 Proceedings of ICCES'08, pp.885-886

Micro-Thermal Functional Assays of Ca^{2+}-Regulated Thin Filaments; Effects of Hypertrophic Cardiomyopathy-Associated Mutants of Human Cardiac Troponin

Nicolas M. Brunet[1,2], Goran Mihajlović[3], Khaled Aledealat[3]
Fang Wang[2], Peng Xiong[3,5], Stephan von Molnár[3] and P. Bryant Chase[1,2,4,5]

Summary

We studied the effect of temperature on the sliding speed of rabbit skeletal F-actin propelled by rabbit skeletal heavy meromyosin (HMM) with and without human cardiac Ca^{2+}-regulatory proteins troponin and αtropomyosin. Temperature was varied rapidly and continuously over a broad temperature range ($\sim 20 - 60^{\circ}$C) using a micro-fabricated thermo-electric controller (1). The sliding speed for regulated thin filaments at saturating [Ca^{2+}] was greater than for unregulated actin (2) over the entire temperature range. Regulated thin filaments, however, exhibited an anomalous decline in sliding speed above 54°C at pCa 5, and a reversible loss of Ca^{2+}-regulation above 45°C at pCa 9, suggesting an effect of temperature on Ca^{2+}-regulatory protein structure and/or affinity for actin. A transition temperature (T_t) was observed for regulated thin filaments at pCa 5 below which the activation energy (E_a) was two-fold higher than E_a for higher temperatures; correction for effects of temperature on solvent viscosity (3) accentuated these differences in E_a, and suggest that sliding speed is determined by a diffusion-limited process at the higher temperature range. Mutations in troponin subunits that are associated with familial hypertrophic cardiomyopathy (FHC) cTnI K206Q (4) and cTnT R278C both increased sliding speed at pCa 5 over most of the temperature range and also increase T_t, while mutation cTnI R145G has little or no effect; T_t was lower in the absence of troponin and tropomyosin. Assays performed under conditions where [ATP], [Pi], or motor density on the surface were changed suggest that T_t can also be modulated by metabolic factors in addition to the Ca^{2+} regulatory proteins. These results demonstrate the utility of our thermo-electric controller for investigating molecular mechanisms underlying biomolecular motor function, and cardiovascular diseases related to altered biomechanics of cardiac myofilament proteins.

[1]Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306 USA
[2]Department of Biological Science, Florida State University, Tallahassee, FL 32306 USA
[3]Department of Physics and Center for Material Research and Technology (MARTECH), Florida State University, Tallahassee, FL 32306 USA
[4]Department of Chemical and Biomedical Engineering, Florida State University, Tallahassee, FL 32306 USA
[5]Integrative NanoScience Institute, Florida State University, Tallahassee, FL 32306 USA

References

1. Mihajlović G, Brunet NM, Trbović J, Xiong P, von Molnár S, Chase PB (2004) *Appl Phys Lett* **85**, 1060-1062.
2. Schoffstall B, Brunet NM, Wang F, Williams S, Barnes AT, Miller VF, Compton LA, McFadden LA, Taylor DW, Dhanarajan R, et al. (2006) *J Physiol* **577**, 935-944.
3. Chase PB, Chen Y, Kulin K, Daniel TL (2000) *Am J Physiol Cell Physiol* **278**, C1088-C1098.
4. Köhler J, Chen Y, Brenner B, Gordon AM, Kraft T, Martyn DA, Regnier M, Rivera AJ, Wang C-K, Chase PB (2003) *Physiological Genomics* **14**, 117-128.