Homodimerization through coiled-coil regions enhances activity of the myotonic dystrophy protein kinase

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Abstract **Myotonic dystrophy protein kinase (DMPK) is the protein product of the human *DM-1* locus on chromosome 19q13.1 and has been implicated in the cardiac and behavioral dysfunctions of the disorder. DMPK contains four distinct regions: a leucine-rich repeat (L), a serine-threonine protein kinase catalytic domain (PK), an α-helical coiled-coil region (H), and a putative transmembrane-spanning tail (T). Multiple protein kinases that participate in cytoskeletal and cell cycle functions share homology with DMPK in the PK and H regions. Here we show that the LPKH and PKH subfragments of DMPK formed dimers of 140 000 molecular weight, whereas the LPK subfragment remained a monomer of 62 000 apparent molecular weight. The H domain thus appeared to be required for dimerization of DMPK subfragments. Caspase 1 cleaved LPKH between the PK and H regions. After cleavage, LPKH dimers became LPK-like monomers, consistent with the H domain mediating dimerization. The V_{max} and k_{cat}/K_m of LPKH with a synthetic peptide kinase substrate were over 10-fold greater than either LPK or caspase-cleaved LPKH. The K_m of dimeric LPKH was over three-fold greater than those of the monomeric proteins. Dimerization appeared to significantly affect the catalytic efficiency and substrate binding of DMPK. These interactions are likely to be functionally significant in other members of the myotonic dystrophy family of protein kinases with extensive coiled-coil domains.**

Key words: Myotonic dystrophy; Protein kinase; Dimer; Coiled-coil; Activity

1. Introduction

Myotonic dystrophy protein kinase (DMPK) is the protein product encoded by the *DM-1* locus on human chromosome 19q13.3 in which expansion mutations of a GCT repeat produce myotonic dystrophy type 1 (DM-1) [1]. Several isoforms may be produced by alternative splicing, primarily affecting the expression of the carboxy-terminal region and putative transmembrane-spanning (T) domain [2]. Although the myotonia and insulin resistance of DM-1 may be explained by gain-of-function effects at the mRNA level unrelated to DMPK expression [3–6], haploinsufficiency of DMPK expression has been demonstrated in material from affected patients [7–9]. Furthermore, knockout mice null for DMPK expression show the cardiac arrhythmias that are life-threatening in human DM-1 patients and altered synaptic plasticity in the hippocampus that is potentially related to the cognitive deficits and mental retardation seen in DM-1 [10–13]. The cardiac arrhythmias appear in mice heterozygous for the DMPK knockout suggesting that even a partial reduction of DMPK may be functionally significant.

DMPK is the canonical member of the myotonic dystrophy family of protein kinases (MDFPK) [14]. Several functional and structural characteristics of DMPK are related to the properties of the other protein kinases of this family including *Neurospora* Cot-1 [15], *Drosophila* Warts [16], rat ROKα [17], human p160^{ROCK} [18], human PK428 [14], *Caenorhabditis elegans* LET-502 [19], *Drosophila* Genghis Khan [20], murine Citron Rho-interacting kinase [21], rat DM kinase-related Cdc42 binding kinase [22] and *C. elegans* DM kinase-related kinase (M. Shimizu and H.F. Epstein, unpublished results). All family members are serine-threonine kinases which show strong conservation in their catalytic protein kinase (PK) domains (Fig. 1). They also have varying lengths of α-helical, coiled-coil-forming sequences. Most have been shown to be regulated by a member of the Ras superfamily of p21 GTPases and to be involved in cytoskeletal and cell cycle-related processes. Previous work from our and other laboratories verified by enzymological studies of recombinant proteins that DMPK is a functional serine-threonine protein kinase [23–25]. We have also shown that DMPK is activated by Rac-1 by cotransfection in Cos-M6 cells [26]. The interaction with Rac-1 and structural similarities to Rho-kinase stimulated studies of the effects of DMPK on enhancing the organization of the actin cytoskeleton [27], which culminated in the finding that DMPK phosphorylates the myosin binding subunit of myosin phosphatase (MYPT1) and inhibits the activity of myosin light chain phosphatase [28]. These studies suggest that DMPK may function in a role complementary to Rho kinase in the regulation of the cytoskeleton.

An interesting structural feature of MDFPK enzymes is the presence of long stretches of α-helix of varying length. DMPK contains residues 461–538 within the α-helical (H) region that are predicted to form a coiled coil [29]. Homologous sequen-

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Abbreviations: DMPK, myotonic dystrophy protein kinase; FLAG peptide, DYKDDDDK; H, α-helical region; HA, hemagglutinin antigen; L, leucine-rich region; MDFPK, myotonic dystrophy family of protein kinases; MYPT1, myosin binding subunit of myosin phosphatase; PK, protein kinase catalytic domain region; SDGS, sucrose density gradient sedimentation; T, transmembrane region

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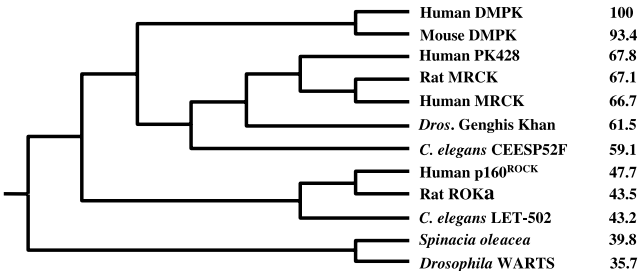


Fig. 1. The myotonic dystrophy family of protein kinases. The catalytic domains of these related protein kinases were compared in terms of identity of aligned amino acid sequences to DMPK. The phylogenetic tree was constructed by pairwise comparisons of sequences [29].

ces to this region have been shown to be necessary for the binding of Rho, a Ras superfamily GTPase [17]. We report here that the α-helical coiled-coil region is necessary for the formation of DMPK homodimers and that the dimeric form enhances the catalytic efficiency of the kinase active centers. These structural and functional findings in DMPK are likely to be significant in understanding the structure–function relationships of other important MDFPK members such as Rho kinase.

2. Materials and methods

2.1. cDNA constructs

Full-length and truncated subfragments of human DMPK (AAC14449) were cloned in-frame into the *Eco*RI and *Bam*HI sites of insect cell expression vector pVL1392 (PharMingen). For purification and detection of DMPK, a poly-His tag and a FLAG/hemagglutinin antigen (HA) epitope tag were added at the N- and C-termini of DMPK respectively. pVL1392 His-LPKHT-FLAG, pVL1392 His-LPKH-FLAG, pVL1392 His-LPK-FLAG, pVL1392 His-PKH-FLAG, pVL1392 His-LPKH-HA and pVL1392 His-PKH-HA were constructed for protein expression. All constructs were sequenced to confirm their correct sequences and reading frames.

2.2. Protein expression and purification

The cDNA constructs of DMPK were transfected into Sf9 cells. Briefly, 0.5 μg baculovirus DNA and 2 μg plasmid mixture were cotransfected into 2×10^6 Sf9 cells. The transfected cells were incubated for 5 days at 27°C. The supernatant which contained recombinant virus for protein expression was collected. 1 ml of amplified virus was added to 2×10^7 Sf9 cells and incubated for 3 days at 27°C. The cells were harvested and spun at $5000\times g$ for 5 min. The pellets were suspended in lysis buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol (DTT), 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride, 3 μM benzoyl-L-arginine ethyl ether, 3 μM *N-p*-tosyl-L-arginine methyl ester, 2 μM each of chymostatin, leupeptin and pepstatin, 2 mM sodium orthovanadate and sodium fluoride). The cells were passed through a 27 gauge needle six times, then centrifuged at $14\,000\times g$ for 10 min. 6 ml lysates were injected into 5 ml Hi-Trap Column. The binding column was washed with lysis buffer with increasing concentrations of imidazole (10, 30 mM) until the UV absorbance of the wash was constant, and then eluted with 200 mM imidazole. The eluted proteins were desalted and injected into a 5 ml DEAE column equilibrated with buffer (20 mM Tris–HCl pH 7.0). The bound protein was washed with buffer with increasing concentrations of NaCl (50 and 150 mM) until the constant UV absorbance of the eluant was reached. The proteins were eluted with 300 mM NaCl in buffer 1. The eluted protein were concentrated and stored in liquid nitrogen.

2.3. Sucrose density gradient sedimentation (SDGS)

A linear 5–20% (w/v) sucrose gradient solution was constructed with lysis buffer with a final volume of 9.0 ml. 1.0 ml of 2×10^7 infected cell lysate or 1.0 ml of 0.8 mg/ml purified protein was layered on the top of the sucrose gradient. The protein sample and sucrose gradient combinations were spun in an SW41.1 swinging-bucket rotor (Beckman) at 29 000 rpm (about $100\,000\times g$) for 16–20 h for LPKH, 16 h for LPK and 20 h for PKH. 1 ml fractions were collected and each fraction was further analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Apparent molecular weights were determined by relative sedimentation with respect to proteins of known molecular weights [30].

2.4. Sedimentation equilibrium analysis

The molecular weights of the DMPK construct were measured in 20 mM Tris–HCl pH 7.5, 150 mM NaCl with a Beckman Coulter XL analytical ultracentrifuge and an An60Ti rotor. Sedimentation equilibrium was performed at 4°C with six channel Epon centerpieces and scanned at 14, 16, 18, and 25 h at 7000 rpm, then scanned at 14, 16, 18, and 25 h at 9000 rpm, and then scanned at 14, 16, 18, and 25 at 11 000 rpm sequentially. The data were analyzed with software (Origin Microcal Software, 1995) provided by Beckman Coulter. The partial specific volume of LPKH was assumed to be 0.725 cm^3/g. All samples were visually checked for clarity after ultracentrifugation, and no indication of precipitation was observed.

2.5. Protein kinase assays

Purified DMPK subfragments were analyzed using histone H1 and the MYPT1 regulatory peptide (residues 690-RQSRRSTQGVTLTD-703) as substrate [28]. DMPK (LPKH, LPK, PKH) subfragments were incubated with 100 μM ATP, 3 μCi of [^{32}P]γ-ATP and 12 μM of MYPT1 peptide or 3 μg of histone H1 in 40 μl of kinase buffer (20 mM Tris–HCl pH 7.5, 0.25 mM $MgCl_2$, 30 mM KCl, 0.5 mM DTT, 0.5 mM EGTA, 1 μM microcystin-LR, 0.5 mg/ml bovine serum albumin (BSA)) for 20 min at 30°C. The reactions were terminated by adding 6 μl of acetic acid to each. 11.5 μl of each reaction was spotted onto Whatman P81 cellulose phosphate filter circles. P81 filter circles were washed three times with 400 ml of cold 0.4% phosphoric acid for 10 min. $^{32}PO_4$ incorporation was measured by counting the pads in a Beckman scintillation counter. Each reaction was repeated three times, and the resulting data were summarized as mean ± S.D. Autophosphorylation assay samples were subjected directly to SDS–PAGE and autoradiographed.

2.6. In vitro protein binding assays

Purified recombinant DMPK subfragments from insect cells were used for the protein binding assays. 1 μM of DMPK-FLAG and 3 μM of DMPK-HA were used in all reactions. DMPK with FLAG-tagged proteins were immobilized by anti-FLAG M2 affinity gel (Sigma) in binding buffer (20 mM Tris–HCl pH 7.5, 30 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, 0.5% Triton X-100, 0.5 mg/ml BSA). Then DMPK with HA-tagged proteins was added and incubated for 3 h at 4°C with rocking. The beads with bound proteins were washed three times with binding buffer and eluted by boiling. The eluted protein was identified by anti-HA-Tag polyclonal antibody (Clontech), followed by secondary goat anti-rabbit antibody conjugated to alkaline phosphatase. The signal was detected using a chemiluminescent detection system.

2.7. Caspase 1 cleavage

Purified LPKH from insect cells was assayed as substrate for caspase 1. 1.6 μg of LPKH and 60 U of caspase 1 (Biomol®) were

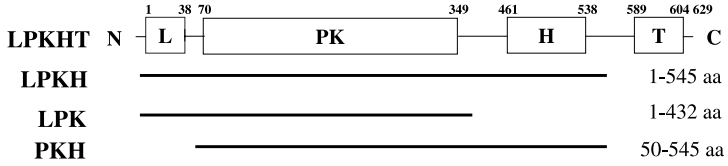


Fig. 2. Protein modules of DMPK and its subfragments used in this study. The LPKH, LPK, and PKH constructs of DMPK were used.

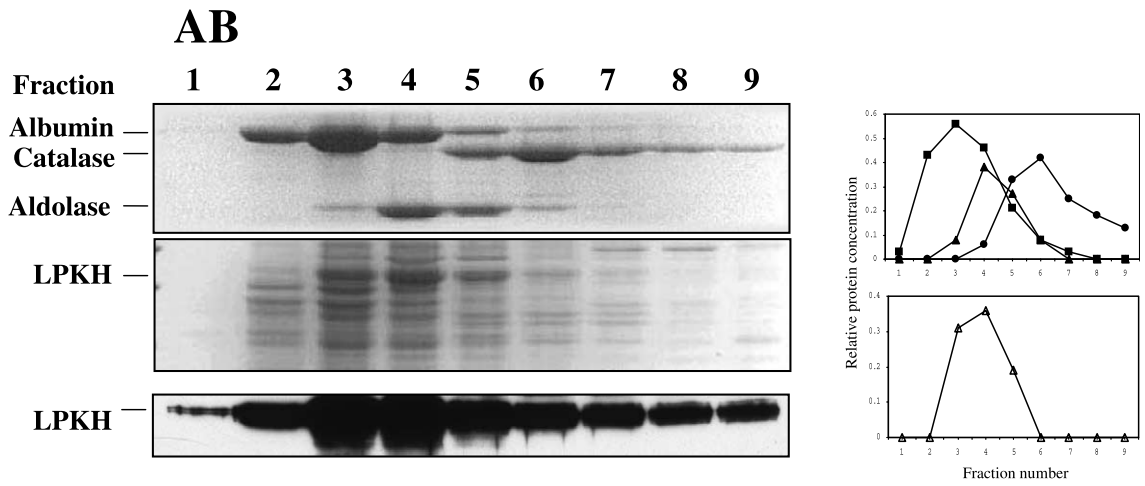


Fig. 3. SDGS shows that LPKH is a dimer. LPKH-containing insect cell lysates were analyzed on 5–20% SDGS. A: Lanes 1–9 were fractions collected from top to bottom. The top panel was an SDGS of marker proteins: albumin, 67 000 (major band shown in lanes 2–4), catalase, 232 000 (major band shown in lanes 5–7), and aldolase, 158 000 (major band shown in lanes 4 and 5). The middle panel shows a parallel SDGS of LPKH in an insect cell lysate. Its major band appeared in lanes 3–5. The major band of LPKH was also detected by Western blot as shown in the lower panel; samples (5 μl) of each fraction were analyzed by Western blot. The apparent molecular weight of LPKH was calculated to be 140 000. Since the molecular weight of the LPKH monomer is 63 200 based upon the predicted amino acid sequence, native LPKH appeared to be a dimer. B: Quantification of marker proteins and LPKH in Coomassie blue-stained SDS–PAGE of the SDGS analyses. Albumin (■), aldolase (▲), catalase (●), LPKH (△).

incubated in buffer (50 mM HEPES pH 7.4, 100 mM NaCl, 1 mM EDTA, 10 mM DTT, 10% glycerol, 0.1% CHAPS) for 3 h at 30°C [34]. The mixture was then subjected to SDS–PAGE with a 5–20% acrylamide gradient.

3. Results

3.1. DMPK constructs and subfragments

Fig. 2 shows the distinct regions of full-length DMPK (LPKHT) and the three subfragments and DMPK used in the experiments of this report. LPKH and PKH were selected because they contain the catalytic PK domain and the α-helical H region whereas LPK like LPKH contains the NH_2-terminal leucine-rich region (L) and catalytic PK structures but lacks the H region. The study of these three subfragments permitted us to analyze the role of the H region in homodimerization and enzymatic activity. LPKHT and the truncated subfragments LPKH, LPK, and PKH were expressed as recombinant proteins in Sf9 cells. All the proteins were poly-His-tagged at their NH_2-termini and FLAG-tagged at their COOH-termini. Their expression as proteins was tested by Western blots with anti-FLAG monoclonal antibody. We purified LPKH, LPK, and PKH by nickel affinity and DEAE anion exchange chromatography on the Amersham/Pharmacia FPLC system.

3.2. LPKH is a homodimer

DMPK is a homooligomer [23,25,31], but whether native DMPK is a homodimer or higher order multimer has not been determined. To examine the native structure of DMPK, LPKHT was expressed at low levels in Sf9 cells and appeared to be insoluble (data not shown). Because the T region appears to be a putative transmembrane domain, LPKHT is probably localized to membranes [24,31]. In separate experiments, LPKHT also appeared to be insoluble when expressed in *Escherichia coli*. In contrast, LPKH was expressed abundantly as a soluble protein in Sf9 cells. Because

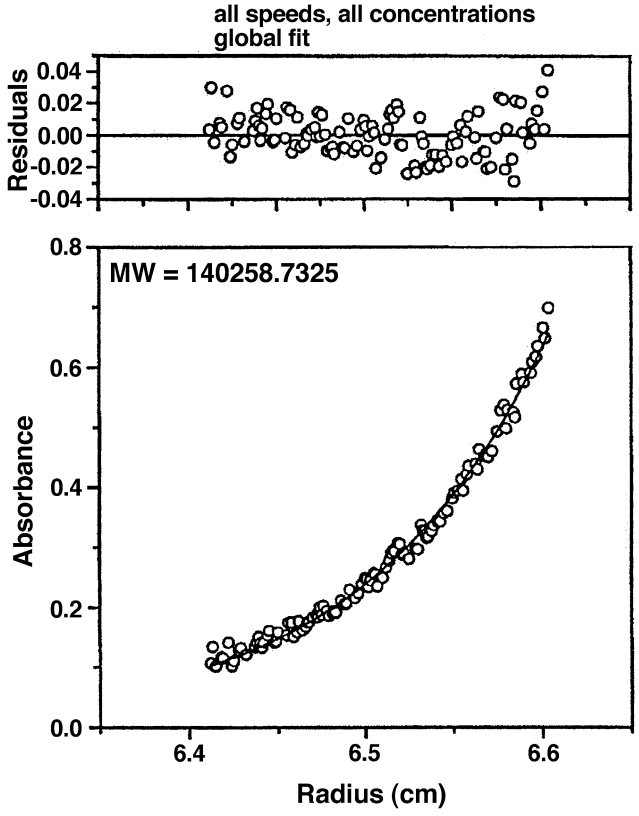


Fig. 4. Determination of the molecular weight of purified LPKH by sedimentation equilibrium. Purified LPKH at three different concentrations, 1.2 μg/μl, 0.6 μg/μl, and 0.3 μg/μl, was spun for 25 h in the Beckman Coulter XL ultracentrifuge at three different speeds (7000 rpm, 9000 rpm and 11 000 rpm). The molecular weight of LPKH was calculated as 140 258 from these experiments. The partial specific volume (v-bar) of LPKH was calculated to be 0.725 cm^3/g based upon the predicted amino acid sequence (Origin Microcal Software, 1995). Therefore, LPKH appeared to be a dimer as a purified protein and in insect cell lysates.

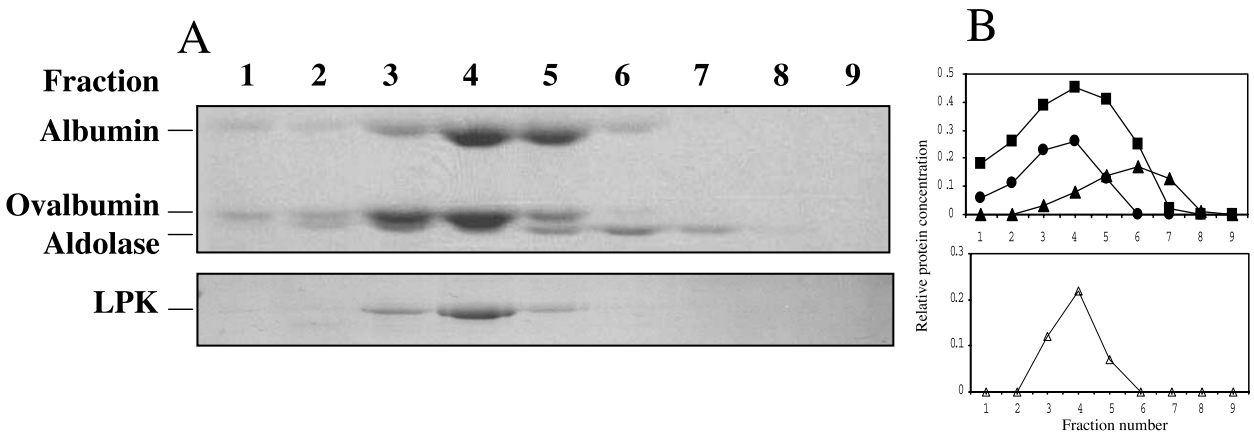


Fig. 5. SDGS of LPK. Purified LPK was analyzed. A: Lanes 1–9 were fractions collected from top to bottom. The upper panel was an SDGS of marker proteins: albumin, 67 000 (major band show in lanes 3–5), ovalbumin, 43 000 (major band shown in lanes 3–4), and aldolase, 158 000 (major band shown in lanes 5–7). The lower panel was a parallel SDGS of LPK (major band shown in lanes 3–5). The apparent molecular weight of 62 000 for LPK was estimated from the results of three experiments. Considering the molecular weight of 51 800 calculated from the predicted amino acid sequence and this result, LPK appeared to be monomeric. B: Quantification of marker proteins and LPK in SDGS. Ovalbumin (●), albumin (■), aldolase (▲) and LPK (△).

LPKH closely resembles an expressed spliceoform of DMPK [2], we considered LPKH to be an appropriate model for native DMPK in cells.

Lysates of infected cells expressing LPKH were subjected to SDGS, and the protein was identified on Western blots by anti-FLAG. The apparent molecular weight of LPKH in the lysates was 140 000 by SDGS (Fig. 3) based upon the method of Martin and Ames [30].

To further confirm these results, purified LPKH was studied by sedimentation equilibrium experiments in the analytical ultracentrifuge. This analysis showed that LPKH behaves as a homogeneous species of 140 259 molecular weight (Fig. 4) which is consistent with the SDGS results with lysates and purified protein. Because the polypeptide molecular weight as calculated from the predicted amino acid sequence is 63 200, these results are most consistent with LPKH being a homodimer.

3.3. The H domain is required for DMPK homodimerization

We next examined the LPK and PKH subfragments in order to determine whether the H domain was required for DMPK homodimerization. In SDGS, purified LPK behaved as a species with the apparent molecular weight of 62 000 (Fig. 5). This result is consistent with LPK being a monomer because its molecular weight is 51 800 as calculated from the predicted amino acid sequence for the LPK subfragment. In contrast, PKH behaved as a dimer, suggesting that the H but not L region is necessary for homodimerization (data not shown).

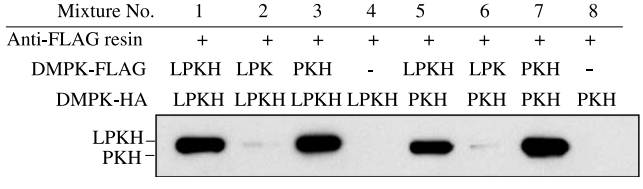


Fig. 6. The H domain is required for DMPK subfragment interactions. FLAG-tagged DMPK protein subfragments (1 μM) were adsorbed by anti-FLAG monoclonal antibody M2 beads to pull down HA-tagged DMPK protein subfragments (3 μM), then the pulled down subfragments were probed with anti-HA polyclonal antibody in a Western blot of the SDS–PAGE. Mixture 1, LPKH-FLAG:LPKH-HA; 2, LPK-FLAG:LPKH-HA; 3, PKH-FLAG:LPKH-HA; 4, anti-FLAG M2 beads:LPKH-HA; 5, LPKH-FLAG:PKH-HA; 6, LPK-FLAG:PKH-HA; 7, PKH-FLAG:PKH-HA; 8, anti-FLAG M2 beads:PKH-HA. Lanes 4 and 8 represent control experiments for the absence of binding of either LPKH-HA and PKH-HA by anti-FLAG beads.

To further confirm that the H domain is required for the interactions between monomers leading to dimer formation, we expressed LPKH and PKH as HA-tagged proteins in Sf9 cells in addition to the FLAG-tagged proteins. FLAG-tagged DMPK subfragments were immobilized on an anti-FLAG M2 affinity gel. HA-tagged DMPK subfragments were tested for binding to the immobilized subfragments. These in vitro pull-down assays were performed at 4°C, and the DMPK subfragments pulled down were identified with anti-HA antibody. As shown in Fig. 6, LPKH pulled down other LPKH and PKH molecules. PKH also pulled down other PKH and LPKH molecules, but LPK did not pull down either LPKH or PKH. Because the H domain was present in both LPKH and PKH but not in LPK, the two DMPK protein subfragments required the H but not the L region for their interaction. The PK domain is clearly not sufficient for homodimerization. The predicted ability of the α-helical H domain sequence from residues 461–538 to form a coiled coil is suggested to be the structural basis for DMPK homodimerization.

3.4. DMPK dimers have greater enzymatic activity than monomers

DMPK phosphorylates the MYPT1 subunit and thereby inhibits myosin phosphatase activity [28]. LPKH and LPK activities were assayed with the 690-RQSRRSTQGVTLTD-703 peptide of MYPT1 as substrate. This sequence contains the phosphorylatable threonine which regulates myosin phosphatase activity [28]. The activity of LPK was less than half that of LPKH under similar conditions (Fig. 7), and the V_{max} and k_{cat}/K_m of LPK were both significantly lower than those of LPKH (Fig. 9A,C). LPKH also showed higher autophosphorylation activity than LPK. The K_m of LPK for the peptide substrate was significantly lower than that of LPKH as well. Therefore, both the catalytic efficiency and the binding

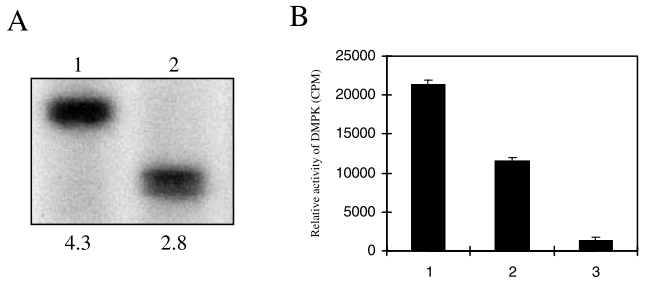


Fig. 7. LPKH has higher activity than LPK. A: Autophosphorylation. 0.5 μM DMPK in a total volume of 30 μl was incubated for 30 min at 30°C, and the mixtures were analyzed by SDS–PAGE. The dried gel was radioautographed. The numbers below reflect relative intensities of the bands: 1, LPKH (4.3); 2, LPK (2.8). B: MYPT1 peptide phosphorylation. 0.12 μM DMPK and 12.5 μM MYPT1 peptide in a total volume of 40 μl were incubated for 20 min at 30°C. 1, LPKH plus MYPT1 peptide; 2, LPK plus MYPT1 peptide; 3, MYPT1 peptide alone.

of substrate by the active center of DMPK were affected by interactions within the homodimer in contradistinction to the monomer.

3.5. *Caspase 1 cleavage of LPKH removes the H domain, produces monomers, and reduces kinase activity*

In order to test whether the observed differences in activity between LPKH and LPK are the result of their dimeric versus monomeric states or are the result of differences in folding

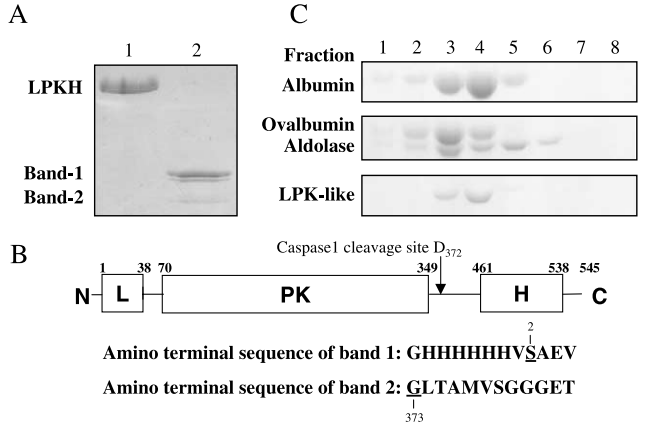
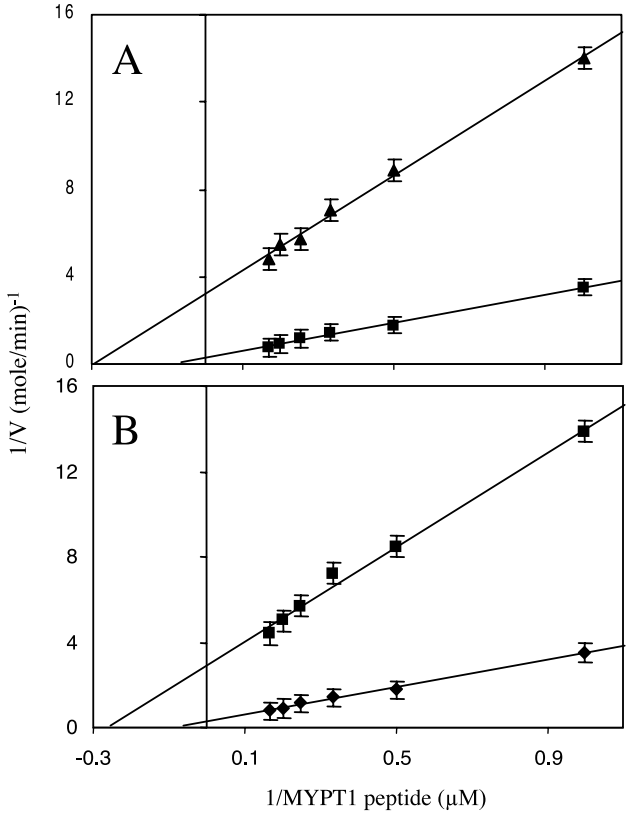


Fig. 8. Caspase 1 cleaves DMPK to form a LPK-like protein and reduces its activity. A: Caspase 1 cleaves LPKH. 1, LPKH; 2, LPKH after caspase 1 incubation. LPKH was cleaved into two bands. B: Caspase 1 cleavage site on DMPK. The NH_2-terminal sequences of band 1 matched the NH_2-terminal six-His tag (GHHHHHHV) and four residues near the N-terminus of DMPK (2-SAEV-5). The NH_2-terminal sequence of band 2 (373-GLTAMVSGGGET-384) matched sequence between the PK and H domains. Caspase 1 cleaved to the NH_2-terminal side of the indicated amino acid residues of band 2. The preceding sequence 369-LVED-372 is probably the caspase 1 substrate binding site [34]. C: SDGS showed that the LPK-like protein was a monomer. Caspase 1-cleaved LPK-like protein was analyzed. Lanes 1–8 were fractions collected from top to bottom. The upper panel contains an SDGS of the marker protein: albumin, 67 000 (major band in lane 4); the middle panel contains an SDGS of the marker proteins ovalbumin, 43 000 (major band in lane 3), and aldolase, 158 000 (major band in lanes 5–6). The lower panel was an SDGS of LPK-like protein (major band shown in lane 4). The apparent molecular weight of LPK-like protein was very close to albumin, 67 000. The LPK-like protein appeared to be monomeric.

during their expression or of perturbations during their purification, we studied the cleavage of LPKH by caspase 1. This reaction produced two subfragments, the larger of which is LPK-like in terms of polypeptide mobility on SDS–PAGE (Fig. 8A). The NH_2-terminal sequences of these two protein subfragments (Fig. 8B) show that the sequence which precedes the cleavage site, 369-LVED-372, is probably the substrate binding site for caspase 1. This sequence shows strong similarity to known sites for the protease [35]. SDGS showed that the larger LPK-like subfragment behaved as a monomer (Fig. 8C). The kinase activity of LPKH was compared before and after incubation with caspase 1. After cleavage, LPKH lost about 60% of its kinase activity under the same assay conditions. Lineweaver–Burk analysis revealed that the caspase 1-cleaved monomer like LPK had reduced V_{max}, K_m and k_{cat}/K_m compared to uncleaved LPKH (Fig. 9B,C). These experiments confirm the structural and functional significance of the α-helical, coiled-coil H region.



C

	LPKH	LPK	LPKH+Caspase1
V_{max} (mole/min)	3.49	0.31	0.34
k_m (μM)	11.23	3.38	3.78
k_{cat}/K_m ($M^{-1}.sec^{-1}$)	1.87×10^7	5.51×10^6	5.42×10^6

Fig. 9. Kinase activities of DMPK subfragments. A: Lineweaver–Burk analysis showed significant differences in the enzyme kinetics of LPKH and LPK. 0.12 μM of each DMPK subfragment were incubated with 1, 2, 3, 4, 5, 6 μM MYPT1 peptide for 20 min at 30°C. ▲ LPK, ■ LPKH. B: Lineweaver–Burk analysis shows that caspase 1 digestion altered the enzyme kinetics of LPKH. ♦ LPKH, ■ LPK-like product of caspase 1 digestion of LPKH. The conditions were similar to those in A. C: V_{max}, K_m and k_{cat}/K_m of LPKH, LPK and LPK-like protein were calculated from the Lineweaver–Burk analyses in A and B.

461 EAEAEVTLRELQEALEEEVLTRQSLSREMEAIRTDNQNFA
SQLREAEARNRDLEAHVRQLQERMELLQAEGATAVTGV 538

Fig. 10. Sequence of predicted coiled-coil in H region of DMPK.

4. Discussion

The MDFPK enzymes show strong conservation both in their catalytic domains and in long stretches of α-helix that are compatible with the formation of coiled coils between monomers. DMPK is the canonical member of the MDFPK and may be considered a model for the general and functional characteristics of this protein kinase family. The α-helical H region between residues 461–538 is predicted by PAIRCOIL to contribute to coiled-coil formation [29] (Fig. 10) with residues 506–534 showing four clear heptad repeats. Our experimental results suggest that this region is critical in the homodimerization of DMPK polypeptides. Furthermore, the homodimeric forms showed higher catalytic efficacy than monomeric forms that lacked the H region. Further research is required to better understand the mechanism of how homodimerization affects the catalytic domain. Because our previous studies suggested that most of the apparent autophosphorylation activity in DMPK showed a marked dependence on enzyme concentration [23], transphosphorylation between the individual monomers in the homodimer may have an activating effect. Clearly in such a model, the catalytic PK domains would interact directly with each other as the basis for this activation.

Kinetic analysis of the dimeric LPKH and monomeric LPK subfragments of DMPK revealed that LPKH showed a three-fold greater k_{cat}/K_m and a 10-fold greater V_{max} than LPK. These differences were also demonstrated when LPKH was cleaved by caspase 1 to produce an LPK-like monomeric species. These differences are very likely to have physiological and clinical significance. The kinetic differences between dimeric LPKH and monomeric LPK reported here reflect their relative effects on actin cytoskeletal organization and membrane blebbing [27]. DM patients show haploinsufficiency in the levels of DMPK mRNA and protein [7,8], consistent with their heterozygous state for the autosomal dominant trait. Moreover, mice that are heterozygous for the DMPK null state show the age-dependent development of cardiac arrhythmias that human DM patients exhibit [12]. These observations suggest that reduced but present levels of the order of a two-fold reduction in DMPK produce significant pathophysiological changes in vivo. These differences in DMPK concentrations would be reflected by comparable, two-fold differences in protein kinase activity. Thus, the enzyme kinetic differences that we have observed between the dimeric and monomeric forms of DMPK are likely to be significant. All of the forms of DMPK observed in vivo possess the H region and would therefore be dimeric and show the higher activity [2].

Several studies from our laboratory indicate that DMPK acts in the regulation of the cytoskeleton. Transfection of DMPK into human and rabbit eye lens epithelial cells produces a striking rearrangement of the cortical actin cytoskeleton [27]. Similar observations have been made in rat PC12 cells that are a well-known model of neural cells (H.F. Epstein, S. Li, Q. He and F. Liu, unpublished results). These rearrangements are similar to the effects of transfecting the Ras family p21 GTPases RhoA and Rac-1. DMPK, indeed, can bind Rac-1 and be activated three-fold by cotransfection with this established regulator of the actin cytoskeleton [26]. Interestingly, the H region contains the binding site for RhoA in several kinases [17,20,22,33]. DMPK shows a very similar pattern of phosphorylation and inhibition of the regulatory MYPT1 subunit of myosin light chain phosphatase to the effects of RhoA-activated protein kinase [28]. Myosin phosphatase directly regulates the assembly and contractile properties of cytoskeletal myosin II which, in turn, are critical to the assembly and function of the cortical actin cytoskeleton. As with DMPK, loss-of-function mutants of myosin II in *C. elegans* show semi-dominance [32]. That is, about two-fold reductions in active myosin produce significant effects in muscle in vivo.

We propose that homodimerization of DMPK and other MDFPK enzymes is important in producing physiologically necessary levels of protein kinase activity. The H region in DMPK and homologous regions in other MDFPK kinases would be necessary for this homodimerization. This region has also been shown to be important for interaction with the p21 GTPases that regulate these kinases. DMPK like other MDFPK enzymes is likely to participate in the regulation of cytoskeletal actin and myosin function.

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