Ca^{2+} and Mg^{2+} Binding to the C-Domain of Different Troponin C Isoforms in Skeletal Skinned Fibers

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The C-domain Ca-Mg binding sites III and IV of troponin C (TnC) control TnC-thin filament (TF) affinity but are difficult to study because they do not cause tension. Here we measured the influence of Mg^{2+} and rigor bonds on the apparent affinity for Ca^{2+} binding to the C-terminus of TnC. Ca^{2+} binding was estimated from TnC efflux from the TF in the presence of different [Ca^{2+}], using skinned skeletal psoas fibers reconstituted with chicken recombinant (r) or native (s) TnC's that differ in C-terminal Ca^{2+} affinity (pCa_{50} 6.7 vs 7.1) due to substitution of Ile for Thr130. TnC loss on exposure to rigor+Ca^{2+} or relax (R)+Ca^{2+} was monitored by testing isometric tensions under standard conditions (pCa 4.4, pMgATP 2.3, K propionate 152 mM, pH 7, 15°C). In relaxed fibers (R: pMgATP 4 and pMg 6), pCa_{50} was 8.45 for rTnC and 8.85 for sTnC and IT130, a recombinant rTnC with Thr130. In rigor, pCa_{50} was 9.1 for all three. Thus Ca^{2+} affinity for the C-domain on the filament increases ~100x compared to isolated TnC; the difference between rTnC and sTnC disappears in fibers in rigor; and formation of rigor crossbridges enhances C-domain Ca^{2+} binding ($p< 0.05$). In R, Mg^{2+} competes with Ca^{2+} at C-domain sites, and pCa_{50} ~7.7 for all proteins. Without Ca^{2+}, Mg^{2+} titration curves differed for sTnC (Mg_{50} 4.6) and rTnC (Mg_{50} 3.6). At pCa 7 (physiological condition), titration with Mg^{2+} (to 10mM) was unable to displace Ca^{2+} from the C-Domain.

Keywords: Ca^{2+} binding, C-Domain, Troponin C

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