Cooperative Formation of a Substrate Binding Pocket by α- and β-Subunits of Mitochondrial Processing Peptidase

(Received for publication, March 23, 1998, and in revised form, September 8, 1998)

Katsuhiko Kojima, Sakae Kitada, Kunitoshi Shimokata, Tadashi Ogishima, and Akio Ito

From the Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka 812-8581, Japan

Mitochondrial processing peptidase (MPP) specifically recognizes a large variety of mitochondrial precur-
sor proteins and cleaves off N-terminal extension pep-
tides. The enzyme is a metalloprotease and forms a
heterodimer consisting of structurally related α- and
β-subunits. To investigate the responsibility of MPP
subunits for substrate recognition, we monitored inter-
action of the fluorescent-labeled peptide substrates with
the MPP and its subunits. The specific binding of the
peptide to the MPP was confirmed by findings of the
direct participation of arginine residues in the binding,
which are located at position −2 and the position distal
to the cleavage site and are essential for the cleavage
reaction. MPP bound the substrate peptides with high
affinity only in the dimeric complex, and each subunit
monomer had about a 30-fold less affinity than the com-
plex. The individual subunit required arginines at differ-
ent positions in the peptide for binding, although
their affinities were much lower than that of MPP. Flu-
orescence quenching analysis showed that the peptide
bound to MPP was buried in the enzyme. Thus, both
subunits of MPP might be required for formation of a
substrate binding pocket with multiple subsites lying
across them.

Most nuclear-encoded mitochondrial proteins are translated
on cytoplasmic ribosomes as the precursor forms carrying the
N-terminal extension peptides. Extension peptides are re-
quired for transport of mitochondrial proteins as the targeting
signals and are proteolytically cleaved off during or after im-
port into the matrix by the mitochondrial processing peptidase
(MPP)1 (1–4). MPP is a metallopeptidase consisting of two
structurally related subunits, α- and β-MPP, that are both
required for enzyme activity (5–10). Mutation study on rat
β-MPP suggested that the metal binding motif in this subunit
that is conserved in a pitrilysin superfamily (11), H

1 The abbreviations used are: MPP, mitochondrial processing pepti-
dase; DAC, 7-diazo-5-oxo-1,2,3,4-tetrahydro-6-azaindole; MDH, malate
dehydrogenase; HPLC, high performance liquid chromatography; Ac, acetyl.

2 Position of the cleavage site from the cleavage site (13). Previous works
suggested the importance of this arginine for specific cleavage by
MPP (14–16). Using synthetic peptides (17–19) and precur-
sor proteins (20, 21) as the substrate, we demonstrated that the
structural element in the substrates required for processing is
not only proximal arginine but also distal basic amino acid
residues from the cleavage site, the flexible linker regions
containing proline and/or glycine between the two basic resi-
dues, and hydrophobic amino acids at position +1. These re-
results indicate that MPP recognizes a higher order structure of
extension peptides.

There are conflicting results concerning the substrate bind-
ing of MPP. The intrinsic tryptophan fluorescence study on
Neurospora MPP demonstrated that both subunits of MPP can
bind substrates with the dissociation constants of the order of
sub-μM (22). In yeast MPP, cross-linking and surface plasmon
resonance analyses showed that α-MPP, but not β-MPP, binds
substrates as efficiently as does the MPP complex (23, 24).
Which subunit functions for substrate recognition has re-
mained to be determined.

To investigate the subunit responsible for substrate recogni-
tion in yeast MPP, we used the fluorescence-labeled peptides. A
coumarin derivative, which is an environment-sensitive fluo-
rescence probe, was covalently introduced into the synthetic
peptides based on the malate dehydrogenase precursor. The
dissociation constants of MPP and the subunits with the fluo-
rescent-labeled peptide were determined by fluorescence emis-
ion intensity. We report here that MPP can bind the substrate
peptides with high affinity but only in the dimeric complex and
that individual subunit interacts with different parts of the
extension peptide of precursor protein.

EXPERIMENTAL PROCEDURES

Purification of the Hexahistidine-tagged Yeast MPP and Its Sub-
units—A hexahistidine tag was introduced into the C termini of yeast α-
and β-MPP using the polymerase chain reaction method. The resultant
cDNAs were inserted into pET3d vector, leading to pET-α-His and
pET-β-His. For co-expression of yeast MPP subunits, a T7 promoter/
nontagged β-MPP cassette was inserted in tandem into pET-α-His. The
resulting construct, pET-α-His-β, contained the histidine-tagged α-MPP and
nontagged β-MPP under the T7 promoters.

BL21(DE3) strain transformed with each plasmid was cultured for
24 h at 25 °C and then harvested by centrifugation at 1,000 × g for 10
min. After sonication of the harvested cells, the suspension was cen-
trifuged at 15,000 × g for 20 min. The resultant supernatant was loaded
on a 1-ml nickel trap-chelating column (Amersham Pharmacia Biotech)
equilibrated with buffer A (10 mM Hepes-KOH, pH 7.4, containing 500
mM NaCl and 0.01% Tween 20). The column was washed with 10 ml of
the buffer A containing 10 mM imidazole and then with 10 ml of the
buffer A containing 100 mM imidazole. The hexahistidine-tagged pro-
teins were eluted using buffer A containing 500 mM imidazole.
The purity was confirmed by SDS-polyacrylamide gel electrophoresis fol-
lowed by Coomassie Blue staining.

Preparation of Coumarin-labeled Peptides—Peptides were synthe-
sized using an EPL221 economy peptide synthesizer (ABIMED Anal-
ysen-Teknik GmbH, Germany), employing Fmoc (N-α-9-fluorenylmeth-
oxycarbonyl) strategy. After acetylation of the α-amino group of the
Substrate Binding Pocket of Mitochondrial Processing Peptidase

N-terminal amino acid with acetic anhydride, deprotection, cleavage of the peptides from the resin, and purification by reverse phase HPLC were performed as described (17).

The purified peptides were labeled with 7-diethylaminocoumarin (DAC) at the ε-amino group of lysine residue in the C-terminal portion of the peptides (see Fig. 1A and Table I). The reaction was conducted in 100 mM Hepes-KOH (pH 7.4), 40% dimethylformamide, 1 mM succinimidy ester of DAC, and 0.1 mg/ml peptide for 2 h at 25 °C. After precipitation of the peptide by adding 10 volumes of acetonitrile, the precipitate was collected by centrifugation at 5,000 × g for 5 min, washed twice with acetonitrile, and then dissolved in distilled water. The coumarin-labeled peptides were purified by reverse phase HPLC on an Asahipak C4P-50 column (Asahi Chemical Industry, Japan) and dissolved in 50% aqueous dimethylformamide. Concentration of the fluorescence peptides was calculated from the absorbance of the DAC at 430 nm using a molar extinction coefficient of 40,000.

**Fluorescence Measurements**—Fluorescence was measured at 25 °C using a Hitachi F-2000 fluorescence spectrophotometer. Excitation was at 390 nm, and the emission intensity was measured at 470 nm. For binding assay, the purified α-MPP, β-MPP, and MPP complex were preincubated with 5 mM EDTA on ice for 30 min. Proteins of a fixed concentration (see legends to tables and figures) were diluted into 20 mM Hepes-KOH (pH 7.4), 30% glycerol, and 1 mM EDTA, then the concentration (see legends to tables and figures) were diluted into 20 mM Hepes-KOH (pH 7.4), 40% dimethylformamide, 1 mM succinimidy ester of DAC, and 0.1 mg/ml peptide for 2 h at 25 °C. After precipitation of the peptide by adding 10 volumes of acetonitrile, the precipitate was collected by centrifugation at 5,000 × g for 5 min, washed twice with acetonitrile, and then dissolved in distilled water. The coumarin-labeled peptides were purified by reverse phase HPLC on an Asahipak C4P-50 column (Asahi Chemical Industry, Japan) and dissolved in 50% aqueous dimethylformamide. Concentration of the fluorescence peptides was calculated from the absorbance of the DAC at 430 nm using a molar extinction coefficient of 40,000.

**Fluorescence Measurements**—Fluorescence was measured at 25 °C using a Hitachi F-2000 fluorescence spectrophotometer. Excitation was at 390 nm, and the emission intensity was measured at 470 nm. For binding assay, the purified α-MPP, β-MPP, and MPP complex were preincubated with 5 mM EDTA on ice for 30 min. Proteins of a fixed concentration (see legends to tables and figures) were diluted into 20 mM Hepes-KOH (pH 7.4), 30% glycerol, and 1 mM EDTA, then the concentration (see legends to tables and figures) were diluted into 20 mM Hepes-KOH (pH 7.4), 40% dimethylformamide, 1 mM succinimidy ester of DAC, and 0.1 mg/ml peptide for 2 h at 25 °C. After precipitation of the peptide by adding 10 volumes of acetonitrile, the precipitate was collected by centrifugation at 5,000 × g for 5 min, washed twice with acetonitrile, and then dissolved in distilled water. The coumarin-labeled peptides were purified by reverse phase HPLC on an Asahipak C4P-50 column (Asahi Chemical Industry, Japan) and dissolved in 50% aqueous dimethylformamide. Concentration of the fluorescence peptides was calculated from the absorbance of the DAC at 430 nm using a molar extinction coefficient of 40,000.

**RESULTS**

**Binding of the Coumarin-labeled Peptides to MPP**—To monitor substrate binding of the MPP and the subunits, a coumarin derivative was introduced to the peptides corresponding to the N-terminal 21 amino acids of rat malate dehydrogenase (MDH) precursor, as shown in Fig. 1A. Yeast MPP was obtained from co-purification of the C-terminally hexahistidine-tagged α-MPP with the nontagged β-MPP by nickel-chelating column. The purified MPP was confirmed to be an equal stoichiometry of both subunits by SDS-polyacrylamide gel electrophoresis and gel filtration and had the same kinetic parameters, $K_a$ and $K_{cat}$, as the native MPP (data not shown). The coumarin-labeled peptide (DAC-MDH5–25) gave a fluorescence emission spectrum with the maximum at 482 nm (Fig. 1B, spectrum 1). The addition of the purified MPP, which was inactivated in the presence of EDTA, led to a large increase in fluorescence intensity and a blue shift in emission spectrum (emission maximum at 470 nm) (Fig. 1B, spectrum 2). When the coumarin-labeled peptide was added to the MPP in the absence of EDTA, the increased fluorescence rapidly disappeared to the level seen with the peptide alone (Fig. 1B, spectrum 3). The processed peptides obtained after incubation of DAC-MDH5–25 with a small amount of the active enzyme gave no change in the fluorescence spectrum (data not shown).

These observations are interpreted to mean that the increase in the fluorescence occurs through a specific interaction of the precursor peptide with MPP and that this interaction is lost with processing of the peptide.

The titration of MPP with DAC-MDH5–25 showed a simple saturation curve (Fig. 2). The inset in the figure shows the Scatchard plot of the data, from which the dissociation constant, $K_d$, was calculated to be 0.13 ± 0.07 μM, as the inverse of the slope. The molar ratio of the peptide to MPP was determined to be 0.83 ± 0.09, suggesting a stoichiometric binding of the peptide to the enzyme.

Our earlier study using synthetic MDH peptides (17) indicated that the arginine residue at position −2 and those distant from the cleavage point are important for cleavage by the MPP. To confirm the specific binding of the fluorescence-labeled peptide to the MPP, we examined the interactions of MPP with DAC-MDH5–25 derivatives (Table I), in which arginine residues were replaced with alanine. DAC-MDH7A, in which the distal Arg7 of the MDH extension peptide was altered to alanine, showed substantially decreased affinity compared with the wild type. Compared with the $K_a$ value of MDH1–25 (0.71 μM) in the cleavage reaction (17), those of MDH7A, MDH14A, MDH15A, and MDH14A15A were 8.7-fold (6.2 μM), 1.8-fold (1.3 μM), 1.8-fold (1.3 μM), and 47.9-fold (34 μM), respectively. The absolute values of the $K_a$ reported previously were about 5-fold larger than those of $K_d$, because of differences in salt concentrations in the reaction medium. The affinity of the enzyme for substrates is markedly affected by the salt concentration, and the $K_a$ value at 10 μM...
Substrate Binding Pocket of Mitochondrial Processing Peptidase

Fig. 2. Titration of yeast MPP with the coumarin-labeled MDH peptide. Titration of yeast MPP (2 μM) with DAC-MDH5–25 (0–4 μM). The fluorescence intensity at 470 nm was corrected, based on the emission of the peptide alone. The inset gives a linear least square plot of 1/(1 – F/F_{\text{max}}) versus [L]/(F/F_{\text{max}}) for titration of MPP with DAC-MDH5–25. F and [L] represent the increased fluorescence intensity and peptide concentration, respectively. F_{\text{max}} was obtained by fitting the saturation curve to a rectangular hyperbola. a.u., arbitrary units.

Table 1

| Peptide      | Sequence                          | K_{d} (μM) |
|--------------|-----------------------------------|------------|
| DAC-MDH5–25  | Ac-LARPVGAAALKS-FSTSAQNK(e-DAC)A | 0.13       |
| DAC-MDH14A   | Ac-LARPVGAAALKS-FSTSAQNK(e-DAC)A | 0.21       |
| DAC-MDH15A   | Ac-LARPVGAAALKS-FSTSAQNK(e-DAC)A | 1.56       |
| DAC-MDH7A    | Ac-LARPVGAAALKS-FSTSAQNK(e-DAC)A | 1.75       |
| DAC-MDH14A15A| Ac-LARPVGAAALKS-FSTSAQNK(e-DAC)A | 7.26       |

NaCl is about 10 times lower than that at 100 mM. For enzyme assays in the previous experiments, we used a large amount of the diluted enzyme solution eluted from columns with high salt, whereas contamination of salts was negligible in the present experiment because we used an extremely high concentration of the recombinant enzyme. Thus, a similar response to amino acid substitution between K_{d} and K_{m} indicates that the distal and proximal arginine residues directly participate in substrate binding of MPP. This method using a fluorescence-labeled peptide allows one to estimate the affinity of MPP for substrates.

Cooperative Formation of a Substrate Binding Pocket by Two Subunits—To determine which subunit is responsible for binding of the substrate, each subunit that was tagged with hexa-histidines at the C terminus was individually purified using a nickel-chelating column. They reconstituted the enzyme activity when mixed together, although the distal and proximal arginines directly participate in substrate binding pocket of MPP for substrates.

The fluorescence intensity at 470 nm increased with increasing concentrations of the peptide, although the intensity for both subunits did not reach the maximum level even at 5 μM peptide (Fig. 3). The K_{d} values for α- and β-MPP were determined to be 4.38 ± 0.75 and 4.45 ± 0.60 μM, respectively, from the Scatchard analysis of the data (Fig. 3, insets). Thus, the affinities of α- and β-MPP for the substrate are about 30-fold lower than that of the MPP complex. The results indicate that only the MPP complex can bind substrates with high affinity. The molar ratio of the peptide to α- and β-subunit were calculated to be 0.92 ± 0.12 and 0.83 ± 0.09, respectively, suggesting specific interaction of the peptide to each subunit, although with low affinity.

The interaction of each subunit with the peptides that lack the distal and proximal arginines was too weak to detect a change of the coumarin fluorescence in the titration experiment. Therefore, the affinities of the subunit monomers with these mutant peptides were estimated by competition between DAC-MDH5–25 and the nonlabeled mutant peptides in binding to each subunit. In the experiment, MDH7A14A was used as a substrate lacking distal arginine, instead of MDH7A, to eliminate the effect of the number of positive charges in the peptides, because the replacement of arginine residue at position 14 had little effect on binding of the peptide to the MPP (Table I). Fig. 4A shows the titration of β-MPP with DAC-MDH5–25 in the presence of 0 to 75 μM competitor peptide, nonlabeled MDH7A14A. The apparent K_{d} values of the subunit for DAC-MDH5–25 were obtained from titration curves at various concentrations of the competitors. The K_{d} value for the competitor peptide could be determined from a plot of the apparent K_{d} values for the labeled peptide in the presence of the competitor versus the concentration of the competitor peptide, and the plot was reasonably linear (Fig. 4B). When nonlabeled wild-type peptide was used as the competitor, the calculated K_{d} values for α-MPP (4.4 μM) and β-MPP (3.6 μM) were substantially the same as those obtained by the direct binding assay described above. The K_{d} value of MDH7A14A for β-MPP was determined to be 43 μM (Fig. 4B), which is about 10 times higher than that of the wild-type peptide. This mutant peptide, however, exhibited practically no inhibition to the binding of DAC-MDH5–25 to α-MPP even at 5 μM. On the other hand, the K_{d} values of MDH14A15A for binding to α- and β-MPP were determined to be 0.46 and 2.67 μM, respectively, indicating that the mutant

---

2 K. Kojima, S. Kitada, K. Shimokata, T. Ogishima, and A. Ito, unpublished results.
peptide has more than 100 and 500 times less affinity to α- and β-subunits, respectively, than MDH5–25. These findings mean that substrate binding with the individual subunits as well as with the whole enzyme (Table I) depends on the presence of the proximal and distal arginines, although dependence varies with the subunits and position of arginines. Thus, α-MPP appears to participate in the interaction with distal arginine residue of the extension peptide, whereas β-MPP seems to be more responsible for interaction with proximal arginine.

The difference in the binding state of peptides between the MPP complex and each subunit was revealed by the quenching of coumarin fluorescence by acrylamide. After binding of the peptide to the MPP complex and the subunits, increasing concentrations of acrylamide were added, and the fluorescence change was measured at 470 nm. Fig. 5 shows the Stern-Volmer quenching plots, and the quenching constants, $K_{sv}$, were calculated to be $1.1 \pm 0.4$, $8.7 \pm 0.6$, and $9.1 \pm 1.3 \text{ M}^{-1}$, for the MPP complex, α-, and β-subunit, respectively. The results indicate that the local environment around the substrate peptide bound to the MPP complex differs from that for each subunit and that in the MPP complex the coumarin fluorophore is partially buried in the enzyme, whereas the one in the subunit is fully exposed to the solvent.

**DISCUSSION**

We presented evidence that MPP can bind the substrate peptides with high affinity but only in the dimeric complex. MPP has an affinity of the dissociation constant of about 0.13 μM to the peptide corresponding to the N-terminal 21 amino acids of rat MDH precursor, whereas each subunit has about a 30-fold less affinity than the dimeric complex. The study using surface plasmon resonance, however, showed that α-MPP bound a substrate with the same affinity as the MPP complex and that the $K_d$ value to the immobilized peptide was determined to be 0.2 μM (24). We obtained similar results showing no clear difference in the affinity between the MPP complex and each subunit, based on the experiments on surface plasmon resonance and on affinity purification of the enzyme using the peptide ligand bound to the Sepharose resin.5 Because our fluorescence-quenching analysis demonstrated that the peptide ligand to the MPP was buried in the enzyme, the finding that the $K_d$ value for the MPP complex is similar to that for individual subunits in these experiments was probably because of steric interference by immobilization of the peptides to the dextran matrix on the sensor chip or Sepharose resin. These findings indicate that dimer formation of the two subunits of MPP is essential for substrate binding.

We also demonstrated that the individual subunit required arginines at different positions in the peptide for binding, although their affinities were much lower than that of MPP. Because β-MPP has a catalytic center (12), the subunit appears to interact with amino acid residues around a scissile bond of the precursor, including proximal arginine at position –2. The interaction between β-MPP and the proximal arginine seems to...
Substrate Binding Pocket of Mitochondrial Processing Peptidase

be more responsible for the initial binding step of substrates to the enzyme rather than the catalytic reaction, because mutation of the proximal arginine caused a drastic increase in the $K_m$ value but not much effect in the $V_{max}$ value (17). $\beta$-MPP may also interact with the upper region, distal to the cleavage site, of the extension peptide. The lack of the distal arginine of the MDH extension peptide led to an increase in the $K_m$ value to this subunit (Table II). Moreover, mutation of Glu$^{79}$ in rat $\beta$-MPP, which is a residue within the conserved acidic amino acid cluster in $\beta$-MPP, decreased the interaction of substrate peptide fluorescent-labeled at the N-terminal portion with this subunit (28). On the other hand, it is likely that $\alpha$-MPP is responsible for the interaction with the upper region of the extension peptide. The present data showed that distal arginine was indispensable for binding of the peptide to $\alpha$-MPP. We recently found that substitution of the conserved acidic amino acid residues in $\alpha$-MPP (Glu$^{353}$ and Glu$^{377}$/Asp$^{378}$ in yeast $\alpha$-MPP) on the processing of the precursor protein was more effective with a longer extension peptide (25). Thus, individual subunits interact with different parts of the extension peptide of the precursor protein.

The subunits of the MPP are homologous to the core proteins of mitochondrial ubiquinol-cytochrome oxidoreductase (bc$_1$ complex), a component of the respiratory chain. The crystal structure of the bc$_1$ complex from bovine heart mitochondria has been determined (26, 27), and one can expect that MPP is similar in structure to the bovine core 1 and core 2 complex. Core 1 and 2 proteins are structurally similar and consist of two domains of roughly equal size with almost identical folding topology. The two bowls representing these proteins were described as coming together in the form of a ball with a crack leading to the internal cavity. From the available data, the middle portion of core 2, which corresponds to the glycine-rich region of $\alpha$-MPP conserved among many species, is close to a site of core 1 that corresponds to the zinc binding site of $\beta$-MPP. These regions might cooperatively form the active site that recognizes the proximal arginine and catalyzes cleavage reaction.

In conclusion, the two subunits of MPP cooperatively form the substrate binding pocket and recognize different structural elements in the extension peptide, including the proximal and distal arginine residues. Individual subunit monomers can interact with distinct structural elements, although with low affinity, and complex formation with the two subunits leads the enzyme to have higher affinity for substrates, the results both of induced structural change at the interface between the two and increase in binding sites. Recognition of the precursors at multiple subsites in MPP make it possible to render strict specificity and high affinity to the enzyme for precursors with structures little in common.

REFERENCES
1. Conboy, J. G., Fenton, W. A., and Rosenberg, L. E. (1982) Biochem. Biophys. Res. Commun. 105, 1–7
2. Sagara, Y., Ito, A., and Omura, T. (1984) J. Biochem. (Tokyo) 96, 1743–1752
3. Schmit, B., Wachtler, E., Sebold, W., and Neuert, W. (1984) Eur. J. Biochem. 144, 581–588
4. Miura, S., Amaya, Y., and Mori, M. (1986) Biochem. Biophys. Res. Commun. 134, 1151–1156
5. Jensen, R. E., and Yaffe, M. P. (1988) EMBO J. 7, 3863–3871
6. Hawlitschek, G., Schneider, H., Schmidt, B., Tropschug, M., Hartl, F.-U., and Neuert, W. (1988) Cell 53, 795–806
7. Pollock, R. A., Hartl, F.-U., Cheng, M. Y., Ostermann, J., Horwich, A., and Neuert, W. (1988) EMBO J. 7, 3493–3500
8. Géli, V., Yang, M., Suda, K., Lustig, A., and Schatz, G. (1990) J. Biol. Chem. 265, 19216–19222
9. Géli, V. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6247–6251
10. Saavedra-Alanis, V. M., Rysavy, P., Rosenberg, L. E., and Kalousek, F. (1994) J. Biol. Chem. 269, 9284–9288
11. Rawlings, N. D., and Barrett, A. J. (1991) Biochem. J. 275, 389–391
12. Kitada, S., Shimokata, K., Niidome, T., Ogishima, T., and Ito, A. (1995) J. Biochem. (Tokyo) 117, 1148–1150
13. Hartl, F.-U., Planner, N., Nicholson, D. W., and Neuert, W. (1989) Biochim. Biophys. Acta 988, 1–45
14. Hendrick, J. P., Hodges, P. E., and Rosenberg, L. E. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 4056–4060
15. Gavel, Y., and von Heijne, G. (1990) Protein Eng. 4, 33–37
16. Arretz, M., Schneider, H., Guiard, B., Brunner, M., and Neuert, W. (1994) J. Biol. Chem. 269, 4959–4967
17. Niidome, T., Kitada, S., Shimokata, K., Ogishima, T., and Ito, A. (1994) J. Biol. Chem. 269, 24719–24722
18. Ogishima, T., Niidome, T., Shimokata, K., Kitada, S., and Ito, A. (1995) J. Biol. Chem. 270, 30322–30326
19. Song, M.-C., Shimokata, K., Kitada, S., Ogishima, T., and Ito, A. (1996) J. Biochem. 120, 1163–1166
20. Ou, W.-J., Kumanoto, T., Mihara, K., Kitada, S., Niidome, T., Ito, A., and Omura, T. (1994) J. Biol. Chem. 269, 24673–24678
21. Shimokata, K., Nishio, T., Song, M.-C., Kitada, S., Ogishima, T., and Ito, A. (1997) J. Biochem. (Tokyo) 122, 1019–1023
22. Boteva, R., and Salvato, B. (1990) Arch. Biochem. Biophys. 332, 323–328
23. Yang, M., Géli, V., Oppliger, W., Suda, K., James, P., and Schatz, G. (1991) J. Biol. Chem. 266, 5013–5020
24. Luciano, P., Geoffrey, S., Brandt, A., Hernandez, J. P., and Géli, V. (1997) J. Mol. Biol. 272, 213–225
25. Shimokata, K., Kitada, S., Ogishima, T., and Ito, A. (1998) J. Biol. Chem. 273, 25355–25363
26. Xia, D., Yu, C.-A., Kim, H., Xia, J.-Z., Kachurin, A. M., Zhang, L., Yu, L., and Deisenhofer, J. (1997) Science 277, 60–66
27. Iwata, S., Lee, J. W., Okada, K., Lee, J. K., Iwata, M., Rasmussen, B., Link, T. A., Ramsawamy, S., and Jap, B. K. (1998) Science 281, 64–71
28. Kitada, S., Kojima, K., Shimokata, K., Ogishima, T., and Ito, A. (1998) J. Biol. Chem. 273, 32547–32553
Cooperative Formation of a Substrate Binding Pocket by α- and β-Subunits of Mitochondrial Processing Peptidase
Katsuhiko Kojima, Sakaie Kitada, Kunitoshi Shimokata, Tadashi Ogishima and Akio Ito

J. Biol. Chem. 1998, 273:32542-32546.
doi: 10.1074/jbc.273.49.32542

Access the most updated version of this article at http://www.jbc.org/content/273/49/32542

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 28 references, 14 of which can be accessed free at http://www.jbc.org/content/273/49/32542.full.html#ref-list-1