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Characterizing the Catecholamine Content of Single Mammalian Vesicles by Collision–Adsorption Events at an Electrode

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Supporting Information

ABSTRACT: We present the electrochemical response to single adrenal chromaffin vesicles filled with catecholamine hormones as they are adsorbed and rupture on a 33 μm diameter disk-shaped carbon electrode. The vesicles adsorb onto the electrode surface and sequentially spread out over the electrode surface, trapping their contents against the electrode. These contents are then oxidized, and a current (or amperometric) peak results from each vesicle that bursts. A large number of current transients associated with rupture of single vesicles (86%) are observed under the experimental conditions used, allowing us to quantify the vesicular catecholamine content.

Approaches for electrochemically detecting soft nanoparticles and, more specifically, the contents of these nanoparticles have been reported in the past few years. One approach developed in our laboratory, termed electrochemical cytometry, involves detecting the content of soft particles such as vesicles or liposomes as they individually lyse on a cylindrical electrode after separation by capillary electrophoresis.[1−3] More recently, Bard and co-workers reported the detection of substances in emulsion droplets as they impact an electrode.[4] Furthermore, Cheng and Compton examined the electrochemistry of ascorbic acid-filled liposomes as they impact electrodes.[5] These are all examples of nanoparticles filled with electroactive molecules, their collisions at electrode surfaces, and the subsequent electrochemistry of the container contents. We show here that it is possible to combine these approaches to detect the contents of nanometer-sized vesicles from living systems following rupture on an electrode. This "vesicle electrochemical cytometry" (VEC) allows us to determine in a rapid fashion the total catecholamine amount in the vesicles and to compare this to the amount released during measurements of exocytosis. Furthermore, it allows us to characterize the rupturing phenomenon in the separations-based electrochemical cytometry experiments examining vesicle content.

An important debate in the community examining exocytosis is whether exocytosis involves full release of the vesicle contents (the traditional view) or if this process involves only release of part of the contents.[2,3,6,7] Vesicle electrochemical cytometry provides a robust approach for obtaining this information and opens the door to a wide variety of experiments for which quantitative knowledge of the contents of vesicles is important.

Exocytosis is a key process in chemical communication between cells, as it is the main mechanism enabling chemical communication between neurons. This phenomenon is based on the fusion of a neurotransmitter-filled vesicle with the cell membrane, inducing the release of its content into the extracellular space.[8] The released neurotransmitters can then stimulate or depress another neuron, thus enabling signal transmission. An important method to measure the material released during exocytosis, and the only truly molecularly quantitative method (allowing counting of molecules), is single-cell amperometry.[9,10] In this method, typically a 5 μm carbon fiber microelectrode is used to oxidize, in a diffusion-limited manner, the neurotransmitters released from the vesicle. Amperometry has been extensively used to investigate the biophysical regulation of exocytosis and the dynamics of the fusion pore formed between the vesicle and the membrane.[11,12] These findings include evidence that the membrane lipid composition can affect the nature of the exocytosis events.[13,14] It has also been found that the vesicles might not release all of their content during exocytosis, perhaps only about 40% in PC12 cells,[2,3,15] and that the pore does not fully dilate during the course of the exocytosis event.[6,7] Open and closed exocytosis leading to partial release, or an extended version of kiss-and-run,[15] might then be the main mode of neuronal communication.

Here we present the electrochemical response to single adrenal chromaffin vesicles filled with hormone transmitters as they rupture on a 33 μm diameter disk-shaped carbon electrode (Figure 1A,C). It appears that the vesicles adsorb onto the electrode surface and sequentially spread out over the electrode surface, trapping their contents against the electrode. This is supported by studies of vesicle adsorption and opening toward the surface on which they are adsorbed,[16,17] in contrast to the mechanism suggested by Cheng and Compton.[5] We have also carried out quartz crystal microbalance experiments to provide evidence for vesicle adsorption and rupture (Figure S1 in the Supporting Information).

In our model, the catecholamine contents of each vesicle are oxidized, and a peak results for each vesicle that bursts (Figure 1). The peaks initially rise quickly and decay at a lower rate. This results from opening of the membrane and initial transfer of material to the electrode surface. After this initial phase, the

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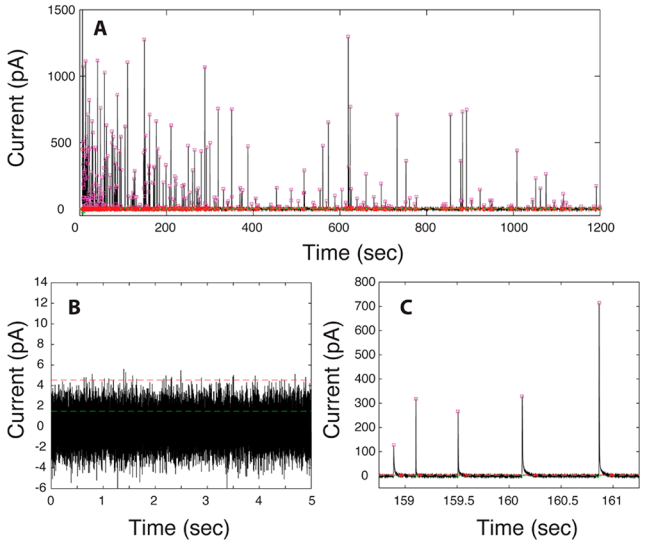

Figure 1. (A) Representative trace from a suspension of chromaffin cell vesicles. (B) A 5 s baseline at 0 mV vs Ag|AgCl in the presence of vesicles. (C) Expanded view of current transients. The pink squares represent the I_{max} of all peak candidates submitted for further analysis. The green lines represents the root-mean-square (RMS) and the red lines 5 times the RMS of the baseline noise.

membrane distention is slower as it spreads over the electrode and the contents of the vesicle diffuse to its surface.

The sensitivity of the method is inversely proportional to the area of the electrode, as larger area leads to larger background capacitance. Therefore, we used 33 μm diameter disk-shaped electrodes for these experiments. A key issue is to discriminate single vesicles from multiple vesicles that burst at the same time. Figure 2 is a plot of the maximum peak current for each

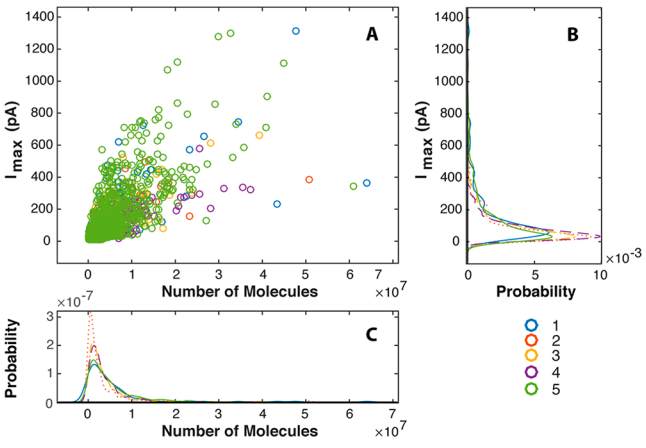

Figure 2. (A) Scatter plot of the number of molecules detected in the vesicles vs the maximum current (I_{max}) of the peak. The different vesicle isolations are represented by different colors (bottom right). (B, C) Histograms of the distributions of (B) I_{max} and (C) the number of molecules (the vesicular content). As these were fit to fill individual bins, the probability plot drops below 0, but this has no real meaning.

spike (I_{max}) versus the number of molecules (the integral of the peak). Alongside this plot are histograms of each quantity per vesicle showing in each case major distributions with much smaller distributions at 2 and 3 times the main distribution. These represent two or three vesicles rupturing at the electrode at the same time. Thus, the vast majority of the events (86%) appear to represent the content from single vesicles. Additionally, the content distribution of vesicles that rupture early is not different from that of vesicles that open late when there are far fewer events per unit time (Figure S2). We quantified (counted) the number of molecules in each vesicle. The characteristic peak shape statistics, which are listed in Table 1, show numbers similar to those expected in adrenal vesicles and event parameters similar to those found in earlier cytometry experiments.[2]

The concentration of catecholamines in the vesicles (C) can be estimated with the following equation:

$$C = \frac{3Q}{4\pi nFr^3} \quad (1)$$

where Q is the average measured charge from the electrochemical measurements, F is the Faraday constant (96485 C mol^{-1}), n is the number of electrons transferred during the reaction (n = 2 for catecholamines), and r is the average radius of the vesicles obtained from nano particle tracking analysis (NTA) experiments. From this equation, the concentration was estimated to be 0.50 M for Q = 0.88 pC and r = 130 nm, which is in agreement with previous studies.[18−21] If we assume that the concentration is the same in all of the vesicles,[20] a distribution of the theoretical vesicle diameters can be constructed from the measured values of Q for the individual spikes using eq 2:

$$d = 2\sqrt[3]{\frac{3Q}{4\pi nFC}} \quad (2)$$

We estimated the diameters of the vesicles on the basis of the number of molecules and the assumption that the intracellular concentration is constant and compared the resulting distribution to a measured vesicular size distribution obtained by the NTA (Figure 3). Both distributions are slightly skewed toward larger diameters. This might be due to aggregation of vesicles, which would decrease the apparent diffusion rate in the NTA measurements. It is also plausible that aggregates can bias the charge Q in the electrochemical measurements. For smaller diameters, the NTA measurements are affected by the presence of small dust particles that cannot be filtered out of the solution. At our lower limit, a vesicle with a radius of 30 nm and a catecholamine concentration of 0.50 M contains <35000 molecules (corresponding to a charge of around 0.01 pC), which most likely will not lead to a peak larger than 5 times the RMS noise (I_{max} = 12.5 pA) at a 33 μm diameter electrode. Thus, we cannot measure the content of the smallest vesicles in synapses; however, adrenal vesicles are not expected to be this small.

Although the results are very preliminary, if we compare the measured average number of catecholamines per vesicle (4.31 × 10^6 molecules) to that observed and published for amperometrically measured release from adrenal cell vesicles (1.8 × 10^6 molecules),[19] we find that the amount released during exocytosis is 42% that in the average vesicle. It should be noted that although this is similar to other measurements, there are issues with signal-to-noise differences between the methods that need to be taken into account before numbers like this can be certain.[2,3,15]

The experiments shown here provide a means to quantify the contents of lipid vesicles in a collision−adsorption protocol at single microelecrodes and to understand the chemical dynamics of the vesicle−electrode adsorption/rupture process. We find

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Table 1. Data from Multiple Vesicle Isolations[a]

vesicle isolation	T_{rise} (ms)[b]	$T_{1/2}$ (ms)[c]	T_{fall} (ms)[d]	I_{max} (pA)[e]	N (10^6)[f]	$Q^{1/3}$ [g]	n[h]
1	1.00	8.03	10.9	167	5.97	1.06	3
2	0.75	6.10	8.88	86.2	3.09	0.83	3
3	0.83	7.59	12.6	93.9	3.59	0.92	4
4	0.90	9.03	15.7	77.7	3.95	0.97	3
5	0.90	7.85	11.2	148	4.93	1.02	6
mean ± SEM	0.88 ± 0.10	7.72 ± 1.06	12.0 ± 2.49	114 ± 40.3	4.31 ± 1.15	0.96 ± 0.09	–

[a]The data are presented as means of means for the individual measurements, and the mean ± standard error of the mean (SEM) of all preparations is also reported. The total numbers of vesicle events measured for groups 1−5 were 126, 157, 225, 433, and 969, respectively. [b]T_{rise} is the rise time for each current transient from 25 to 75% of the peak signal. [c]$T_{1/2}$ is the width at half maximum of each peak. [d]T_{fall} is the time from 75% to 25% of the backside of each peak. [e]I_{max} is the maximum current for each event. [f]N is the number of molecules oxidized from each vesicle. [g]$Q^{1/3}$ is the cube root of the charge under the peak. [h]n is the number of measurements for each isolation.

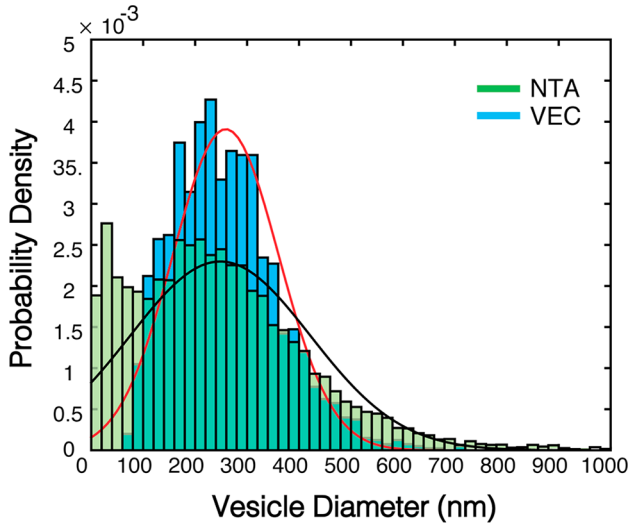

Figure 3. Histograms of vesicle sizes measured by nanoparticle tracking analysis (NTA) (green) and the theoretical size distribution calculated from the vesicle electrochemical cytometry (VEC) data (blue). The solid lines are Gaussian fits to the VEC (red) and NTA (black) data.

that under the conditions used here only one vesicle opens on the electrode surface in 86% of the events, leading to the ability to quantify single-vesicle events. Comparison with release from vesicles in cells suggests that the average vesicle contains considerably more catecholamine than observed in a single exocytotic release event.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and procedures for cell culture, electrochemical measurements, and data processing. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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