Modeling Ca-Polyanion Crosslinking in Secretory Networks. Assessment of Charge Density and Bond Affinity in Polyanionic Secretory Networks

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Summary: Materials released by secretory cells are stored inside intracellular membrane-bound vesicles. These moieties are not freely diffusible in the vesicle but remain immobilized in a Ca^{2+}-crosslinked condensed-phase polyanionic polymer matrix. During exocytosis a Na^{+}/Ca^{2+} ion exchange process triggers a volume phase transition resulting in massive swelling and release of the materials to the extracellular space. Here we formulate a simple model to assess Ca^{2+}-ion binding from the swelling kinetics of polymer networks. We found the diffusivity of the networks (D) exhibits a power-law dependency on the Ca^{2+} concentration where $D \propto [Ca^{2+}]^{-2/3}$. The model yields an estimate of charge density and ionic affinity of the polymer chains. Studies of post-exocytic swelling kinetics in airway mucin granules, mast cell granules and granules from the microalga (*Phaeocystis globosa*) were used to validate predictions from our model. These results suggest that independent of the cell type, from animal to plant cells, a single polyelectrolyte interaction mechanism appear to be responsible for product release in exocytosis.

Keywords: exocytosis; gel; secretory granule; swelling kinetics

Introduction

The concentration of moieties in secretory granules, including hormones, neurotransmitters, Ca^{2+}, ATP, etc, can reach near molar concentrations.[1-3] Since granules are membrane-bound water-permeable vesicles, if secretory products were in a free diffusible phase, a large luminal/cytosol osmotic gradient would drive massive swelling of these vesicles. A stable swelling equilibrium is reached because the diffusional mobility of secretory product inside the granules is virtually suppressed. These products remain

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entrapped in a condensed polymer gel network that forms the matrix of secretory granules. Although the chemical nature of the granular matrix varies with the cell type, a general feature of these networks is that they are all strongly polyanionic, and that condensed phase is stabilized by high concentrations of intragranular Ca^{2+}[4]. Release of the secretory products takes place by the fusion of the secretory vesicle membrane and the cell plasma membrane. The formation of a secretory pore—which follows membrane fusion—allows Na^+ from the extracellular space to diffuse into the granular space. Na^+/Ca^{2+} ion-exchange triggers a volume phase transition that expands the intragranular polymer matrix releasing the secretory material in a process called exocytosis[5-8]. The swelling kinetics of poly-anionic exocytic networks has been characterized on the basis of a Donnan equilibrium process[5, 4]. However, de Gennes scaling theory and the work of Tokita and Tanaka[9,10] provide an excellent paradigm to relate the matrix diffusivity to counter-ion crosslinking in secretory networks. The following is an attempt to formulate a simple relationship between Ca^{2+} crosslinking and diffusivity in exocytic networks of secretory granules. We used results from three different secretory cell types: (a) mouse mast cells that secrete histamine and have a matrix made of heparin; (b) rabbit goblet cells that secrete a variety of antimicrobial peptides and their matrix is made of mucins; and (c) *Phaeocystis*—a unicellular microalga that secretes a complex set of still not well identified small moieties—releases a matrix made of an alginate-like polyanionic polysaccharide that form the characteristic marine mucilage found in algae blooms[6-8].

We found that the diffusivity of the networks (D) and the concentration of $[Ca^{2+}]$ in the swelling medium follow a power law of the form:

$$D \propto [Ca^{2+}]^{-2/3}$$

Materials and Methods

Isolation of mast cell granules. Mast cells were isolated via a peritoneal lavage of beige (Bg^j/Bg^j) mice from Jackson Laboratory (Bar Harbor, ME) as described before[11,12,13]. Exocytosis of mast cell was induced by compound 48/80 (Sigma, St. Louis, MO).

Swelling kinetics. The swelling of newly released granules was monitored and video-recorded using phase contrast optics. The swelling kinetics was obtained by digitizing the diameter of exocytosed secretory networks at a 30 frames/sec sampling rate. In animal

secretory cells, the swelling medium was Hanks' buffer containing Ca^{2+} concentrations ranging from 1 to 4 mM. In the case of *Phaeocystis*, the swelling medium was artificial seawater (ASW) containing 2.5 to 15 mM $CaCl_2$.

Measurements of the radius of the exocytosed polymer gels as a function of time in solutions containing different Ca^{2+} concentrations were fitted to a first-order kinetic of the form[14]:

$$r(t) = r_f - (r_f - r_i)\, e^{-t/\tau} \qquad (1)$$

Where r_i and r_f are the initial and final radius of the granule respectively, and τ is the characteristic relaxation time of the swelling process[5]. According to Tanaka's theory of swelling of polymer gels[14], in the case of spherical gels, the characteristic time (τ) of the granule swelling kinetic is linearly related to the second power of the final radius $(r_f)^2$ of the swollen granules. The diffusivity of the gel matrix (D) can then be calculated from the slope of $(r_f)^2$ as a function of τ .

Modeling Ca^{2+} ionic bonding and network diffusivity (D) in exocytic gels. The friction (f) of gel in a good solvent is inversely proportional to the square of the correlation length of the networks (the average distance between the neighboring contact points of the polymers) (ξ)[9,10].

$$f \propto \xi^{-2} \qquad (2)$$

The diffusivity (D) is inversely proportional to f[10].

$$D = (K + 4/3\ \mu)/f \qquad (3)$$

Where K is the osmotic bulk modulus and μ is the shear modulus.

Assuming that for the same polymer gel in a swelling medium containing equally high $[Na^+]$ and various Ca^{2+} concentration, changes of K and μ are negligible, we get

$$D \propto f^{-1} \quad (4)$$

From (1) and (2), we get

$$D \propto \xi^2 \quad (5)$$

Since exocytic networks are stabilized by Ca^{2+} cross-links via ionic bonding between polymers in the network.

$$\xi \propto [Ca^{2+}]^{-1/3} \quad (6)$$

$$\xi = \xi_0 + C\,[Ca^{2+}]^{-1/3} \quad (7)$$

where C is a constant and ξ_0 is the correlation length of the networks saturated with Ca^{2+} bonds, hence reflecting the charge density of the polymer.

Therefore, from (3) and (5), the relationship between $[Ca^{2+}]$ and D is

$$D \propto (\xi_0 + C\,[Ca^{2+}]^{-1/3})^2 \quad (8)$$

$$D \propto (\xi_0^2 + 2\,C\,\xi_0\,[Ca^{2+}]^{-1/3} + C^2\,[Ca^{2+}]^{-2/3}) \quad (9)$$

Notice that 1/C is an indicator of affinity of the crosslinker, Ca^{2+} in this case, to the polymer chains.

Results and Discussion

Swelling kinetics for secretory granules.

Typical swelling kinetics of secretory granules from mast cell is shown in Fig. 1. Swelling kinetics of rabbit mucin granules and *Phaeocystis* secretory granules was obtained from previous published data[6,8]. The relationship between diffusivity (D) and extracellular Ca^{2+} concentration of three different secretory granules (mast, *Phaeocystis* and goblet cell) is shown in Figure 2-4. Notice that the diffusivity of the different secretory matrixes exhibits a drastic decrease as Ca^{2+} concentration increases.

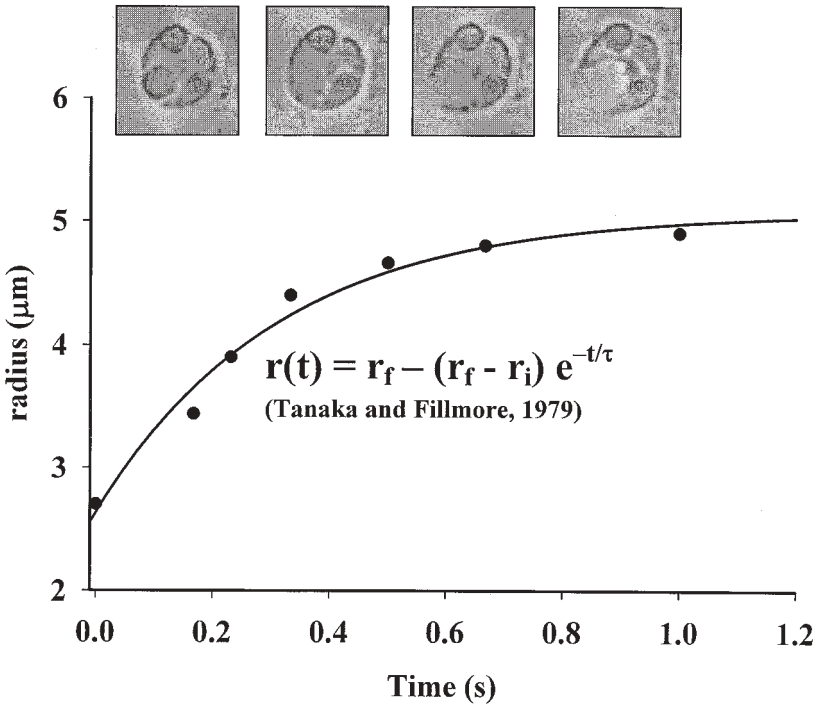

Figure 1. Swelling kinetics of secretory granules. Data points of typical swelling kinetics of secretory granules of mast cell were fit with eq. (1).[14] Exocytosis was induced by compound 48/80. The swelling of newly released granules was monitored and video-recorded using phase contrast optics. The swelling kinetics was obtained by digitizing the diameter of exocytosed secretory networks at a 30 frames/sec sampling rate.

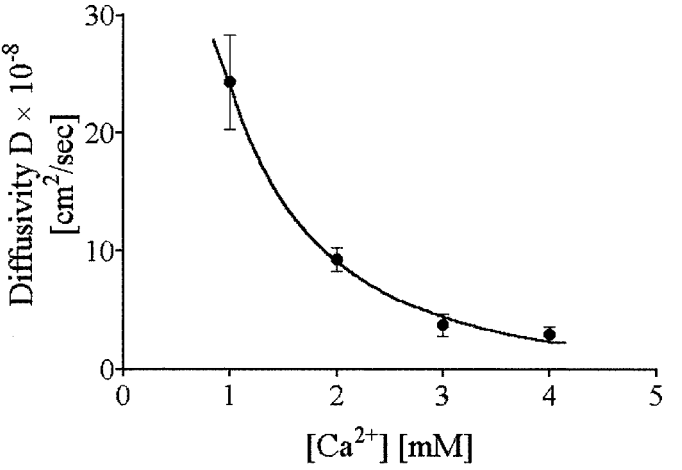

Figure 2. Ca^{2+}-dependent diffusivity change in mast cell secretory granule. Diffusivity of secretory matrix (D) decreases with the increase of extracellular Ca^{2+} concentration. Data points were fit with eq. (10). The swelling medium was Hanks' buffer (pH 7.2) containing Ca^{2+} concentration ranging from 1 to 4 mM.

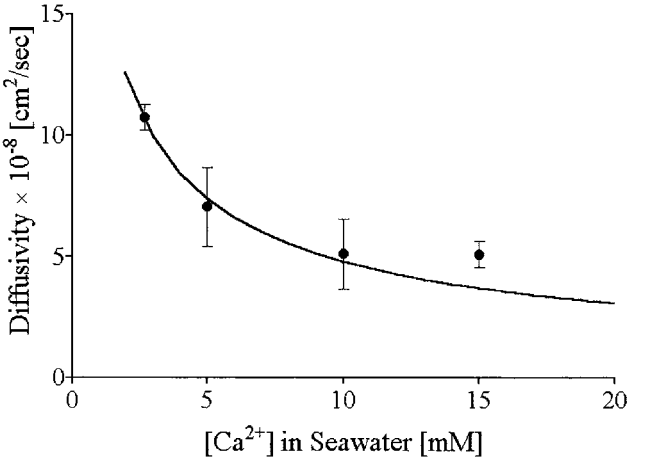

Figure 3. Ca^{2+}-dependent diffusivity change in *phaeocystis* secretory granule. Diffusivity of secretory matrix (D) decreases with the increase of seawater Ca^{2+} concentration. Data points were fit with eq. (10). The swelling medium was artificial seawater (pH 8.2) containing 2.5 to 15 mM $CaCl_2$.

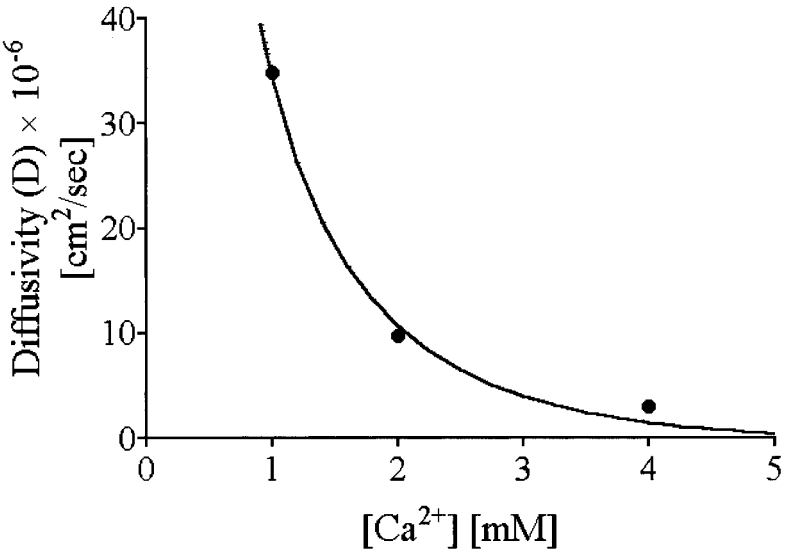

Figure 4. Ca^{2+}-dependent diffusivity change in rabbit airway goblet mucin granule. Diffusivity of secretory matrix (D) decreases with the increase of extracellular Ca^{2+} concentration. Data points were fit with eq. (9).

Prediction of the model and experimental results.

The fitting of eq. (9) to the changes of diffusivity in function of Ca^{2+} concentration for different secretory polymer matrices is illustrated in Figure 2-4, yielding the ξ_0 and C values listed below (Table 1).

Table 1. ξ_0 and C value for different secretory granules.

	Mast Cell Granule	*Phaeocystis* Granule	Mucin Granule
ξ_0	-3.008×10^{-8}	6.929×10^{-9}	-2.952×10^{-6}
C	6.488×10^{-8}	3.589×10^{-8}	5.525×10^{-6}

Although in physical networks a larger ξ_0 indicate lower charge density of the polyanionic chains, in chemical networks this inference does not hold since in this case ξ_0 might reflect pre-existing covalent cross-links other than transient Ca^{2+} ionic bonds. The larger C is, the smaller the affinity of polyvalent ions (in this case, Ca^{2+}) to the polymer chains.

This proposed model successfully predicts the relationship of extracellular Ca^{2+} and D in all three cases (Figure 2, 3 & 4). From simple measurement of swelling kinetics, this model provides a straight forward experimental approach to estimate the relative charge density and Ca-ionic affinity in polyanionic secretory networks.

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