$ (n, refractive index of the medium; λ, wavelength of light in a vacuum; θ, scattering angle), k_B is the Boltzmann constant, T the absolute temperature and η the solvent viscosity [9,10]. Some of the technical problems in application of DLS to osmotic swelling experiments have been discussed elsewhere [12]. Swelling experiments were performed at 10°C for granules within 48 h after preparation and filtered through a 3 μm pore size filter just before measurements. The granule suspension (~100 μg/ml protein) after adjustment of pCa with a Ca^{2+}-EGTA buffer was diluted with a solution containing 5 mM Hepes (pH 7.0) and the same amount of Ca^{2+}-EGTA as that of the suspension. After every addition of 50 μl dilution buffer at a speed of 10 μl/min, the average diameter was determined from ten successive measurements at a scattering angle of 90° [10].

3. RESULTS AND DISCUSSION

Fig.1 shows a video-enhanced optical micrograph of the suspension of our chromaffin granules, where neither mitochondria nor other organelles were observed. Although an image-processing technique made it possible to visualize the distribution of granules in suspension, a quantitative estimation of size and size distribution was not possible for such small particles as the present granules. On the other hand, a quantitative characterization of the granules could be made by DLS; the number-average diameter d_n = 380–400 nm at 0.3 M sucrose, and the relative dispersion of size distribution $\sigma/d_n \sim 0.2$ [13]. Our suspension was substantially monodisperse. The diameter of chromaffin granules determined by DLS is close to and, in some preparations, a little larger than that in the literature; the mean diameter of about 380 nm at 0.27–0.32 M osmolarity [11]. The sizes measured in whole mount electron microscopic preparations are measurements of only the core diameters [11], whereas those by DLS are measurements of the hydrodynamic diameters [12].

A linear relationship between $(d_f - d_0)$ and $(C_0 - C_e)$ was observed over a certain range of osmolarity (fig.2a). As $[Ca^{2+}]$ was increased, the change in diameter deviated from a linear relation at 0.27–0.28 M osmolarity (also in fig.2c,e). At the same time, the light-scattering intensity began to decrease. This suggests that at higher $[Ca^{2+}]$, the granule membrane underwent lysis. Chromaffin granules are in a stable state in a hypertonic condition (pH 7.0) with divalent cations but free from Cl^-. However, they begin to undergo lysis in a

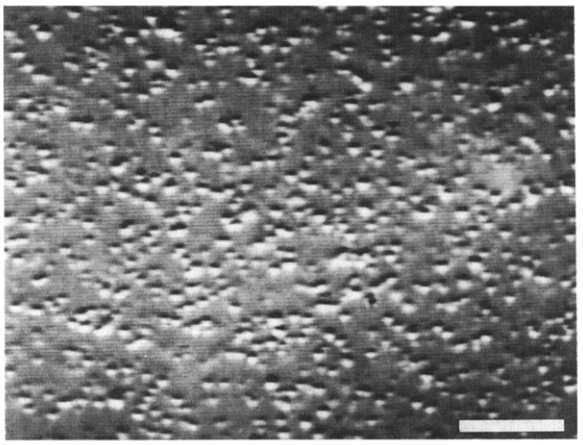

Fig.1. Optical images of chromaffin granules in a stock suspension of preparation 1. Scale bar: 5 μm. Note that the concentration of granules was 50-times higher than for osmotic swelling experiments.

hypotonic condition [11]. From the initial linear portion of osmotic swelling data, the elastic modulus of the granule membrane was obtained as a function of pCa (fig.2b,d). The magnitude of the elastic modulus decreases from about 24 dyn/cm at $pCa > 7$ to about 4 dyn/cm at $pCa = 6$. It is almost zero at $pCa \leqq 5$, which corresponds to easy fusion and/or aggregation. The free $[Ca^{2+}]$ in secretory cells has been determined via fura-2 and quin-2 fluorescence measurements [14,15]. Cytosolic $[Ca^{2+}]$ of the chromaffin cell rises from the 100 nM level in an unstimulated state to the 1 μM level on synaptic stimulation or chemical stimulations of secretagogues. The process of exocytosis is triggered after this Ca^{2+} rise. The transitional change in elastic modulus just occurred in the same $[Ca^{2+}]$ range as cytosolic transient. This suggests that immediately before fusion with the plasma membrane, the granule membrane must become flaccid and that the change in membrane elasticity must be regulated by $[Ca^{2+}]$.

It has been shown that many F-actin molecules become attached to the surface of chromaffin granules and are present in the cytosol [4,16,17]. Since granules for fig.2a–d were prepared at a low ionic strength, F-actin was considered to be depolymerized into globular actin (G-actin). However, particular amounts of Ca^{2+} and/or Mg^{2+} may or may not induce repolymerization of G-actin, if it remains in the suspension. It is well

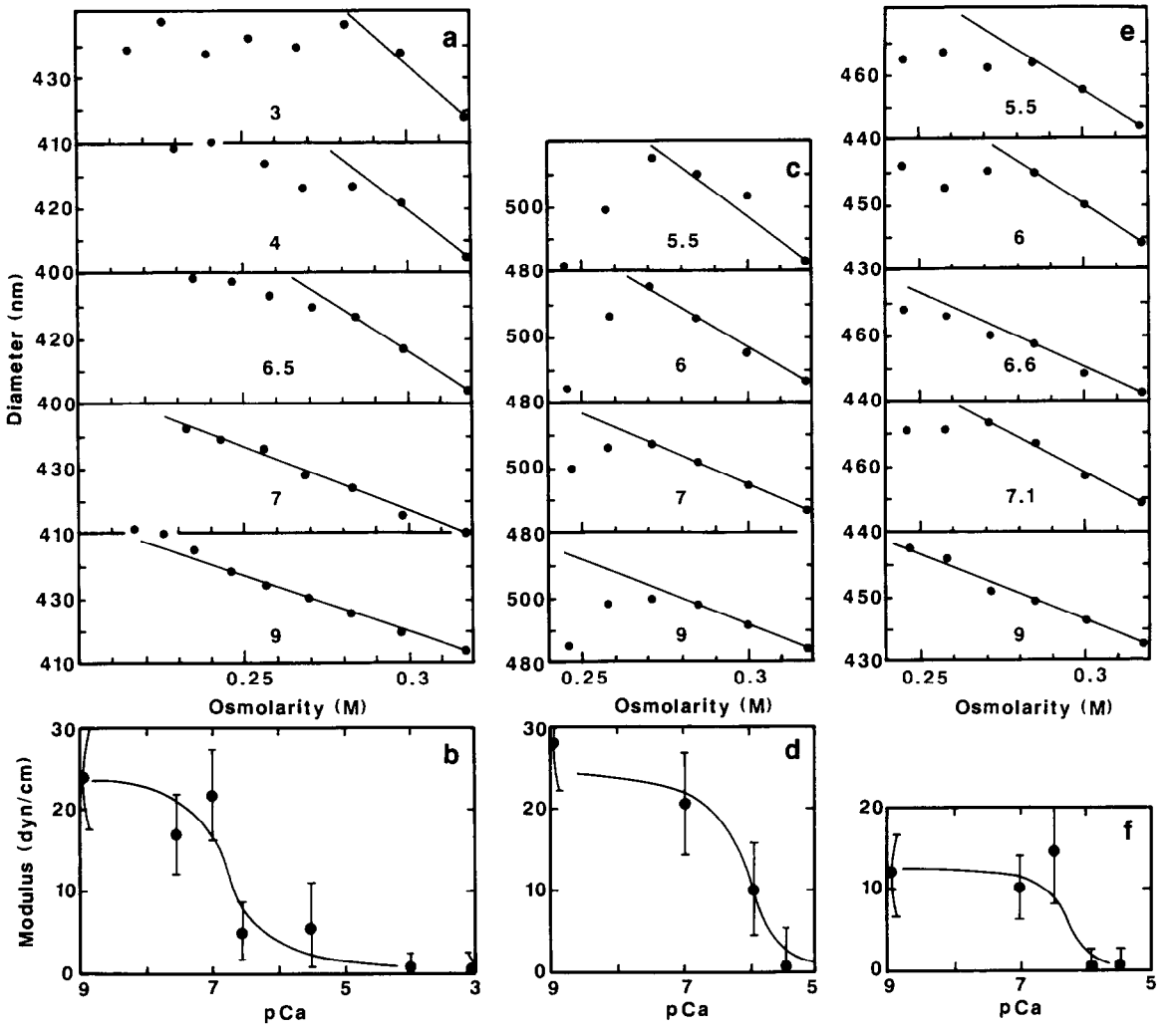

Fig.2. Osmotic swelling data (upper) and membrane elastic modulus (lower) of chromaffin granules of preparation 1 at 0 mM (a,b) and 1 mM MgATP (c,d), and preparation 2 at 1 mM MgATP (e,f). The lines and numbers in upper panels indicate, respectively, the initial slope of swelling data and p*Ca* value at measurement; curves in lower panels are merely a visual guide. The standard deviation was within twice the size of the symbol in the upper panels.

known that DNase 1 induces depolymerization of F-actin, and G-actin complexed with DNase 1 loses its polymerizability. To avoid the possible involvement of F-actin in the change of elastic modulus with $[Ca^{2+}]$, granules of preparation 2 were also used, and a drastic change in elastic modulus was again observed at p*Ca* between 7 and 6 in both the presence of 1 mM MgATP (fig.2e,f) and its absence (not shown).

The initial diameter of granules was almost independent of p*Ca*, suggesting the absence of Ca^{2+}-induced aggregation of granules which often interfered with a quantitative interpretation. Absolute sizes of the granule diameter and elastic modulus under given conditions differed from preparation to preparation, probably because of slight differences in the state, mean size and/or size distribution of granules for each preparation; however, the general trends of many other results were all the same as those in fig.2. It is thus suggested that the transitional reduction in the elastic modulus of the granule membrane with a

physiological rise of $[Ca^{2+}]$ is solely due to a change in the structure of the granule membrane itself. Here, the granule membrane signifies the lipid bilayer membrane decorated with cytoskeleton and embedded proteins. It remains to be elucidated, however, which biochemical events are most responsible for the great reduction in membrane elasticity with $[Ca^{2+}]$.

In some endocrine cells, 'degranulation' occurs even at a low $[Ca^{2+}]$ after a fast cytosolic Ca^{2+} transient [18]. Some factors other than $[Ca^{2+}]$ may facilitate a change in flexibility of the granule membrane. In this respect, it is noteworthy that a large reduction of the elastic modulus with slight increase in pH (at $pCa > 8$) as well as with pCa from 7 to 6 (at fixed pH) has been observed for zymogen granules isolated from rat pancreas acinar cells (unpublished). The hypothesis that the granule membrane should become flaccid just before fusion can then be generalized further. The conclusion is that the membrane of secretory granules undergoes an elastic transition from a rigid state at rest to a very flexible one as a prelude to exocytosis.

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