3.5: Extracellular Calcium Ion-binding Proteins

The Ca^{2+} concentration in extracellular fluids is usually orders of magnitude higher than intracellular concentrations. In mammalian body fluids, the "free" Ca^{2+} concentration is estimated to be 1.25 mM (total Ca^{2+} is ~2.45 mM) with only minor variations.[140] We would thus expect that Ca^{2+} ions in extracellular fluids playa very different role from that inside cells. To ensure Ca^{2+} binding the macromolecular binding sites need have only a modest Ca^{2+} affinity ($K_B^{Ca2+} \approx 10^3$ to 10^4 M^{-1}), and since extracellular Ca^{2+} does not seem to have a signaling function, the rates of Ca^{2+} association or dissociation in protein-binding sites need not be very high.

One particularly important aspect of Ca^{2+} in mammals is its role in the blood coagulation system. Here we will meet a new type of amino acid, γ-carboxyglutamic acid ("Gla")-see Figure 3.29, that seems to have been designed by Nature as a Ca^{2+} ligand with rather special functions. Gla-containing proteins are also encountered in some mineralized tissues. The formation of bone, teeth, and other calcified hard structures is an intriguingly complicated phenomenon that will be dealt with in Section VII. We start, however, with a brief discussion of the role of Ca^{2+} in some extracellular enzymes.

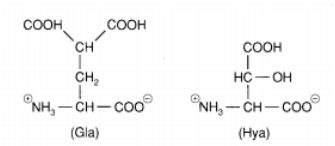

Figure 3.29 - Chemical structures of two novel amino acids believed to bind calcium in, e.g., blood-clotting proteins.

Ca^{2+}-binding in Some Extracellular Enzymes

Several extracellular enzymes have one or more Ca^{2+} ions as integral parts of their structure. In a very few of them the Ca^{2+} ion is bound at or near the active cleft, and appears necessary for maintaining the catalytic activity (phospholipase A_2 , α-amylase, nucleases), whereas other enzymes show catalytic activity even in the absence of Ca^{2+} (trypsin and other serine proteases). In the latter proteins, the Ca^{2+} ion is usually ascribed a "structural" role, although its function may be rather more related to "dynamics" and so be more subtle and complex.

Trypsin has one Ca^{2+}-binding site with four ligands (two side-chain and two backbone oxygens) donated by the protein (Glu-70, Asn-72 , Val-75, and Glu-80) and two ligating water molecules, making the site roughly octahedral.[141] The binding constant of Ca^{2+} to trypsin and its inactive precursor "proenzyme," *trypsinogen*, has been measured (see Table 3.2). The binding constant is slightly smaller for the precursor, as is also true for *chymotrypsin* and *chymotrypsinogen*.[142] The Ca^{2+} affinities of the serine proteases and their proenzymes are such that their Ca^{2+} sites will be largely occupied in extracellular fluids, but would be unoccupied inside a cell. It has been suggested that this phenomenon constitutes a safeguard against unwanted conversion of the proenzymes into the active enzymes as long as they still are inside the cells where they are synthesized.

The rates of Ca^{2+} dissociation of the above enzymes and proenzymes have been measured by ^{43}Ca NMR and stopped-flow techniques,[142] and are collected in Table 3.4. We note that the values of k_{on} and k_{off} are generally much smaller than in the intracellular regulatory EF-hand proteins discussed in Section VI. Whereas the latter have dynamic and equilibrium properties similar to those of flexible low-molecular- weight chelators such as EDTA and EGTA, the serine proteases are more similar to the more-rigid cryptates, such as the macrobicyclic amino cryptate [2.2.2] (see Tables 3.2 and 3.4).

As mentioned above, there are a few enzymes in which a Ca^{2+} ion is present in the active cleft and essential for activity. Pancreatic *phospholipase* A_2 ($M_r \approx 14$ kDa) is an enzyme of this type. The x-ray structure is known to high resolution, and a single Ca^{2+} ion is found to be surrounded by six ligands, four presented by the protein (Tyr-28, Glu-30, Glu-32, and Asp-49) and two water molecules.[143] A mechanism for the action of phospholipase A_2 has been proposed[144] and is shown in Figure 3.30.

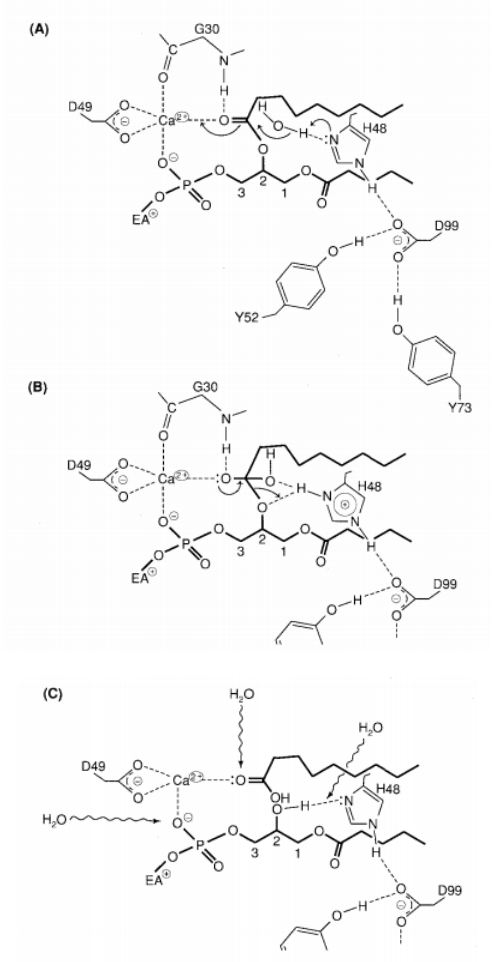

Figure kindly provided by P. B. Sigler.

This mechanism is based on three high-resolution x-ray crystal structures of phospholipase A_2 with and without transition-state analogues bound. The binding constant for Ca^{2+} together with the rate of dissociation found from variable-temperature ^{43}Ca NMR studies[145] can be used to calculate $k_{on} \approx 4 \times 10^6\ M^{-1}s^{-1}$, again lower than in EF-hand proteins. Recent ^1H NMR studies indicate that the global structure of the lipase is very much the same in the Ca^{2+}-free and the Ca^{2+}-bound forms. Structural changes upon Ca^{2+} binding appear primarily located in the region of the binding site.[112,146]

The mammary glands produce, among other substances, a Ca^{2+}-binding enzyme activator, *α-lactalbumin*, that has about 40 percent sequence identity with lysozyme. This protein, which is involved in the conversion of glucose into lactose, is secreted in large quantities, and in human milk constitutes some 15 percent of total protein. The Ca^{2+}-binding constant of bovine or human α-lactalbumin is on the order of $10^7\ M^{-1}$ under physiological conditions. In addition to Ca^{2+}, the enzyme also binds Zn^{2+}. It appears that Ca^{2+}-ion binding affects enzymatic activity, and somehow controls the secretion process, but the biological role of metal-ion binding to a-lactalbumin needs to be studied further. The x-ray structure of a-lactalbumin from baboon milk ($M_r \approx 15$ kDa) has been determined[147] to a high resolution (~1.7 Å). The Ca^{2+}-binding site has an interesting structure. The ion is surrounded by seven oxygen ligands, three from the carboxylate groups of aspartyl residues (82, 87, and 88), two carbonyl oxygens (79 and 84), and two water molecules. The spatial arrangement is that of a slightly distorted pentagonal bipyramid with the carbonyl oxygens at the apices, and the five ligands donated by the proteins are part of a tight "elbow"-like turn. The α-lactalbumin site has a superficial structural similarity to an "EF-hand," although the enzyme presumably has no evolutionary relationship with the intracellular Ca^{2+}-binding regulatory proteins.

Blood clotting proceeds in a complicated cascade of linked events involving many enzymes and proenzymes. About a decade ago it was shown that several of these proteins contained a previously unknown amino acid, γ-carboxyglutamic acid (Gla), and more recently yet another new amino acid, β-hydroxyaspartie acid (Hya), has been discovered (see Figure 3.29). The former is formed postribosomally by a vitamin-K-dependent process in the liver.[148] Presently the most-studied Gla protein in the blood-clotting system is *prothrombin* ($M_r \approx 66$ kDa). Ten Gla residues are clustered pairwise in the N-terminal region, essentially lining one edge

of the molecule, forming a highly negatively charged region.[149] A small (48 residues) proteolytic fragment (F1) that contains all ten Gla amino acids can be prepared. Prothrombin can bind about 10 Ca^{2+} ions, but F1 binds only 7. Binding studies to F1 show that the Ca^{2+} ions bind at three high-affinity cooperative sites and four noninteracting sites,[150] and that this binding takes places in conjunction with a spectroscopically detectable conformational change (see Table 3.1).

In the presence of Ca^{2+} ions, prothrombin and other vitamin-K-dependent proteins in the blood-coagulation system will bind to cell membranes containing acidic phospholipids, in particular, the platelet membrane, which is rich in phosphatidylserine. A proposed model for the prothrombin-membrane interaction is shown in Figure 3.31.

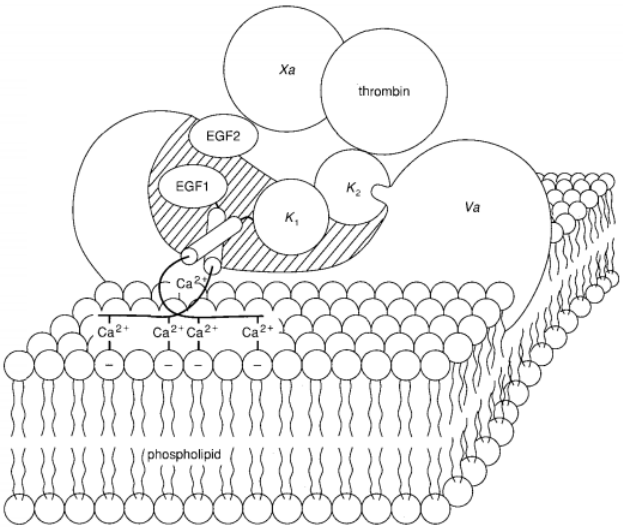

Figure kindly provided by C. C. F. Blake

It has long been known that calcium ions are involved in cell-to-cell and cell-to-extracellular matrix interactions, but the molecular details largely remain to be unraveled. In the late 1980s a large, adhesive, calcium-binding matrix glycoprotein (M_r ~ 420 kDa) named thrombospondin was characterized. This multifunctional adhesion molecule is composed of three polypeptide chains, each with 38 amino-acid-Iong repeats that are homologous with the calcium-binding helix-loop-helix sites of the calmodulin superfamily.[152] Each thrombospondin molecule is reported to bind 12 calcium ions with an affinity of about 10^4 M^{-1}, and the removal of calcium is accompanied by a conformational change.[153,154]