Evidence for Structural Differences between the Two Highly Homologous Actin-regulatory Proteins, Destrin and Cofilin*

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The amino acid sequences of destrin and cofilin are very similar (84% homology) throughout the entire range of proteins, but they have different functions. In this study, we constructed a new cofilin expression plasmid, which had high expression frequency, and the structures of destrin and cofilin were analyzed by limited proteolysis and circular dichroism (CD). When destrin was digested by trypsin, two fragments of 17.0 kDa and 9.2 kDa were obtained, whereas only one 8.4 kDa fragment was obtained from cofilin. In spite of the overall sequence homology, an N-terminal amino acid sequence analyses of the fragments revealed the cleavage sites on destrin and cofilin to be different. These results suggest that destrin and cofilin differ in their overall tertiary folds. Cofilin showed activity similar to destrin at high pH values, although no pH-dependent structural change in cofilin was confirmed by using limited proteolysis and CD.

Key words: destrin; cofilin; actin-regulatory protein

Destrin and cofilin are related, low-molecular-mass, actin-binding proteins.\(^1-4\) The amino acid sequences of the two proteins are very similar (84% homologous), but they are unique proteins derived from different mRNAs and genes.\(^4\) Both proteins exist in a single tissue at the same time,\(^9\) suggesting that they have distinct physiological roles.

Destrin depolymerizes actin filaments in a stoichiometric manner.\(^2\) It is capable of interacting with the actin monomers in the filament and removing them from the filament, thereby cutting and depolymerizing the actin filaments.\(^2\) Consequently, the depolymerization caused by destrin is much faster than that caused by other actin-monomer-sequestering proteins such as profilin.\(^2\)

In vitro experiments have revealed that cofilin binds to actin filaments in a 1:1 molar ratio of cofilin to actin monomer in the filament.\(^9\) The actin filaments are apparently thickened, due to the binding of cofilin along the entire length. The characteristics of the actin filaments are altered by the binding of cofilin:\(^1,5\) cofilin inhibited actin-myosin interaction, actin-tropomyosin interaction, and actin-caldesmon interaction. The fragility of the actin filaments is also affected by the binding of cofilin.\(^9\)

The activity of cofilin is pH-sensitive.\(^9\) Cofilin causes partial depolymerization of actin in the neutral pH range,\(^9\) but at higher pH, the actin filaments rapidly depolymerize in the presence of cofilin.\(^6\)

Cofilin shows an action apparently similar to that of destrin at high pH, yet the two proteins have clearly different functions in vitro at neutral pH: destrin is a depolymerization factor,\(^2\) while cofilin is an actin filament side-binding factor.\(^1,6\) Although their primary structures are very similar, the differences in their functions result from the subtle differences in their amino acid sequences, rather than from possible post-translational modifications.\(^4\) Therefore, it would be interesting to clarify the structural differences between the two proteins, Moriyama et al., having constructed and characterized several cofilin-destrin chimeric proteins.\(^7\)

In this study, we analyzed the structures of these proteins by limited proteolysis. The results suggest that destrin and cofilin differ in their overall tertiary folds. We also examined the effect of pH on the conformation of cofilin by using limited proteolysis and circular dichroism (CD). Any structural change of cofilin at different pH values was not evident, but the spectral profile revealed a difference in the secondary structure contents between destrin and cofilin.

Materials and Methods

Construction of a New Expression Plasmid for Cofilin. The entire region of the structural gene for cofilin was amplified by the polymerase chain reaction with cofilin expression plasmid pKIC-w, which had been constructed by Moriyama et al.,\(^6\), being used as the template and the following two oligonucleotides as primers: P1: 5'-AAAACAGCTATGACCAGGTGTACGCCCAA-3'
P2: 5'-GGGGCTGAGGAGTCTCACAAGGCTTG-3'
P1 and P2 contain a new Ncol site and BamH1 site (underlined), respectively. Both primers also have some mismatched bases (boxed). The primers were purchased from Biologica Co. (Aichi, Japan), and the polymerase chain reaction product was electrophoresed on a 1.0%
agarose gel.

The DNA fragment was recovered from the agarose gel by using GENE CLEAN II (BIO101, La Jolla, CA, USA) and digested with NcoI and BamHI. The product was ligated into the pTV119-N vector (Takara Shuzo Co., Shiga, Japan) and was sequenced by the dideoxy chain termination method to check for possible mutations. The sequence analysis revealed that no mutations had occurred. A new expression plasmid, pET-C, was constructed by ligating the NcoI-BamHI fragment into expression vector pET-21d (Takara Shuzo Co., Shiga, Japan).

**Expression of the Proteins.** The pUC18-based cDNA expression plasmid (pKID-w) was transformed into E. coli MV1184, while the pET-C plasmid was transformed into E. coli BL21(DE3). Culture of the MV1184 cells and the expression of cofilin were performed as described. BL21 (DE3) cells were cultured for 9 h at 37°C in the presence of 50 mg/ml of ampicillin. Nothing was added to induce cofilin expression.

**Purification of the Proteins.** The cells were harvested, suspended in a lysis buffer containing 20 mM Tris-HCl (pH 7.0), 10 mM EGTA and 1 mM dithiothreitol (DTT) at 0°C, sonicated, and then centrifuged at 17,000 rpm for 10 min. The supernatant was supplemented with 0.01 volume of 2 M HEPES (pH 7.0), before being centrifuged for 30 min at 17,000 rpm. From this supernatant, either cofilin or actin was purified by sequential chromatography as described.

**Co-sedimentation Analysis.** Rabbit skeletal muscle actin was prepared by the method of Spudich and Watt.  

The pH-dependent activity change of recombinant cofilin was checked by allowing actin (0.21 mg/ml) to polymerize in the presence of 2 mM MgCl₂ and 80 mM KCl while incubating for 40 min at 25°C with (+) or without (−) cofilin (0.21 mg/ml) under neutral (25 mM PIPES, pH 7.0) or alkaline (25 mM Tris-HCl, pH 8.3) conditions. The polymerized actin was collected by 31,000 rpm spinning for 15 min, and the precipitate was resuspended in 0.1 N NaOH containing 0.2% Na₂CO₃. The supernatant and precipitate were electrophoresed on 15% polyacrylamide gel. 2 mM PIPES (pH 7.0), and then aliquots of 1 mM PIPES (pH 7.0) or 1 mM Tris-HCl (pH 8.3) buffer were added to a final concentration of 25 mM.

**Limited Proteolysis.** Trypsin (type II), chymotrypsin (type VII) and subtilisin (type VIII) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Desmin and cofilin were separately mixed with each protease and were incubated at 30°C for digestion. The reaction was stopped by heating (100°C for 2 min). Each sample was electrophoresed on 17% polyacrylamide gel containing SDS by the method of Laemmli, the gel being stained with Coomassie Brilliant Blue R-250. The Daichi peptide molecular weight marker (Daichi Pure Chemicals Co., Tokyo, Japan; myoglobin (17.0 kDa), myoglobin I&II (14.4 kDa), myoglobins I&III (10.7 kDa), myoglobin I (8.2 kDa), myoglobin II (6.2 kDa), and myoglobin III (2.5 kDa)) was used as a molecular mass standard.

**Blotting Peptide Fragments from the Polyacrylamide Gel on a Polyvinylidene Difluoride (PVDF) Membrane.** The peptides on each SDS-polyacrylamide gel were blotted onto a polyvinylidene difluoride (PVDF) membrane by using AE-6670 semidy-blotting apparatus (ATTO Co., Tokyo, Japan) according to the method of Hirano. The PVDF membrane was then stained with Coomassie Brilliant Blue R-250.

**Electrophoretic Elution of the Peptides from the Polyacrylamide Gel.** The peptide fragments were electrophoretically eluted from each SDS-polyacrylamide gel with a Maxyflex-NP AE6580 unit (ATTO Co., Tokyo, Japan) for 60 min at 10 mA. To remove glycerine, the peptide samples were treated by adding sodium deoxycholate to a final concentration of 2.4% (w/v). After 15 min, trichloroacetic acid (TCA) was added to a final concentration of 6% (w/v) and 30 min later, the sample was centrifuged at 4,000 rpm for 20 min.

**Other Procedures.** Samples for the partial amino acid sequence analysis were obtained by either blotting or by electrophoretic elution, and were analyzed with a model 477A gas-phase protein sequencer (Applied Biosystems, Foster City, CA, USA). CD spectra were measured with a model J-720 spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan). The contents of α-helix and β-sheet for each protein were calculated according to the assumption of Chen et al., using bovine serum albumin as the standard.

**Results**

**Construction of a New Plasmid for Cofilin Expression**

The expression plasmid for cofilin, pKIC-w, had previously been constructed by Moriyama et al. Unfortunately, the efficiency of cofilin production was not adequate when using pKIC-w, so a new plasmid for cofilin was constructed as described in the experimental procedures section. The reconstructed plasmid, pET-C, contains the T7 promoter which has high expression efficiency. We obtained a sufficient amount of cofilin for limited proteolysis by using pET-C, the yield being about 4.5 mg of protein/g of cells and 3 times greater than that by the previous method.

As described in the materials and methods section, the new plasmid had no mutations in its coding region. Therefore, the recombinant cofilin is expected to have the same activity as that of authentic cofilin from porcine brain, this being confirmed in Fig. 1. The polymerization of actin itself was not affected by the change in pH (Fig. 1, lanes 1, 2, 5 and 6). Most of the actin was polymerized and precipitated under both pH conditions. At pH 7.0, a small amount of actin depolymerized and appeared in the supernatant (Fig. 1, lane 3), while a large amount of cofilin co-precipitated with ac-
Fig. 1. pH-Dependent Activity Change of Recombinant Cofilin.
Actin (0.21 mg/ml) was incubated at 25°C for 40 min with (+) or without (−) cofilin (0.21 mg/ml) in the presence of 2 mM MgCl₂ and 80 mM KCl under neutral (pH 7.0) and alkaline (pH 8.3) conditions. After a 31,000 rpm spin for 15 min, the supernatant and precipitate were electrophoresed on 15% polyacrylamide gel. S, supernatant; P, precipitate.

Fig. 2. Limited Proteolysis of Destrin and Cofilin by Three Different Proteases.
Destrin (0.5 mg/ml; lanes 1, 2 and 3) and cofilin (0.5 mg/ml; lanes 1', 2' and 3') were each digested with proteases at 20 μg/ml for 50 min at 30°C. The buffer consisted of 25 mM PIPES (pH 6.9) and 0.1 mM DTT, with trypsin (lanes 1 and 1'), chymotrypsin (lanes 2 and 2'), and subtilisin (lanes 3 and 3') being used. The trypsin and undigested destrin/cofilin bands are indicated by arrows. The arrowheads indicate the fragments obtained by trypsin digestion.

Fig. 3. Time-dependent Tryptic Digestion of the Two Proteins.
A. Destrin (0.5 mg/ml) was digested with trypsin (20 μg/ml) at 30°C. The incubation times were as follows: lane 1, 0 min; lane 2, 15 min; lane 3, 25 min; lane 4, 35 min; lane 5, 60 min; lane 6, 80 min; lane 7, 100 min; lane 8, 120 min. The 17.0 kDa fragment is indicated by a solid arrowhead, and the 9.2 kDa fragment is indicated by the open arrow. The molecular mass standard was run in lane 9.
B. Cofilin (0.5 mg/ml) was digested with trypsin (20 μg/ml) at 30°C. The incubation times were as follows: lane 1, 0 min; lane 2, 5 min; lane 3, 25 min; lane 4, 50 min; lane 5, 75 min; lane 6, 100 min; lane 7, 150 min. The 8.4 kDa fragment is indicated by the open arrow.
C. Cofilin (0.5 mg/ml) was digested with a low concentration (5 μg/ml) of trypsin at 30°C. The incubation times were as follows: lane 1, 15 sec; lane 2, 30 sec; lane 3, 1 min; lane 4, 2 min; lane 5, 4 min; lane 6, 6 min. The 17.5 kDa fragment is indicated by the solid arrowhead, and undigested destrin/cofilin bands are indicated by arrows.

tin filaments (Fig. 1, lane 4). The depolymerization of actin was more pronounced at pH 8.3 (Fig. 1, lane 7), while co-polymerization of cofilin with actin was not as evident at higher pH values (Fig. 1, lanes 7 and 8). These activities are identical to those of porcine brain cofilin (6) and recombinant cofilin (9).

In the subsequent study, we used pET-C as the cofilin expression plasmid and pKID-w (1) as the destrin expression plasmid.

Limited Proteolysis with Three Proteases
Destrin and cofilin were digested with three different proteases: trypsin, chymotrypsin, and subtilisin. Destrin and cofilin were each digested with varying concentrations (10, 20, 30, or 40 μg/ml) of each protease, and the time-dependent digestion process was observed. Typical results are shown in Fig. 2.

Trypsin digested destrin and cofilin into a limited number of fragments (Fig. 2, lanes 1 and 1'). Destrin was digested into two fragments with apparent molecular masses of 17.0 kDa and 9.2 kDa (Fig. 2, lane 1, arrowheads). Cofilin was digested into one fragment with a molecular mass of 8.4 kDa (Fig. 2, lane 1', arrowhead). On the other hand, chymotrypsin digested destrin and cofilin into several low-molecular-mass fragments (Fig. 2, lanes 2 and 2'). Subtilisin rapidly digested the two proteins, and new bands appeared in a broad molecular mass range (Fig. 2, lanes 3 and 3'). These results indicate that distinct fragments were not obtained when either chymotrypsin or subtilisin was used. For these reasons, trypsin was used for further analyses as a suitable protease for limited proteolysis.

Time-dependent Tryptic Digestion of Destrin and Cofilin
Destrin was mixed with trypsin to reveal the proteolytic pattern (Fig. 3A). Two bands, one with a molecular mass of 17.0 kDa, and the other smaller than 6.0 kDa, appeared after a short time of digestion (Fig. 3A, lanes 2 and 3). The amount of the 17.0 kDa fragment was maximal at 25 min (Fig. 3A, lane 3, arrowhead). A new 9.2 kDa fragment appeared after 25 min (Fig. 3A, lane 3, arrow). The amount of the 9.2 kDa fragment increased with decreasing amount of the 17.0 kDa fragment. The
digestion pattern suggests that the 17.0 kDa fragment was further digested into the 9.2 kDa fragment, which was more stable.

The proteolytic pattern of coflin was different from that of desmin (Fig. 3B). Only one fragment of 8.4 kDa was produced (Fig. 3B, arrow). The amount of the 8.4 kDa fragment was smaller than that of the 9.2 kDa desmin fragment. Fragments similar to the 17.0 kDa fragment desmin were not obtained under these conditions. The amino acid sequence of each of these three peptides (the 17.0 kDa and the 9.2 kDa desmin fragments, and the 8.4 kDa coflin fragment) were determined in the following section for further comparison.

When coflin had been digested with a lower concentration of trypsin (5 μg/ml), a 17.5 kDa fragment was produced after a 15-sec incubation (Fig. 3C, lane 1, arrowhead). The 17.5 kDa fragment was almost completely digested at 6 min (Fig. 3C, lane 6). The molecular mass of this fragment is similar to the 17.0 kDa desmin fragment, but the two fragments differed in their stability. It was anticipated that the domain structure of coflin might be different from that of desmin.

**Amino Acid Sequence Analysis**

The N-terminal sequence analysis was accomplished by blotting the proteolytic fragments of desmin on the SDS-polyacrylamide gels on to a PVDF membrane, and then excising them from the membrane. The 8.4 kDa coflin fragment was extracted from the gels by electrophoretic elution, because it could not be blotted on to the PVDF membrane under any of the conditions tested (data not shown).

The N-terminal side amino acid sequence of each fragment is shown in Table 1. It appears that the 17.0 kDa fragment of desmin is a single band in Figs. 1 and 2A, but in practice, three kinds of peptides were contained in this fragment (Table 1, 17.0 kDa desmin fragment [A], [B] and [C]). When a larger quantity of the sample was electrophoresed, a minor band with a mobility slightly higher than that of the 17.0 kDa desmin fragment was observed (data not shown). The sequence analysis indicated that the amount of peptide [A] was much smaller than those of peptides [B] and [C], so we consider that the minor band corresponds to peptide [A]. Next, the N-terminal amino acid sequences of the 9.2 kDa desmin fragment and the 8.4 kDa coflin fragment were determined (Table 1).

The N-terminal amino acid sequences of the tryptic fragments were compared with the primary structures of the proteins in order to determine the cleavage sites (Fig. 4). The sequence of peptide [A] corresponds to that of the original N-terminus of desmin. Since the amount of peptide [A] was small, this fragment was not characterized any further. Peptides [B] and [C] correspond to the sequences from Lys$^{22}$ to Lle$^{30}$, and from Cys$^{23}$ to Lys$^{30}$, respectively (Fig. 4, arrow [1]). It was noted that peptide [B] was only one residue longer than peptide [C]. In the subsequent text, we refer to both fragments as the 17.0 kDa desmin fragment.

The N-terminal sequence of the 9.2 kDa desmin fragment indicate that the peptide bond between residues Arg$^{81}$ and Tyr$^{82}$ was cleaved (Fig. 4, arrow [2]). The sequence of the 8.4 kDa coflin fragment indicates that it had Asn$^{46}$ as the N-terminal amino acid residue (Fig. 4, arrow [3]).

**Comparison of the Proteolytic Patterns and CD Spectra of Desmin and Coflin under neutral and basic conditions**

The activity of coflin is pH-dependent, and coflin showing the same activity as that of desmin under basic conditions such as pH 8.3. From this point of view, it is possible that coflin could have a desmin-like conformation at pH 8.3. The structures of desmin and coflin under neutral and basic conditions were examined by tryptic digestion and CD spectroscopy.

The two proteins were digested with trypsin, and the time-dependent digestion was examined at pH 7.0 and pH 8.3 (Fig. 5). The activity of trypsin is also pH-sensitive, so we carried out some preliminary experiments (da-

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**Table 1. N-terminal sequences of the proteolytic fragments obtained by limited proteolysis with trypsin**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Fragment</th>
<th>N-terminal sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Destrin</td>
<td>17.0 kDa [A]</td>
<td>TMITPSSG</td>
</tr>
<tr>
<td></td>
<td>17.0 kDa [B]</td>
<td>KCSTPEEI</td>
</tr>
<tr>
<td></td>
<td>17.0 kDa [C]</td>
<td>CSTPEEIK</td>
</tr>
<tr>
<td></td>
<td>9.2 kDa</td>
<td>YAL-</td>
</tr>
<tr>
<td>Coflin</td>
<td>8.4 kDa</td>
<td>NIILE-</td>
</tr>
</tbody>
</table>

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**Fig. 4. Alignment of the Sequences of Destrin and Coflin, and Partial Amino Acid Sequences of the Proteolytic Fragments.**

The sequences of desmin and coflin are taken from Yonezawa et al. Asterisks indicate the positions at which the aligned residues are identical between desmin and coflin. Arginines and lysines, the preferred cleavage sites of trypsin, are indicated by dots. "17.0", "9.2" and "8.4" denote the N-terminal side sequence of the 17.0 kDa desmin fragment, the 9.2 kDa desmin fragment, and the 8.4 kDa coflin fragment, respectively (Table 1). Arrows [1], [2] and [3] indicate cleavage sites.
Fig. 5. Partial Digestion of Dextrin and Coflin under Alkaline Conditions.
A. Dextrin (0.5 mg/ml) was digested by trypsin (2.5 µg/ml) at pH 7.0 (lanes 1a, 2a, 3a, 4a, and 5a) and at pH 8.3 (lanes 1b, 2b, 3b, 4b, and 5b). The 17.0 kDa fragment is indicated by an arrowhead. Incubation times were as follows: lane 1, 10 min; lane 2, 2 min; lane 3, 4 min; lane 4, 6 min; lane 5, 10 min. The pH value of each reaction mixture was controlled according to Yonezawa et al. Briefly, the protein samples were dialyzed against 2 mM PIPES (pH 7.0), and then aliquots of 1 M PIPES (pH 7.0) or 1 M Tris-HCl (pH 8.3) buffer were added to a final concentration of 25 mM.
B. Coflin (0.5 mg/ml) was digested by trypsin (2.5 µg/ml) at pH 7.0 (lanes 1a, 2a, 3a, 4a, and 5a) and at pH 8.3 (lanes 1b, 2b, 3b, 4b, and 5b). The 17.5 kDa fragment is indicated by an arrowhead. Incubation times were as follows: lane 1, 10 sec; lane 2, 30 sec; lane 3, 1 min; lane 4, 2 min; lane 5, 4 min. Undigested dextrin/coflin bands are indicated by arrows.

Fig. 6. Circular Dichroism Spectral Profiles.
Curve A, spectral profile of dextrin at pH 7.0; curve B, that of dextrin at pH 8.3; curve C, that of coflin at pH 7.0; curve D, that of coflin at pH 8.3. A K+, Na+-phosphate buffer (25 mM) was used. Each sample was dialyzed against a phosphate buffer at either pH 7.0 or pH 8.3 to control the pH-conditions. Spectra were measured at 25°C.

Discussion
The purpose of this study was to compare the structure of dextrin with that of coflin. Our results show that dextrin and coflin had different tertiary structures and that the pH-dependent conformational change of coflin was minor.

When pKIC-w was used as the expression plasmid for coflin, we could not obtain a sufficient amount of coflin to analyze the detailed conformation. The newly reconstructed plasmid, pET-C, elicited a yield 3 times larger than that by the previous method, and consequently enabled this study to be continued.

When dextrin and coflin were digested with trypsin, three fragments were obtained (Fig. 3). In spite of the high degree of homology (84%) in their primary structures, the cleavage sites in dextrin and coflin clearly differed (Fig. 4). The arginine and lysine residues, which are the preferred cleavage sites of trypsin, were largely conserved in dextrin and coflin, dextrin having 26 arginine and lysine residues out of 165 residues, while coflin had 28 out of a total of 166 residues. Among them, 24 residues, including the actual cleavage sites (i.e., Arg21, Lys22 and Arg41 of dextrin and Lys40 of coflin; Fig. 4, arrows [1], [2] and [3]), were completely conserved in the two proteins. Some residues (Lys69 and Arg145 of dextrin we could not obtain enough evidence for the proteolytic pattern of coflin being pH-dependent (data not shown).

CD spectra were obtained under the two pH conditions, the spectral profiles of dextrin at pH 7.0 and pH 8.3 being similar (Fig. 6, curves A and B). Those of coflin were also similar, in contrast to its activity change at both pH values (Fig. 6, curves C and D). However, dextrin and coflin exhibited their own characteristic spectral profiles in the wavelength range from 190 to 208 nm at both pH values. The contents of α-helix and β-sheet were 26% and 51% in dextrin, and 17% and 44% in coflin, respectively.
and Lys\textsuperscript{144}, Arg\textsuperscript{146} and Lys\textsuperscript{164} of coflin) are unique to each protein.

Even if the cleaved residues are conserved, extreme differences in the adjacent sequences may affect the trypsin substrate specificity. It has been reported that three residues on the N-terminal side of Arg or Lys are a prerequisite for hydrolysis.\textsuperscript{15} However, we could ignore this possibility on the grounds that, although the adjacent sequences of cleavage site [2] in destrin were highly conserved between destrin and coflin, the digestion patterns differed (Fig. 4, arrow [2]).

We surmise that the 9.2 kDa destrin fragment and the 8.4 kDa coflin fragment contain their core structures. Judging from the molecular mass, the 9.2 kDa destrin fragment apparently contains the actin binding sequence.\textsuperscript{10} The 9.2 kDa destrin fragment could be blotted on to a hydrophobic PVDF membrane, but the 8.4 kDa coflin fragment could not (data not shown). Consequently, the micro-environment of the actin binding site may be different between destrin and coflin. There is room for further investigation on this point.

Moriyama et al. have characterized a series of destrin/coflin chimeric proteins.\textsuperscript{5} They substituted the local sequences of destrin for those of coflin, but they failed to specify any defined sequences that were responsible for destrin-like or coflin-like activity. Their results suggest that the functional differences between the two proteins were based on differences in the overall tertiary structure, which is in agreement with our conclusions derived from this study. Hatanaka et al. have determined the tertiary structure of destrin by triple-resonance multi-dimensional nuclear magnetic resonance and reported a similarity between the folding of destrin and the two actin-regulating proteins, the coflin and gelsolin family.\textsuperscript{7} But a comparison between the structure of destrin and coflin has not yet been sufficiently made.

In general, proteins with homologous amino acid sequences have similar three-dimensional structures.\textsuperscript{18} The sequences of destrin and coflin are 84% homologous,\textsuperscript{11} and their predicted secondary structures were also very similar (data not shown). However, trypsin cleaved destrin and coflin at different sites, indicating that the residues exposed on the surfaces of the two proteins are different, and that the overall tertiary folds are different. The CD analysis also revealed differences in the secondary structural contents. The results of these experiments prove that a subtle variation in the primary structure can affect the entire tertiary structure. This study will thus provide useful working hypotheses for protein design and structural prediction.

A comparisons of the proteolytic patterns and CD spectral profiles at neutral pH revealed a distinction between destrin and coflin. This result agrees with the fact that they have different functions at neutral pH. Though the CD spectral profiles were different under higher pH conditions, coflin showed an activity similar to that of destrin.\textsuperscript{6} There thus arises a second question as to whether the structure of coflin resembles that of destrin under basic conditions. In this regard, we did not obtain enough evidence for the pH-dependent conformational change of coflin to be drastic when compared with destrin. The stability of the 17.0 kDa destrin fragment and the 17.5 kDa coflin fragment was markedly different: the 17.0 kDa destrin fragment remained without being digested after 80 min (Fig. 3A), while the 17.5 kDa coflin fragment was almost completely digested after 4 min (Fig. 3C). This protease resistance was not affected by pH (Fig. 5). The CD spectral profile of coflin at pH 8.3 did not show any marked difference from the profile at pH 7.0 (Fig. 6). Therefore, the activity change of coflin is unlikely to be accompanied by a dynamic conformational change. In this study, the fine conformational change is noteworthy, and it would be wrong to assume that coflin has a destrin-like structure under basic conditions.

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References