The motor function of smooth muscle myosin is activated by phosphorylation of the regulatory light chain (RLC) at Ser\(^{19}\). However, the molecular mechanism by which the phosphorylation activates the motor function is not yet understood. In the present study, we focused our attention on the role of the central helix of RLC for regulation. The flexible region at the middle of the central helix (Gly\(^{95}\)-Pro\(^{98}\)) was substituted or deleted to various extents, and the effects of the deletion or substitution on the regulation of the motor activity of myosin were examined. Deletion of Gly\(^{95}\)-Asp\(^{97}\), Gly\(^{95}\)-Thr\(^{96}\), or Thr\(^{96}\)-Asp\(^{97}\) decreased the actin-translocating activity of myosin a little, but the phosphorylation-dependent regulation of the motor activity was not disrupted. In contrast, the deletion of Gly\(^{95}\)-Pro\(^{98}\) of RLC completely abolished the actin translocating activity of phosphorylated myosin. However, the unregulated myosin long subfragment 1 containing this RLC mutant showed motor activity the same as that containing the wild type RLC. Since long subfragment 1 motor activity is unregulated by phosphorylation, i.e. constitutively active, these results suggest that the deletion of these residues at the central helix of RLC disrupts the phosphorylation-mediated activation mechanism but not the motor function of myosin itself. On the other hand, the elimination of Pro\(^{98}\) or substitution of Gly\(^{95}\)-Pro\(^{98}\) by Ala resulted in the activation of actin translocating activity of dephosphorylated myosin, whereas it did not affect the motor activity of phosphorylated myosin. Together, these results clearly indicate the importance of the hinge at the central helix of RLC on the phosphorylation-mediated regulation of smooth muscle myosin.

The motor activity of vertebrate smooth muscle as well as non-muscle cell myosin is regulated by phosphorylation of the 20,000 dalton light chain (LC20) of myosin (1–5). While the phosphorylation of myosin at LC20 occurs at several sites which are catalyzed by various protein kinases, the activation effect is rather specific to the site of phosphorylation, and only the phosphorylation at serine 19 and/or threonine 18 can activate the motor activity of myosin (6–8). Recent three-dimensional structural analysis of the skeletal muscle myosin head domain greatly facilitated the understanding of the structure-function relationship of the myosin motor (9). It was revealed that the ATPase active site and actin binding site, two functionally essential regions of the myosin motor, exist toward the top of the myosin head while the regulatory light chain (i.e. LC20) associates with the myosin heavy chain at the lower end of myosin head, i.e. adjacent to the head-rod junction. This structural feature indicates that it is unlikely that the regulation is achieved by the direct interaction between the motor effector sites and the phosphorylation site. This leads to the hypothesis that the change in the conformation of LC20 induced by phosphorylation is transmitted to the motor effector sites via intersubunit communication that would be critical for the regulation of myosin motor activity (10). While the detail of such a mechanism is not yet understood, some progress toward this problem has been made. It has been shown using various probes that phosphorylation of LC20 changes the conformation at the head-rod junction of myosin (10–13). It was shown recently that the two-headed structure is critical for the phosphorylation-mediated regulation of myosin motor activity (14–16). Furthermore, it was found that the chimeric myosin which consisted of the skeletal globular motor domain, smooth muscle light chain-associated domain and S2, is completely regulated by phosphorylation (17), suggesting that the regulatory domain solely confers the phosphorylation-dependent regulation. Based on these findings, it is plausible that the interaction between the two myosin heads at the C-terminal regulatory domain of S1 is altered by phosphorylation that is critical for regulation. The question is how the phosphorylation changes the conformation of RLC (regulatory light chain), thus activating the myosin motor activity. Using myosin molecules containing the chimeric or mutated LC20, it was revealed that: 1) the negative charge introduced by the phosphate moiety is important for the activation of myosin motor activity because the substitution of Ser\(^{19}\)/Thr\(^{19}\) of LC20 by the acidic residues partially mimicked the phosphorylation-induced activation of myosin motor activity (18–19); and 2) the C-terminal domain of LC20 is critical for both heavy chain binding and phosphorylation-dependent regulation because an N-terminal smooth RLC/C-terminal skeletal RLC chimera failed to activate myosin motor activity by phosphorylation (20), the deletion of the C-terminal residues diminished the binding to the heavy chain (21–22), and the deletion or substitution of the C-terminal residues of LC20 disrupted the phosphorylation-dependent activation of smooth muscle myosin (21). These results suggest that the
phosphorylation at the N-terminal region of RLC influences the conformation at the RLC-heavy chain interface at the C-terminal domain of RLC and thus achieves the regulation of myosin motor function. For calmodulin, it has been suggested that there is a cross-talk between the N- and C-terminal domains, and the long central helix which connects the two domains plays a role on the cross-talk. Since three-dimensional structure of RLC is homologous to calmodulin (9), it is plausible that the long central helix of RLC also plays a role on the cross-talk between the N- and C-terminal domains of RLC, thus involved in regulation of smooth muscle myosin motor activity.

In the present study, we produced a series of mutant LC20s in which the hinge region at the middle of the long central helix of LC20 is modified. The motor activity and the phosphorylation dependence of myosin containing these mutant LC20s were examined. The results clearly indicate that the hinge region at the central helix of LC20 plays a critical role for the phosphorylation-induced regulation of myosin motor activity.

**EXPERIMENTAL PROCEDURES**

**Materials**—Restriction enzymes and modifying enzymes were purchased from New England Biolabs (Beverly, MA). The P7−7 E. coli expression vector containing the T7 promotor (23) was provided by Dr. S. Tabor (Harvard Medical School). Smooth muscle myosin was prepared from frozen turkey gizzards as described (24). Actin was prepared from rabbit skeletal muscle actomyosin powder according to Spudich and Watt (25). Smooth muscle myosin light chain kinase was prepared from frozen turkey gizzards (26). Recombinant calmodulin was expressed in E. coli and prepared as follows. Calmodulin cDNA from Xenopus oocytes (27) in the pTnCo2 vector was kindly supplied by Dr. C. Klee (National Institutes of Health). Two oligonucleotide primers, 5′-GAA ATC TTA CAT ATG GCT GAC CAA CTG ACA G3′, containing an NdeI site as well as an initiation codon, and 5′-TAC CGC AAG CGA CAG GCC G3′, downstream of the polylinker region of the vector, were synthesized and used for polymerase chain reaction amplification of calmodulin cDNA. The amplified cDNA was digested with NdeI and HindIII and subcloned into P7−7 using unique NdeI and HindIII sites at the polylinker region. BL21(DE3) was transformed with the calmodulin expression vector and then cultured in LB medium supplemented with 50 μg/ml ampicillin overnight at 37 °C. 0.1 mM isopropyl-β-D-thiogalactopyranoside was added to the liquid culture, and the cells were harvested 10 h later. The packed cells (3 g) were sonicated in buffer containing 8M urea, 5 mM DTT, 10 mM imidazole-HCl, pH 7.0, and then centrifuged at 35,000 g for 15 min at 4 °C, and 5% trichloroacetic acid was added to the supernatant. The precipitates were collected by centrifugation for 15 min at 4 °C, and then the precipitates were dissolved in 50 mM Tris-HCl, pH 7.5. The fractions containing calmodulin were determined by SDS-PAGE analysis, concentrated, and subjected to Sephacryl S200 gel filtration chromatography (3 cm × 90 cm) in buffer containing 10 mM NaCl, 1 mM DTT, and 30 mM Tris-HCl, pH 7.5. The fractions containing calmodulin were collected, dialyzed against buffer B (30 mM NaCl, 1 mM DTT, and 30 mM Tris-HCl, pH 7.5), and stored at −80 °C.

**Production of the Mutant LC20**—The mutant cDNAs were made by the site-directed mutagenesis strategy as described previously using the LC20 expression vector pT7-LC20 as a template (18, 21). PT7-LC20 does not contain a tag sequence, to avoid having artificial amino acid residues in the expressed recombinant LC20. After confirming the mutation by direct sequencing analysis, the mutant LC20 expression vectors were transferred to BL21(DE3). The expression and purification of the mutant LC20s was done as described previously (21).

**Incorporation of the Mutant LC20s into Myosin**—The regulatory light chain was removed from myosin according to Trybus et al. (22) with the following modification. Myosin (5 mg/ml) was incubated with buffer C containing 0.4 mM NaCl, 5 mM EDTA, 2 mM EGTA, 3 mM Na3PO4, 10 mM DTT, 5 mM TFP, 0.1% Triton X-100, 1.5 mM ATP, and 20 mM imidazole-HCl, pH 7.0, for 1 h at 0 °C and then applied to a Sephrose CL4B column (3 × 90 cm) equilibrated with buffer D containing 0.4 mM NaCl, 5 mM EDTA, 2 mM EGTA, 3 mM Na3PO4, 0.5 mM ATP, 1 mM DTT, and 20 mM imidazole-HCl, pH 7.0. The myosin-containing fractions were combined and a three-fold molar excess of LC20 was added to them. The mixture was dialyzed for 90 min against buffer F containing 0.4 mM NaCl, 1 mM EGTA, 5 mM MgCl2, 0.2 mM ATP, 1 mM DTT, and 10 mM imidazole-HCl, pH 7.0. The mixture was further dialyzed for 2 h against buffer G containing 15 mM MgCl2, 2 mM DTT, and 20 mM Tris-HCl, pH 7.5, and then centrifuged for 2 min at 10,000 × g. The precipitates were washed twice with buffer D and then dissolved with buffer G (0.3 mM KCl, 5 mM DTT, and 50 mM Tris-HCl, pH 7.5). To prepare myosin containing the mutant LC20, the purified LC20s were thio phosphorylated before adding to the LC20-deficient myosin. The extent of phosphorylation was monitored by urea-gel electrophoresis (29), and complete phosphorylation of LC20 was confirmed (not shown).

**Expression of the Recombinant Truncated Smooth Muscle Myosin**—The baculovirus transfer vectors of smooth muscle heavy chain and light chains were produced as described (16). Recombinant baculovirus for the heavy chain and the light chains were produced according to the protocols described by O’Reilly et al. (30). To express truncated smooth muscle myosin, S9 insect cells were infected with three separate viruses expressing the heavy chain and two light chains. The recombinant smooth muscle myosin was purified as described previously (16).

**Determination of Myosin Motor Function**—Actin-activated ATPase activity was measured at 25 °C in an assay mixture containing 0.1 mg/ml myosin, 10 mM MgCl2, 30 mM KCl, 1 mM EGTA, 0.2 mM ATP, and 30 mM Tris-HCl, pH 7.5 with or without 5 mg/ml F-actin. The ATPase activity of myosin or actomyosin was determined by measuring the liberated 32P as described previously (24). SDS-PAGE was carried out on 7.5–20% polyacrylamide gradient slab gels by using the discontinuous buffer system of Laemmli (31).

**Actin-translocating velocity was measured by an in vitro motility assay as described previously (16). The Student’s t test was used for statistical comparison of mean values. A value of p < .01 was considered to be significant.**

**Analytical Ultracentrifugation**—Sedimentation velocity analysis was performed at 20 °C on a Beckman model-E analytical ultracentrifuge. Sedimentation patterns were acquired with the on-line Rayleigh system and converted into concentration versus radius every 20 s. The camera lens was focused at the 2/3 plane of the cell equipped with a sapphire windows. The apparent sedimentation coefficient distribution function were computed as described by Stafford (33, 34).

**RESULTS**

**Hybridization of Smooth Muscle Myosin with Exogenous LC20**—The hybrid myosin containing various mutant LC20

**Table I**

| LC20 mutant | Dephosphorylated + Actin | Dephosphorylated − Actin | Phosphorylated + Actin | Phosphorylated − Actin |
|-------------|--------------------------|--------------------------|------------------------|------------------------|
| Wild        | 6.5 ± 1.7                | 3.1 ± 1.1                | 3.4 ± 0.9              | 60.3 ± 7.1             |
| GTPD/AAAA   | 13.0 ± 3.2               | 6.6 ± 1.7                | 6.5 ± 1.6              | 73.0 ± 2.3             |
| ΔGTPD       | 22.5 ± 3.5               | 11.8 ± 4.3               | 10.7 ± 5.0             | 35.6 ± 7.7             |
| ΔGTD        | 13.2 ± 2.3               | 4.7 ± 0.7                | 8.5 ± 1.6              | 61.8 ± 15.9            |
| ΔTD         | 8.1 ± 0.0                | 4.0 ± 1.6                | 4.0 ± 1.6              | 55.8 ± 11.6            |
| ΔP          | 20.1 ± 2.6               | 5.9 ± 1.0                | 14.2 ± 3.6             | 64.3 ± 4.7             |

**Mg2+-ATPase activity in nmol/mg · min**

The activities were measured with three independent preparations of myosin. All data are presented as mean activity ± S.D. ATPase activity was measured as described under “Experimental Procedures.”
was prepared by adding exogenous LC20 to the LC20-deficient myosin. The exogenous LC20 was properly bound to the LC20-deficient myosin at the LC20 binding site based on the following findings. 1) The stoichiometry of the heavy chain and LC20 in the reconstituted myosin was identical to that of naturally isolated smooth muscle myosin based upon gel densitometry analysis (data not shown, see also Ref. 16). 2) The reconstituted myosin with wild type LC20 showed phosphorylation-dependent actin-activated ATPase activity and actin-translocating activity, which were indistinguishable to naturally isolated myosin (Table I, see Fig. 2).

Six mutant LC20s were produced in which the four amino acid residues corresponding to the hinge region at the middle of the central helix of LC20 were deleted or substituted to various extents (Fig. 1). These mutant LC20s were introduced to the LC20-deficient myosin as described above. All mutant LC20s bound stoichiometrically to myosin heavy chain as judged by gel densitometry (not shown). To produce phosphorylated myosin, LC20s were first thiophosphorylated with ATP in the presence of Ca²⁺/calmodulin/MLC kinase to avoid possible dephosphorylation during the preparations and then introduced to the LC20-deficient myosin. For all LC20 mutants tested in this study, the rate of phosphorylation was indistinguishable from that of wild type LC20 (data not shown). The LC20s were completely phosphorylated as judged by the mobility shift due to the phosphate group in urea-gel electrophoresis (not shown, see also Ref. 21).

Actin-activated ATPase Activity of Smooth Muscle Myosin Containing LC20 Mutants—The actin-activated ATPase activity of myosins was determined in the presence of 5 mg/ml F-actin, in which the extent of actin activation was saturated (maximum ATPase activity). The actin-activated ATPase activity of myosin, containing phosphorylated ΔP in which Pro⁹⁸ was deleted, and GTDP/AAAA, in which all four residues were substituted by Ala, were practically the same as that of myosin containing wild type LC20 (Table I), whereas the myosins containing other phosphorylated LC20 mutants showed lower ATPase activity (Table I). The decrease in the activity was moderate for ΔTD and ΔGT and more extensive for ΔGTDP and ΔGT. These results suggest that the hinge at the central helix of LC20 is involved in the phosphorylation-induced activation mechanism of the actomyosin ATPase activity. On the other hand, the actin-activated ATPase activity of dephosphorylated myosin containing mutant LC20s was higher than that of myosin containing wild type LC20, particularly for ΔGTDP and ΔP (Table I).

Actin Sliding Velocity—The effect of the mutation of LC20 at the hinge of the central helix on myosin motor function was evaluated more directly by using an in vitro motility assay. Myosin containing wild type LC20 showed completely phosphorylation-dependent actin translocating activity, and an actin sliding velocity of 0.67 ± 0.04 μm/s at 25 °C was obtained for phosphorylated LC20, which is comparable with the value previously obtained for naturally isolated smooth muscle myosin (16). While myosin containing GTDP/AAAA, ΔP, or ΔTD showed virtually the same phosphorylation-activated actin-translocating activity, the activity was significantly lower for the myosins containing ΔGTD and ΔGT (Fig. 2). The most striking effect was observed for ΔGTDP-containing myosin in which actin-translocating activity was completely abolished (Fig. 2). There are two possibilities to account for this observation. First, the deletion of the four residues at the hinge of LC20, i.e. ΔGTDP, disrupts the motor function of myosin. Second, the mutation does not abolish the motor activity itself but disrupts the phosphorylation-dependent activation mechanism. To distinguish between these two possibilities, we util-
lized a smooth muscle myosin mutant having phosphorylation-independent, constitutively active motor activity. Previously, we produced truncated mutants of smooth muscle myosin containing various lengths of the S2 portion and showed that the monomeric myosins have phosphorylation-independent motor activity while the dimeric (double-headed) myosins have the phosphorylation-dependent activity (16). Fig. 3 shows the actin translocating activity of the 108-kDa truncated smooth muscle myosin (a monomeric myosin). The 108-kDa myosin containing ΔGTDP showed motor activity indistinguishable from that of the myosin containing wild type LC20. Virtually the same results were obtained for the 108-kDa myosin containing phosphorylated ΔGTDP and phosphorylated wild type LC20 (not shown). The results clearly indicate that the deletion of the four residues at the hinge of the central helix of LC20 disrupts the phosphorylation-induced activation process of smooth muscle myosin motor activity but does not diminish the myosin motor activity itself (Fig. 3). The result suggests that ΔGTDP cannot support the activation by phosphorylation when bound to myosin heavy chain and is thus trapped in the off state. To further evaluate this possibility, in vitro motility assay was performed using the mixture of phosphorylated myosin and ΔGTDP-containing myosin (Fig. 4). The significant reduction of the velocity was not found until the majority myosin was a ΔGTDP-containing one. Previously, Cuda et al. (35) reported that more than 60% of dephosphorylated myosin is required until significant reduction of the velocity occurs. The present results are consistent with the earlier observation and suggest that ΔGTDP light chain traps phosphorylated myosin in the off state. It should be noted that the observed actin translocating velocity for the 108-kDa myosin (single-headed) was approximately 40% of that of the full-length myosin (Fig. 3). This is consistent with the previous finding (16) and suggests that the mechano-chemical activity is affected by the interaction between the two heads. Another important finding is that the myosins containing GTDP/AAAA or ΔP showed significant phosphorylation-independent actin translocating activity (Fig. 5). The actin sliding velocity of the dephosphorylated myosins containing these two mutants was approximately 30% of that of

Fig. 2. Sliding velocity of actin filaments on smooth muscle myosins containing various phosphorylated mutant RLCs. Actin movement was observed in 30 mM KCl, 5 mM MgCl₂, 25 mM imidazole-HCl, pH 7.5, 1 mM EGTA, 1% 2-mercaptoethanol, 0.5% methylcellulose, 4.5 μg/ml glucose, 216 μg/ml glucose oxidase, 36 μg/ml catalase, and 2 mM ATP at 25 °C. Measurements were made with three independent preparations, and 11–21 actin filaments were measured to obtain an average velocity for each preparation. All values are mean velocity ± S.D.

Fig. 3. Sliding velocity of actin filaments on smooth muscle long S1 containing either wild RLC or ΔGTDP. Actin movement was observed as described in Fig. 3, except a monoclonal antibody (mm9) which recognizes the S2 portion (Ala⁷⁷⁵-Ser⁹⁴⁴) of myosin was used to bind the long S1 to the nitrocellulose surface as described previously (16).
the phosphorylated myosins. Myosin containing the dephosphorylated GTDP/AAAA or ΔP light chain supported continuous actin filament movement and majority of the filaments moved. This result suggests that the inhibitory activity of dephosphorylated LC20 is diminished by mutation at the hinge of the central helix of LC20.

The Effect of the Mutation at the Hinge of the Central Helix of LC20 on Myosin Conformation—It has been known that smooth muscle/non-muscle myosins exist in two interchangeable conformations, a folded conformation and an extended conformation (36–38) that is directly related to the filament forming ability of myosin (39, 40). The equilibrium between the two conformations is sensitive to ionic strength (41) and phosphorylation of LC20 (38, 41–43). The conformational transition of myosins containing various mutant LC20s was examined using analytical ultracentrifugation. Sedimentation patterns for myosin were measured at 0.2 and 0.4 M KCl (Fig. 6). The sedimentation pattern of myosins containing various mutant LC20s revealed a single symmetric peak under both conditions. The sedimentation coefficient (\(s_{20,w}\) value) of myosin containing dephosphorylated wild type LC20 increased from 6.81 to 9.89 with decreasing KCl concentration, which is consistent with the previous reports (36, 39, 41) indicating that myosin forms an extended and a folded conformation (36–38) at 0.4 M KCl and 0.2 M KCl, respectively. While the sedimentation coefficient of myosins containing various LC20 mutants did not differ from each other at 0.4 M KCl, the values at 0.2 M KCl were significantly different among the myosins containing different LC20 mutants. The sedimentation velocities of the myosins containing dephosphorylated ΔGTDP, ΔP, and GTDP/AAAA were 6.6 ± 0.05 s, 7.3 ± 0.05 s, and 6.5 ± 0.05 s, which indicated that myosin having these mutant LC20s failed to form a folded conformation. On the other hand, the sedimentation constants of the myosins containing ΔGT and ΔTD were similar to that of the myosin having wild type LC20 (Fig. 6).

FIG. 5. Activation of sliding velocity of actin filaments on smooth muscle dephosphorylated myosin by disruption of the hinge at the central helix of RLC. Measurements were made with three independent preparations, and 11–20 actin filaments were measured to obtain an average velocity for each preparation. All values are mean velocity ± S.D.

FIG. 6. Apparent sedimentation coefficient distributions for myosins having various mutant LC20s. Sedimentation velocity was determined in solution containing 1 mM MgCl₂, 1 mM ATP, and 30 mM Tris·HCl, pH 7.5, 0.5 mg/ml myosin containing various LC20 mutants, and either 0.2 M KCl (A) or 0.4 M KCl (B). The velocity run was carried out at 56,000 rpm at 20 °C.

DISCUSSION
While it is well known that the phosphorylation at serine 19 of LC20 activates smooth muscle or non-muscle myosin motor activity, it is not clear how the phosphorylation can activate the motor activity at a molecular level. According to the three-dimensional structural analysis of skeletal muscle S1, the regulatory light chain consists of two domains that are segregated by a long central α-helix (9). We hypothesized that the central long α-helix of LC20 plays a role in the phosphorylation-mediated regulation mechanism. Particularly, we focused our attention on the middle of the central helix where the helix is
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distorted (Fig. 1). To verify our hypothesis, we produced six mutant LC20s in which the four amino acid residues (Gly-Thr-Asp-Pro) corresponding to the hinge of the long central α-helix of LC20 were modified (Fig. 1). We found that the change in the structure of the hinge region significantly altered the motor function of smooth muscle myosin. The most notable findings were: 1) the deletion of all four residues (ΔGTDP) abolished the activation effect of phosphorylation on myosin motor activity; and 2) two mutant LC20s in which the four residues were substituted for Ala (GTDP/AAA) or a proline residue in the hinge was deleted (ΔP) conferred the phosphorylation-independent motor activity on smooth muscle myosin, i.e. the inhibitory function of dephosphorylated LC20 was diminished. These findings clearly show that the hinge region of the central helix of LC20 is critical for phosphorylation-mediated regulation of smooth muscle myosin. It is expected that the deletion or substitution of the proline residue at the hinge would eliminate the distortion at the middle of the long α-helix; therefore, the results obtained in the present work suggest that the flexed nature of the central shaft of the LC20 is important for phosphorylation-mediated regulation of myosin motor activity. Previously, it was reported that the C-terminal domain of LC20 is responsible for the binding of LC20 to the heavy chain (21). Furthermore, the deletion of the C-terminal residues of LC20 abolishes the phosphorylation-mediated regulation of smooth muscle myosin despite the fact that the phosphorylation site is located in the N-terminal domain (21, 22). This raised the hypothesis that there is an interaction between the N- and the C-terminal domains of LC20, which is a critical component for the regulation of smooth muscle myosin motor function (21). The present results are consistent with this notion and suggest that the flexed region in the central helix plays a critical role in the phosphorylation-dependent interaction between the N- and the C-terminal domains.

LC20 shares considerable structural homology with calmodulin and tropomyosin C (9, 44, 45) in which the two domains are linked with a long central connecting α-helix. It has been shown that the deletion of several amino acid residues at the central helix of calmodulin significantly disrupts the activation of target enzymes by calmodulin (46), suggesting the importance of the central connecting linker region in calmodulin function. The importance of the central helix of calmodulin in its function is supported by analyzing the three-dimensional structure of the calmodulin/MLC kinase peptide complex. Upon formation of the ternary complex with the calmodulin binding peptide in the presence of Ca\(^{2+}\), the long central helix of calmodulin is disrupted into two helices connected by a flexible loop (47, 48). In the case of myosin, the N-terminal domain of LC20 wraps around the C terminus of the S1 heavy chain while the C-terminal domain of LC20 interacts with the long helix portion of the S1 heavy chain (9). Therefore, it is plausible that the change in the distortion at the hinge of the central helix of LC20 would influence the bend at the C-terminal end of S1 heavy chain. In scallop myosin, in which the myosin function is regulated by the Ca\(^{2+}\) binding to the essential light chain, a change in the bend at the C-terminal end of S1 by Ca\(^{2+}\) binding is observed (49). Consistent with this idea, the substitution of LC20 by ΔGTDP, in which the flex at the hinge region of the central helix is disrupted, abolishes the formation of a folded structure (Fig. 6) that is likely to be related to the bend at the C-terminal end of S1.