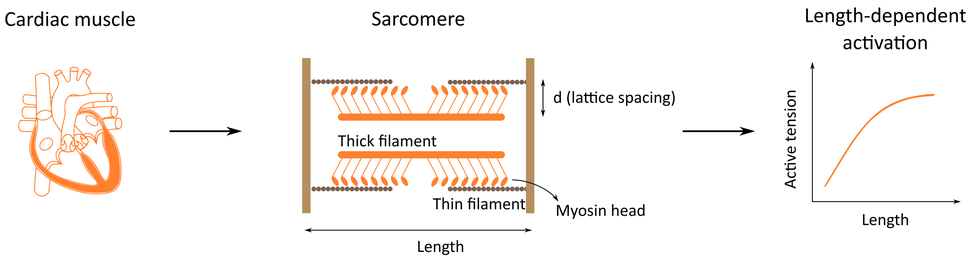
Length-dependence of cardiac fibers EPR spectra: a new experimental setup

S. Kosta[1], M. Hoebeke[2], P.C. Dauby[1]
[1] GIGA - In silico Medicine - University of Liège, Belgium
[2] Laboratory of Biomedical Spectroscopy, University of Liège, Belgium

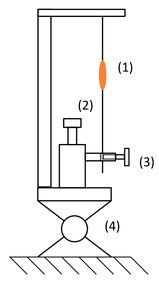
Length-dependent activation (LDA) is a fundamental property of the cardiac muscle that underlies the Frank-Starling law of the heart. However, the molecular mechanisms responsible for LDA are still not fully understood. EPR is a powerful technique to analyse the dynamics of a molecular process and it may provide new insight in our understanding of cardiac contraction properties at the molecular scale . A new experimental setup is proposed to study the length-dependence of EPR spectra obtained from spin-labeled cardiac fibers.

Length variation



What are the molecular processes that underlie LDA ? What is the contribution of lattice spacing to LDA ?

Experimental setup:



(1) Papillary fibers: cardiac fibers (about 2-3 mm in diameter and 1cm long) are extracted from pig hearts, cut along the principal axis of contraction and skinned with glycerol solutions. They are attached to both ends with nylon threads.

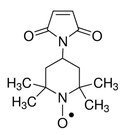
(2) Micrometer screw (ThorLabs): this screw allows for a fine tuning of fibers length (one rotation correponds to 0.5 mm extension).

(3) Flat-bottom screw: fixation of the inferior thread. A similar screw is used for the other thread (not represented).

(4) Lab jack: once the fiber is correclty attached, the whole device is driven down so that the fiber can enter the EPR cavity.

EPR study

Spin labeling

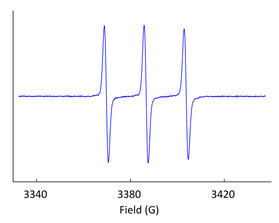


4-Maleimido-2,2,6,6-tetramethyl-1-piperidinyloxy (Sigma-Aldrich) , or MSL, is used to label the SH-1 thiol group of the myosin head. The spin labeling (SL) procedure was roughly similar to D. Lorinczy and J. Belagyi (1996). The fibers were skinned with osmotic shocks between rigor solution (100 mM KCl, 5mM $MgCl_2$, 1 mM EGTA, 10 mM histidine.HCl, pH 7.0) and 50% rigor/50% glycerol solution. Then they were incubated at 4 °C in 6 ml of SL solution (100 mM KCl, 5mM $MgCl_2$, 1 mM EGTA, 10 mM histidine.HCl, 2 mM pyrophosphate, pH 7.0 + 2 mg MSL) for two hours. After that they were transfered in a rigor solution containing 25 mM $K_3Fe(CN)_6$ for 16 hours to remove the unreacted labels and to reduce labels bound to weakly immobilizing sites. The spin-labeled fibers were stored at 4 °C in rigor solution before the EPR measurements.

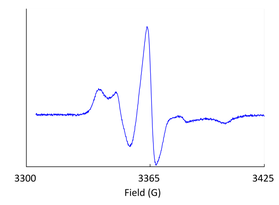
EPR spectra

All ESR spectra were acquired for spin-labeled cardiac fibers in relaxing solution (rigor solution + 5 mM ATP). They were recorded using a Bruker EMX ESR spectrometer. The typical instrument settings were: 9.5 GHz microwave frequency, 3365 G center field strength with 120 G as sweep width, 12 mW microwave power and 6 G modulation amplitude.

Free MSL in relaxing solution

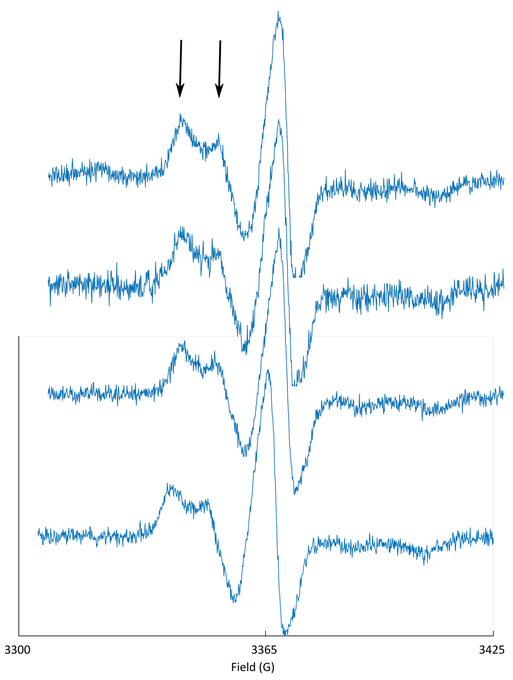


Spin-labeled cardiac fiber in relaxing solution



EPR spectra were acquired for a progressive stretching of the fibers. A two-component spectrum is obtained, one component being more ordered than the second one, as already observed by Naber et al. (2007). The relative contribution of the two components to the spectrum varies with the stretching state of the fiber.

This figure represents consecutive EPR spectra obtained during a progressive stretch of a labeled cardiac fiber. From bottom to top: prestretched state, +0.5mm, +1mm, +1mm. Arrows indicate the two components.



We have validated an experimental protocol that enables the EPR study of cardiac fibers during a progressive stretch. The question that motivated this work remains open: how does the sarcomere length influence cardiac fibers dynamics at the molecular level ? This question will be addressed in future work with contracting fibers. A quantitative analysis of the EPR spectra is planned. Furthermore, the influence of lattice spacing will also be considered in order to establish a comparison between length and lattice spacing effects on molecular dynamics.

References:
[1] Kobirumaki-Shimozawa, F., Inoue, T., Shintani, S.A., Oyama, K., Terui, T., Minamisawa, S., Ishiwata, S., Fukuda, N., 2014. Cardiac thin filament regulation and the Frank-Starling mechanism. J. Physiol. Sci. 64, 221–32.
[2] Belágyi, J., Röth, E., 1987. Orientation dependence and motional properties of spin labels in cardiac and skeletal muscle fibres. Gen. Physiol. Biophys. 6, 571–581.
[3] Lorinczy, D., Belágyi, J., 1996. Internal flexibility of cardiac myosins. J. Therm. Anal. 47, 503–514.
[4] Thomas, D.D., Cooke, R., 1980. Orientation of spin-labeled myosin heads in glycerinated muscle fibers. Biophys J 32, 891–906.
[5] Naber, N., Purcell, T.J., Pate, E., Cooke, R., 2007. Dynamics of the Nucleotide Pocket of Myosin Measured by Spin-Labeled Nucleotides. Biophys J 92, 172–184.

S.K. acknowledges ULg for financial support.

Contact : sarah.kosta@ulg.ac.be