Review Article
Sarcomere Imaging by Quantum Dots for the Study of Cardiac Muscle Physiology

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We here review the use of quantum dots (QDs) for the imaging of sarcomeric movements in cardiac muscle. QDs are fluorescence substances (CdSe) that absorb photons and reemit photons at a different wavelength (depending on the size of the particle); they are efficient in generating long-lasting, narrow symmetric emission profiles, and hence useful in various types of imaging studies. Recently, we developed a novel system in which the length of a particular, single sarcomere in cardiomyocytes can be measured at ∼30 nm precision. Moreover, our system enables accurate measurement of sarcomere length in the isolated heart. We propose that QDs are the ideal tool for the study of sarcomere dynamics during excitation-contraction coupling in healthy and diseased cardiac muscle.

1. Mechanisms of Contraction and Relaxation of Cardiac Muscle

First, we briefly summarize the cellular mechanisms of the excitation-contraction (EC) coupling of cardiac muscle, based on the literature (i.e., [1–3] and references therein). Upon membrane depolarization, Ca2+ enters myocytes via sarcolemmal L-type Ca2+ channels, which induces the release of Ca2+ from the sarcoplasmic reticulum (SR) (i.e., Ca2+-induced Ca2+ release mechanism; CICR), resulting in an increase in [Ca2+]i, and subsequently the binding of Ca2+ to troponin (Tn), resulting in the subsequent formation of cross-bridges (see Figure 1 for sarcomere structure).

Tn is a heterotrimer of distinct gene products: that is, TnC, TnI, and TnT (see [3, 4] and references therein). Ca2+ binds to the regulatory Ca2+-binding site of TnC during systole, the C-terminal domain of TnI is dissociated from actin and binds to the N-terminal domain of TnC, due to the enhanced binding of TnC and TnI (“on” state). The transition from the “off” to “on” state is associated with a movement and conformational change of tropomyosin on the thin filament, facilitating the binding of myosin molecules to actin (see [3, 4] and references therein). Similar to Ca2+, the strongly bound cross-bridges such as the rigor complex or the actomyosin-ADP complex can “turn on” the thin filament, as if Ca2+ were bound to TnC, further promoting the formation of cycling cross-bridges (e.g., [5–7]). Therefore, under physiologic conditions, both Ca2+ and strongly bound cross-bridges regulate the state of the thin filament in a coordinated fashion.

After the peak of contraction, [Ca2+]i is lowered via, mostly, four Ca2+-transport systems: that is, (i) sequestration by the SR Ca2+-ATPase pump, (ii) efflux via the sarcolemmal Na+/Ca2+ exchanger, (iii) extrusion by the sarcolemmal...
Ca$^{2+}$-ATPase pump, and (iv) uptake into mitochondria via the Ca$^{2+}$ uniporter [see [1, 2] and references therein].

Recent advances in molecular imaging technologies have enhanced our understanding of the EC coupling of cardiac muscle. In particular, the processes of the local changes of various ions at/near the T-tubules (such as the dynamics of Ca$^{2+}$ sparks) in isolated myocytes have been elucidated (e.g., [1, 2]). In addition, a recent study greatly improved the quantification of Ca$^{2+}$ sparks [8]. It should be stressed that compared to ions/currents, our knowledge is still limited regarding the sarcomere dynamics in localized, focal areas of cardiomyocytes. Indeed, although it is generally thought that all sarcomeres within a cardiomyocyte uniformly respond to a change in [Ca$^{2+}$], to produce mechanical force, this notion has yet to be verified. Therefore, careful experimentation employing advanced nanotechnologies is needed to fully reveal the mechanism by which EC coupling is regulated in localized areas at/near T-tubules in a cardiomyocyte, and the subsequent transmission throughout the myocyte.

2. Sarcomere Length Dependence of Ca$^{2+}$ Activation: Underlying Mechanism for Frank-Starling’s Law of the Heart

It is well established that active force production of cardiac muscle is more sarcomere length-(SL-) dependent than that of skeletal muscle (e.g., [3, 9-13]). Allen and Kurihara [14] applied the Ca$^{2+}$-sensitive photoprotein aequorin to cardiac muscle and simultaneously measured [Ca$^{2+}$], and twitch force at varying muscle lengths. Accordingly, they discovered that the increase in twitch force was not associated with a rise in [Ca$^{2+}$], but with activation of myofilament proteins per se. Indeed, a number of later studies using various types of preparations have provided solid evidence that a change of only ~0.1 μm in SL causes a dramatic change in mechanical properties, especially under physiologic partial activation states where the average [Ca$^{2+}$], increases to ~10$^{-6}$ M (e.g., [15, 16]).

What is the molecular basis for this pronounced SL dependence? It has been demonstrated that SL elongation causes a decrease in interfilament lattice spacing via the radial component of titin-based passive force in the sarcomere, promoting cross-bridge attachment [17-19]. More recently, we reported that the binding of myosin-ATP to actin upon reduction in the lattice spacing depends highly on the thin filament “on-off” state [7, 20]. It is therefore considered that interfilament lattice spacing and thin filament “on-off” switching are the two essential factors that regulate length-dependent activation.

3. Use of Quantum Dots (QDs) for the Detection of Sarcomere Length

Because of the large length dependence of active force, accurate measurement of SL is the key to unveiling the contracting functions of cardiac muscle at the molecular level. A number of studies have been conducted on cardiomyocytes, cardiac strips, and whole heart of various animal species, but SL is usually averaged along the longitudinal axis of the myocyte (e.g., [21, 22] and references therein). Indeed, the averaging of SLs allows researchers to obtain stable and consistent values with minimal deviation, hence useful for quantification. However, it is likely that the SL value varies even within the same myocyte during cardiac beat, due to variations in the magnitude of the local rise in [Ca$^{2+}$] at/near the T-tubules, especially in diseased tissues (see [1, 2] and references therein). Therefore, the variance in SL may be transmitted over a long distance along the longitudinal axis, influencing the myocyte’s mechanical properties (e.g., length-dependent activation). Thus, it is critical to establish a method by which one can visualize the motions of a single sarcomere at high spatial and temporal resolution in various regions of the myocyte.

In various fields within biological sciences, QDs are widely used to view the motions of molecules, because they are extremely efficient in generating long-lasting fluorescence, with their intrinsic brightness; in fact, they are many times more efficient than other classes of fluorophores (see e.g., [23, 24]). These characteristics of QDs are favorable for long-term imaging experiments in cells as well as in vivo. Indeed, Tada et al. [23] successfully conducted the tracking of the HER2 molecule in living mice. Later, Gonda et al. [24] dramatically enhanced the quality of the positioning of QDs and tracked the movement of cancer cells in anesthetized living mice at a precision of ~7 nm.

By taking advantage of the nature of QDs, we recently developed a novel technique to measure the length of individual sarcomeres in isolated cardiomyocytes and in the whole heart [25] (see also Figure 1). In that study, we analyzed the movement of QDs (Qdot 655 Invitrogen, Carlsbad, CA, USA) conjugated with anti-α-actinin antibody attached to the Z-disks of sarcomeres in skinned rat ventricular myocytes during spontaneous sarcomeric oscillations (SPOC) that occur under partial activation states (i.e., ADP-SPOC and Ca-SPOC; see [26-30]), and in intact cardiomyocytes under electric field stimulation. It should be stressed that compared to a widely used organic dye (i.e., Alexa 488), QDs enable the measurement of the length of a single sarcomere at a resolution of ~30 nm for a relatively long period (i.e., 1 min) [25]. Here, as shown in Figure 2, even when the myocyte was in motion (due to ADP-SPOC), we found that the QD fluorescence successfully provided clear striations along the myocyte compared to that obtained under the bright field (see also Supplementary Videos 1 and 2, see in Supplementary Material available online at doi:10.1155/2012/313814).

One interesting finding in our previous work [25] is that intact cardiomyocytes showed periodic outlines after treatment with a mixture of anti-α-actinin antibody-QDs and FuGENE HD (a lipid reagent; Roche Ltd., Basel, Switzerland), indicating the infiltration of the antibody-QDs into the myocytes and their subsequent attachment to the Z-disks. The myocytes treated in this way were shown to normally respond to electric field stimulations at various frequencies (1–5 Hz), indicating the usefulness of QDs for various physiological experiments with living myocytes. Our previous finding that the waveform properties upon
Figure 1: Schematic illustration indicating the structure of a cardiac sarcomere associated with T-tubules. As discussed in [33], T-tubules and Z-disks are considered to run in parallel in cardiac muscle, causing Ca\(^{2+}\) sparks at/near the Z-disks. Thick and thin filaments, and titin are shown in this illustration (for simplicity, only two titin molecules per half thick filament are shown) (cf. [37] for electron microscopic images of titin molecules in the sarcomere). Also, troponin and tropomyosin are bound to the thin filaments. As described in detail in earlier papers (e.g., [12, 13]), I-band titin is in a contracted state at the slack SL; straightening of the tandem Ig segment and, then, extension of the PEVK and N2B segments are thought to occur (resulting in passive force generation) in response to stretch. In the lower part of the figure, the antibodies we used in the present work and the structure of the QD (Qdot 655 Invitrogen, Carlsbad, CA, USA) are shown (number indicating the emission wavelength). The anti-α-actinin antibody-QDs bind to Z-disks, and anti-α\(_B\)-adrenergic receptor (AR) antibody-QDs bind to the T-tubules.

Figure 2: (A) Epi-illumination of a rat skinned myocyte treated with anti-α-actinin antibody-QDs excited by blue light during ADP-SPOC (see [25] for composition of the ADP-SPOC solution). Note the clear striations along the myocyte. (B) Same myocyte as in the (A) during ADP-SPOC, observed under the bright field. Observations were conducted under the same optics system as described in [25], and the temperature was maintained at 25°C. See Supplementary Videos 1 and 2.
electric field stimulation became similar to those obtained during SPOC at high stimulation frequencies of 3–5 Hz (i.e., relatively slow shortening followed by quick re-lengthening) suggests that SPOC may facilitate the organization of sarcomeric waveform to efficiently produce mechanical force in living myocytes. Future studies should be directed to determine whether or not SPOC indeed occurs in the normal physiological setting.

Figure 3(A) shows the epi-fluorescent image of an intact cardiomypocyte treated with QDs conjugated with anti-α1B-adrenergic receptor antibody (details given in the legend of Figure 3; see also Figure 1 for binding of the antibody-QD complex to the T-tubule). We observed periodic fluorescent signals along the longitudinal axis of the myocyte, indicating the positions of the T-tubules. Figure 3(B) shows the results of the analyses of the distances between the peaks of the QD signals at different locations in the myocyte shown in Figure 3(A). We found that the distances between the T-tubules (even two sequential distances) differ within the myocyte to a magnitude greater than that found in skinned myocytes (i.e., as much as ∼0.2 μm; cf. [25]), suggesting relatively large variance of SL, as previously reported in an earlier study [31]. It has been reported that, albeit a probability lower than that during systole, Ca^{2+} sparks occur during
diastole in intact cardiomyocytes (e.g., [1, 2]). Consistent with this notion, we observed that \( \text{Ca}^{2+} \) sparks indeed randomly occurred under our experimental condition in various regions in an intact myocyte, followed by a marked \( \text{Ca}^{2+} \) wave, coupled presumably with autonomous depolarization of the sarcolemma (see Supplemental Video 3). These results may suggest that during diastole, differing magnitudes of \( \text{Ca}^{2+} \) sparks occurring at varying localized areas of the myocyte result in different magnitudes of contractions in sarcomeres within the cell, hence different lengths of the sarcomere.

It is worthwhile noting that FuGENE HD (cf. [25]) may cause irreversible damage to the sarcolemmal functions of cardiomyocytes, and this damage, albeit only a slight magnitude, may result in abnormal depolarization and/or repolarization. In contrast, we consider that the conjugation magnitude, may result in abnormal depolarization and/or repolarization. Therefore, in future studies, QDs may be a valuable tool to detect the length of a single sarcomere in a particular myocyte in the heart in vivo, after resolution of the problems associated with focus adjustment in the Z-direction during cardiac beat.

5. Conclusion

In this article, we summarized the molecular mechanisms of the EC coupling of cardiac muscle, focusing on the contractile function. We highlighted the recent advances in the measurement of single sarcomere dynamics with QDs in skinned and intact myocytes, as well as in the whole heart. We also presented new data showing that QDs revealed differences in the length of sarcomeres within the same myocyte even during diastole, presumably due to the “on/off” of \( \text{Ca}^{2+} \)-sparks in localized regions. We therefore consider that QDs are a useful, powerful tool to quantitatively analyze the dynamics of cardiac muscle under various experimental settings.

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