A calcium- and calmodulin-dependent kinase that represents the majority of the myosin heavy chain kinase activity in chicken intestinal brush borders has been highly purified. The purification steps include gel filtration, high performance chromatography on anion and cation exchangers, and affinity chromatography on calmodulin-Sepharose. The purified kinase consists of a single major, apparently autophosphorylatable polypeptide of 50,000 daltons. The Stokes radius (68 Å) and sedimentation coefficient (17.5 S) indicate that it has a molecular weight of approximately 490,000. The kinase catalyzed the incorporation of a maximum of 0.8 mol of phosphate/mol of heavy chain, and essentially no phosphate was incorporated into the light chains. This kinase is distinct from other myosin kinases, but has a number of properties in common with the type II calmodulin-dependent protein kinases.

Phosphorylation of the heavy chains of myosin has recently been reported to occur in a variety of vertebrate non-muscle cells, including brain (1), macrophage (2), lymphocytes (3), leukemic myeloblasts (4), and fibroblasts (5); in several lower eukaryotes (6–8); and in scallop smooth muscle (9). Functions of the light chains of myosin by activation of CaM'-dependent myosin light chain kinase (reviewed in Refs. 12–14). Phosphorylation by this kinase has been shown to regulate the actin-activated ATPase activity and filament assembly of some smooth muscle and non-muscle myosins (12–14).

We report here studies of the role of calcium in regulation of heavy chain phosphorylation of myosin from chicken intestinal brush borders. Brush border myosin has been shown to exhibit physical and enzymatic properties very similar to those of many smooth and non-muscle myosins, including regulation of its activity and assembly by light chain phosphorylation (15). In addition, fractones brush borders can contract in vitro (16–18), and this event has been shown to correlate with calcium- and CaM-dependent myosin light chain phosphorylation (19). We recently showed that the large majority of myosin heavy chain kinase activity in chicken intestinal brush borders is calcium- and CaM-dependent (20) and have reported the partial purification of this activity (21). Here we describe methods for the purification of the CaM-dependent myosin heavy chain kinase to near-homogeneity and show that it has several properties in common with the widely distributed family of type II CaM-dependent protein kinases (reviewed in Ref. 22).

EXPERIMENTAL PROCEDURES

All procedures were carried out at 2–4 °C unless specified otherwise. Materials—Electrophoresis chemicals and molecular weight standards were from Bio-Rad and all chromatography media were from Pharmacia Biotechnology, Inc. Chicken skeletal muscle myosin, rabbit skeletal muscle myosin, rabbit gizzard myosin was purified according to Sellers et al. (24). Rat cardiac myosin was a gift of Dr. Irwin Klein, University of Pittsburgh. Rabbit skeletal muscle F-actin was prepared according to Eisenberg and Kielley (25). [γ-32P] ATP, 2–10 Ci/mmol, was from Du Pont-New England Nuclear. Water freshly treated on the Milli-Q system (Millipore Corp., Bedford, MA) was used in all solutions.

Purification of Brush Border Myosin—Isolation of brush borders from chicken small intestines, preparation of the 100,000 × g supernatant of the brush border extract, and gel filtration chromatography of the supernatant on Sepharose CL-4B were performed as described under "Results" for purification of the CaM-dependent myosin heavy chain kinase. Sepharose CL-4B fractions containing myosin were identified by SDS-polyacrylamide gel electrophoresis, pooled, and dialyzed in 10 mm imidazole chloride, 40 mm sodium pyrophosphate, 1 mm ATP, 1 mm EDTA, 10% sucrose, 1 mm DTT, 0.2 mm PMSF, pH 7.5. This material was chromatographed on a Pharmacia Mono Q column (0.5 × 5 cm) equilibrated in the same buffer using a Pharmacia high performance FPLC system. Myosin was eluted with a 40-mL linear gradient of 0–0.4 M NaCl in equilibration buffer. Myosin-containing fractions were identified by SDS-polyacrylamide gel electrophoresis and those with no detectable contaminants were pooled, concentrated by dialysis in solid sucrose, and dialyzed in 10

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The abbreviations used are: CaM, calmodulin; LC20, myosin 20,000-dalton light chain; PMSF, phenylmethyisulfonyl fluoride; DTT, dithiothreitol; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetate acid; SDS, sodium dodecyl sulfate; TES, N-tris(hydroxy-methyl)methyl-2-aminoethanesulfonic acid.
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mm imidazole chloride, 0.5 M NaCl, 10% sucrose, 1 mM DTT, 0.02% NaN₃, pH 7.5. The myosin preparation maintained its original K⁺, EDTA-ATPase, and Ca²⁺-ATPase activities, as determined according to Korn et al. (26), and was used for up to 2 weeks with storage on ice. Approximately 0.5–1.0 mg of myosin was obtained per 20–50 g of intestine from each chicken.

Kinase Assays—Assays for myosin heavy and light chain kinase activity were carried out under standard conditions as described below, unless otherwise noted. Assays were conducted at 30 °C for the times indicated and were initiated by adding 5–10 μl of kinase and 0–5 μl of 10 mM imidazole chloride, 25 mM NaCl, 10% sucrose, 1 mM EDTA, 1 mM EGTA, 1 mM MgCl₂, pH 7.5, to 25 μl of an assay premixture containing components to give a final incubation mixture of 10 mM imidazole chloride, 60–100 mM NaCl, 6 mM MgCl₂, 0.5 mM [γ-³²P]ATP, 0.25 mM DTT, 0.14 mM EDTA, pH 7.5, and 28.6 μg/ml brush border myosin, and either 1 mM EGTA (minus calcium), 0.71 mM CaCl₂, and 0.28 mM EGTA (plus calcium), or 0.71 mM CaCl₂, 0.28 mM EGTA, and 2 μg/ml bovine brain CaM (plus calcium and CaM). In some experiments, the free calcium concentration was varied and its value was calculated with the program of Perrin and brush border myosin, and either 1 mM EGTA (minus calcium), 0.71 mM CaCl₂, and 0.28 mM EGTA (plus calcium), or 0.71 mM CaCl₂, 0.28 mM EGTA, and 2 μg/ml bovine brain CaM (plus calcium and CaM).

In some experiments, the free calcium concentration was varied and its value was calculated with the program of Perrin and Sayce (27), using stability constants from Martell and Smith (28).

Radioactivity was quantitated by liquid scintillation spectrometry.

Other Methods—The sedimentation coefficient (sₐ₀,d) of the kinase was determined by sucrose density gradient centrifugation as described by Martin and Ames (30) with ovalbumin (sₐ₀,d = 3.5 S), human immunoglobulin G (sₐ₀,d = 7.0 S), catalase (sₐ₀,d = 11.3 S), and thyroglobulin (sₐ₀,d = 18.2 S) as standards. Partition coefficients (K₉₀) were calculated and Stokes radii were determined by gel filtration on Sepharose CL-4B calibrated with proteins with known Stokes radii by using the analysis of Laurent and Killander (31). Protein concentrations were determined (32) using the Bio-Rad protein assay kit with bovine serum albumin as the standard, or by densitometry of SDS-polyacrylamide gels run with bovine serum albumin standard.

RESULTS

Purification of Brush Border Myosin Heavy Chain Kinase: Isolation and Extraction of Brush Borders—All solutions used for the kinase preparation contained 1 mM DTT, 0.02% NaN₃, and the protease inhibitors aprotinin (5 μg/ml), leupeptin (1 μg/ml), pepstatin A (5 μg/ml), PMNS (0.25 mM), and dipyridyl fluorophosphate (1 mM). Brush borders were isolated from the small intestines of four adult white Leghorn chickens, as previously described (33). Freshly prepared brush borders in 10 mM imidazole chloride, 75 mM KCl, 5 mM MgCl₂, 1 mM EGTA, pH 7.3, were pelleted by centrifugation at 800 × g for 10 min and resuspended in 2 volumes of 10 mM imidazole chloride, 0.2 M KCl, 5 mM MgCl₂, 5 mM ATP, 1 mM EGTA, pH 6.8 (Buffer E). Centrifugation of this material at 109,000 × g gave a supernatant (95% of protein in 102 ml) containing about 85% of the myosin heavy chain kinase activity present in the suspension. ATP is not necessary for kinase extraction, but was included to extract myosin, which was then purified as described under "Experimental Procedures."

Sepharose CL-4B Gel Filtration Chromatography—The 109,000 × g supernatant was adjusted to 0.6 M KCl by the addition of 3 M KCl and chromatographed on a Sepharose CL-4B column as shown in Fig. 1. A single peak of CaM-dependent myosin heavy chain kinase activity eluted from the column with a partition coefficient (K₉₀) of 0.62. This kinase was well separated from brush border myosin, which eluted with a K₉₀ of 0.24. Fractions 65–77 in the first two-thirds of the peak were of higher specific activity and contained less light chain kinase (not shown) than the following fractions and were pooled for further purification. The pool (17 mg of protein in 216 ml) was dialyzed for a total of 12 h against two changes of 4 liters each of buffer containing 10 mM imidazole chloride, 25 mM NaCl, 10% sucrose, 1 mM EDTA, 1 mM EGTA, pH 7.5 (Buffer K).

Myosin heavy chain kinase activity that was not dependent on calcium and CaM was distributed throughout the peak of CaM-dependent myosin heavy chain kinase activity and was also present in fractions eluting after this peak. The CaM-dependent and CaM-independent activities can be separated by high performance gel filtration chromatography, as described previously (20), or by anion exchange chromatography, as described below.

High Performance Anion Exchange Chromatography on Mono Q—The dialyzed Sepharose CL-4B pool was filtered through a Millipak 20 filter unit (Millipore Corp.) and chromaffin tissue. The void volume (Vo) and elution positions of standard proteins determined in separate runs are indicated. Standard proteins were: 1, rabbit skeletal myosin; 2, fibrinogen (107 A); 3, thyroglobulin (85 A); 4, human immunoglobulin G (58 A); 5, bovine serum albumin (35 A); and 6, ovalbumin (29 A). Details are given under "Experimental Procedures.

FIG. 1. Gel filtration chromatography of brush border extract supernatant on Sepharose CL-4B. 119 ml of brush border extract supernatant adjusted to 0.6 M KCl was chromatographed on a Sepharose CL-4B column (5 x 90 cm) equilibrated and eluted in a solution containing 10 mM TEIS, 0.6 M KCl, 1 mM EDTA, 1 mM ATP, 5% sucrose, pH 7.5, at a flow rate of 120 ml/h. Prior to sample application, 250 ml of Buffer E adjusted to 0.6 M KCl was applied to the column. Fractions of 18 ml were collected and 0.2 ml of the indicated fractions were dialyzed in Buffer K in a flow microdialyzer apparatus (Bethesda Research Laboratories) for 3 h. 5-μl aliquots of the dialyzed samples were assayed for myosin heavy chain kinase activity in the presence of myosin (1 μg) and calcium and CaM ( ) or EGTA ( ) in a volume of 35 μl for 15 min. Protein concentrations (X) were also determined. The fractions pooled for CaM-dependent myosin heavy chain kinase activity are indicated by the solid bar. The inset shows the elution position of a 50-kDa phosphorylated polypeptide ( ) determined by densitometry of autoradiographs of SDS gels of the phosphorylation assay mixtures. The void volume (Vo) and elution positions of standard proteins determined in separate runs are indicated. Standard proteins were: 1, rabbit skeletal myosin (290 A); 2, fibrinogen (107 A); 3, thyroglobulin (85 A); 4, human immunoglobulin G (58 A); 5, bovine serum albumin (35 A); and 6, ovalbumin (29 A). Details are given under "Experimental Procedures."
matographed on a Mono Q high performance anion exchange column with a Pharmacia FPLC system, as described in Fig. 2. A single peak of CaM-dependent myosin heavy chain kinase activity eluted between 0.14 and 0.27 M NaCl. The recovery of CaM-dependent myosin heavy chain kinase activity in the pool (3.3 mg of protein in 7.5 ml) was 88% of the activity applied to the column, which represents a 4.5-fold purification. The calcium- and CaM-independent myosin heavy chain kinase activity was recovered in the unbound fractions (not shown).

**High Performance Ion Exchange Chromatography on Mono S**—The Mono Q pool was diluted 8-fold with Buffer K to reduce the NaCl concentration and applied to a Mono S column. A single peak of myosin heavy chain kinase activity eluted between 0.16 and 0.34 M NaCl (Fig. 3). About 76% of the activity applied to the Mono S column was recovered in the pool (0.9 mg of protein in 7 ml) with a 2.8-fold purification. This step removes most of the CaM-dependent myosin light chain kinase activity, which does not bind to the Mono S column.

**Affinity Chromatography on CaM-Sepharose**—The final step of purification is affinity chromatography on a column of CaM-Sepharose 4B (Fig. 4). Prior to chromatography, the Mono S pool was diluted 2-fold with Buffer K and dialyzed against Buffer K for 2 h. Immediately before application to the CaM-Sepharose column, 2.5 mM CaCl₂ and 3 mM MgCl₂ were added to the sample. Essentially all of the myosin heavy chain kinase activity bound to the column in the presence of calcium and less than 5% of the activity applied eluted in the presence of calcium and 0.2 M NaCl. About 10–20% of the myosin heavy chain kinase activity applied to the column was eluted with buffer containing 1 mM EGTA and 0.2 M NaCl. No additional kinase activity was eluted with buffer containing EGTA and 1 M NaCl. Fractions 36–40 were pooled and concentrated 4–5-fold by dialysis against solid sucrose and dialyzed in Buffer K. This step resulted in a 2-fold purification.

Most of the myosin light chain kinase activity present in the Mono S pool was not dependent on calcium and CaM and was removed during the elution of the CaM-Sepharose column with calcium plus 0.2 M NaCl. A small fraction of the light chain kinase activity usually bound to the column and was eluted with the heavy chain kinase (not shown).

Table I summarizes the recovery and activity of the CaM-dependent myosin heavy chain kinase at the various steps of the purification. The CaM-Sepharose pool represented an 84-fold purification over the 109,000 x g supernatant with a 5.7%...
**Table I**

Summary of purification of chicken intestinal brush border calmodulin-dependent myosin heavy chain kinase

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Protein concentration</th>
<th>Total activity</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract supernatant</td>
<td>102</td>
<td>0.93 (95)</td>
<td>714 (100)</td>
<td>7.5</td>
</tr>
<tr>
<td>Sepharose CL-4B</td>
<td>216</td>
<td>0.08 (17)</td>
<td>426 (59.7)</td>
<td>25.1</td>
</tr>
<tr>
<td>Mono Q</td>
<td>7.5</td>
<td>0.44 (3.3)</td>
<td>375 (52.3)</td>
<td>113.6</td>
</tr>
<tr>
<td>Mono S</td>
<td>7.0</td>
<td>0.13 (0.9)</td>
<td>286 (39.9)</td>
<td>316.7</td>
</tr>
<tr>
<td>CaM-Sepharose</td>
<td>1.5</td>
<td>0.043 (0.065)*</td>
<td>41 (5.7)</td>
<td>630.8</td>
</tr>
</tbody>
</table>

*Protein determination based on densitometry of SDS gels, with bovine serum albumin as standard.

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Overall recovery of activity. Similar results have been obtained for four preparations of the kinase. The poor recovery of activity from the CaM-Sepharose step is not likely to be due to removal of some required factor, since no stimulation of activity was seen when the kinase was recombined with the other column fractions. One possible reason for the low recovery is denaturation of the enzyme during and after the removal of some required factor, since no stimulation of other column fractions. One possible reason for the low recovery of activity within 1 day unless it is immediately concentrated in solid sucrose. After concentration of the kinase, the activity is stable for several days on ice. The kinase purified through the Mono S step can be stored on ice with full retention of activity for at least 1 month. The enzyme is inactivated by freezing and thawing.

**Structure and Properties of Brush Border Myosin Heavy Chain Kinase**—Myosin heavy chain kinase isolated as described above contained a single major polypeptide, as shown by gel electrophoresis in the presence of SDS (Fig. 5, lane a). Comparison with proteins of known M, gave an estimated M, of 50,000 for this polypeptide (see Fig. 5 and Table II). The 50-kDa polypeptide was phosphorylated upon incubation of the CaM-Sepharose pool under our standard kinase assay conditions, as shown in Fig. 5, lane c. The extent of phosphorylation after 60 min was about 1.3 mol of phosphate/mol of 50-kDa polypeptide. The results in Fig. 5, lanes e-f, show that this phosphorylation is highly stimulated by calcium and CaM and that only a trace amount of phosphorylation occurred in the presence of EGTA or calcium alone. The 50-kDa polypeptide is likely to be the catalytic subunit of the kinase because it co-eluted with the CaM-dependent myosin heavy chain kinase activity at each column step of the kinase purification (Figs. 1-4, inset). There was also an approximate correlation between the amount of CaM-dependent myosin heavy chain kinase and the amount of 32P-labeled 50-kDa polypeptide for each of the columns (data not shown).

The molecular weight of the enzyme was estimated from its hydrodynamic properties (Table II). The Stokes radius of the kinase, as determined by gel filtration on Sepharose CL-4B (Fig. 1) and Superose 6 (see Fig. 1, Ref. 24), was 68 Å; and the sedimentation coefficient (s20,w) as determined by sucrose density gradient centrifugation, was 17.5 S. From these values, a molecular weight of 490,000 and a frictional coefficient (f/f₀) of 1.31 were calculated for the kinase, as described in Table II. The kinase is therefore a large, multisubunit enzyme composed of about 10 copies of the 50-kDa polypeptide. The kinase is somewhat asymmetric, with an axial ratio calculated for a prolate ellipsoid of 6.1 (34).

**Regulation of Myosin Heavy Chain Kinase by Calcium and Calmodulin**—The dependence of the phosphorylation of brush border myosin by the purified kinase on calcium and CaM is shown in Figs. 5 and 6. The brush border myosin used in these studies was purified by a new procedure described under overall recovery of activity. Similar results have been obtained for four preparations of the kinase. The poor recovery of activity from the CaM-Sepharose step is not likely to be due to removal of some required factor, since no stimulation of activity was seen when the kinase was recombined with the other column fractions. One possible reason for the low recovery is denaturation of the enzyme during and after the removal of some required factor, since no stimulation of other column fractions. One possible reason for the low recovery of activity within 1 day unless it is immediately concentrated in solid sucrose. After concentration of the kinase, the activity is stable for several days on ice. The kinase purified through the Mono S step can be stored on ice with full retention of activity for at least 1 month. The enzyme is inactivated by freezing and thawing.

![Fig. 5. SDS-polyacrylamide gel electrophoretic patterns and autoradiographs of brush border myosin phosphorylated by the brush border CaM-dependent myosin heavy chain kinase.](image-url)
The figure shows the total phosphate incorporation into heavy and phosphorylation is most probably catalyzed by another kinase the heavy chain kinase since it is not dependent on calcium about 0.04 mol of phosphate/mol of myosin was incorporated into myosin heavy chain kinase, CaM-dependent myosin heavy chain kinase.

The light chains of rabbit skeletal myosin were phosphorylated in a calcium- and CaM-dependent manner to about 0.7 mol of phosphate/mol of heavy chain after 2 h of incubation.

**Specificity for Myosins**—The specificity of the brush border CaM-dependent myosin heavy chain kinase for myosins from several sources was studied. Phosphorylation of rabbit and chicken skeletal myosins, rat cardiac myosin, and chicken gizzard smooth muscle myosin was determined under the standard assay conditions and 37 μg/ml of myosin. The heavy chains of rabbit skeletal myosin were phosphorylated in a calcium- and CaM-dependent manner to about 0.7 mol of phosphate/mol of heavy chain after 2 h of incubation. The phosphorylation of rabbit skeletal myosin occurred at a rate approximately 10% that of phosphorylation of brush border myosin. Rabbit skeletal myosin was not phosphorylated in the absence of added kinase. Neither phosphorylation of the light chains of rabbit skeletal myosin nor of the heavy or light chains of the other myosins was observed after 2 h of incubation.

Studies with rabbit skeletal muscle myosin subfragments were conducted to determine whether the brush border kinase phosphorylates the head or tail portion of this myosin. Incubation of rabbit skeletal myosin, heavy meromyosin, or light meromyosin at a concentration of 37 μg/ml under the standard assay conditions resulted in phosphorylation of myosin and light meromyosin at the rate of about 60 pmol/min/mg in a calcium- and CaM-dependent manner. In contrast, no phosphorylation of heavy meromyosin occurred in a 2-h incubation. Therefore, the site of phosphorylation of rabbit skeletal myosin must be located within the tail.

**DISCUSSION**

The myosin heavy chain kinase described in this report from chicken intestinal epithelial cell brush borders is the first heavy chain kinase found to be activated by calcium and CaM and the first to be highly purified from a vertebrate tissue. In the brush border extract, the CaM-dependent enzyme represents the large majority of myosin heavy chain kinase activity. In addition, a lower molecular weight, calcium- and CaM-dependent myosin heavy chain kinase is also present (see Fig. 1 and Ref. 24). Although it is possible that the CaM-independent enzyme is derived from CaM-dependent myosin heavy chain kinase by proteolysis or auto-phosphorylation (see below), no conversion of CaM-dependent kinase to a CaM-independent form has been observed during purification or storage. CaM-dependent myosin heavy chain kinase would not have been detected in earlier studies in other cell types because myosin heavy or light chain kinases were identified and isolated based on their ability to phosphorylate either myosin in the absence of calcium and CaM, or isolated light chains.

The CaM-dependent myosin heavy chain kinase is a mul-
tisubunit complex with a molecular weight of about 490,000. It contains one major polypeptide of 50 kDa that is most likely to be the catalytic subunit, since it co-purifies with the kinase through each fractionation step, including affinity chromatography on CaM-Sepharose. The brush border kinase is thus postulated to be composed of about 10 subunits. It has several properties, in addition to regulation by calcium and CaM, in common with the type II CaM-dependent kinases. These enzymes have been purified from brain, liver, skeletal muscle, and other vertebrate tissues based on their ability to phosphorylate synapin I, microtubule-associated protein 2, glycogen synthetase, or other proteins (reviewed in Ref. 22). The native and subunit molecular weights of the brush border kinase are within the respective ranges of 500,000–800,000 and 50,000–60,000 reported for the type II kinases. Type II kinases have recently been shown to undergo conversion to a calcium- and CaM-independent form by CaM-dependent autophosphorylation (36–38). The brush border kinase described here is phosphorylated in a CaM-dependent manner upon incubation with Mg$^{2+}$ and ATP. We do not yet know whether the phosphorylation of the kinase is due to an autocatalytic reaction or to phosphorylation by another CaM-dependent kinase possibly present, but undetected, in the kinase preparation, or what effect the phosphorylation has on the kinase. Chemical and immunological comparisons of the brush border CaM-dependent myosin heavy chain kinase with the type II CaM-dependent protein kinases are in progress.

An intriguing possibility raised by these results is that myosin heavy chain phosphorylation in other tissues is catalyzed, at least in part, by the type II kinases and is thus subject to regulation by calcium and CaM. The finding that CaM-dependent protein kinase II from bovine brain phosphorylates the heavy chains of brain myosin (39) suggests that heavy chain phosphorylation in this tissue may be CaM-dependent. One difference between the results reported for brain and brush border is that, whereas the brush border kinase phosphorylated only the heavy chains of brush border and skeletal myosins and did not phosphorylate the light chains of other myosins tested, the brain kinase phosphorylated both the heavy and light chains of brain myosin. However, phosphorylation of the light chains occurred at a much lower rate than that of the heavy chains and may have been due to the possible presence of CaM-dependent myosin light chain kinase. Type II kinases from other tissues have not been tested for their ability to phosphorylate myosin. Some have been reported to phosphorylate the isolated LC$_{20}$s of gizzard myosin (40–42), but, as has been found for some kinases (1, 43, 44), this does not necessarily indicate that they can phosphorylate the light chains in native gizzard or other myosins. Our finding that rabbit skeletal muscle myosin can be phosphorylated on its heavy chains by the brush border kinase may only be a reflection of structural similarities in the heavy chains of the myosins, since there is no evidence that heavy chain phosphorylation occurs in skeletal muscle.

The brush border CaM-dependent myosin heavy chain kinase catalyzed incorporation of up to 0.8 mol of phosphate/mol of heavy chain of brush border myosin. For several myosins, the heavy chain phosphorylation site(s) have been localized very near the tip of the 150-nm-long, α-helical coiled coil tail of the myosin molecule (45–48). Studies to characterize the heavy chain phosphorylation site(s) of brush border myosin are in progress. For rabbit skeletal myosin, phosphorylation by the brush border kinase was shown to occur in the tail portion of the molecule.

The effects of heavy chain phosphorylation on brush border and other vertebrate non-muscle myosins have not been established. Also, the effects of heavy chain phosphorylation may vary for myosins from different tissues, as do the effects of light chain phosphorylation catalyzed by CaM-dependent myosin light chain kinase (48–50). The effects of heavy chain phosphorylation may also depend on the state of phosphorylation of the myosin light chains. Heavy chain phosphorylation of myosins from the lower eucaryotes Dictostelium (6, 51) and Acanthamoeba (7, 52) has been shown to inhibit their actin-activated ATPase activity and assembly into bipolar filaments. These effects have been shown to be mediated by changes in the supramolecular structure of the myosin filament in the case of Acanthamoeba myosin (53). Some evidence suggests that heavy chain phosphorylation may also inhibit the actin-activated ATPase activity of leucemic myeloblast myosin (4), but not of brain myosin (39). Studies conducted under a limited set of conditions, using either a partially purified preparation of the brush border kinase (20) or heavy chain kinase purified as described in this paper, have shown no effect of heavy chain phosphorylation on brush border myosin ATPase activity. However, more extensive studies under a range of conditions that vary in parameters that strongly affect myosin ATPase activity and filament assembly, including Mg$^{2+}$ concentration, pH, and ionic strength (see Ref. 54), may be required to reveal the possible effects of heavy chain phosphorylation on brush border myosin. Heavy chain phosphorylation may regulate other properties of brush border myosin, including its molecular conformation and association with actin and other cytoskeletal proteins. We are currently beginning comprehensive studies of the effects of both heavy and light chain phosphorylation on brush border myosin.

Acknowledgment—We thank Judith Montibeller for expert technical assistance in many of the experiments.

REFERENCES

Brush Border Calmodulin-dependent Myosin Heavy Chain Kinase