a common antigenic determinant. The effect of C135 on the Ca^{2+} dependence of the myofibrillar ATPase activity was also investigated. At low Ca^{2+} concentrations, where the ATPase was inhibited, the antibody had no effect. However, in the presence of Ca^{2+}, where the myofibrils were fully activated, the antibody greatly inhibited the ATPase activity. These results suggest that the binding of the antibody to STnI in the myofibrils 'locks' the conformation of this troponin subunit in an inhibitory state, similar to the one in which STnI is still bound to actin in the absence of Ca^{2+}. Work is in progress to determine the binding site of C135 on fragments of STnI generated by CNBr cleavage.

These two areas will be discussed in terms of our current overall view of the mechanism of Ca^{2+} regulation of muscle contraction.

C4

Calcium Involvement in the Protein-Membrane Systems of Blood Coagulation

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Blood coagulation is an extracellular process that occurs in a medium containing millimolar concentrations of calcium and utilizes this ion in several ways. One major utilization is by the vitamin K-dependent, γ-carboxyglutamate-containing proteins. Four of these proteins are known to function as enzymes and/or substrates for membrane-bound enzyme-catalyzed reactions of the blood-clotting cascade. Three additional vitamin K-dependent proteins are known in the plasma, one of which probably acts as an enzyme in a membrane requiring reaction that decreases coagulation. All of these plasma proteins have homologous amino terminal sequences of about 40 residues which contain 10 to 12 γ-carboxyglutamic acid residues.

Calcium functions to aid binding of these vitamin K-dependent proteins to membranes containing acidic phospholipids. Acidic phospholipids are found primarily in cytosolic membranes and are nearly absent on the external surface of the plasma membranes. Exposure of cytosolic membranes through cell damage may therefore constitute an important signal enabling blood coagulation to occur. Calcium binding to γ-carboxyglutamic acid-containing plasma proteins causes a protein conformational change which is prerequisite to protein–membrane binding. In the case of prothrombin, the protein conformational change apparently involves a trans to *cis* proline isomerization (Pro_{22}) which has a characteristic reaction half-time of 90 minutes at 0 °C and an activation energy of 21 kcal/mol. The conformational changes for all of these proteins result in changes in intrinsic protein fluorescence as well as other spectral properties and are cooperative with respect to calcium. Very little selectivity for multivalent metal ions is displayed by the proteins and the sites have an average K_D of 10^{-4} to 10^{-3} *M* calcium which varies slightly for the different proteins. Prothrombin, the most thoroughly studied protein, binds 6 calcium ions in parallel with the protein conformation change with an average K_D of 0.25 m*M* calcium. Many other ions show site specificity and three bound ions (Mn, Cd, Lanthanides) are sufficient to cause the conformational change.

Calcium serves a second role in these protein–membrane interactions which appears to involve the acidic phospholipid residues. This second calcium-dependent process is also cooperative with respect to metal ion and shows a similar affinity for calcium. Approximately four calcium ions and eight acidic phospholipid residues are required for this second process. Metal ion specificity is observed in this step with calcium and strontium functioning fully while magnesium and manganese fail to function at all.

The precise structure of the protein–membrane interface is not known but studies with phospholipid bilayers and monolayers indicate involvement of the phospholipid headgroup only. The favored model is calcium bridging between ligands of the protein and the phospholipid. Rapid kinetic studies indicate that the metal ions responsible for maintaining protein conformation are in rapid exchange with solvent ions even in the membrane-bound state.

C5

Binding and Transport of Calcium by Sarcoplasmic Reticulum ATPase

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Sarcoplasmic Reticulum (SR) membrane can be isolated from striated muscle in the form of sealed vesicles containing a high density of ATPase protein that accounts for approximately half the membrane mass. The ATPase polypeptide units are composed of polar segments protruding from the outer surface of the membrane into the aqueous medium, and hydrophobic segments intruding the membrane bilayer. The specific function of the SR ATPase is to take up Ca^{2+} from the medium *outside* the vesicles, and release it into the aqueous medium *inside* the vesicles. Thereby a transmembrane Ca^{2+} gradient is formed, deriving free energy from ATP hydrolysis.

Activation of the enzyme is totally dependent on calcium binding to high affinity sites which are exposed to the medium outside the vesicles. In the absence of ATP, the calcium binding properties of the SR ATPase can be characterized at equilibrium by measuring the distribution of radioactive calcium tracer, or measuring ensuing changes of protein intrinsic fluorescence. Specific calcium binding (producing enzyme activation) involves two sites per enzyme unit, with a $\sim 10^6\ M^{-1}$ apparent association constant, at neutral pH. The relationship of Ca^{2+} binding to Ca^{2+} concentration is pH dependent, inasmuch as higher affinity for Ca^{2+} and higher cooperativity are observed as the H^+ concentration is reduced. The experimental data can be fitted satisfactorily assuming competition of one Ca^{2+} with one H^+ for each site, and cooperative interaction of binding sites.

When ATP is added to Ca_2 ATPase, the ATP terminal phosphate is rapidly transferred to an aspartyl residue of the catalytic site, forming an anhydride bond. Following the phosphorylation reaction the affinity of the sites for Ca^{2+} is reduced of ~3 orders of magnitude ($K \cong 10^3 M^{-1}$), and their orientation is changed to permit dissociation of Ca^{2+} inside the vesicles. The phosphoenzyme then undergoes hydrolytic cleavage, and the enzyme is free to undergo a new catalytic and transport cycle. When the Ca^{2+} concentration inside the vesicle is increased ~3 orders of magnitude, Ca^{2+} accumulation reaches an asymptote which is *permitted* by the free energy of ATP, and *limited* by the dissociation constant acquired by the calcium sites when the enzyme is phosphorylated. Therefore, it is clear that a basic feature of the transport mechanism is a special relationship between binding sites and catalytic site, whereby occupancy of binding sites by Ca^{2+} is required for the utilization of ATP by the catalytic site, and phosphorylation of the catalytic site reduces the affinity of the binding sites for Ca^{2+}. Thereby ATP phosphorylation potential is transformed into Ca^{2+} concentration potential.

The ATPase catalytic site can be phosphorylated not only by ATP, but also by orthophosphate. The latter reaction, however, occurs only in the *absence* of Ca^{2+}, indicating that phosphorylation of the catalytic site does not require free energy input (*i.e.*, ATP) if the binding sites are free of Ca^{2+}. It is of interest that orthovanadate can form a stable complex with the catalytic site even in the *presence* of Ca^{2+} and, in analogy to ATP, it reduces the affinity of the binding sites causing dissociation of Ca^{2+}. Contrary to ATP, however, the vanadate interaction with the catalytic site is rather stable and does not produce Ca^{2+} fluxes. It is possible that the vanadate trigonal bipyramidal structure is a stable stereo-analogue of a pentacovalent transition state in the phosphoryl transfer reaction. In addition to the anhydride bond with the aspartyl residue, the vanadate enzyme complex is likely to be stabilized by conformational fit in the protein site, and acceptance of electrons by the vanadate d orbitals from neighboring oxygens. The resulting free energy well prevents cycling of free enzyme and Ca^{2+} fluxes. The vanadate reaction demonstrates that it is possible to affect the binding characteristics of the calcium binding sites by means other than ATP. The uniqueness of ATP however, resides in its kinetic as well as its thermodynamic adequacy.