

# Calcium and its Protein Binding in Lactation in Mammary Gland and Blood Clotting

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**Abstract** -  $\text{Ca}^{2+}$  ions are central to a complex intracellular messenger system that is mediating a wide range of biological processes: muscle contraction, secretion, glycolysis and gluconeogenesis, ion transport, cell division and growth.  $\text{Ca}^{2+}$  ions are also known to play various roles outside cells.  $\text{Ca}^{2+}$  ions are instrumental in joining certain proteins in the blood-clotting system with membrane surfaces of circulating cells. An important class of transport proteins is the  $\text{Ca}^{2+}$ -ATPases, which are particularly abundant in muscle cells. In higher organisms, the  $\text{Ca}^{2+}$  concentration in extracellular fluids generally is considerably higher than the intracellular concentrations. The mammary glands produce, among other substances, a  $\text{Ca}^{2+}$ -binding enzyme activator,  *$\alpha$ -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.  $\text{Ca}^{2+}$  ions are involved in the cascade of enzymatic events that results in blood clotting in mammals.

**Keywords:** Calcium, Blood Clotting, Mammary Gland, Protein Binding, Lactation.

## I. DISCUSSION

Today it is widely recognized that  $\text{Ca}^{2+}$  ions are central to a complex intracellular messenger system that is mediating a wide range of biological processes: muscle contraction, secretion, glycolysis and gluconeogenesis, ion transport, cell division and growth. A prerequisite for the proper function of the calcium messenger system in higher organisms is that the cytosolic  $\text{Ca}^{2+}$  concentration in a "resting" cell be kept very low, on the order of 100 to 200 nM. Transitory increases in the  $\text{Ca}^{2+}$  concentration that may result from hormonal action on a membrane receptor must rapidly be reduced. Several transport proteins, driven either by ATP hydrolysis or by gradients of some other ion like  $\text{Na}^+$ , are involved in this activity.  $\text{Ca}^{2+}$  ions are also known to play various roles outside cells. In the blood plasma of mammals, in which the  $\text{Ca}^{2+}$  concentration exceeds the intracellular by a factor of about  $10^4$ ,  $\text{Ca}^{2+}$  ions are instrumental in joining certain proteins in the blood-clotting system with membrane surfaces of circulating cells. Many

extracellular enzymes also contain  $\text{Ca}^{2+}$  ions, sometimes at the active site but most often at other locations. It is generally believed that  $\text{Ca}^{2+}$  ions confer on proteins an increased thermal stability, and indeed proteins in heat-tolerant microorganisms often hold many such ions. Vertebrates require much calcium in their food; in the USA the recommended daily allowance (RDA) for adult humans is 800 mg, and most other countries have comparable recommendations. During gestation in mammals, calcium must be transported across the placenta into the fetus, in particular during those phases of pregnancy when bone formation is most rapid.

Concentrations of uncomplexed, or "free,"  $\text{Ca}^{2+}$  can be measured by  $\text{Ca}^{2+}$ -selective microelectrodes, bioluminescence and complexing agents with  $\text{Ca}^{2+}$ -dependent light absorption, fluorescence, or NMR spectra. Total  $\text{Ca}^{2+}$  concentrations, uncomplexed and complexed, can be measured by a variety of physical techniques. Some techniques, like atomic absorption, are sensitive but give poor spatial resolution. An important class of transport proteins is the  $\text{Ca}^{2+}$ -ATPases, which are particularly abundant in muscle cells. These proteins translocate  $\text{Ca}^{2+}$  ions against large activity (or concentration) gradients through the expenditure of ATP. Transport of  $\text{Ca}^{2+}$  ions against activity gradients across membranes may also be accomplished by coupled transport of other ions, like  $\text{Na}^+$ , with a gradient in the opposite direction. As a result of some external stimulus—the action of a hormone, for example—the "free"  $\text{Ca}^{2+}$ -ion concentrations in the cytoplasm of many cell types may transiently increase several orders of magnitude. This increase largely results from the release of  $\text{Ca}^{2+}$  from intracellular stores (Endoplasmic Reticulum, Sarcoplasmic Reticulum) in response to the initial formation of a new type of messenger, 1,4,5- $\text{IP}_3$ . The activity of  $\text{Ca}^{2+}$ -transport proteins eventually restores the  $\text{Ca}^{2+}$  concentration levels to resting levels. This sequence of events forms the basis for  $\text{Ca}^{2+}$ 's role in the regulation of a wide variety of cellular activities. In higher organisms, the  $\text{Ca}^{2+}$  concentration in extracellular fluids generally is considerably higher than the intracellular concentrations. In mammalian body fluids, the  $\text{Ca}^{2+}$  concentration is typically on the order of a few mM. The extracellular concentration levels are highly regulated and undergo only minor variations. A consequence of these high levels of  $\text{Ca}^{2+}$  in extracellular fluids is that the binding

constant need be only  $10^3$  to  $10^4$   $M^{-1}$  in order for a protein site to be highly occupied by  $Ca^{2+}$ . Several extracellular enzymes and enzyme activators have one or more  $Ca^{2+}$  ions as integral parts of their structures. Some  $Ca^{2+}$  ions are bound at, or near, the active cleft and may take part in the enzymatic reactions (e.g., phospholipase  $A_2$ ,  $\alpha$ -amylase). In other molecules, for example, serine proteases like trypsin and chymotrypsin, the  $Ca^{2+}$  ion is not essential for enzymatic activity, and may play more of a structural role. Calcium is, along with iron, silicon, and the alkaline earth metals, an important constituent of mineralized biological tissues. Some  $Ca^{2+}$ -based biominerals, like bone or mother-of-pearl, can be regarded as complex composites with microscopic crystallites embedded in a protein matrix. The formation of calcified biominerals is a highly regulated process, and human bone, for instance, is constantly being dissolved and rebuilt. When the rates of these two counteracting processes are not in balance, the result may be decalcification, or *osteoporosis*, which seriously reduces the strength of the bone. 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,  $\gamma$ -carboxyglutamic acid ("Gla") 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. Discussion of the role of  $Ca^{2+}$  in some extracellular enzymes. In humans, uptake occurs in the small intestine, and transport is regulated by a metabolite of vitamin D, calcitriol (1,25-dihydroxy vitamin  $D_3$ ). The uptake process is not without loss; roughly 50 percent of the calcium content in an average diet is not absorbed. To maintain homeostasis and keep the calcium level in blood plasma constant, excess  $Ca^{2+}$  is excreted through the kidney. The main factor controlling this phenomenon in vertebrates is the level of the parathyroid hormone that acts on kidney (increases  $Ca^{2+}$  resorption), on bone, and, indirectly, via stimulated production of calcitriol, on the intestinal tract (increases  $Ca^{2+}$  uptake). Calcium enters the cells from the outside world, i.e., the intestinal lumen, by traveling through the brush-border membrane of the intestinal **epithelial cells**, through the cytosolic interior of these cells, and into the body fluids through the **basal lateral membranes** of the same cells.

## II. CALCIUM ROLE IN LACTATION

The mammary glands produce, among other substances, a  $Ca^{2+}$ -binding enzyme activator,  *$\alpha$ -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  *$\alpha$ -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  *$\alpha$ -lactalbumin* needs to be studied further. The x-ray structure of  *$\alpha$ -lactalbumin* from baboon milk ( $M_r \approx 15$  kDa) has been determined to a high resolution ( $\sim 1.7$  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  *$\alpha$ -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.

## III. BLOOD CLOTTING

Blood clotting proceeds in a complicated form of linked events involving many enzymes and proenzymes. The several of these proteins contained a previously unknown amino acid,  $\gamma$ -carboxyglutamic acid (Gla), and more recently yet another new amino acid,  $\beta$ -hydroxyaspartic acid (Hya), has been discovered. The former is formed postribosomally by a vitamin-K-dependent process in the liver. 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. 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 and that this binding takes places in conjunction with a spectroscopically detectable conformational change.  $Ca^{2+}$  ions are involved in the cascade of enzymatic events that results in blood clotting in mammals. Several of the proteins in this system contain two new amino acids,  $\gamma$ -carboxyglutamic acid (Gla) and  $\beta$ -hydroxyaspartic acid (Hya), which are strongly suspected to be involved as ligands in  $Ca^{2+}$  binding. In the presence of  $Ca^{2+}$  ions, prothrombin and other Gla-containing proteins will bind to cell membranes containing acidic phospholipids, in particular, the platelet membrane. It appears likely that  $Ca^{2+}$  ions form a link between the protein and the membrane surface. 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.

#### IV. CONCLUSION

The mammary glands produce, a  $\text{Ca}^{2+}$ -binding enzyme activator,  $\alpha$ -lactalbumin, 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.  $\text{Ca}^{2+}$  ions are involved in the cascade of enzymatic events that results in blood clotting in mammals. Several of the proteins in this system contain two new amino acids,  $\gamma$ -carboxyglutamic acid (Gla) and  $\beta$ -hydroxyaspartic acid (Hya), which are strongly suspected to be involved as ligands in  $\text{Ca}^{2+}$  binding. In the presence of  $\text{Ca}^{2+}$  ions, prothrombin and other Gla-containing proteins will bind to cell membranes containing acidic phospholipids, in particular, the platelet membrane. Several extracellular enzymes and enzyme activators have one or more  $\text{Ca}^{2+}$  ions as integral parts of their structures.

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