Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 653–657, February 1985
Biochemistry

Phosphorylation of lens fiber cell membrane proteins

(main intrinsic membrane protein/MP26 protein/protein kinase/cAMP)

DONITA GARLAND AND PAUL RUSSELL

Laboratory of Vision Research, National Eye Institute, National Institutes of Health, Bethesda, MD 20205

Communicated by Earl R. Stadtman, September 14, 1984

ABSTRACT **Two intrinsic membrane proteins of calf lens fiber cells can be phosphorylated by a soluble bovine lens cAMP-dependent protein kinase and rabbit muscle cAMP-dependent protein kinase. After electrophoresis of the phosphorylated membranes, ^{32}P comigrates with the lens main intrinsic protein at 26–27 kDa and with a minor band of protein that migrates at 19–20 kDa. ^{32}P is also found with proteins that, based on the molecular sizes, are likely multimers of the 19-kDa and 26-kDa proteins. Upon boiling in $NaDodSO_4$, all the radioactivity is found at the top of the gel, suggesting that both phosphoproteins are intrinsic membrane proteins. Serine is the only phospho amino acid detected in both proteins regardless of the source of protein kinase. The phosphorylation sites of both proteins are lost upon cleavage with trypsin and chymotrypsin. The smaller phosphoprotein is likely not a crystallin, because antibodies directed against α-, β-, or γ-crystallins do not cross-react with the 19-kDa protein. The 19-kDa ^{32}P-labeled protein does not migrate coincident with calf α-crystallin.**

Recent studies have demonstrated a link between disruption of lens fiber membranes and formation of cataracts (1–5). As a result, there is increased interest in the structure and function of lens membranes.

Numerous studies have elucidated the composition of the lens fiber cell membrane (6–13). One protein of 26–28 kDa (MP26) constitutes ≈50% of the intrinsic proteins of these membranes. Several minor species, including one of 18–20 kDa, also constitute the intrinsic membrane proteins. Approximately 60% of the fiber cell membrane is involved in the intercellular communicating junctions, commonly referred to as gap junctions. Since MP26 is the most abundant protein in the fiber membranes, it is probably the major protein in these regions (8–10). MP26 is localized in and outside the junctional regions (14–16). This suggests that, in addition to some specialized function in the gap junction, the MP26 protein may play another role.

In tissues other than the lens there is considerable evidence that intercellular communication is mediated by the opening and closing of gap junctions in response to variations in the concentration of calcium (17–22). In the lens, however, it is thought that the junctions are always open and are, therefore, insensitive to environmental stimuli such as the calcium level (23). Nevertheless, recent studies suggest that the uncoupling of cell communication in the lens can be provoked by shifts in the calcium concentration (24). In view of the fact that the activity of some proteins (enzymes) is altered by mechanisms involving phosphorylation, the present study was undertaken to explore the possibility that the phosphorylation of the MP26 protein might be involved in its biological function. In this report, we describe results of studies showing that the phosphorylation of two intrinsic membrane proteins is catalyzed by an endogenous lens cAMP-dependent protein kinase and a cAMP-dependent protein kinase from rabbit muscle.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Preparation of Lens Fiber Cell Membranes and Lens Protein Kinase. Lens fiber cell membranes were prepared from 4-month-old calf lenses according to the procedure of Russell *et al.* (25). Bovine lens protein kinase was prepared from lens cortex as follows: extracts were prepared by homogenizing cortical material in 50 mM Tris·HCl, pH 7.5/2 mM EDTA/100 mM NaCl/10 mM 2-mercaptoethanol. After centrifugation at 35,000 × *g* for 20 min, the supernatant fraction was chromatographed on Ultragel AcA 34. Cyclic AMP-dependent protein kinase activity coeluted from this column with the first peak of β-crystallin. These fractions were used as bovine protein kinase. Rabbit antisera obtained using purified calf α-, β-, and γ-crystallins were provided by J. S. Zigler.

Phosphorylation of Len Membranes. Phosphorylation experiments were done at 30°C. Incubation mixtures contained 50 mM Pipes, pH 7.0/1 mM $MgCl_2$/100 μM [γ-^{32}P]ATP (60–300 cpm/pmol)/10 μM cAMP, and a source of protein kinase in a vol of 50 μl. The reactions were stopped by adding 500 μl of cold buffer. The membranes were centrifuged at 25,000 × *g* for 40 min. The membranes were resuspended in cold Laemmli sample buffer minus the $NaDodSO_4$. $NaDodSO_4$ was added to a final concentration of 2%, and the samples were electrophoresed in 13% polyacrylamide slab gels according to the Laemmli procedure (26). Phosphorylated proteins were localized by autoradiography.

Phospho Amino Acid Determinations. Phospho amino acid determinations were done on ^{32}P-labeled proteins eluted from polyacrylamide gels after electrophoresis or after extraction from the membranes by chloroform/methanol (10). After hydrolysis in 6 M HCl, the sample was electrophoresed on a cellulose thin-layer plate in acetic acid/formate/water (39:25:436) (pH 2.0) at 1000 V for 90 min. Phosphoserine and phosphothreonine (Sigma) were included as internal standards.

RESULTS

Phosphorylation of Lens Fiber Cell Membranes. After removal of extrinsic proteins, calf lens fiber cell membranes have protein profiles as seen in Fig. 1. During electrophoresis in 13% polyacrylamide gels, a major band of protein migrates at a position of 26–27 kDa. This is in agreement with previous studies. Several minor bands are always present in these preparations, and some of these are more abundant in membranes prepared from the nuclear region of the lens. One minor band that migrates at a position of 19–20 kDa is consistently present in these preparations and those of others (18 kDa) (10, 23, 27–30). When electrophoresis was performed using gradient gels, these proteins migrated at 28–29 kDa and at 19–22 kDa, respectively. In this report, the major protein band will be referred to as MP26, in keeping with the literature, and the smaller protein will be referred to as MP19. As previously reported (31), when the membranes are

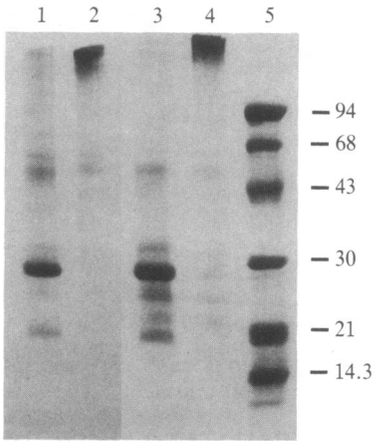

FIG. 1. Protein profile of lens cortical and nuclear membranes. Membranes were prepared as described. $NaDodSO_4$/polyacrylamide gel electrophoresis was performed in 13% polyacrylamide gels. Prior to electrophoresis, cortical membranes and nuclear membranes were incubated with 2% $NaDodSO_4$ at room temperature (lanes 1 and 3, respectively) or at 100°C (lanes 2 and 4, respectively). Lane 5, molecular size standards (kDa).

boiled in $NaDodSO_4$ prior to electrophoresis, MP26 aggregates and remains at the top of the gel. We find that MP19 also aggregates when boiled in $NaDodSO_4$ (Fig. 1, lanes 2 and 4).

Cortical membranes were incubated with [γ-^{32}P]ATP, Mg^{2+}, cAMP, and a source of protein kinase, and then electrophoresed and analyzed by autoradiography. ^{32}P was found associated with MP26, the minor band of protein at 19 kDa and at positions corresponding to 40, 52, 60, and 80 kDa and at the top of the gel (Fig. 2). The labeled bands at positions >26 kDa are likely multimers of the 26- and 19-kDa proteins and for now will be considered such. The focus of this report will be on the 26- and 19-kDa proteins.

The same pattern of phosphorylation was observed whether membranes prepared from the cortical or nuclear region of the lens were used (data not shown). As seen in Fig. 2, the same proteins were phosphorylated whether the source of protein kinase was lens or rabbit muscle. No phosphorylation was observed in the absence of added protein kinase. Phosphorylation of membrane proteins by the lens cortex protein kinase was only observed when cAMP was added. The phosphorylation of both MP26 and MP19 was time (data not shown) and temperature dependent (Fig. 2*B*). Essentially no labeling was seen at 4°C. When the membranes were boiled in the presence of $NaDodSO_4$ prior to electrophoresis, all of the radioactivity remained at the top of the gel.

Phosphorylation of Membranes Before Treatment with Denaturants. To test whether these two proteins become substrates for the protein kinase only after membranes are treated with urea and alkali during purification, the membranes prepared only by extensive washing with buffer were used as substrates for phosphorylation. Membranes prepared from cortical and nuclear regions were incubated with [γ-^{32}P]-ATP, $MgCl_2$, and cAMP, and in the presence or absence of added protein kinase. These results are shown in Fig. 3. In the cortex, several high molecular weight bands become phosphorylated (lanes 4 and 5). In addition, in both the cortex and nucleus samples, ^{32}P is incorporated into MP19 when cAMP is added, even though protein kinase is not added. Phosphorylation of MP19 and the higher molecular weight proteins is enhanced with the addition of protein kinase to both the cortex and nuclear membranes. These results indicate these membranes contain an endogenous cAMP-stimulated protein kinase. Furthermore, MP19 is a substrate for this protein kinase even before removal of extrinsic proteins by urea and alkali treatment. There is, however, very little ^{32}P found associated with MP26. In the cortex, other membrane-associated proteins, particularly the cytoskeletal proteins, are probably preferential substrates for the protein kinase. However, even though the cytoskeletal proteins are diminished in the nucleus there is still little ^{32}P incorporated into MP26. Since MP26 is readily phosphorylated after removing the extrinsic proteins it is possible that the phosphorylation site is not accessible to the kinase because extrinsic proteins may be bound to MP26.

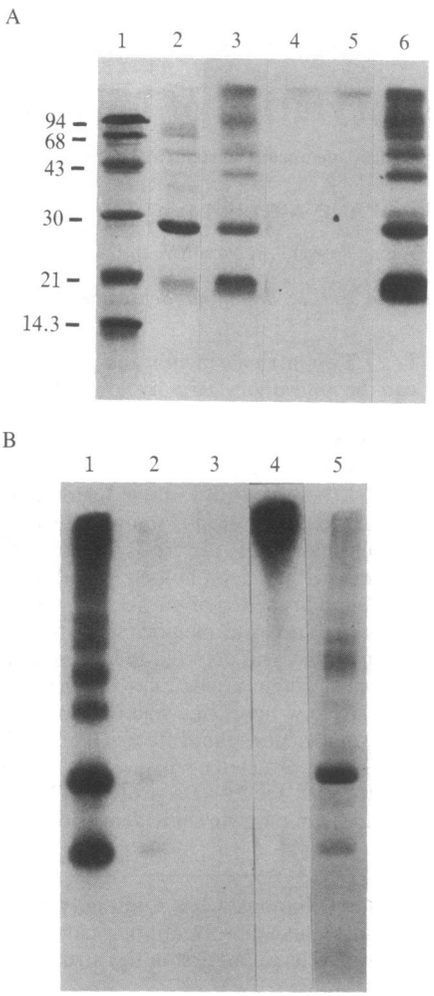

FIG. 2. Autoradiograms of lens membranes after phosphorylation and electrophoresis. (*A*) Lane 1, molecular size standards (kDa); lane 2, Coomassie stain; lane 3–6, autoradiograms of membranes after incubation at 30°C with 100 μM [γ-^{32}P]ATP/1 mM $MgCl_2$ with lens protein kinase and 10 μM cAMP (lane 3), with lens protein kinase and no cAMP (lane 4), with 10 μM cAMP and no protein kinase (lane 5), with 10 μM cAMP and rabbit muscle protein kinase (lane 6). (*B*) In a separate experiment, membranes were incubated in 100 μM [γ-^{32}P]ATP/1 mM $MgCl_2$/10 μM cAMP, and rabbit muscle protein kinase (lanes 1, 2, and 4) or in the absence of protein kinase (lane 3). Phosphorylation was done at 30°C (lanes 1, 3, and 4) or at 4°C (lane 2). After phosphorylation, the samples were incubated in $NaDodSO_4$ at 25°C (lanes 1–3) or at 100°C (lane 4) for 10 min prior to electrophoresis. Lane 5, Coomassie stain.

Phospho Amino Acid Determinations. Phospho amino acid determinations were done on phosphoproteins eluted from polyacrylamide gel slices after electrophoresis (Fig. 4) and on phosphoproteins extracted by chloroform/methanol from calf cortex fiber membranes (data not shown). For both preparations, phosphoserine was the only phospho amino acid detected for both the 26- and 19-kDa proteins. This was true even after long exposure times for the autoradiograms.

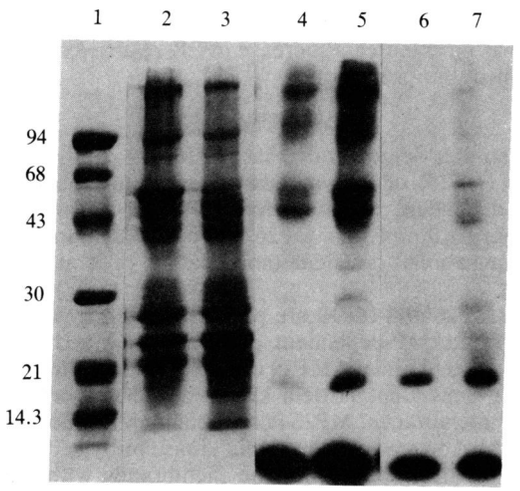

FIG. 3. Phosphorylation of membranes before treatment with urea or NaOH. Lens fractions were homogenized and centrifuged according to Russell *et al.* (25). Pellet was rehomogenized in the same buffer 3 times and centrifuged. The final pellets were resuspended in Pipes buffer and phosphorylated. Lanes: 1, molecular size markers (kDa); 1–3, Coomassie stain; 4–7, autoradiograms. Cortical (lanes 2, 4, and 5), and nuclear (lanes 3, 6, and 7) fractions were incubated with 100 μM [γ-^{32}P]ATP/10 μM cAMP/1 mM $MgCl_2$, and rabbit muscle protein kinase (lanes 5 and 7) or without added protein kinase (lanes 4 and 6).

Phosphoserine was found whether the bovine lens protein kinase or rabbit muscle protein kinase was used to phosphorylate the membrane proteins. Even though both kinases phosphorylate serine it remains to be determined whether the same serine is phosphorylated by each protein kinase.

Comparison of MP19 with α-Crystallin. With increasing age of lens cells, cytoplasmic proteins, particularly α-crystallin, become associated with the membranes. Furthermore, there are reports that α-crystallin binds to these membranes (12, 32, 33) and α-crystallin is a component of the membranes (34). All the experiments reported here were done using 4-month-old calf lenses to minimize the age-related association of crystallins with the membranes. Even when only the outer cortex region, which contains the youngest fiber cells in the lens, was used there did not appear to be a decrease in the 19-kDa band of protein or a decrease in the phosphorylation of this band. When electrophoresis was performed using gradient gels to increase resolution of proteins (Fig. 5), the 19-kDa protein did not comigrate with added α-crystallin. Furthermore, the α-crystallin could not be phosphorylated to any significant extent by added rabbit muscle protein kinase. These results suggest that the 19-kDa phosphoprotein is likely not α-crystallin. This is further substantiated by immunoblot experiments.

Immunoblot Using Antibodies Against Bovine Crystallins. After electrophoresis, the proteins were electrophoretically transferred to nitrocellulose and reacted with antibodies made against α-, β-, and γ-crystallin. The results are shown in Fig. 5. There are no bands that react with antibodies to γ-crystallin. There are several bands that react with antibodies to α-crystallin. Three of these migrate between 21 and 23 kDa. These bands are not coincident with the band of radioactivity. Antibodies to β-crystallin react with two bands of protein that migrate at the front edge of the MP26, but none in the region of MP19. The slower moving β-crystallin band is within the region containing radioactivity. However, in numerous experiments the radioactivity always corresponds to the width of the MP26 band and does not appear to be associated with an edge of the MP26 band. Thus, it is unlikely that the β-crystallin is the phosphorylated protein rather than MP26.

Stoichiometry of Phosphorylation. As seen in Fig. 2 *A* and *B*, the specific radioactivity of the MP19 is higher than that of MP26. The protein ratio of MP26 and MP19 in these two experiments is about the same and is the same in all preparations. Yet, the incorporation of ^{32}P is more into MP19 in Fig. 2*A* and about equal into MP26 and MP19 in Fig. 2*B*. In other experiments (data not shown), there is more ^{32}P incorporated into MP26 than into MP19. The reason for this has not been fully elucidated. The source of protein kinase is not a factor because, in several experiments, the same relative incorporation was always observed with either lens or rabbit muscle protein kinase. Determination of the maximum stoichiometry of phosphorylation of either of these proteins awaits elucidation of those factors responsible for determining which species is preferentially phosphorylated. In an experiment in which there was little label in MP19, the maximum incorporation of ^{32}P into MP26 was $\approx$0.2 mol per mol of protein. However, the stoichiometry of the protein re-

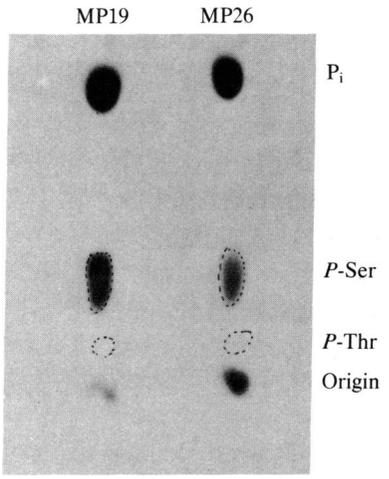

FIG. 4. Identification of phosphorylated amino acid. Membranes were phosphorylated with rabbit muscle protein kinase and electrophoresed. Radioactive bands corresponding to MP26 and MP19 were eluted from the gel, hydrolyzed at 155°C for 5 min in 6 M HCl, and electrophoresed. Phosphoserine and phosphothreonine added as standards were visualized by ninhydrin (dotted lines). ^{32}P-labeled amino acids were located by autoradiography. Comparable results were obtained when lens protein kinase was used to phosphorylate the membranes.

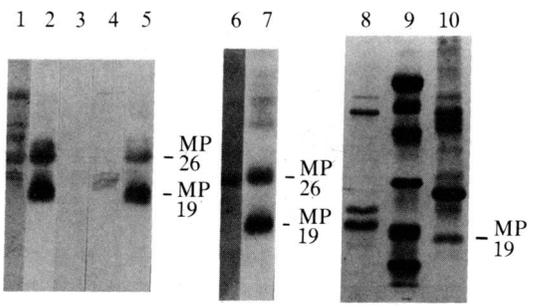

FIG. 5. Immunoblots using antisera for bovine α-, β-, and γ-crystallins. Phosphorylated membranes were electrophoresed in $NaDodSO_4$ according to Laemmli (26). The proteins were electrophoretically transblotted onto nitrocellulose and reacted with antisera to crystallins. The antigen–antibody complexes were localized using horseradish peroxidase-labeled goat anti-rabbit IgG according to Hawkes *et al.* (35). Lanes 1, protein pattern of blotted proteins stained with amido black; 2 and 5, corresponding autoradiograms; 3, reacted with antibodies to γ-crystallin; 4, reacted with antibodies to α-crystallin; 6, reacted with antibodies to β-crystallin; 7, corresponding autoradiograms; 8–10, Coomassie-stained bands; 8, α-crystallin; 9, molecular size standards; 10, membrane proteins.

maining at the top of the gel was considerably higher, suggesting the state of aggregation of these proteins may influence the phosphorylation or vice versa. Pretreatment of the membranes with protein phosphatase; incubation for 19 hr; varying the concentrations of kinase, Mg^{2+}, cAMP, and ATP; and adding calmodulin and calcium did not significantly increase the molar ratio of ^{32}P into MP26.

Protease Treatment of Phosphorylated Membranes. Membranes were phosphorylated and then incubated with trypsin, chymotrypsin, or *Staphylococcus aureus* V8 to determine (*i*) whether the phosphorylation site of MP26 was within the terminal segments cleaved by these proteases and (*ii*) whether the phosphorylation site could be removed from MP19. As shown in Fig. 6*A*, when membranes are incubated with trypsin or chymotrypsin all of the radioactivity is removed coincident with the cleavage of 4–5 kDa from MP26. All of the ^{32}P is also lost from MP19. However, because MP19 generally migrates as a rather diffuse band, it is not clear how much is removed by protease treatment. Using gradient gels (Fig. 6*B*, lanes 4 and 9) in which MP19 ran as a relatively sharp band, there was little change in the molecular size of MP19 after trypsin treatment even though there is no ^{32}P remaining with the band. The size of the phosphopeptide removed from MP19 must be relatively small.

Staphylococcus aureus V8 protease cleaves MP26 in two steps. A segment of ≈2 kDa is removed and with increased time (data not shown) or concentration of protease, another segment of about the same size is removed (lanes 2 and 3). The final polypeptide is essentially the same size as that after trypsin or chymotrypsin treatment. The larger of the MP26 cleavage products still contains ^{32}P, although the level is reduced relative to the untreated sample. The smaller cleavage product appears to have lost all of the radioactivity. With increasing concentrations of V8 protease, ^{32}P is slowly lost from MP19. But there is little observable decrease in the size of MP19. These results are consistent with the phosphorylation site on MP19 being close to a terminus of the protein such that the site can be removed by any of the three proteases while not drastically changing the molecular size of the protein.

$^{32}P_i$ was assayed during the incubations with all three proteases to rule out the possibility that the ^{32}P was removed by esterases present in the protease preparations. In each case, there was no significant increase in $^{32}P_i$ released over that of the control (no protease).

DISCUSSION

These studies demonstrate that MP26 and another intrinsic protein (MP19) of lens membranes are phosphorylated by cAMP-dependent protein kinase. To our knowledge, the phosphorylation of lens MP26 is the first demonstration of post-translational covalent modification of a gap junction protein.

Both MP26 and MP19 are phosphorylated by an endogenous lens cAMP-dependent protein kinase and by rabbit muscle protein kinase. MP19 can also be phosphorylated by a cAMP-dependent protein kinase associated with buffer-washed membranes. MP26 is not readily phosphorylated by the membrane-associated protein kinase or by added protein kinase before treatment of the membranes with urea and NaOH to remove extrinsic proteins. For the MP26, it is possible that a portion of the protein must be denatured to expose the phosphorylation site, but it is more likely that the phosphorylation site is not available to the kinase because of the binding of extrinsic proteins to this region.

Kawaba *et al.* (36) have reported the phosphorylation of 26-kDa proteins in Triton X-100-extracted protein fractions from bovine lens epithelial cells and cortex nucleus. Since MP26 has not been identified as a component of lens epithelial cell membranes, it remains to be determined whether the 26-kDa phosphoproteins in that study are the main intrinsic lens fiber cell membrane proteins. A preliminary report also describes the phosphorylation of MP26 (37).

The limited cleavage of MP26 observed here after incubation of membranes with chymotrypsin, trypsin, or V8 protease is consistent with previous reports (27, 28). In each case, there is a total loss of 4–5 kDa primarily from the carboxyl terminus. With this cleavage, the ^{32}P is removed. With V8 protease, some ^{32}P is still found on the polypeptide after only ≈2 kDa is removed. These results are consistent with the phosphorylation site(s) being between 15 and 35 residues from the carboxy terminus of the protein. This is confirmed by examination of the sequence of MP26 (42), which reveals the presence of multiple serines that are potential sites for

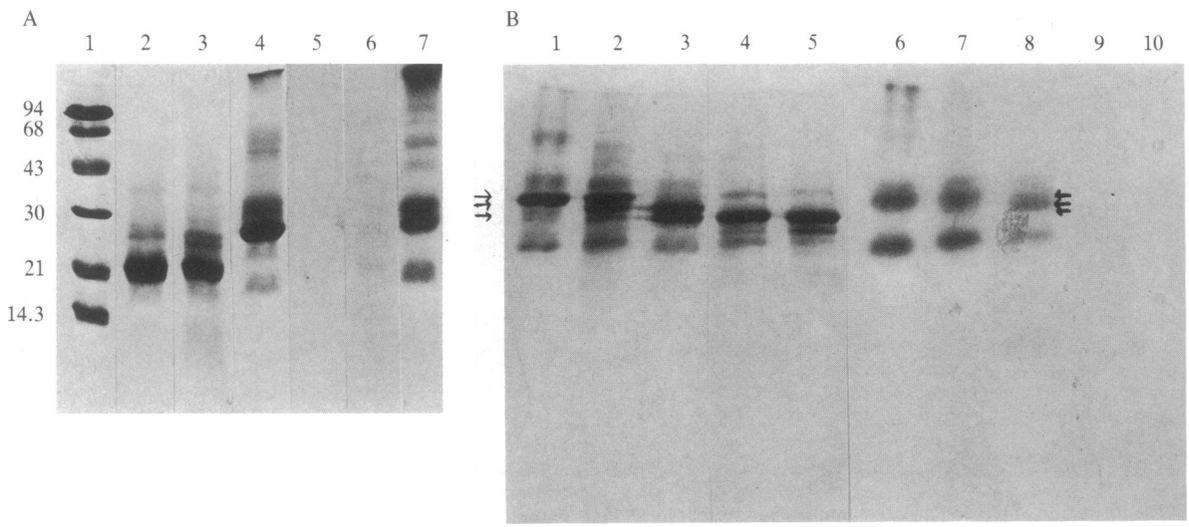

FIG. 6. Electrophoresis and autoradiography of phosphorylated membranes after incubation with trypsin, chymotrypsin, or *Staphylococcus aureus* V8 protease. Digestion was stopped by adding 0.1 M NaOH and centrifuging the membranes. Membranes were resuspended in Laemmli buffer and electrophoresed in 13% polyacrylamide gels (*A*) or 17%–27% gradient gels (*B*). (*A*) Lanes: 1–4 protein stain; 5–7, autoradiograms; 1, protein molecular size standards (kDa); 2 and 5, membranes and trypsin; 3 and 6, membranes and chymotrypsin; 4 and 7, membranes and no protease. (*B*) Lanes: 1–5, protein stain; 6–10, autoradiograms; 1 and 6, membranes and no protease; 2 and 7, membranes and V8 protease (1:40); 3 and 8, membranes and V8 protease (1:20); 4 and 9, membranes and trypsin (1:40); 5 and 10, membranes and trypsin (1:20). Arrows indicate position of MP26 and the two cleavage products and their corresponding position on the autoradiogram.

phosphorylation by cAMP-dependent protein kinases (38) between 21 and 35 residues from the carboxyl terminus.

The identity of MP19 is not known. The present studies support the conclusion that it is an intrinsic membrane protein, but it is not a crystallin. In addition, preliminary experiments show that antibodies directed against MP26 do not react with MP19 (data not shown). The biological role of the phosphorylation of the MP26 and MP19 remains be elucidated. If phosphorylation or dephosphorylation regulates the function of either, it will likely be by altering the interaction with other proteins, such as calmodulin and cytoskeleton proteins. Interactions of both with MP26 and the fiber cell membranes have been reported (39, 41). Since MP26 is thought to represent a major protein in the communicating junction of the lens, the state of phosphorylation of the MP26 may influence the cell to cell communication within the lens. The exact function and role of the MP19 remains unclear, but the ease with which it is phosphorylated by the endogenous kinase suggests it may play a very active role in the cytostructure of the lens fibers.

We wish to thank Dr. Jin Kinoshita and Dr. Earl Stadtman for their continued support during the course of this work and Dr. Sam Zigler for providing the antisera. This work was supported in part by the National Heart, Lung, and Blood Institute.

1. Philipson, B. (1973) *Exp. Eye Res.* **16,** 29–39.
2. Dilley K. J., Bron, A. J. & Habgood, J. D. (1976) *Exp. Eye Res.* **22,** 155–167.
3. Unakar, N. J., Genyea, C., Reddan, J. R. & Reddy, V. (1978) *Exp. Eye Res.* **26,** 123–133.
4. Farnsworth, P. N., Burke, P. A., Wagner, B. J., Fu, S.-C. J. & Regan, T. J. (1980) *Metab. Pediatr. Opthalmol.* **4,** 31–42.
5. Garner, M. H., Roy, D., Rosenfeld, L., Garner, W. H. & Spector, A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1892–1895.
6. Broekhuyse, R. M. & Kuhlmann, E. D. (1974) *Exp. Eye Res.* **19,** 297–302.
7. Bloemendal, H., Zween, A., Vermorken, F., Dunia, I. & Benedetti, E. L. (1972) *Cell Differ.* **1,** 91–106.
8. Alcala, J., Lieska, N. & Maisel, H. (1975) *Exp. Eye Res.* **21,** 581–595.
9. Lasser, A. & Balazs, E. A. (1972) *Exp. Eye Res.* **13,** 292–308.
10. Broekhuyse, R. M., Kuhlmann, E. D. & Stols, A. L. H. (1976) *Exp. Eye Res.* **23,** 365–371.
11. Benedetti, E. L., Dunia, I., Bentzel, C. J., Vermorken, A. J. M., Kibbelaar, M. & Bloemendal, H. (1976) *Biochim. Biophys. Acta* **457,** 353–384.
12. Alcalá, J., Valentine, J. & Maisel, H. (1980) *Exp. Eye Res.* **30,** 659–677.
13. Roy, D., Rosenfeld, L. & Spector, A. (1982) *Exp. Eye Res.* **35,** 113–129.
14. Bok, D., Dockstader, J. & Horwitz, J. (1982) *J. Cell Biol.* **92,** 213–220.
15. Fitzgerald, P. G., Bok, D. & Horwitz, J. (1983) *J. Cell Biol.* **97,** 1491–1499.
16. Paul, D. L. & Goodenough, D. A. (1983) *J. Cell Biol.* **96,** 625–632.
17. Loewenstein, W. R. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **40,** 49–63.
18. Deleze, J. & Loewenstein, W. R. (1976) *J. Membr. Biol.* **28,** 71–86.
19. DeMello, W. C. (1975) *J. Physiol.* **250,** 231–245.
20. Loewenstein, W. R. (1967) *J. Colloid Interface Sci.* **25,** 34–46.
21. Oliveira-Castro, G. M. & Loewenstein, W. R. (1971) *J. Membr. Biol.* **5,** 51–77.
22. Rose, B. & Loewenstein, W. R. (1975) *Nature (London)* **254,** 250–252.
23. Goodenough, D. A. (1979) *Invest. Ophthalmol. Vis. Sci.* **18,** 1104–1122.
24. Bernardini, G., Peracchia, C. & Venosa, R. A. (1981) *J. Physiol.* **320,** 187–192.
25. Russell, P., Robison, W. G., Jr., & Kinoshita, J. H. (1981) *Exp. Eye Res.* **32,** 511–516.
26. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
27. Horwitz, J. & Wong, M. M. (1980) *Biochim. Biophys. Acta* **622,** 134–143.
28. Keeling, P., Johnson, K., Sas, D., Klukas, K., Donahue, P. & Johnson, R. (1983) *J. Membr. Biol.* **74,** 217–228.
29. Freidlander, M. (1980) *Curr. Top. Dev. Biol.* **14,** 321–358.
30. Broekhuyse, R. M. & Kuhlmann, E. D. (1978) *Exp. Eye Res.* **26,** 305–320.
31. Wong, M. M., Robertson, N. P. & Horwitz, J. (1978) *Biochem. Biophys. Res. Commun.* **84,** 158–165.
32. Bloemendal, H., Hermsen, T., Dunia, I. & Benedetti, E. L. (1982) *Exp. Eye Res.* **35,** 61–67.
33. Ramaekers, F. C. S., Selten-Versteegen, A.-M. E. & Bloemendal, H. (1980) *Biochim. Biophys. Acta* **596,** 57–63.
34. Bracchi, P. G., Carta, F., Fassela, P. & Maraini, G. (1971) *Exp. Eye Res.* **12,** 151–154.
35. Hawkes, R., Niday, E. & Gordon, J. (1982) *Anal. Biochem.* **119,** 142–147.
36. Kawaba, T., Takamura, R., Matsuda, H. & Hayashi, S. (1982) *Acta Soc. Ophthalmol. Jpn.* **86,** 578–586.
37. Johnson, K. R. & Johnson, R. G. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41,** 755 (abstr.).
38. Kemp, B. E., Graves, D. J., Benjamini, E. & Krebs, E. G. (1977) *J. Biol. Chem.* **252,** 4888–4894.
39. Welsh, M. J., Aster, J. C., Ireland, M., Alcalá, J. & Maisel, H. (1982) *Science* **216,** 642.
40. Hertzberg, E. L. & Gilula, N. B. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **46,** 639–645.
41. Bloemendal, H. (1982) *CRC Crit. Rev. Biochem.* **12,** 1–38.
42. Gorin, M. B., Yancey, S. B., Cline, J., Revel, J.-P. & Horwitz, J. (1984) *Cell* **39,** 49–59.