The eukaryotic multicatalytic proteinase complex (proteasome) is a high molecular mass enzyme which contains 13–15 nonidentical subunits of similar size (molecular masses of 21–31 kDa), but differing widely in net charge (isoelectric points ranging from 3 to 10). At least four catalytic components termed chymotrypsin-like, trypsin-like, peptidylglutamyl peptide-hydrolyzing, and caseinolytic are associated with the proteinase. The catalytic nature of the components is unknown, since sequences of cloned subunits bear no homology to known proteinases and proteolytically active subunits have not been isolated. Analysis of the relationship between structure and catalytic function would be greatly facilitated if a means for reversibly dissociating and reassociating the proteinase were available. We provide the first evidence of reassembly of dissociated multicatalytic proteinase complex into a functional molecule. Incubation with the organic mercurial, p-chloromercuribenzoic acid disrupts in a concentration-dependent manner the quaternary structure of the enzyme, leading to formation of a heterogeneous population of subunits. Dissociation of the complex coincides with progressive loss of chymotrypsin-like, trypsin-like, and peptidylglutamyl peptide-hydrolyzing activities. The caseinolytic activity of the residual undissociated enzyme is markedly activated. Exposure of the dissociated enzyme to dithiothreitol restores the catalytic profile and reassociates the enzyme. Evidence for catalytically active subcomplexes was not obtained indicating that structural integrity may be necessary for expression of all defined activities.

The multicatalytic proteinase complex (MPC)\(^1\) (EC 3.4.99.46), also known as the proteasome, is an extralysosomal high molecular mass proteinase (\(\sim 700\) kDa) found in all eukaryotic cells examined (reviewed in Refs. 1–3). MPC is composed of 13–15 nonidentical subunits of similar size (molecular masses ranging from 21 to 31 kDa), but widely varying isoelectric points ranging from 3 to 10 (1–3). Electron microscopy provided evidence that the subunits are arranged in four stacked rings forming a cylindrical structure (4), each ring containing seven subunits (5), and that the eukaryotic complex may be a symmetrical dimer (6). Assembly may be facilitated by similarity in subunit size, and stability may be conferred by complementary interactions among heterogeneous net charges of subunits (7). A striking observation is that MPC purified from archaeobacteria contains only two nonidentical subunits, but has a similar quaternary structure to the eukaryotic enzyme (8). This underlines the importance of the quaternary structure to the catalytic properties of MPC.

Although the number of distinct activities associated with MPC is unknown, at least four catalytic components have thus far been defined. MPC hydrolyzes peptide bonds on the carboxyl side of basic (trypsin-like activity), acidic (peptidylglutamyl peptide-hydrolyzing or PGP activity) and hydrophobic (chymotrypsin-like activity) amino acids (9, 10). A fourth activity, the specificity of which remains to be established, is responsible for the initial degradation of \(\beta\)-casein (11, 12). Evidence for still other activities has been presented (13, 14). The mechanistic classification of MPC remains undefined. The primary structures of cloned subunits bear no homology to known proteinases (3). Some inhibitors of both cysteine and serine proteinases affect the multiple activities identified. However, catalytic components resistant to or even activated by the serine proteinase inhibitor 3,4-dichloroisocoumarin (DCI) were described (12, 13, 15), and the cysteine proteinase inhibitor Ep-775 does not alter any of the activities of the complex (16).

Catalytic activities of the proteinase are associated with separate components, as activation or inhibition of one activity has a positive or negative effect on the others (9, 10). Attempts to isolate proteolytically active subunits failed. Dissociation of the complex after treatment with SDS (17), urea (18), or certain divalent cations (19) produced loss of chymotrypsin-like, trypsin-like, and PGP activities. These results suggest that the three originally defined catalytic components depend on the structural integrity of the complex. However, loss of the heaviest subunit of the proteinase (molecular mass \(\approx 31\) kDa) coincides with activation of the caseinolytic activity (12, 20, 21). Moreover, chymotrypsin-like, trypsin-like, and PGP activities are readily measured in preparations lacking the heaviest subunit (12). It is not known if the loss of the 31-kDa subunit affects the quaternary structure of the proteinase.

Structure-function relationship studies would be facilitated if a means of reversibly dissociating and reassociating the complex were available. Organic mercurials such as \(p\)-chloromercuribenzoate (pCMB) and \(p\)-mercuriphenylsulphonate are strong inhibitors of hydrolysis of chromogenic substrates.
Dissociation-Reassociation of the Multicatalytic Proteinase Complex

by MPC (13, 22, 23). We present evidence that changes in the enzymatic activities of MPC produced by pCMB coincide with dissociation of the complex. Dithiothreitol restores the original catalytic profile and reassociates the complex. These studies provide the first evidence of reassembly of dissociated MPC into a functional molecule.

EXPERIMENTAL PROCEDURES

Materials—MPC was isolated from bovine pituitaries as described (24). Frozen bovine pituitaries were from Pel-Freez Inc. (Rogers, AK). Chz-Gly-Gly-Leu-pNA and Chz-d-Ala-Leu-Arg-2NA were synthesized as described (9, 25). Chz-Leu-Leu-Glu-2NA, succinyl-Leu-Leu-Val-Tyr-AMC, dephosphorylated β-casein, and pCMB were from Sigma. Other reagents were of highest purity available.

Treatment of the Enzyme with pCMB—Different concentrations of pCMB dissolved in dimethyl sulfoxide alone were preincubated with the enzyme for the desired times at 23 °C on a shaking platform. When indicated, 1 mM DTT was added to the samples, and the reaction continued for 30 min. The concentration of dimethyl sulfoxide in the preincubation mixture was 1.3%. Reactions were stopped by rapidly freezing on dry ice.

Enzyme Assays—Enzyme activities were measured with Chz-Gly-Gly-Leu-pNA, Chz-d-Ala-Leu-Arg-2NA, and Chz-Leu-Leu-Glu-2NA for chymotrypsin-like, trypsin-like, and PGP activities, respectively (9, 10). Degradation of dephosphorylated β-casein (caseinolytic activity) was determined by a gel electrophoretic method (11).

Polyacrylamide Gel Electrophoresis—Nondissociating PAGE was performed on 5% acrylamide slab gels in 0.25 M Tris glycin buffer, pH 8.3, at 4 °C and stained for protein with either Coomassie Blue or silver stain. When appropriate, individual lanes were sliced into 19 5-mm consecutive horizontal sections. Enzymatic activities were measured by incubating each slice separately for 20 h at 37 °C in buffers containing the respective substrates. In other experiments, protein was eluted from disc gel slices by incubation for 20 h at 4 °C in 0.1 M Tris-EDTA, pH 7.5, on a shaking platform. Aliquots of the eluate were subjected to SDS-PAGE on 12.5% gels (26) and stained for protein with silver stain.

HPLC Analysis—HPLC was conducted on a Waters 600E liquid chromatograph. Two Spherogel-TSK 3000SW columns (7.5 mm × 60 cm, dp 10 μm) were connected in tandem, preceded by a Spherogel TSK, SW-PRE guard column. The columns were equilibrated with 0.01 M Tris-EDTA, pH 7.5. The flow rate was 0.4 ml/min. Proteins were monitored by UV detection at 280 nm. The gel filtration system was calibrated with blue dextran (Mr = 2 × 10^6) to determine the void volume and with bovine serum albumin (Mr = 66,000) and dephosphorylated β-casein (Mr = 24,000). Nineteen fractions (1 ml/fraction) were collected, the volumes reduced by evaporation, and aliquots subjected to SDS-PAGE on 12.5% gels and stained for protein with silver stain or transferred onto a polyvinylidene difluoride membrane for immunoblotting.

Immunoblotting—Antisera against purified MPC was raised in rabbits (9). Electrophoretic transfer of proteins was as described (27). Polyvinylidene difluoride membranes were treated with 5% bovine serum albumin and then with MPC antiserum in a dilution of 1:5000. For visualization, an affinity-purified goat anti-rabbit horseradish peroxidase conjugate (Bio-Rad) was used following manufacturer’s recommendations.

Protein Determination—Protein concentrations were measured by the Lowry method with bovine serum albumin as standard (28).

RESULTS

Gel Electrophoretic Examination of pCMB-treated MPC—The effect of pCMB on the migration pattern of the enzyme under nondissociating gel electrophoresis was studied. As shown in Fig. 1A, lane 1, MPC migrated as a single band in the presence of dimethyl sulfoxide alone. Coomassie Blue staining revealed a gradual disappearance of native enzyme when incubated for 10 min with increasing concentrations of pCMB (Fig. 1A, lanes 2–4). A diffuse protein band migrating faster than the native enzyme was most apparent at 100 μM pCMB (lane 3), diminishing when MPC was incubated with 400 μM pCMB (lane 4).

Silver staining (Fig. 1B) revealed discrete subcomplexes of MPC visible as protein bands migrating more rapidly than native enzyme (Fig. 1B, lanes 3 and 4). Formation of dissociated protein migrating with the dye front depended upon the concentration of pCMB (Fig. 1B, lanes 2–4). Similar results were obtained with aqueous solutions of the sodium salt of pCMB (data not shown). Freezing and thawing had no effect on dissociation of MPC by pCMB (data not shown). A small amount of silver-stained leading material was detected under control conditions (Fig. 1B, lane 1). This material was only visible after intense silver staining (Fig. 1B) and was not detected by Coomassie Blue staining (Fig. 1A, lane 1).

Reversal by DTT of the Effect of pCMB on the Structure of MPC—Reaction of organic mercurials with sulfhydryl groups of proteins can be reversed by sulfhydryl compounds. Therefore, the effect of exposure of pCMB-treated MPC to DTT was studied. As seen in Fig. 1, A and B, incubation of pCMB-treated enzyme with 1 mM DTT produced a normal gel pattern.
Changes in the Catalytic Properties of MPC Produced by pCMB—Low concentrations of pCMB (5–10 μM) mildly activated the chymotrypsin-like activity (Fig. 2A), but higher concentrations progressively inhibited this activity. PGP and trypsin-like activities declined even in the presence of 5–10 μM pCMB, with the trypsin-like activity being the most sensitive. For example, after a 10-min exposure to 20 μM pCMB the enzyme preparation contained 96% chymotrypsin-like activity, 50% PGP activity, but only 2% trypsin-like activity (Fig. 2A). By contrast, the caseinolytic activity of pCMB-treated MPC was markedly activated by 20 μM pCMB. A maximal stimulation of 8-fold occurred when the enzyme was preincubated for 10 min with 50 μM pCMB. As the concentration of pCMB was increased further, the degree of stimulation decreased (Fig. 2B).

The effect of 50 μM pCMB on the hydrolysis of chromogenic substrates and degradation of β-casein was seen even without preincubation. Trypsin-like, PGP, and chymotrypsin-like activities of pCMB-treated enzyme at t = 0 were 0, 42, and 45% of control values, respectively, and the caseinolytic activity was stimulated 5-fold (Fig. 3A). The final pCMB concentration in the incubation mixture was 2 μM, a concentration that cannot account for the large changes in catalytic activities observed at t = 0 (see Fig. 2A and B). After 30-min exposure to 50 μM pCMB, the MPC preparation contained no measurable trypsin-like activity, only trace amounts of PGP and chymotrypsin-like activities, whereas the caseinolytic activity increased approximately 7-fold (Fig. 3A). A small amount of MPC remained undissociated in the presence of 50 μM pCMB at all times (Fig. 3A, bottom panel).

The increase in caseinolytic activity in the presence of pCMB was not the result of changes in substrate. Preincubation of β-casein with or without 100 μM pCMB, followed by dialysis to remove unbound pCMB, produced no difference in the rate of degradation of native or pCMB-treated β-casein by MPC (data not shown).

Effect of DTT on the Catalytic Activities of the pCMB-treated Enzyme—After 30-min incubation with DTT, the catalytic activities of the pCMB-treated MPC were restored to nearly control values (Table I). The trypsin-like activity of the pCMB-treated enzyme showed the greatest recovery. Rescue of the markedly depressed catalytic activities resulting from incubation of MPC for 10 min with 400 μM pCMB (Table I) or for 60 min with 50 μM pCMB (Fig. 3B) was only partially successful. Nonetheless, the migration pattern of the latter enzyme preparations on nondenaturing PAGE was indistinguishable from that of control MPC (Figs. 1A and 3B, lower panel). Of particular interest is the observation that treatment of MPC with 100 or 400 μM pCMB followed by DTT produced a form of enzyme having a permanently activated caseinolytic component. This effect was particularly marked with the 400 μM pCMB-treated enzyme.

Effect of Substrates on Enzymatic Inactivation by pCMB—Cbz-Gly-Gly-Leu-pNA, Cbz-D-Ala-Leu-Arg-2NA, and Cbz-Leu-Leu-Glu-2NA at a final concentration of 0.4 mM were separately added to incubation mixtures containing MPC and buffer and the assay mixtures adjusted to a final concentration of 10, 50, or 100 μM pCMB. None of the substrates protected the enzyme from the action of pCMB (data not shown).

Identification of Products of Dissociation and Measurement of Their Enzymatic Activity—Enzyme preparations were run under nondissociating conditions on polyacrylamide disc gels, the gels cut into 5-mm slices, and proteins eluted overnight as described under “Experimental Procedures.” The subunit pattern on SDS-PAGE of the eluted samples was analyzed. Most of native and reassociated enzyme migrated to slices 4 and 5 (Fig. 4, A and C). Evidence for MPC subunits in the pCMB-treated preparation could be found throughout the gel, indicating dissociation to heterogeneous populations of oligomers (Fig. 4B). The pattern and relative abundance of subunits in most fractions were clearly different from native enzyme. A homogeneous MPC subfraction containing only 28-kDa subunits migrated with the dye front (Fig. 4B, lane 19). After exposure of pCMB-treated enzyme to DTT, this 28-kDa subunit subfraction was no longer visible, indicating that it had reassociated with other components into the native form of the enzyme (Fig. 4C).

Slab gel slices from pCMB treated enzyme were incubated overnight with substrates, as described under “Experimental Procedures.” Enzymatic activities including caseinolytic activity were only detected at the position of native protein (Table II). Evidence for catalytically active sub-species was not obtained.

Determination of Enzymatic Activity in Native Gels—Aliquots of enzyme before and after pCMB treatment were subjected to gel electrophoresis under nondissociating conditions as described above. It was reasoned that treatment with DTT of gels containing pCMB-treated enzyme should remove pCMB from the protein in situ. Subsequent incubation of...
Fig. 3. Effect of time of preincubation of pCMB (50 μM) on the catalytic activities and structure of MPC. At different times of preincubation an aliquot of the reaction mixture was removed and enzymatic activities measured with synthetic substrates and dephosphorylated β-casein (inset), as described under “Experimental Procedures.” Each point represents the mean ± S.E. of four determinations. In B the pCMB-treated enzyme was incubated for an additional 30 min in the presence of 1 mM DTT prior to measurement of enzymatic activities. All activities are expressed relative to the value shown in B (100%) corresponding to time 0 pretreatment with pCMB and 30-min exposure to 1 mM DTT. Bottom panels display nondissociating gel electrophoresis migration patterns of pCMB-treated MPC before (A) and after (B) exposure to 1 mM DTT. Lanes 1–5; 0, 5, 10, 30, and 60-min incubation with 50 μM pCMB, respectively. O, Cbz-Gly-Gly-Leu-pNA; ⋆, Cbz-Leu-Leu-Glu-2NA; ⋆, Cbz-D-Ala-Leu-Arg-2NA; ■, dephosphorylated β-casein.

Table I

Catalytic activities of pCMB-treated MPC in the absence and presence of 1 mM DTT

MPC was preincubated for 10 min with pCMB and, when indicated, for 30 min with 1 mM DTT as described under “Experimental Procedures.” Enzymatic activities were measured with synthetic substrates and dephosphorylated β-casein, as described under “Experimental Procedures.” All activities are expressed relative to zero pCMB treatment. Each value represents the mean of two experiments.

| pCMB μM | Chymotrypsin-like | Trypsin-like | PGP | Caseinolytic |
|---------|-------------------|-------------|-----|--------------|
| 0       | 100               | 100         | 100 | 100          |
| 10      | 97                | 95          | 24  | 100          |
| 50      | 37                | 93          | 1   | 95           |
| 100     | 17                | 82          | 0   | 82           |
| 400     | 3                 | 43          | 0   | 51           |
| DTT     | 100               | 100         | 100 | 100          |

Fig. 4. SDS-PAGE of samples eluted from nondissociating disc gel slices. 50 μl of each of 19 eluted samples were subjected to SDS-PAGE as described under “Experimental Procedures.” Samples from 15 μg of native (A) and pCMB (400 μM) treated MPC before (B) and after (C) exposure to 1 mM DTT. The numbers above A represent disc gel slice # (number 1 first slice from top of gel). On the left of each panel, the position of a molecular mass marker (29 kDa) is shown.

Table II

Catalytic activities of pCMB-treated MPC subjected to nondissociating gel electrophoresis (numbers represent activities of gel slices incubated overnight)

MPC was preincubated for 10 min with 50 μM pCMB and subjected to nondissociating PAGE as described under “Experimental Procedures.” Gel lanes were cut into 19 5-mm sections, and catalytic activities of each slice measured overnight with synthetic substrates (0.4 mM) or β-casein as described under “Experimental Procedures.” Activities are expressed as absorbance at 540 nm for chymotrypsin-like activity and 580 nm for trypsin-like and PGP activities. Only values for the 6 top slices are shown. No activities could be measured in the other 13 slices. Caseinolytic activity was only detected in gel slices 2 and 3.

| Slice # | Chymotrypsin-like | Trypsin-like | PGP | Caseinolytic |
|---------|-------------------|-------------|-----|--------------|
| 1       | 0.050             | 0.009       | 0.004 | –            |
| 2       | 0.261             | 0.788       | 0.069 | +++          |
| 3       | 0.497             | 1.856       | 0.835 | +++          |
| 4       | 0.004             | 0.007       | 0.125 | –            |
| 5       | 0.000             | 0.002       | 0.000 | –            |
| 6       | 0.000             | 0.000       | 0.000 | –            |

DTT-treated gels with fluorogenic substrate should reveal whether any partially dissociated MPC was catalytically active. Gels were incubated with solutions of succinyl-Leu-Leu-Val-Tyr-AMC, Cbz-Leu-Leu-Glu-2NA, and Cbz-D-Ala-Leu-Arg-2NA at a final concentration of 0.4 mM and examined on
a UV-illuminated plate. Enzymatic activity was only detected in the protein band corresponding to undissociated MPC.

**HPLC Analysis of Native, Dissociated, and Reassociated MPC**—Reaction with pCMB introduces negatively charged groups into the protein molecule. Some material migrating more rapidly under gel electrophoresis may therefore still be fully associated protein. To eliminate the confounding effect of charge, enzyme preparations before and after treatment with 100 μM pCMB were subjected to gel filtration on HPLC Spherogel columns (Fig. 5). The absorbance at 280 nm of a void volume peak corresponding to native MPC (Fig. 5A) was reduced approximately 70% in the pCMB-treated sample (Fig. 5B). Other discrete peaks eluting after the intact enzyme were detected. Exposure of the pCMB-treated preparation to DTT reversed the pCMB effect, restoring an elution pattern almost identical to the untreated enzyme (Fig. 5C).

SDS-PAGE analysis of the lower molecular weight forms visible in Fig. 5B revealed subcomplexes lacking some MPC subunits (Fig. 6A, lanes 4 and 5). Subunit patterns of HPLC fractions differed from those of nondissociating PAGE slices (Fig. 4B). This observation may be accounted for by resolution accordingly to size by HPLC, as opposed to resolution as a function of both size and charge by gel electrophoresis. Moreover, each fraction analyzed may contain more than one discrete component.

**Fig. 5.** HPLC gel filtration chromatography of 42 μg of native (A) and pCMB (100 μM)-treated MPC before (B) and after (C) exposure to 1 mM DTT. Chromatographic and incubation conditions as described under “Experimental Procedures.” Detector sensitivity: 0.01 absorbance units at full scale. Arrows above A depict the eluting positions of blue dextran (void volume), bovine serum albumin (66,000), and β-casein (24,000).

Immunoblots of HPLC fractions of pCMB-treated MPC (Fig. 6B) showed a pattern of subunits recognized by the polyclonal antiserum similar to that identified by silver stain (Fig. 6A). HPLC fractions 2 and 3 represent the void volume of the HPLC gel filtration column. Most likely these fractions correspond to a mixed population of MPC subspecies with molecular masses between 700 and 200 kDa, the lowest limit of the void volume of the column.

**DISCUSSION**

The experiments described here provide evidence that the organic mercurial pCMB, dissociates MPC, and markedly changes the profile of its enzymatic activities. We demonstrate for the first time that dissociated species of MPC including a 26-kDa monomer can be reassociated with little loss of original activity. Treatment with pCMB leads to a marked loss of chymotrypsin-like, trypsin-like, and PGP activities. All residual activities are associated with the fraction of enzyme that remains undissociated. When the pCMB-treated enzyme is incubated with DTT, the products of dissociation disappear, a protein with similar electrophoretic and gel filtration properties to the native enzyme appears, and the catalytic activities are largely restored to control levels. These changes are dependent on the concentration of pCMB used. At the highest concentration tested, i.e. 400 μM, the enzyme can be reassociated but catalytic activities are not fully restored. Reversal of the effects of pCMB especially at lower concentrations indicates that the organic mercurial disrupts the quaternary structure of the proteinase without significantly denaturing the resulting products.

In contrast to inactivation of the catalytic activities directed toward chromogenic substrates in the pCMB-treated enzyme, degradation of β-casein was stimulated. Enhanced caseinolytic activity was only present in the enzyme fraction remaining undissociated. The pCMB-treated enzyme degraded dephosphorylated β-casein without significant accumulation of intermediate products despite its very low trypsin-like, chymotrypsin-like, and PGP activities. A similar effect was seen when MPC was treated with the serine proteinase inhibitor DCI (12). These experiments demonstrate that the chymotrypsin-like, trypsin-like, and PGP activities are not required for complete degradation of β-casein. One or more DCI-
pCMB-resistant enzymatic components catalyze the secondary degradation of β-casein to smaller peptides. Stimulation of hydrolysis of β-casein by pCMB or DCI may be due to a relaxation of the structure of the complex, facilitating access of the protein substrate to the caseinolytic active site. The observation that the caseinolytic activity of the reassociated enzyme is still somewhat stimulated suggests that the conformation of the reassociated enzyme is in a more relaxed form than native MPC.

Organic mercurials were shown previously to affect not only the catalytic activity of proteins, but also to disrupt their protein structure. For example, pCMB was found to totally inhibit muscle phosphorylase a and to split the protein into four fractions of equal molecular weight (29). Cysteine reactivated and restored the structure of phosphorylase a. E. coli ribosomes (70 S) were dissociated into 30 and 50 S subunits after exposure to pCMB. Dissociation was accompanied by the formation of an intermediate component and was partially reversed by sulphydryl reagents such as DTT and β-mercaptoethanol (30). Reaction of enzymes with pCMB does not always lead to inhibition of catalytic activities. For example, chicken duodenal adenosine deaminase was activated by pCMB, and activation was reversed by DTT. Addition of substrate to the incubation mixtures did not affect the activation, suggesting that -SH groups reacting with pCMB are not at the substrate binding site (31).

Dissociation of MPC brought about by pCMB is dependent upon the concentration of the reagent and time of preincubation. The progressive reaction of -SH groups with pCMB resulted in destabilization of the quaternary structure of MPC, facilitating dissociation. Dissociation gave rise to smaller subcomplexes, lacking some subunits. Evidence for formation of a homogeneous MPC subfraction of 26-kDa subunits was obtained by gel electrophoresis. In addition to appearance of discrete subcomplexes by protein staining, Western blotting also provided clear evidence of dissociation. The methods used do not allow for calculation of percent dissociation. The facile dissociation of MPC by pCMB suggests either a critical role of -SH groups in maintaining the quaternary structure of the complex or that introduction of the bulky mercurial is itself sufficient to destabilize the complex.

No enzymatic activities were detected in any of the subfractions. Incubation of substrates in the presence or absence of DTT either with gel slice eluates or with intact native gels only revealed enzymatic activities associated with intact protein. Others have reported that mild dissociation of MPC by Zn²⁺ and Cu²⁺ was accompanied by loss of all catalytic activities measured. No separately active components were identified (32). Unlike the pCMBl effect which is reversed by DTT, dissociation, and inactivation induced by Zn²⁺ and Cu²⁺ was not reversed even by addition of excess EDTA (32). Apparently, pCMB, unlike other treatments previously found to irreversibly dissociate MPC, did not denature the resulting products, making it possible for the enzyme to be reversed back to its initial state when exposed to dithiothreitol.

These results suggest the possibility that in vivo the quaternary structure of MPC is not static. Assembly into the 700-kDa complex may be a dynamic process allowing for exchange of subunits in response to specific conditions. The method of dissociating and reassociating MPC reported here should prove to be of value in studies exploring the architecture of this unique macromolecule and in assigning catalytic activities and regulatory functions to particular subunit subsets or individual subunits. Moreover, this method may provide a means for incorporation of an overexpressed subunit into the complex.

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