A Proposed Common Structure of Substrates Bound to Mitochondrial Processing Peptidase*

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Mitochondrial processing peptidase (MPP), a metalloendopeptidase consisting of α- and β-subunits, specifically cleaves off the N-terminal presequence of the mitochondrial protein precursor. Structural information of the substrate bound to MPP was obtained using fluorescence resonance energy transfer (FRET) measurement. A series of the peptide substrates, which have distal arginine residues required for effective cleavage at positions −7, −10, −14, and −17 from the cleavage site, were synthesized and covalently labeled with 7-diethylamino-3-carboxylic acid at the N termini and N,N-dimethyl-N-(iodoacetyl)-N′-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IANBD) at position +4, as fluorescent donor and acceptor, respectively. When the peptides were bound to MPP, substantially the same distances were obtained between the two probes, irrespective of the length of the intervening sequence between the two probes. When 7-diethylamino-3-(4′-maleimidyl phenyl)-4-methyl coumarin was introduced into a single cysteine residue in β-MPP as a donor and IANBD was coupled either at the N terminus or the +4 position of the peptide substrate as an acceptor, intramolecular FRET measurements also demonstrated that distances of the donor-acceptor pair were essentially the same among the peptides with different lengths of intervening sequences. The results indicate that the N-terminal portion and the portion around the cleavage site of the presequence interact with specific sites in the MPP molecule, irrespective of the length of the intervening sequence between the two portions, suggesting the structure of the intervening sequence is flexible when bound to the MPP.

Numerous mitochondrial proteins are translated on cytoplasmic ribosomes as larger precursors. An N-terminal presequence of the mitochondrial protein precursor functions as a targeting signal for their transport to mitochondria (1–3). During import of precursors into mitochondria, the presequences are recognized by multiple proteins (4, 5), such as molecular chaperones, translocases of the mitochondrial outer and inner membranes, and peptidases from inside mitochondria. Despite the identification of various proteins that interact with the mitochondrial precursors, the mechanism of recognition of the presequence by these components has not yet been understood.

The lack of sequence homology of the presequences, even though they are characterized by the positively charged residues and the formation property of amphiphilic α-helices, has inhibited clarification of the recognition mechanism (6).

Mitochondrial processing peptidase (MPP), located in the matrix of the mitochondria, cleaves off most presequences of the imported precursors. MPP consists of two structurally related subunits, α-MPP and β-MPP. Complex formation with the two subunits is essential for both enzymatic activity (7, 8) and substrate binding (9).

Earlier studies indicated that some structural elements of the presequence are required for recognition by MPP. An arginine residue at position −2, the so-called “proximal arginine,” from the cleavage site, which is usually found among most precursor proteins, plays a critical role in cleavage reaction (10–12). Distal basic amino acid residue(s) around position −10 are also important for effective cleavage (10–12). The length between the proximal arginine and the distal basic residues is not so fixed, and 4–10 amino acids are allowed (13). Our more recent studies have shown a requirement for effective cleavage of flexible linker sequences containing proline and glycine between the two basic residues (13, 14), a hydrophobic residue at position +1 (12, 13), and serine or threonine residues at position +2 and/or +3 (12, 15).

Some functional amino acid residues in MPP were determined using mutational analysis: His-101, Glu-104, and His-105 in rat β-MPP, which form a metal binding site, HxxEH, conserved among a pitrilysin metalloendopeptidase superfamily (16), are the catalytic center of MPP (17, 18). Glu-181 is the third metal-binding residue (19). Glu-174 may participate in the catalytic reaction (18). Glu-124, which is in a characteristic acidic amino acid cluster conserved in β-MPP, may interact with the N-terminal portion of the presequence in the cleavage reaction (18). On the other hand, Glu-390 and Asp-391 in yeast α-MPP interact with the distal arginine residues, which are required especially for cleavage of precursors with a longer presequence (19). Deletion of three residues in the glycine-rich segment characteristic of α-MPP resulted in a drastic reduction in affinity to the substrate (20).

Findings on functional amino acid residues both in precursor

1 The abbreviations used are: MPP, mitochondrial processing peptidase; CPM, 7-diethylamino-3-(4′-maleimidylphenyl)-4-methylcoumarin; DAC, 7-diethyl aminoacoumarin-3-carboxylic acid; FRET, fluorescence resonance energy transfer; IANBD, N,N-dimethyl-N-(iodoacetyl)-N′-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine; MDH, malate dehydrogenase; α- and β-MPP, α- and β-subunits, respectively, of the mitochondrial processing peptidase.

2 In our previous works, the residues of MPP were numbered from the N terminus of the mature protein reported in the data base. In this studies, we numbered the residues according to the full-length MPP precursors. For instance, His-101, Glu-104, and His-105 in rat β-MPP were represented as His-56, Glu-59, and His-60, respectively, in our previous papers.
proteins and MPP required for the processing reaction, especially for precursor recognition by MPP, suggest that the two subunits of MPP cooperatively form the substrate binding pocket and that they have several substrate binding sites to cope with different structural elements in the extension peptide. To elucidate the recognition mechanism that makes feasible strict substrate specificity for MPP, it is necessary to determine the structure of the precursor sequence bound to the enzyme.

In the present study, fluorescence resonance energy transfer (FRET) experiments provide the first evidence that the distal arginine and the portion around the cleavage site of the precursor are located at specific sites in the MPP molecule, irrespective of the position of the distal arginine. An induced-fit mechanism of substrate recognition of MPP seems likely.

**EXPERIMENTAL PROCEDURES**

**Preparation of Fluorescence-labeled Peptides**—The fluorescent dyes, 7-diethylaminocoumarin-3-carboxylic acid (DAC), N,N′-dimethyl-N-(iodoacetyl)-N′-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IANBD), and 7-diethylaminomino-3-(4′-maleimidylphenyl)-4-methylcoumarin (CPM) were purchased from Molecular Probes, Inc. (Eugene, OR). Peptide synthesis and DAC labeling were done as described (9). IANBD amide labeling with the cysteine residue of peptides for intramolecular FRET experiments was done essentially the same as DAC labeling. Peptide authenticity was identified by MALDI-TOF mass spectrometry (Voyager, PerSeptive Biosystems). The concentrations of the fluorescence peptides were calculated from the molar extinction coefficient of 45,000 (M<sup>–1</sup> cm<sup>–1</sup>) for DAC or 23,500 (M<sup>–1</sup> cm<sup>–1</sup>) for IANBD amide.

**Preparation of Fluorescence-labeled MPP**—A hexahistidine-tagged yeast α-MPP and yeast α/β-MPP complex were purified as described (9). Purification and fluorescent labeling of a hexahistidine-tagged yeast β-MPP were done as follows. The supernatant from the BL21(DE3) strain carrying pET-βE73QHis was loaded on a 5-ml Hi-trap chelating column (Amersham Pharmacia Biotech) equilibrated with buffer A (20 mM Hepes-KOH, pH 7.4, containing 500 mM NaCl). The column was washed with 50 ml of buffer A containing 50 mM imidazole. The β-MPP was eluted with buffer A containing 200 mM imidazole. To the fractions containing β-MPP were added 0.1 mM CPM, and then the sample was left to react for 1 h at 25 °C. The reaction was terminated using 1 mM cysteine for 30 min at 25 °C. The free dye was removed on a PD-10 desalting column equilibrated with 20 mM Hepes-KOH, pH 7.4, containing 500 mM NaCl. Labeled β-MPP was then applied onto 2 ml of Q-Sepharose FF (Amersham Pharmacia Biotech) equilibrated with 20 mM Hepes-KOH, pH 7.4, containing 200 mM NaCl. The column was washed with 30 ml of the same buffer, and then the protein was eluted with 20 mM Hepes-KOH, pH 7.4, containing 100 mM NaCl. The purity of CPM-labeled β-MPP was confirmed by SDS-polyacrylamide gel electrophoresis followed by UV transillumination and Coomassie Blue staining. The labeling efficiency was calculated from a molar extinction coefficient of 33,000 (M<sup>–1</sup> cm<sup>–1</sup>) for CPM. The labeling procedure resulted in the incorporation of 0.11 ± 0.01 mol of CPM/mol of β-MPP.

**Fluorescence Measurements**—Fluorescence was measured at 25 °C using a Hitachi F-4500 fluorescence spectrophotometer. Steady-state fluorescence and anisotropy measurements were performed with a Hitachi F-4500 fluorescence spectrophotometer equipped with automatic fluorescence polarization system. In the intramolecular FRET measurements, excitation of DAC was measured at 390 nm and the emission intensity at 470 nm. MPP (0.5 μM) was diluted into 20 mM Hepes-KOH, pH 7.4, containing 30% glycerol, and then various concentrations of the double-labeled peptides were added. The emission spectra were taken after each sample and the blank had been thoroughly mixed and allowed to equilibrate for 1–2 min. In the intermolecular FRET, the excitation of CPM was measured at 390 nm and the emission intensity at 480 nm. To the IANBD-labeled peptides (0.5 μM), CPM-labeled MPP was added at the indicated concentrations, and then the emission spectra were taken, and the fluorescence intensity at 480 nm was read.

The dissociation constant, K<sub>D</sub>, was determined as follows: F = (F<sub>max</sub> – [Enz])/K<sub>D</sub> + ([Enz]), where F<sub>max</sub> and F<sub>max</sub> are the measured and maximal fluorescence intensity of the peptides, respectively, and [Enz] represents the concentrations of the enzyme. A plot of [Enz]/F versus [Enz] yields a linear function with a slope of 1/F and an ordinate intercept of K<sub>D</sub>.

From the decrease in fluorescence of donor DAC or CPM, induced in the presence of acceptor IANBD, the energy transfer efficiency E was calculated from

$$E = 1 - \frac{Q_D}{Q_{DA}}$$  \hspace{1cm} (Eq. 1)

where Q<sub>D</sub> stands for the unquenched quantum yield of the donor and Q<sub>DA</sub> is the quantum yield in the presence of the acceptor. Quantum yield was substituted for emission maximum intensity F. From the energy transfer efficiency results, the distance between donor and acceptor was calculated according to the Förster theory (21),

$$R = \frac{R_0}{(1 - E)^{1/6}}$$  \hspace{1cm} (Eq. 2)

where R is the calculated distance and R<sub>0</sub> is the distance at which 50% energy transfer would occur between the donor-acceptor pair; it is given in angstroms, as shown in Equation 3,
$R_0 = 9790(J^2Qa \lambda^{-4})^{1/6}$  
(Eq. 3)

where $J$, the overlap integral, is the degree of spectral overlap of donor emission $F_D(\lambda)$ and acceptor absorbance $e_A(\lambda)$, as defined by Equation 4.

\[
J = \text{Integral} F_D(\lambda)e_A(\lambda)\lambda^4d\lambda/\text{Integral} F_D(\lambda)d\lambda
\]

$\kappa^2$ is assumed to be 2/3. The refractive index of the solvent, $n$, is used at a value of 1.4. $Q_D$, the quantum yield for the donor, was given as

\[
Q_D = Q_0 \times F_D / A_D / (F_A \times A_D)
\]

where $Q_0$ is the quantum yield for the reference dye, $F_D$, and $F_A$ are the fluorescence intensities for the donor and reference dye, respectively, and $A_D$, and $A_A$ are the fluorescence intensity for the donor and reference dye, respectively. Fluorescein was used as the reference dye and was assumed to have a quantum yield of 0.92 in 0.1 N NaOH.

Although $\kappa^2$ was taken as 2/3 for the calculation of distances, the maximum and minimum values of $\kappa^2$ were estimated according to the method of Dale et al. (21).

\[
\kappa^2_{\text{max}} = (2/3) \times (1 + d_0 + d_A + 3d_s^2(\lambda))
\]

\[
\kappa^2_{\text{min}} = (2/3) \times (1 - d_0 + d_A)/2
\]

where $d_0 = (r_D(0.4))^{1/2}$, $d_A = (r_A(0.4))^{1/2}$; $r_D$ and $r_A$ are the limiting anisotropies of the donor and acceptor, respectively. Using these values for the orientation factor, the maximum and minimum distances between probes were calculated and were regarded as the probable error limits of the distance (R-limits).

RESULTS

Distance between the N-terminal End and the C-terminal Portion of the Peptide Substrates Bound to MPP—Sequence data on the presequence of mitochondrial protein precursors show that position of the distal basic acid is not so fixed among the extension peptides and is located from 7 to 17 from the cleavage site. To elucidate the structure of the substrate peptide bound to the enzyme, it is vital to determine whether the distal arginine residue at position to be arginine (10). This peptide has a length of 14 from the N-terminal end) was replaced with alanine in one of the MDH presequence.

**Fig. 2. Titration of DAC- and DAC/IANBD-labeled peptides with the MPP.** The single- or double-labeled MDH-AAL (A), MDH14A (B), MDH-AdAR (C), and MDH-AdRA (D) (all at 0.5 $\mu$M) were titrated with yeast MPP (0–3 $\mu$M). The fluorescence intensities at 470 nm of DAC- and DAC/IANBD-labeled peptides (circles and squares, respectively) were plotted as a function of the concentration of MPP. The solid lines are nonlinear least-squares fits of the plots to the equation, $F = (F_{\text{max}} \times [\text{Enz}] + [\text{Enz}])$, where $F_{\text{max}}$, and [Enz] represent increased fluorescence intensity, the calculated maximum of F, and the enzyme concentration, respectively. The excitation wavelength of DAC was 390 nm. a.u., arbitrary units.

The spectral characteristics of all the peptides were essentially the same. For the intermolecular FRET measurements, fixed concentrations (0.5 $\mu$M) of the DAC or DAC/IANBD-labeled peptides were added to various concentrations (usually 0–3 $\mu$M) of the purified a/βE73Q, in which the ratio of the peptides for binding to MPP increases with environmental change around the dye (Fig. 1). Titration of the DAC-labeled peptides gave the dissociation constant, $K_D$, of peptides for binding to MPP (Fig. 2). All of the peptides bound to MPP with a high affinity to the same extent.

Introduction of IANBD into the peptides led to a drastic suppression of increase in DAC fluorescence through FRET in all of the peptides studied (Fig. 2). In the titration of DAC- or DAC/IANBD-labeled peptides with MPP, the fitting curves showed a biphasic nature (Fig. 2). Because fluorescence anisotropy change of the peptides was saturated at a stoichiometric amount of the enzyme (data not shown), the biphasic fluorescence change in the titration experiments might be due to increased scattering by increasing the concentration of the enzyme. For calculation of FRET efficiency, $E$, the fluorescence intensities, $F_D$ and $F_A$, for DAC- and DAC/IANBD-labeled peptides, respectively, were taken by extrapolating the second phase curve to the ordinate. The calculated FRET efficiencies of all the peptides showed a range of 80–85% (Table II). The quantum yield, $Q$, of the fluorescence donor DAC showed a gradual decrease with the increasing length of the peptides; this also resulted in a decrease in the distance at which 50% FRET occurred between the donor-acceptor pair, $R_0$, suggest-
The histidine-tagged 2118 emission spectrum of CPM, which has a fluorescence maximum would occur between the donor-acceptor pair; E, FRET efficiency; R, calculated distance between the donor-acceptor pair; R-limits, probable error limits of the distance including the orientation factor. See “Experimental Procedures” for the calculation of these parameters.

### Table II

<table>
<thead>
<tr>
<th>Peptides</th>
<th>$K_d$</th>
<th>Q</th>
<th>$R_0$</th>
<th>$R$</th>
<th>R-limits</th>
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<tr>
<td></td>
<td>µM</td>
<td></td>
<td>Å</td>
<td>Å</td>
<td></td>
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<tr>
<td>MDH-AAAL</td>
<td>0.20</td>
<td>0.217</td>
<td>38.1</td>
<td>0.850</td>
<td>28.5</td>
</tr>
<tr>
<td>MDH-14A</td>
<td>0.21</td>
<td>0.203</td>
<td>37.7</td>
<td>0.850</td>
<td>28.2</td>
</tr>
<tr>
<td>MDH-AdAR</td>
<td>0.38</td>
<td>0.158</td>
<td>36.2</td>
<td>0.797</td>
<td>28.8</td>
</tr>
<tr>
<td>MDH-AdRA</td>
<td>0.33</td>
<td>0.148</td>
<td>35.8</td>
<td>0.834</td>
<td>27.4</td>
</tr>
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**FIG. 3. Specific modification of Cys-252 in β-MPP by CPM.** A Coomassie Blue-stained gel (left panel) and a UV-transilluminated (right panel) SDS-polyacrylamide gel (8%) after electrophoresis are shown. βE73Q (lane 1) and βE73Q/C252S (lane 2) following nickel-chelating column chromatography were reacted with CPM. Then, each sample was finally purified by Q-Sepharose chromatography. Lanes 3 and 4 represent the final eluate of βE73Q (6 µg) and βE73Q/C252S (6 µg), respectively. The purification and CPM labeling are described in detail under “Experimental Procedures.”

**DISCUSSION**

We found that when mitochondrial protein precursors are bound to MPP, distal basic amino acids in its precursors interact with the specific site in the enzyme if the basic residues are present at positions −7 to −17. This means that the intervening sequence between the proximal arginine and the
TABLE III
Amino acid sequences of the fluorescence-labeled peptides for intermolecular FRET measurements

<table>
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<tr>
<th>Peptides</th>
<th>Position of the distal Arg</th>
<th>Sequences</th>
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</thead>
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<tr>
<td>Labeled at position +4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDH-ΔAAL</td>
<td>–7</td>
<td>Ac-LAEFGGASS-FSTC(S-IANBD)AQNN</td>
</tr>
<tr>
<td>MDH-14A</td>
<td>–10</td>
<td>Ac-LAEFGGASS-FSTC(S-IANBD)AQNN</td>
</tr>
<tr>
<td>MDH-AdAR</td>
<td>–14</td>
<td>Ac-LAEFGGASS-FSTC(S-IANBD)AQNN</td>
</tr>
<tr>
<td>MDH-AdRA</td>
<td>–17</td>
<td>Ac-LAEFGGASS-FSTC(S-IANBD)AQNN</td>
</tr>
<tr>
<td>Labeled at the N terminus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDH-ΔAAL</td>
<td>–7</td>
<td>Ac-C(S-IANBD)ARGPGLGGGAVATRSFSCTSAQNN</td>
</tr>
<tr>
<td>MDH-14A</td>
<td>–10</td>
<td>Ac-C(S-IANBD)ARGPGLGGGAVATRSFSCTSAQNN</td>
</tr>
<tr>
<td>MDH-AdAR</td>
<td>–14</td>
<td>Ac-C(S-IANBD)ARGPGLGGGAVATRSFSCTSAQNN</td>
</tr>
<tr>
<td>MDH-AdRA</td>
<td>–17</td>
<td>Ac-C(S-IANBD)ARGPGLGGGAVATRSFSCTSAQNN</td>
</tr>
</tbody>
</table>

FIG. 4. Spectral change of the emission of CPM-labeled MPP with the addition of IANBD-labeled MDH14A. The emission spectra of CPM-labeled MPP (CPM-βE73Q/a) (0.5 μM) were taken in the presence of various concentrations (0–1.5 μM) of IANBD-labeled MDH14A. The excitation wavelength of CPM was 390 nm. The spectra changed from the spectrum 1 to the spectrum 2 with the addition of the peptide. a.u., arbitrary units.

FIG. 5. Fluorescence quenching of CPM attached to MPP with the addition of IANBD-labeled peptides. Plots of the fluorescence quenching of CPM attached to MPP, 1 – (F<sub>L</sub>/F<sub>0</sub>), versus the concentration of the peptides labeled at position +4 (A) or at the N terminus (B). F<sub>L</sub> and F<sub>0</sub> represent the fluorescence of CPM in the presence or absence of IANBD-labeled peptides, respectively. CPM-labeled MPP (0.5 μM) was titrated with various concentrations (0–1.5 μM) of IANBD-labeled MDH-ΔAAL (squares), MDH-14A (diamonds), MDH-AdAR (circles), and MDH-AdRA (triangles). The excitation wavelength of CPM was 390 nm. The fluorescence intensity at 480 nm of CPM-labeled MPP was read. The solid lines are nonlinear least-squares fits of the plots to the equation, 1 – (F<sub>L</sub>/F<sub>0</sub>) = (E × [L])/([E] × [K<sub>D</sub>] + [L]), where E and [L] represent the calculated maximum of 1 – (F<sub>L</sub>/F<sub>0</sub>) with regard to the FRET efficiency between the CPM-IANBD pair and the concentration of the IANBD-labeled peptides, respectively. a.u., arbitrary units.
The amino acid sequences of the peptides are shown in Table III. The quantum yield of DAC, Q, and the distance at which 50% FRET would occur between the donor-acceptor pair, \( R_0 \), were calculated to be 0.90 ± 0.02 and 48.6 ± 0.3 Å, respectively. \( K_d \), dissociation constant; E, FRET efficiency; \( R \), calculated distance between donor-acceptor pair; \( R \)-limits, probable error limits of the distance including the orientation factor. See “Experimental Procedures” for the calculation of these parameters.

### Table IV

<table>
<thead>
<tr>
<th>Peptides</th>
<th>( K_d )</th>
<th>E</th>
<th>( R )</th>
<th>( R )-limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labeled at</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>position +4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDH-ΔAAL</td>
<td>0.38</td>
<td>0.600</td>
<td>45.5</td>
<td>37.2–58.7</td>
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<tr>
<td>MDH-14A</td>
<td>0.78</td>
<td>0.662</td>
<td>44.8</td>
<td>37.1–57.4</td>
</tr>
<tr>
<td>MDH-AdAR</td>
<td>0.93</td>
<td>0.597</td>
<td>45.6</td>
<td>36.9–59.0</td>
</tr>
<tr>
<td>MDH-AdRA</td>
<td>0.91</td>
<td>0.585</td>
<td>45.9</td>
<td>37.5–59.3</td>
</tr>
<tr>
<td>Labeled at</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N terminus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDH-ΔAAL</td>
<td>0.28</td>
<td>0.669</td>
<td>43.2</td>
<td>34.5–56.3</td>
</tr>
<tr>
<td>MDH-14A</td>
<td>0.85</td>
<td>0.626</td>
<td>44.6</td>
<td>37.2–57.2</td>
</tr>
<tr>
<td>MDH-AdAR</td>
<td>0.53</td>
<td>0.623</td>
<td>44.7</td>
<td>36.5–57.8</td>
</tr>
<tr>
<td>MDH-AdRA</td>
<td>0.55</td>
<td>0.643</td>
<td>44.1</td>
<td>36.1–56.9</td>
</tr>
</tbody>
</table>

During import into mitochondria, multiple proteins, including molecular chaperones, receptors, and processing peptides, recognize the presences of mitochondrial protein precursors. The formation of an \( \alpha \)-helix of the presquence is required apparently for interaction with these components (25–27). The NMR structure of the cytosolic domain of Tom20, a component of the translocase complex in mitochondrial outer membrane, with a synthetic peptide based on the aldehyde dehydrogenase precursor has recently been resolved (28). The peptide that forms an amphiphilic \( \alpha \)-helix in a crack of the core structure of Tom20 consists of four helices. The present results indicate that structures required for targeting and processing differ and that a flexible structure is required for the processing, although basic residues in the sequences functions as recognition signals for both processes.

Structural convergence between MPP and thermolysin, a \( \text{Zn}^{2+} \)-peptidase with a typical metal-binding motif, HExxH, has been discussed recently (29). Superimposition between the N-terminal domain of core 1 protein of the bc1 complex and the portion around the active site of thermolysin showed a similar arrangement of secondary structural elements but with different topological connections and in a reverse main chain orientation. This structural architecture is based on four helices, which contain metal ligands and the catalytic glutamate residue, and the neighboring five strands of a \( \beta \)-sheet. A main chain of substrates of thermolysin interacts with the edge of the \( \beta \)-sheet through hydrogen bonds. Like thermolysin, the \( \beta \)-sheet structure around the active center of MPP might interact with a nonhelical structure around the cleavage site of the precursors through hydrogen bonding, to present the scissile bond of the substrate to the active center in \( \beta \)-MPP. Further studies, especially on the crystal structure of MPP, should reveal the precise structure of the presquence bound to MPP and the mechanisms of strict recognition and specific cleavage of precursor proteins by the enzyme.

**REFERENCES**