

# Casein Kinase from the Golgi Apparatus of Lactating Mammary Gland

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## SUMMARY

A casein kinase that catalyzes the phosphorylation of dephosphorylated  $\alpha_{s1}$ -casein by ATP has been found in the Golgi apparatus of lactating rat mammary gland. Dephosphorylated  $\beta$ - and  $\kappa$ -caseins are also phosphorylated by this enzyme, while other milk proteins ( $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, native  $\alpha_{s1}$ -,  $\beta$ -, and  $\kappa$ -casein, and proteins of the fat globule membrane) are phosphorylated to a limited extent. Histones, phosvitin, and lysozyme are not appreciably phosphorylated. The optimum pH for phosphate incorporation into dephosphorylated casein is 7.6. The  $K_m$  for dephosphorylated  $\alpha_{s1}$ -casein is 12  $\mu$ M (.27 mg per ml), whereas the  $K_m$  for ATP is 80  $\mu$ M. The casein kinase requires a divalent cation for maximum activity; both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  can satisfy this requirement. Adenosine 3':5'-monophosphate (cyclic AMP) has no effect on casein kinase activity. The protein inhibitor from rabbit skeletal muscle, which inhibits adenosine 3':5'-monophosphate-dependent protein kinases, is also without effect. These findings suggest that phosphorylation of casein, a food protein, is quite different from the phosphorylation of cellular enzymes, which requires cyclic AMP, is inhibited by  $\text{Ca}^{2+}$  and is involved in control mechanisms.

Although phosphorylation of proteins has been studied in many species and tissues, very little is known about the phosphorylation of casein, a group of phosphoproteins secreted in large amounts by the lactating mammary gland. Through sequence analysis (1), it is known that  $\alpha_{s1}$ -casein, the major protein of cow's milk, has eight phosphate groups attached to specific serine residues and 8 additional serine residues that are not phosphorylated. Because this protein has been well characterized, it is an appropriate choice for studies on the phosphorylation of casein.

Gaye *et al.* (2) showed that the biosynthesis of casein occurs on the polyribosomes of the endoplasmic reticulum and in this respect resembles the biosynthesis of other proteins. Turkington and Topper (3) provided evidence that the synthesis of the polypeptide chain of casein occurs prior to phosphorylation and that phosphorylation of casein takes place by utilizing a pool of unphosphorylated casein. Bingham *et al.* (4, 5) tested this theory by using dephosphorylated  $\alpha_{s1}$ -casein as a substrate.

They examined subcellular fractions from the lactating mammary glands of rats seeking to find an enzyme with a preference for dephosphorylated casein over native casein. An enzyme from Golgi apparatus appeared to have the required characteristics. These preliminary studies have been extended to better characterize the casein kinase from Golgi membranes.

## EXPERIMENTAL PROCEDURES

**Materials**—The following proteins were isolated from cow's milk of known genetic composition.  $\alpha_{s1}$ -Casein B and dephosphorylated  $\alpha_{s1}$ -casein B were prepared as described by Bingham *et al.* (6).  $\beta$ -Casein A<sup>1</sup> was a gift from Dr. R. F. Peterson (Eastern Regional Research Center).  $\kappa$ -Casein B was isolated by the method of Zittle and Custer (7).  $\beta$ -Casein and  $\kappa$ -casein were dephosphorylated by the same procedure used for  $\alpha_{s1}$ -casein.  $\beta$ -Lactoglobulin A and  $\alpha$ -lactalbumin were prepared by the method of Aschaffenburg and Drewry (8). The fat globule membrane protein was a gift of Dr. E. B. Kalan (Eastern Regional Research Center).

Calf thymus histone, arginine- and lysine-rich histones and cyclic AMP<sup>1</sup> were purchased from Sigma.<sup>2</sup> Phosvitin was obtained from Schwarz-Mann and lysozyme was obtained from Difco. [ $\gamma$ -<sup>32</sup>P]ATP was obtained from Amersham-Searle. The protein inhibitor from rabbit skeletal muscle (9), which inhibits cyclic AMP-dependent protein kinases, was a gift of Dr. Donald A. Walsh (University of California, Davis).

The protein kinase of rabbit skeletal muscle was partially purified by the procedure of Walsh *et al.* (10) through the first DEAE-cellulose chromatography step.

**Isolation of Golgi Apparatus Fraction**—Golgi membranes from lactating mammary glands were prepared by the procedure of Keenan *et al.* (11). Sprague-Dawley rats, 10 to 15 days postpartum, were killed by exsanguination following ether inhalation. Mammary glands from three rats were excised, chilled, and minced. The cold minced tissue (20 g) was suspended in 60 ml of 0.5 M sucrose containing 37.5 mM Tris-maleate buffer (pH 6.5), 1 mM EDTA, and 1% dextran (homogenization buffer). All operations were performed at 0–4°. Following homogenization for 1 min at medium speed with a Polytron 10 ST homogenizer (Brinkman Instruments, Westbury, N.Y.), the homogenate was squeezed in rapid succession through two, four, and six layers of cheesecloth. The filtrate was centrifuged for 15 min at 4000  $\times g$  in a Sorvall HB-4 rotor. The floating lipid and supernatant were removed by aspiration and discarded. The loose, upper one-third of the pellet was removed, suspended in 10 ml of the homogeniza-

<sup>1</sup> The abbreviations used are: cyclic AMP, cyclic adenosine 3':5'-monophosphate; MES, 2-(*N*-morpholino)ethanesulfonic acid; EGTA, ethylene glycol bis( $\beta$ -aminoethyl ether)-*N,N'*-tetraacetic acid.

<sup>2</sup> Reference to brand or firm name does not constitute endorsement by the United States Department of Agriculture over others of a similar nature not mentioned.

tion buffer and resuspended with one stroke in a glass-Teflon homogenizer. The suspension was layered over 1.5 volumes of homogenization buffer containing 1.25 M sucrose and centrifuged for 30 min at  $100,000 \times g$  in a Beckman SW 39 rotor. The material at the interface of the two sucrose solutions was removed, diluted with the 0.5 M sucrose homogenization medium and centrifuged at  $4000 \times g$  for 15 min in a Beckman SW 39 rotor to obtain the Golgi membranes as a pellet. The pelleting procedure was repeated twice. A sample of the pellet was then examined with the electron microscope (Fig. 1).

For enzyme studies, the pellet containing the Golgi membranes was suspended in approximately 0.4 ml of 37.5 mM Tris-maleate buffer (pH 6.5) containing 1 mM EDTA and was diluted with an equal volume of 1% Triton X-100. The extract was sonicated for 30 s at 3 ma using a Branson Sonifier and centrifuged at  $50,000 \times g$  for 30 min in a Beckman SW 56 rotor. The supernatant solution, containing the casein kinase, was stored at  $-20^\circ$  and retained full activity for 2 months. The casein kinase preparation contained 1 to 5 mg of protein from 20 g of mammary gland tissue.

**Enzyme Assays**—Casein kinase was measured at pH 7.6 in a 100- $\mu$ l reaction mixture, containing 10  $\mu$ moles of Tris-HCl buffer (pH 7.6), 0.8  $\mu$ mole of  $MgCl_2$ , 0.3 mg of dephosphorylated  $\alpha_{s1}$ -casein, 5  $\mu$ l of enzyme solution, and 0.02  $\mu$ mole of ATP having  $4 \times 10^6$  cpm of  $[\gamma\text{-}^{32}P]\text{ATP}$ . The reaction was started by the addition of ATP. After incubation for 20 min at  $30^\circ$ , a 50- $\mu$ l aliquot of reaction mixture was pipetted onto squares ( $2 \times 2$  cm) of Whatman No. 31ET chromatography paper. The papers were washed according to the procedure described by Reimann *et al.* (12), transferred to scintillation vials containing 15 ml of Aquasol (New England Nuclear), and counted. The endogenous protein kinase activity was measured in the absence of casein and this value was subtracted from the value obtained in the presence of casein to determine the amount of phosphate incorporated. When the enzyme was omitted, the amount of phosphate incorporated into casein was insignificant. A unit of protein kinase activity is defined as the amount of enzyme which catalyzes the incorporation into protein of 1 pmole of phosphate per 20 min.

For rabbit muscle protein kinase, the assay mixture described by Reimann *et al.* (12) was used with minor modifications. The protein kinase was measured at pH 6.0 in a 100- $\mu$ l reaction mixture containing the following: 5  $\mu$ moles of glycerol-P, 2  $\mu$ moles of NaF, 0.03  $\mu$ mole of EGTA, 0.2  $\mu$ mole of theophylline, 0.3 mg per ml of casein (vitamin-free casein from Nutritional Biochemicals), 1  $\mu$ mole of  $MgCl_2$ , 0.2 nmole of cyclic AMP, 5  $\mu$ l of rabbit muscle protein kinase, and 0.02  $\mu$ mole of ATP having  $4 \times 10^6$  cpm of  $[\gamma\text{-}^{32}P]\text{ATP}$ .

**Protein Concentration**—Protein was estimated according to Lowry *et al.* (13) with bovine serum albumin as the standard.

## RESULTS

Fig. 1 shows the appearance of Golgi membranes as viewed with the electron microscope. On the basis of morphological analyses, 80 to 90% of the fraction consists of material derived from the Golgi apparatus. Enzyme assays, previously reported, showed that the Golgi membranes have a high specific activity for lactose synthetase and casein kinase (4). Lactose synthetase has been shown to be a marker enzyme for Golgi membranes from lactating mammary glands (14).

The general characteristics of the casein kinase reaction are depicted in Table I.  $Mg^{2+}$  is required for full activity, while  $Na^+$  has very little effect. Sulfhydryl reagents, such as  $\beta$ -mercaptoethanol and dithiothreitol, have little effect. Cyclic AMP does not stimulate the casein kinase activity, which confirms previous results (4), indicating that this enzyme is not a cyclic AMP-dependent protein kinase.

**Time Course**—When dephosphorylated  $\alpha_{s1}$ -casein is used as a substrate for the casein kinase, reaction rates are linear for 25 min (Fig. 2). When casein is omitted from the reaction mixture, a small but measurable amount of phosphate is incorporated into the Golgi apparatus preparation (Fig. 2), indicating endogenous phosphorylation.

**Enzyme Concentration**—The effect of enzyme concentration on

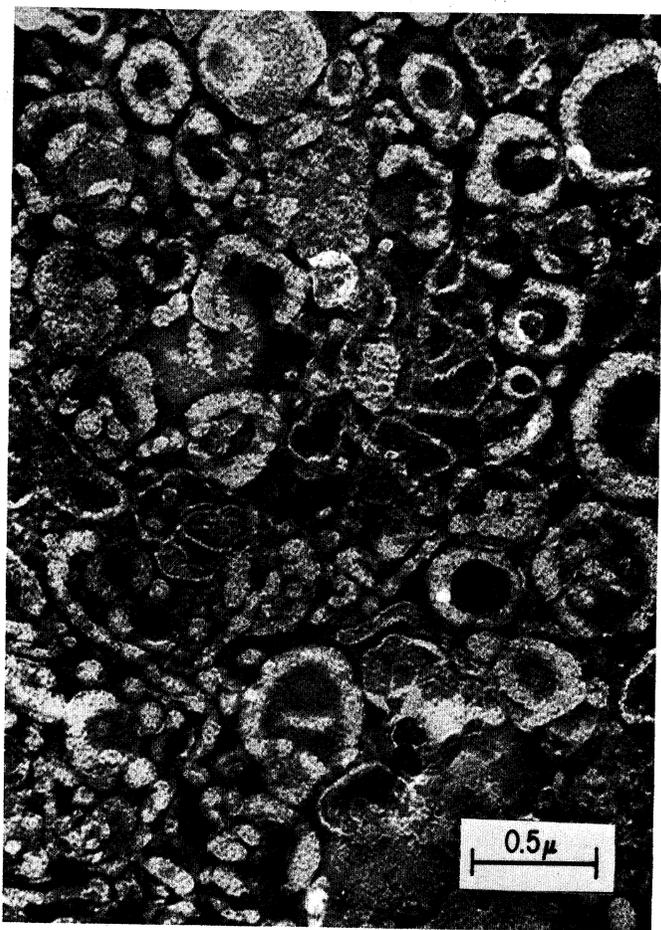


FIG. 1. Golgi apparatus fraction, prepared as described under "Experimental Procedures." The pellets were suspended in water and diluted with an equal volume of 1% phosphotungstic acid (pH 7.0) on carbon-coated grids. Micrographs were taken with an RCA EMU 3-G microscope using a 50- $\mu$ m aperture at 100-kv acceleration voltage.

TABLE I

General properties of casein kinase from Golgi apparatus extract of lactating mammary gland

Incubation conditions were as described under "Experimental Procedures."

Reaction mixture	Relative activity
	%
Complete.....	100
- $MgCl_2$ .....	1
- $MgCl_2$ + NaCl (7.5 mM).....	4
- $MgCl_2$ + NaCl (30 mM).....	5
+ cyclic AMP ( $10^{-6}$ M).....	100
+ cyclic AMP ( $10^{-5}$ M).....	105
+ cyclic AMP ( $10^{-4}$ M).....	94
+ cyclic AMP ( $10^{-3}$ M).....	67
+ $\beta$ -mercaptoethanol (10 mM).....	103
+ dithiothreitol (1 mM).....	84

the rate of casein phosphorylation is shown in Fig. 3. The reaction rate is linear up to 130  $\mu$ g of enzyme protein per 100  $\mu$ l of reaction mixture.

**pH Optimum**—Fig. 4 illustrates the effect of pH on phosphate incorporation into dephosphorylated casein, using 50 mM buffers (Tris and MES). Maximum activity was observed at pH 7.6.

**Apparent  $K_m$  for Casein**—The effect of varying casein concentration on enzyme activity is shown in Fig. 5. The concentration of dephosphorylated  $\alpha_{s1}$ -casein needed for half-maximum activity is 0.27 mg per ml (12  $\mu$ M) and was calculated from the double reciprocal plot.

**Apparent  $K_m$  for ATP**—The effect of ATP concentration on the incorporation of phosphate into dephosphorylated casein is depicted in Fig. 6. The  $K_m$  value for ATP is 80  $\mu$ M, which was evaluated from the double reciprocal plot.

**Effect of Divalent Metal Ions**—There is an absolute requirement for divalent cations as shown in Table I. At 2 mM concentration this requirement can be satisfied by four cations,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Ca}^{2+}$  (Table II). At a higher cation concentration (10 mM) only  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  are effective. The activities in the presence of several cations at a concentration of 10 mM were not reported because dephosphorylated  $\alpha_{s1}$ -casein precipitated in the

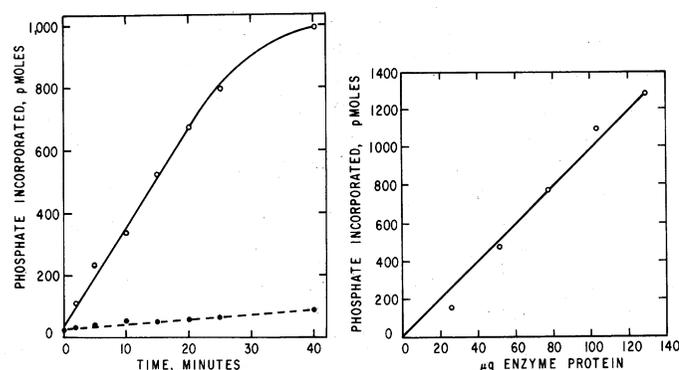


FIG. 2 (left). Effect of incubation time on phosphate incorporation into dephosphorylated  $\alpha_{s1}$ -casein. Casein kinase assays were performed as described under "Experimental Procedures." Samples were removed at the times indicated and tested for phosphate incorporation. The experiment was performed with dephosphorylated casein (O—O) and without dephosphorylated casein (●—●) in the reaction mixture, using 15  $\mu$ g of enzyme protein per 100  $\mu$ l of reaction mixture.

FIG. 3 (right). Effect of casein kinase concentration on the rate of incorporation of phosphate into dephosphorylated  $\alpha_{s1}$ -casein. The enzyme was assayed by the method described under "Experimental Procedures," except that the amount of casein kinase was varied.

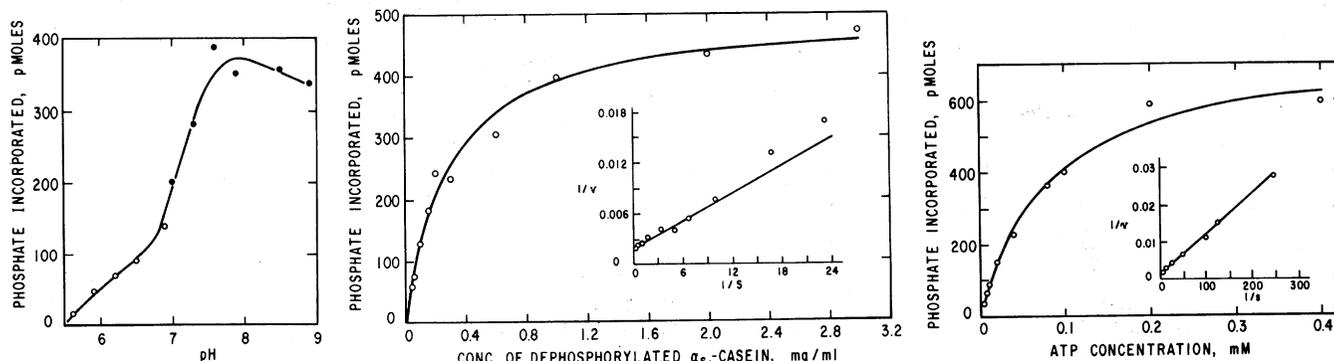


FIG. 4 (left). Effect of pH on casein kinase activity. Assay conditions are described under "Experimental Procedures." Each assay contained 10  $\mu$ g of enzyme. Buffers were 50 mM MES (O—O) and 50 mM Tris (●—●).

FIG. 5 (center). Effect of the concentration of dephosphorylated  $\alpha_{s1}$ -casein on phosphate incorporation into casein. Each assay contained 15  $\mu$ g of enzyme per 100  $\mu$ l of reaction mixture. Casein kinase activity was assayed by the method described under "Experimental Procedures," except that the concentration of dephosphorylated casein was varied. Inset shows a double reciprocal

presence of these metals. The cations causing precipitation at the higher concentrations were  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$ .

**Effect of Varying Calcium and Magnesium**—The fact that calcium ions as well as magnesium ions can activate the casein kinase prompted us to examine this effect in more detail. The effect of cation concentration on the incorporation of phosphate into dephosphorylated  $\alpha_{s1}$ -casein is shown in Fig. 7. Although the curves for calcium and for magnesium differ slightly, it would be difficult to interpret the difference at this time. It is significant that both cations ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) activate the casein kinase.

**Effect of Protein Inhibitor from Rabbit Skeletal Muscle**—As shown previously, the protein kinase inhibitor is very effective when used with the cyclic AMP-dependent protein kinase of skeletal muscle (9). At four times the concentration, which completely inhibits the skeletal muscle enzyme, the inhibitor has little effect on the casein kinase from the Golgi membranes (Table III).

**Substrate Specificity**—The ability of the casein kinase to phosphorylate different protein substrates with ATP as the phosphate donor was tested (Table IV). It is interesting to note that the

TABLE II

*Effects of divalent cations on protein kinase activity*

Incubation conditions were as described under "Experimental Procedures," except that  $\text{Mg}^{2+}$  was omitted and was replaced with the cations listed in the table. Each assay contained 15  $\mu$ g of protein kinase.

Divalent cation	Enzyme activity	
	2 mM cation	10 mM cation
	<i>units</i>	
$\text{Mg}^{2+}$ .....	283	808
$\text{Ca}^{2+}$ .....	218	846
$\text{Co}^{2+}$ .....	566	
$\text{Mn}^{2+}$ .....	422	
$\text{Cu}^{2+}$ .....	37	
$\text{Ba}^{2+}$ .....	35	32
$\text{Sr}^{2+}$ .....	42	25
$\text{Zn}^{2+}$ .....	49	

plot of the data. The line was drawn from a least squares analyses of the data, using the weighting methods of Wilkinson (15).

FIG. 6 (right). Effect of ATP concentration on phosphate incorporation into dephosphorylated  $\alpha_{s1}$ -casein. Each assay contained 15  $\mu$ g of casein kinase. Assay conditions are similar to those described under "Experimental Procedures," except that the concentration of ATP was varied. Inset shows a double reciprocal plot of the data. The slope and the intercept was computed according to the method described in Fig. 5.

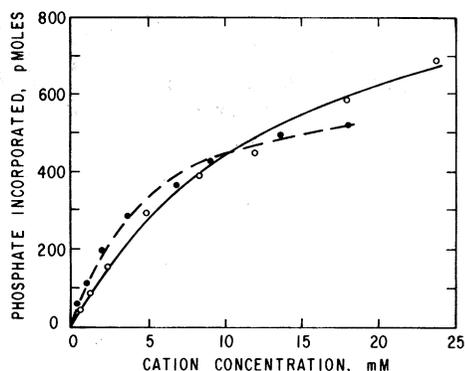


Fig. 7. Effect of cation concentration ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) on casein kinase activity. Enzyme,  $10 \mu\text{g}$ , was used in each  $100\text{-}\mu\text{l}$  reaction mixture. The enzyme activity was measured by the procedure described under "Experimental Procedures," except that the cation concentration was varied. Phosphate incorporation was measured in the presence of  $\text{Mg}^{2+}$  ( $\bullet\text{---}\bullet$ ) and in the presence of  $\text{Ca}^{2+}$  ( $\circ\text{---}\circ$ ).

TABLE III

Effect of rabbit muscle protein inhibitor on protein kinase of rabbit muscle and on casein kinase of Golgi membranes

The assays of rabbit muscle protein kinase and Golgi apparatus casein kinase are described under "Experimental Procedures."

Inhibitor added	Relative activity	
	Rabbit muscle	Golgi
$\mu\text{g}$		
0	100	100
1.75	0.4	
3.5	0.0	104
7.0		93

TABLE IV

Phosphorylation of proteins by casein kinase

Each assay contained  $15 \mu\text{g}$  of enzyme. Incubation conditions were as described under "Experimental Procedures," except that the dephosphorylated  $\alpha_{s1}$ -casein was replaced with a variety of protein substrates.

Protein substrate	Enzyme activity
	<i>units</i>
$\alpha_{s1}$ -Casein	129
Dephosphorylated $\alpha_{s1}$ -casein	645
$\beta$ -Casein	38
Dephosphorylated $\beta$ -casein	486
$\kappa$ -Casein	19
Dephosphorylated $\kappa$ -casein	128
$\beta$ -Lactoglobulin	31
$\alpha$ -Lactalbumin	17
Fat globule membrane proteins	92
Histone	5
Histone-arginine rich	1
Histone-lysine rich	1
Phosvitin	4
Lysozyme	0

dephosphorylated caseins were the best substrates and were phosphorylated at much higher rates than the phosphorylated native caseins. The relative activity of the enzyme with both

caseins and dephosphorylated caseins seemed to vary with the phosphate content. Native  $\alpha_{s1}$ -casein has eight phosphate groups,  $\beta$ -casein has five phosphate groups, and  $\kappa$ -casein, one phosphate group. Whether this correlation is significant cannot be said at this time. Other milk proteins ( $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, and the fat globule membrane) are phosphorylated to a limited extent. Histones, lysozyme, and phosvitin are poor substrates.

#### DISCUSSION

Since Walsh *et al.* (10) isolated a cyclic AMP-dependent protein kinase from rabbit skeletal muscle, work in this area has progressed rapidly. Because of the widespread distribution of this enzyme in various tissues and species, it seemed relevant to examine the casein kinase of lactating mammary gland and compare its properties to cyclic AMP-dependent protein kinases.

The casein kinase from the Golgi apparatus of lactating mammary glands catalyzes phosphate incorporation into dephosphorylated casein using ATP as a phosphate donor in the presence of divalent cations.  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ca}^{2+}$  can stimulate the casein kinase at a concentration of 2 mM, whereas only  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  are effective when the concentration is raised to 10 mM due to the precipitation of the dephosphorylated casein in the presence of  $\text{Co}^{2+}$  and  $\text{Mn}^{2+}$ . It is significant that  $\text{Ca}^{2+}$  activates the enzyme as well as  $\text{Mg}^{2+}$ . Kuo *et al.* (16) examined cyclic AMP-dependent protein kinases from 15 bovine tissues and showed that in the presence of  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ , or  $\text{Co}^{2+}$  enzyme activity was stimulated, whereas  $\text{Ca}^{2+}$  inhibited the activity. The inhibition of cyclic AMP-dependent protein kinases by  $\text{Ca}^{2+}$  has been confirmed by others (17, 18). Thus, the activation of casein kinase by  $\text{Ca}^{2+}$  suggests that this enzyme has unusual properties.

The mammary gland casein kinase was not affected by cyclic AMP. However, by this criteria alone, one cannot conclude that the enzyme is not a cyclic AMP-dependent kinase. Tao (19) has pointed out that the degree of stimulation by cyclic AMP seems to depend on the phosphoryl acceptor used and that stimulation can be low in the presence of casein, phosvitin, and protamine. He showed that protamine alone without cyclic AMP can react with the regulatory subunit, thereby releasing the catalytic subunit. Thus, it could be suggested that dephosphorylated casein reacts with the casein kinase to release the catalytic subunit or that casein kinase is a catalytic subunit devoid of its regulatory subunit. However, if the casein kinase were a catalytic subunit of a holoenzyme which is normally cyclic AMP-dependent, then the protein inhibitor from rabbit skeletal muscle would probably inhibit its activity (9, 20), but the inhibitor has no effect. Therefore, it could be suggested that casein kinase is not activated by cyclic AMP nor is it similar to the catalytic subunit of cyclic AMP-dependent protein kinases.

The substrate requirements of the casein kinase are unusual. Dephosphorylated caseins are preferred. In fact the activity increases dramatically when the caseins ( $\alpha_{s1}$ -,  $\beta$ -, and  $\kappa$ -) are dephosphorylated. There is a small amount of activity with other milk proteins as substrates, *viz.*  $\beta$ -lactoglobulin, fat globule membrane protein and  $\alpha$ -lactalbumin, all unphosphorylated. Although casein kinase was isolated on the basis of its activity toward dephosphorylated  $\alpha_{s1}$ -casein, it is surprising that it is so specific in its substrate requirements. Other mammary gland protein kinases have different properties. Majumder and Turkington (21) isolated two protein kinases from the cytosol of rat mammary gland, which showed a high specificity for histone,

one of which was activated by cyclic AMP. Waddy and MacKinlay (22) found similar results using bovine mammary glands.

Our results indicate that the Golgi apparatus casein kinase differs from cyclic AMP-dependent protein kinases in several of its properties. There could be a reasonable explanation for these differences. Many of the cyclic AMP-dependent protein kinases are involved in control mechanisms mediated through hormones. These kinases phosphorylate enzymes, such as phosphorylase *b* kinase (23), glycogen synthetase (24), lipase from adipose tissue (25); as well as proteins, such as histones (26, 27). These phosphorylations are often countered by the action of specific phosphatases. On the other hand, proteins such as casein and phosvitin possess a storage function for phosphorus in milk and in egg yolk. It therefore seems likely that these proteins are phosphorylated by a different mechanism that does not require cyclic AMP-dependent protein kinases. Recently, Goldstein and Hasty (28) characterized the phosvitin kinase from the livers of roosters treated with estrogen. Although this enzyme differs from casein kinase in several of its properties the phosvitin kinase, like casein kinase, is not activated by cyclic AMP.

The biological significance of the casein kinase remains to be fully elucidated. From the evidence available, casein synthesis in the mammary gland proceeds by a mechanism similar to protein synthesis in other tissues (2, 29). After completion of the synthesis of the polypeptide chain, phosphorylation of casein takes place, utilizing a pool of unphosphorylated casein (3). Our results provide evidence that the polypeptide chain of casein is phosphorylated by the Golgi apparatus enzyme. The phosphorylated casein monomers are formed into colloidal complexes (micelles), containing up to 30,000 monomers, held together by calcium bonds (30) and the casein micelles are secreted by the Golgi vacuoles (31). In view of the role of  $\text{Ca}^{2+}$  in micelle formation it is significant that  $\text{Ca}^{2+}$  is not inhibitory to the casein kinase as both casein kinase and micelle formation require  $\text{Ca}^{2+}$ . Thus, our results seem to corroborate current views on the biosynthesis of casein.

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#### REFERENCES

- MERCIER, J.-C., GROSCLAUDE, F., AND RIBADEAU-DUMAS, B. (1971) *Eur. J. Biochem.* **23**, 41-51
- GAYE, P., HOUEBINE, L., AND DENAMUR, R. (1973) *Biochem. Biophys. Res. Commun.* **51**, 637-644
- TURKINGTON, R. W., AND TOPPER, Y. J. (1966) *Biochim. Biophys. Acta* **127**, 366-372
- BINGHAM, E. W., FARRELL, H. M., JR., AND BASCH, J. J. (1972) *J. Biol. Chem.* **247**, 8193-8194
- BINGHAM, E. W., FARRELL, H. M., JR., AND CARROLL, R. J. (1973) *Fed. Proc.* **32**, 2425
- BINGHAM, E. W., FARRELL, H. M., JR., AND CARROLL, R. J. (1972) *Biochemistry* **11**, 2450-2454
- ZITTLE, C. A., AND CUSTER, J. H. (1963) *J. Dairy Sci.* **46**, 1183-1188
- ASCHAFFENBURG, R., AND DREWRY, J. (1957) *Biochem. J.* **65**, 273-277
- ASHBY, C. D., AND WALSH, D. A. (1972) *J. Biol. Chem.* **248**, 1255-1261
- WALSH, D. A., PERKINS, J. P., AND KREBS, E. G. (1968) *J. Biol. Chem.* **243**, 3763-3765
- KEENAN, T. W., HUANG, C. M., AND MORRÉ, D. J. (1972) *J. Dairy Sci.* **55**, 1577-1585
- REIMANN, E. M., WALSH, D. A., AND KREBS, E. G. (1971) *J. Biol. Chem.* **246**, 1986-1995
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- KEENAN, T. W., MORRÉ, D. J., AND CHEETHAM, R. D. (1970) *Nature* **228**, 1105-1106
- WILKINSON, G. N. (1961) *Biochem. J.* **80**, 324-332
- KUO, J. F., KRUEGER, B. K., SANES, J. R., AND GREENGARD, P. (1970) *Biochim. Biophys. Acta* **212**, 79-91
- LABRIE, F., LEMAIRE, S., AND COURTE, C. (1971) *J. Biol. Chem.* **246**, 7293-7302
- RUBIN, C. S., ERLICHMAN, J., AND ROSEN, O. M. (1972) *J. Biol. Chem.* **247**, 6135-6139
- TAO, M. (1972) *Biochem. Biophys. Res. Commun.* **46**, 56-61
- ASHBY, C. D., AND WALSH, D. A. (1972) *J. Biol. Chem.* **247**, 6637-6642
- MAJUMDER, G. C., AND TURKINGTON, R. W. (1971) *J. Biol. Chem.* **246**, 2650-2657
- WADDY, C. T., AND MACKINLAY, A. G. (1971) *Biochim. Biophys. Acta* **250**, 491-500
- WALSH, D. A., PERKINS, J. P., BROSTROM, C. O., HO, E. S., AND KREBS, E. G. (1971) *J. Biol. Chem.* **246**, 1968-1976
- SCHLENDER, K. K., WEI, S. H., AND VILLAR-PALASI, C. (1969) *Biochim. Biophys. Acta* **191**, 272-278
- CORBIN, J. D., REIMANN, E. M., WALSH, D. A., AND KREBS, E. G. (1970) *J. Biol. Chem.* **245**, 4849-4851
- LANGAN, T. A. (1968) *Science* **162**, 579-580
- JERGIL, B., AND DIXON, G. H. (1970) *J. Biol. Chem.* **245**, 425-434
- GOLDSTEIN, J. L., AND HASTY, M. A. (1973) *J. Biol. Chem.* **248**, 6300-6307
- BEITZ, D. C., MOHRENWEISER, H. W., THOMAS, J. W., AND WOOD, W. A. (1969) *Arch. Biochem. Biophys.* **132**, 210-222
- THOMPSON, M. P., AND FARRELL, H. M., JR. (1974) in *Lactation: A Comprehensive Treatise* (LARSON, B. L., AND SMITH, V. R., eds) Vol. 3, pp. 109-134, Academic Press, New York
- HEALD, C. W., AND SAACKE, R. G. (1972) *J. Dairy Sci.* **55**, 621-628