CHAPTER II

BACKGROUND INFORMATION

Milk synthesis

Milk synthesis or lactogenesis in the mammary gland is the continuous process of synthesis and accumulation of milk both before and after calving. The synthesis of milk is the composite result of the many chemical reactions taking place in the secretory cell of this gland during lactation period.

Production of milk in this sense is dependent on the status of this cellular biosynthetic factory, that is on the smooth functioning of the interdependent chemical reactions of the synthetic pathways and their control mechanisms in the secretory cell. Milk synthesis involves millions of secretory cells, each one a separate factory but each which similar genetic plans, fabricating into milk the various precursors from the blood stream, such as the amino acid into milk proteins, blood sugar into milk sugar, fatty acid into milk fat and so force on a myriad of different pathways. (Linzell and Peaker, 1971). Milk is produced by mammary glands that having wide variation in the composition of milk. In the basic components, there are water, fat, protein, lactose and minerals.

Milk protein synthesis and secretion.

The mechanism of protein synthesis in the mammary secretory cell is the same as in other cells that amino acid derives from two sources. Some sources are synthesized de novo in the mammary gland and others are derived from the plasma (Linzell and Peaker, 1971).

In the mammary gland, protein synthesis consists of sequence of nucleotides which under appropriate conditions, It will serve as a template for the synthesis of ribonucleic acid (RNA) molecule. Each of the types of RNA synthesize messenger-RNA (mRNA), transfer-RNA (tRNA) and ribosomal-RNA (rRNA) has a specific role in directing the polymerization of amino acids which take place on ribosomes on endoplsmic reticulum in the cytoplasm. Protein leaves the polyribosome on the outer surface of the endoplasmic reticulum enters the intercistern space, and then it is transported to the golgi apparatus region where aggregation of the peptide chain into the casein micelles occurs prior to secretion. The vesicles containing protein move to the apex of the cell and then fuse with the apical plasmalemma and releasing their contents by reverse pinocytosis (Mepham, 1977). (Figure1)

The lactating mammary gland synthesizes and secretes large amounts of phosphoproteins that mainly are associated with the casein fraction of milk. The association characteristics of the different casein molecules are dependent on a number of the modifications which they undergo in golgi vesicle (Bingham and farrell, 1977). In addition, this area has other transportations of many constituents between inside the vesicle and the cytosol (Holt,1985). (Figure 2)

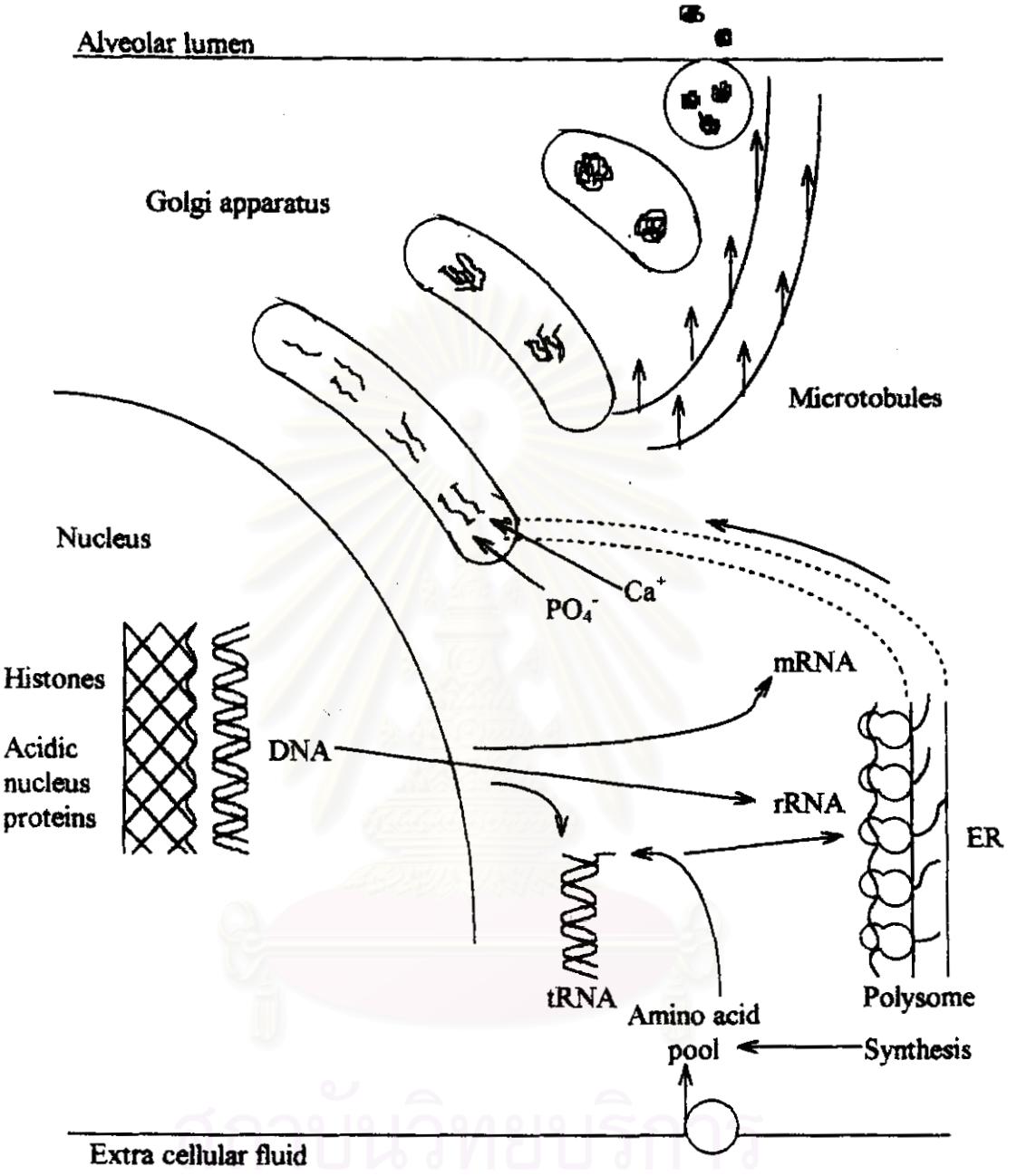


Figure 1. Protein synthesized on the endoplasmic reticulum conveyed to the golgi apparatus and aggregation to casein in micelle and release by reverse pinocytosis. (Mepham, 1977)

Physicochemical properties of casein

Milk protein is 3 to 5 percentage in total milk. The true principle types of milk proteins are casein and whey protein. The casein constituents are accounted for 76-86% of total milk protein (Hui, 1993 ; Varnum and Sutherland, 1994). Caseins present as a colloidal and whey present as solution in milk.

It is known that in manufacture of cheese or fermented dairy uses the action of rennet or acid coagulation of the casein in milk (Okigbo, et al, 1985 ; Fox,1989). Meanwhile other reports about milk process by prolonged heating at high temperature will definitely change the properties of the casein complex (Berge, 1988). Evaporated milk and sweetened evaporated milk (condensed milk) have been reporeted to depend on the behavior of the milk proteins, especially casein (White and Davies, 1960).

In addition casein is the main protein in testing quality or stability of milk. Horne (1987) reported that ethanol stability was due to the major milk protein family, the caseins existing in milk in an aggregated form. According to Davies and White (1958) showed that when equal volume of milk and aqueous ethanol solution were mixed, when it was sufficiently strong or the milk was not good quality, clots of proteins was apparent from mainly of denature caseins. However, whey proteins are not involved the determining the ethanol stability of milk (Horne and Parker ,1981c).

Casein is above 80 percentages of protein in bovine milk consist of mixture of four phosphoprotein as α_{s1}-casein, α_{s2}- casein, β- casein and κ- casein .

(Varnum and Sutherland, 1994; Hui, 1993). There are α_{s1}- casein , α_{s2}- casein , β- casein and κ- casein in the approximate ratio 40:10:35:12. (Davies and Law, 1980 ; Barry and Donnelly, 1980). Mostly casein is on the form of polydisperse distribution of colloidal particles, the casein micelle (Holt, Davies and Law, 1986). The casein micelle system of bovine milk is composed by protein aggregation of similar spherical shape by self assembly subunits (Slattery, 1976).

The caseins are globular proteins and have an amino acid content similar to that of other types, although cysteine is present, in small quantities, in only α_s- casein and κ- casein (Varnum and Sutherland, 1994). In the formation and structure of casein micelles are based on the most fundamental of biostructure formation as the highly specific sequences of protein-protein and protein-ion interactions (McKenzie, 1971).

In forming micelle of each submicelle or subunit uses hydrophobic force and many noncovalent interactions that can be classified as electrostatic forces and playing roles in micelle stability (Fox,1989). Electrostatic forces are the force between charge-charge, charge-dipole, dipole-dipole and also hydrogen bond (Bloomfield, 1979). In each properties of casein designated to α_{s1}-casein, α_{s2}-casein and β- casein are hydrophobic while κ-casein portion of the submicelle surface is hydrophilic (Slattery, 1976).

In formation of structure of casein micelles, there are α_s- casein and β-casein in core unit while κ- casein is outside to protective colloid (Brunner, 1981). By κ- casein has C-terminal region of the surface that to be hydrophilic and protude from micelle giving them a hairy appear and contribute to micelle

stabilizer (Fox,1989). Structure and forming micelle in milk protein is shown in Figure 3.

Phosphorus

Phosphorus is a macromineral in the plasma and an important mineral for many organs. Phosphorus as in the form phosphate is the major anion of intercellular fluids. Phosphates have the ability to combine reversebly with many coenzyme systems and also with multiple other compounds that are necessary for operation of the metabolism process. Many important reactions of phosphate have been due to the function of ATP,ADP, phosphocreatine and so forth (Guyton, 1991). There is phosphorus in both inorganic and organic form, the latter as a constituent of the lipids, in blood serum. Total serum phosphorus concentration under normal conditions in most species is 6 to 9 mg/dl.(Pon, et al.,1995).

Phosphorus from blood serve as building a material for milk protein synthesis. Inorganic phosphate from blood appeared in milk about 1.5 to 2 hours (Bingham and Farrell, 1977). Phosphorus is one component of protein in milk by phosphorylation into phosphoprotein ,casein micelle (Gangnair, et al. 1996; Rusmussen, et al. 1997) and to be some constituent forming in the structure of casein micelle (Fox,1989). In cow's milk, 20% of phosphorus is esterified to casein, a further 40% is presented as colloidal inorganic calcium phosphate, 30% occures as phosphate ion in solution and about 10% is associated with lipid fraction (Fox, 1985).

Effect of phosphorus on phosphorylation of casein

Phosphorylation is the process of introducing a phosphate group into an organic molecule. Phosphorylation of milk protein into phosphoprotein appear in the mammary gland. The lactating mammary gland synthesizes and secretes large amounts of phosphoprotein that mainly are associated with the casein fraction of milk. Inorganic phosphorus of blood serves as building materials for casein, and the final product appears in milk as a colloidal-sized particle, the casein micelle. This step is contributed by protein kinase from golgi apparatus that incorporation into casein using ATP as the phosphate donor (Bingham and Farrell, 1977).

All the caseins are phosphorylated but to variable extents. The phosphate is esterified to the polypeptides as monoesters of serine, rarely threonine. Phosphorus may attach to the hydroxyl oxygen of serine and threonine residues, the amino nitrogen of lysine residues or the imidazole nitrogen of histidine residues (Fox, 1989).

There is variable extents the phosphorylation of casein, α_{s1}- casein usually contains 8-9 moles P per mole protein, α_{s2}- casein consists 10-13 moles P per mole protein. β- casein consists of 5 moles P per mole protein and κ- casein consists of 1 mole P per mole protein (McKenzie, 1971; Fox, 1989). The contributions from the phosphorylated serine residues of κ- casein , locate in the C-terminal portion of the molecule, to the mobile constituents of the micelles.

Casein phosphorylation is important to milk both as a food and as a raw material for the dairy processing industry (Aoki and Kako, 1986). Through interactions with phosphorylated serine residues, colloidal calcium phosphate is regarded as fundamental for the integrity of the casein micelle (Rusmussen, et.al, 1997). The higher the number of phosphorylation residues is the stronger interaction strength with colloidal calcium phosphate in forming micelle (Gangnaire, et.al, 1996).

Effect of phosphorus on structure and stability of casein

The structure of casein micelle has inorganic phosphorus in the part as the bridge between submicelle to maintain the structure. By the phosphorus is a main inorganic constituent of casein micelle that combine with calcium as colloidal calcium phosphate (Payen, 1979 ; Aoki and Kako, 1986).

There are many reports about inorganic phosphorus on micelle structure and its stability. These have the inconsistent of inorganic phosphorus on micelles and stability of them. Horne and Parker (1981a) found that when inorganic phosphorus 2mM and 5mM were added into milk without any effect on milk stability. This may lead to more calcium phosphate being precipitated and destabilization of micelle.

Horne and Parker (1981b) found in the same way that when inorganic phosphorus 5mM was added into milk and it had no effect on milk stability. This report has inclusion of phosphate into a milk previously destabilized by the addition

of calcium made the milk more stable to EtOH induced coagulation. It did not restore the stability to that of the original milk. Meanwhile, Horne (1987) found that when micelles were diluted into non- phosphate buffer for reducing phosphate concentration and when the phosphate was removed from milk by dialysis then it should increase micelle stability.

McMahon and Brown (1984) found that the precipitation occured at higher phosphate was due to insufficient casein to maintain stability of colloidal.

Inorganic phosphorus shows another effect on micelle structure and its stability. The unstable milk to EtOH induced coagulation has been shown to contain less soluble inorganic phosphorus than that of milk more stable to EtOH induced coagulation (Davies and Law, 1958). The presence of inorganic phosphorus causes formation and growth casein polymer to submicelle size and then promotes micelles formation (Slattery, 1979).

Meanwhile, Abbassy and Wahba (1986) found that the addition of phosphte increased alcohol test value or EtOH induced coagulation and rennet coagulation which these were promotion of inorganic phosphorus to the stability of milk.

However, there is evidence that inorganic phosphorus is not involve stability and maintain structure of micelle. Holt, Davies and Law (1986) found that when removed inorganic phosphorus by dialysis against phosphate free buffer to lower 30 percentage from original milk did not effect to dissociation of the casein micelle.

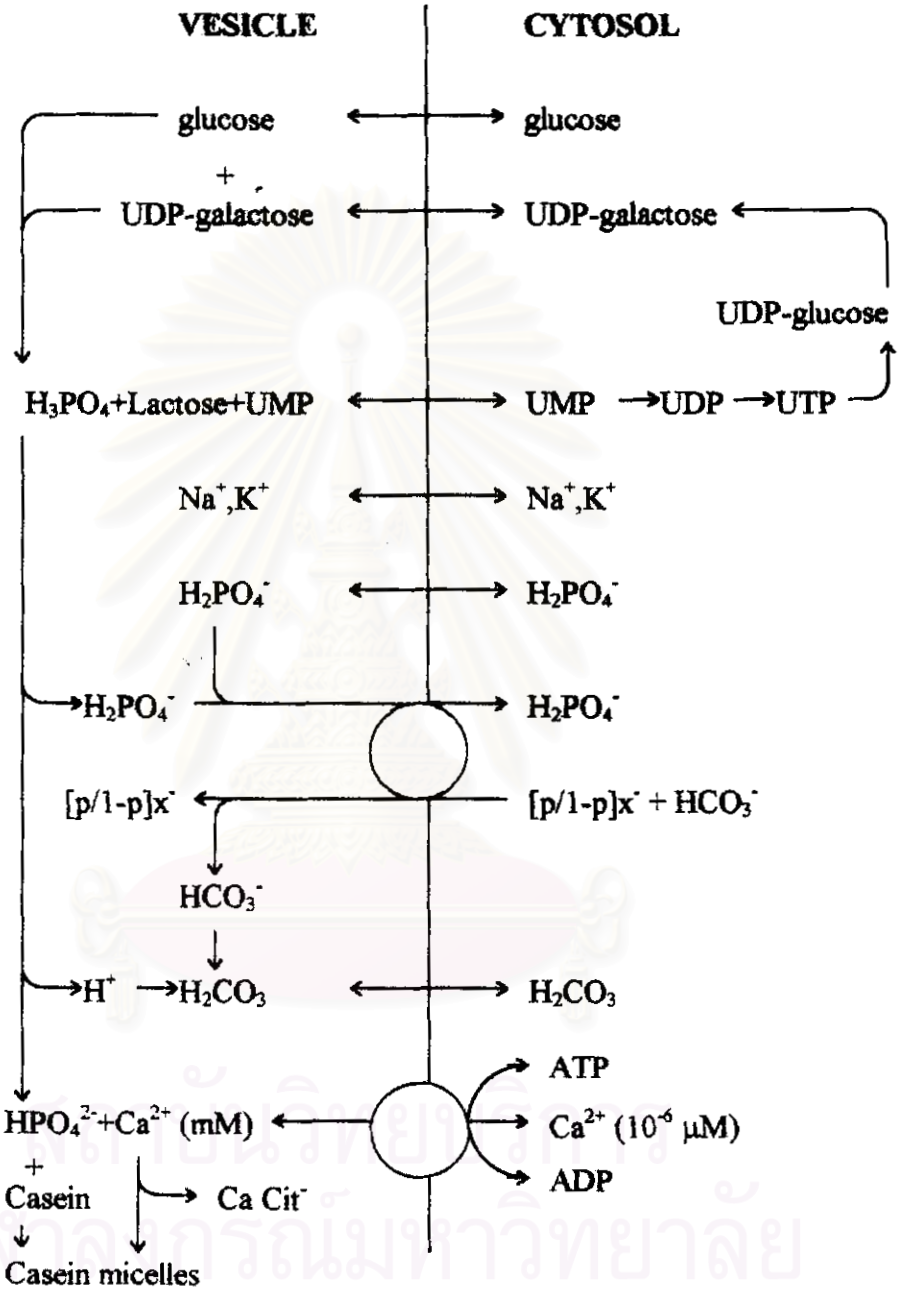
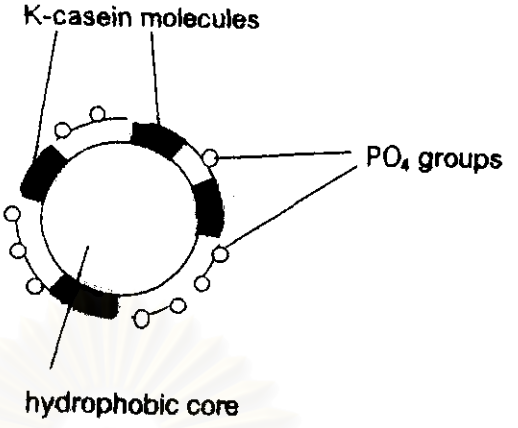


Figure 2.Transport mechanism for milk salt and other components between the cytosol of secretory cell and the inside of golgi vesicle. (Holt,1985)

(A)



(B)

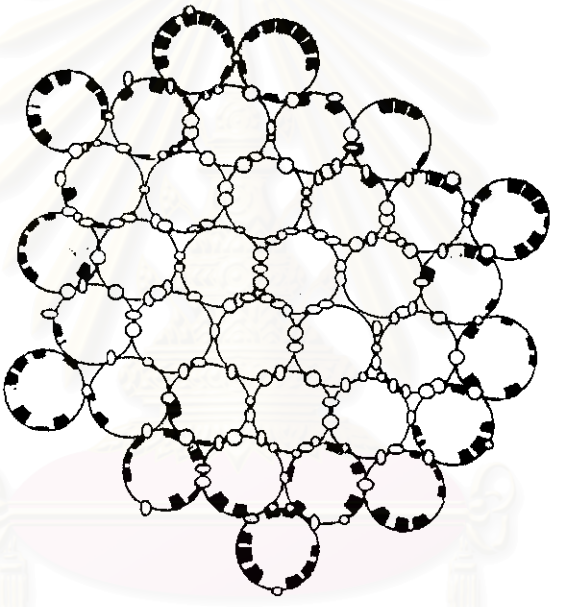


Figure 3. Schematic representation of a sub micelle (A) and a casein micelle, composed of submicelle in a spherical form (B). (Fox,1989)