Molecular Cloning and Expression of a γ-Interferon-inducible Activator of the Multicatalytic Protease*

Claudio Realini, Wolfgang Dubiel*, Greg Pratt, Katherine Ferrell, and Martin Rechsteiner  
From the Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah 84132 and the Institut für Biochemie, Medizinische Fakultät (Charité), Humboldt Universität Berlin, Hessische Straßle 3-4, 0-1040 Berlin, Federal Republic of Germany

The multicatalytic protease (MCP) can be activated by two distinct multisubunit complexes. One is the regulatory component of the 26 S protease, which contains at least 15 distinct subunits. The other is a hexameric activator composed of 31- and 29-kDa subunits. A cDNA for the smaller subunit has been cloned and sequenced. The cDNA encodes a protein of 249 amino acids. Embedded in the smaller subunit are sequences typical of globular protein domains. Similar regions, which we call KEKE motifs, are also found in two MCP subunits, in subunit 12 of the 26 S protease and in a variety of chaperonins including hsp90, hsp70, and calnexin.

Expression of the activator cDNA in Escherichia coli produced a functional protein virtually indistinguishable from MCP activator purified directly from rodent cells. The recombinant protein formed three iso-electric species on two-dimensional polyacrylamide gel electrophoresis, and it reacted with antibodies to rodent blood cell activator. Recombinant activator also bound the multicatalytic protease and stimulated cleavage at the carboxyl terminus of hydrophobic or charged residues. Synthesis of the activator subunit was induced by γ interferon treatment of HeLa cells. These last two findings have implications for antigen presentation by class I major histocompatibility receptors.

Multimeric, ATP-dependent proteases serve important regulatory functions in both prokaryotic and eukaryotic cells. Two distinct Escherichia coli proteases, Lon and Clp, have been shown to degrade specific regulatory proteins, thereby controlling a variety of bacterial processes (1). Only one ATP-dependent protease has been identified in nuclear or cytosolic extracts from eukaryotes. This large (26 S) protease is the regulatory component of the 26 S protease, which contains at least 15 distinct subunits (2). The 26 S enzyme is formed from a proteolytic core provided by the 26 S protease and in a variety of chaperonins including hsp90, hsp70, and calnexin.

The 26 S enzyme is formed from a proteolytic core provided by the 26 S protease (7, 8). In the presence of ATP, the cylindrical MCP associates with a particle containing 15 or more different polypeptides to form the 26 S enzyme (9). Assembly generates an enzyme capable of degrading Ub conjugates and results in elevated peptidease activity. Recently, we and others (10, 11) described a simpler protein complex capable of stimulating the MCP peptidease activity. As isolated from human red blood cells, this activator is an apparent hexamer composed of two distinct ~30-kDa subunits. It binds the MCP reversibly in the absence of nucleotides and it stimulates cleavage of some fluorogenic peptides as much as 50-fold (11). Here we report the isolation and sequencing of a full-length cDNA for the smaller subunit. We also show that a functional MCP activator is formed by expression of the cDNA in E. coli, and we demonstrate that synthesis of the protein is induced by γ interferon treatment of HeLa cells.

**EXPERIMENTAL PROCEDURES**

**Materials**—The fluorogenic peptides Suc-Leu-Leu-Val-Tyr-MCA, Suc-Arg-Pro-Phe-His-Leu-Leu-Val-Tyr-MCA, Suc-Gly-Pro-Leu-Gly-Pro-MCA, glutaryl-Gly-Gly-Phe-MCA, and Pro-Phe-Arg-MCA were obtained from Peninsula Laboratories Inc. (Belmont, CA). Cbz-Leu-Leu-Glu-p-nitroanilide was from Sigma. Affinity-purified goat anti-mouse IgG used as secondary antibody was obtained from Dako Corp. (Santa Barbara, CA). The Sequenase DNA sequencing kit was from U. S. Biochemical Corp., and the Gene Amp polymerase chain reaction (PCR) components were purchased from Perkin-Elmer Corp. Recombinant human γIFN was from Chemicon International, Inc. (Temecula, CA). Sequencing grade ATP (1000 Ccp/mmole), d(Na)GdNTP (100 Ccp/mmole), carrier-free phosphorus-32 and [γ-32P]ATP were from DuPont NEN. The λZAP II cDNA library, the pBluescript KS, and XL1-Blue E. coli were all from Stratagene, whereas the BL21(DE3) E. coli strain was from Novagen (Madison, WI). Ndel, BamHI, EcoRI, HindIII, T4-polynucleotide kinase, and isopropyl-β-d-thiogalactopyranoside (IPTG) were from Boehringer Mannheim. Amplolines were from Pharmacia Biotech Inc.

**Isolation and Characterization of Activator cDNAs**—Activator was partially purified from outdated human blood as described earlier (11). The partially purified activator was separated by SDS-PAGE and submitted to V8 protease or CNBr cleavage. The resulting peptides were fractionated by high pressure liquid chromatography, and specific peaks were sequenced using an ABI automated gas phase sequencer (12). Peptide sequences were used to design synthetic sense and anti-sense degenerate PCR primers for PCR screening of the sequence (5) of TCGAAAATCCATATTGG-TATGGA(A/G)A-3' and 5'-ATAAGCTTTC(A/G)TA(m/G)TCATTC(T/C/T'T)-3', respectively. The oligonucleotides were combined in PCR reactions with 1-2 μg of CcC1-purified total RNA derived from HeLa cells. Amplified DNA was separated on 1% agarose gels, and the appropriate product was identified upon Southern blot analysis using the γP-labeled oligonucleotide (5'-AAGGCTTCTTGATCATTGG-TATGGA(A/G)A-3' and 5'-ATAAGCTTTC(A/G)TA(m/G)TCATTC(T/C/T'T)-3', located between the two PCR primers. Hybridizing DNA was subcloned into EcoRI and HindIII sites of pBluescript KS and sequenced using the Sequenase kit. The sequences were used to design nondegenerate oligonucleotides to screen cDNA libraries. Approximately 107 phage recombinants from a λ ZAP II cDNA library from HeLa cells and a λ gt 11 cDNA library from human tonsils were screened with nondegenerate 70-80-nucleotide-long hybridization probes.
probes end-labeled with \(^{32}\text{P}\)ATP using T4-polynucleotide kinase. The pBluescript KS phagemid was excised and recircularized from the linear λ ZAP II of positive recombinant bacteriophages. Insertas of positive λ gt 11 clones were subcloned into EcoRI sites of pBluescript KS phagemids. Both constructs were amplified in E. coli XLI-Blue and propagated using M13 DNA sequencing. The PC primer algorithm and database were used to analyze the nucleotide and deduced amino acid sequences.

### In Vivo Expression of Activator Gene

The full-length cDNA for the human red blood cell activator was ligated into the NdeI and BamHI site of the T7 polymerase-dependent expression vector pAE4. Ligation products were transformed into BL21(DE3) prepared for CaCl\(_2\)-dependent transformation (12). Recombinants were amplified or selective plates, and purified vectors were sequenced using the Sequenase products. Recombinant E. coli carrying the full-length cDNA were either induced for 2 h with 0.5 mM IPTG or grown in the absence of IPTG. The soluble protein fraction was obtained from induced or non-induced recombinant cells by sonication in 10 mM Tris, pH 7.0, 25 mM KCl, 10 mM NaCl, 1.1 mM MgCl\(_2\), 0.1 mM EDTA, and 1.0 mM dithiothreitol, followed by centrifugation at 39,000 \(\times g\) for 30 min at 4°C to remove insoluble proteins.

#### Western Blot Analysis of Recombinant Activator

Soluble proteins obtained from recombinant BL21(DE3) E. coli were electrophoresed on a 12% denaturing SDS-polyacrylamide gel (see below) and transferred to nitrocellulose for 12 h at 4°C and at a constant current of 100 mA. The nitrocellulose filter was blocked for 60 min in 5% dried milk in Tris, pH 7.5, 0.9% NaCl, and 0.02% sodium azide, incubated with polyclonal mouse anti-activator serum (1/2000 dilution) for 12 h at 4°C, washed in Tris-buffered saline, incubated for 6 h in the presence of \(^{125}\text{I}\)-labeled rabbit anti-mouse IgG, extensively washed in Tris-buffered saline, and exposed for 3 days to X-Omat AR film (Kodak) at -20°C. Polyclonal antibodies were raised in BALB/c mice injected intraperitoneally with purified human red blood cell activator purified as described (11).

### Electrophoresis

Native gels consisted of 2.5% stacking gel in 50 mM Tris-HCl, pH 6.8 and 4.5% or 8% resolving gel in TBE (90 mM Tris, pH 8.3, 1.6 mM borate, and 0.08 mM EDTA) as described previously (9). Gels were run for 2.5 h at 4°C at a constant voltage of 100 V/cm. Denaturing SDS-polyacrylamide gels were composed of a 4% stacking gel and a 0.75% resolving gel in Tris-HCl, pH 6.8, 1% SDS, and a 10 or 12% separating gels in 37.5 mM Tris-HCl, pH 8.8, and 0.1% SDS. Gels were run at room temperature in 25 mM Tris, pH 8.6, 200 mM glycerol, and 0.1% SDS using a Mini-Protean apparatus (Bio-Rad). For two-dimensional native/SDS-gel electrophoresis, MCP and recombinant activator were mixed and electrophoresed for 6 h at 4°C on an 8% native polyacrylamide gel as described above. After native electrophoresis, an individual lane of the gel was incubated for 10 min in 30 mM Tris-HCl, pH 6.8, 1% SDS, 5% glycerol, and 5 mM β-mercaptoethanol, and the proteins separated on a 10% SDS-polyacrylamide gel. Following electrophoresis, proteins were visualized by staining in 0.2% Coomassie Brilliant Blue in 22.5% methanol and 7.5% acetic acid. For determination of isoelectric points, proteins were separated on a first dimension consisting of isoelectric focusing in the pH range 4.2–6.5 followed by separation on a 10% SDS-polyacrylamide gel as described (11).

### Purification of Multicatalytic Proteases

MCP was purified from human lymphoblastoid cell lines 721.45 and 721.174 as follows. Cells were grown in the presence of MCP and various fluorogenic peptides incubated in the presence of MCP and various amounts of recombinant or purified human activator in a final volume of 100 μl. The source of MCP and incubation conditions are specified in the figure legends. The two components were incubated 10 min prior to the addition of the fluorogenic peptide. Reactions were initiated by the addition of 200 μl of cold 100% ethanol. Fluorescence was measured on a Perkin-Elmer fluorometer using an excitation wavelength of 380 nm and emission wavelength of 440 nm for Suc-Arg-Pro-Phe-His-Leu-Leu-Val-Tyr-MCA, Suc-Gly-Pro-Leu-Gly-Pro-MCA, Suc-Leu-Leu-Val-Tyr-MCA, and Pro-Phe-Arg-MCA. For Cbz-Leu-Leu-Glu-pNA, excitation and emission wavelengths were 355 and 410 nm, respectively. The stimulation of MCP peptidase activity is defined as 1 - F\(_{\text{MCP,act}}/F\(_{\text{MCP,act}}\) where F\(_{\text{MCP,act}}\) is the rate of change in the fluorescence (F) in the presence of a given amount of activator, and F\(_{\text{MCP}}\) is the fluorescence measured in the absence of activator.

### Substrate Overlay

Mixtures of MCP and recombinant or purified human activator were electrophoresed on 4.5% native gels for 2.5 h as described above. The gel was overlaid with 200 μl Suc-Leu-Leu-Val-Tyr-MCA and incubated for 10–20 min at 37°C, and the released MCA was localized by transilluminating on a UV light box. Recombinant activator was prepared as described above. Purified human activator and MCP were prepared from outbred human blood (12).

Analysis of γIFN-induced Proteins—Proteins synthesized in HeLa cells grown in the presence or absence of γIFN were analyzed by two-dimensional PAGE. Cells of the human HeLa line, D8A4H2, were plated at 2 x 10⁴ cells/cm² in McCoy’s medium plus or minus 200 units/ml recombinant γIFN. After 72 h the cells were rinsed with F-12 medium and further cultured in F-12 medium lacking methionine but containing 50 μCi/ml [³²S]methionine. Three hours later, the cells were recultured in McCoy’s medium for 1 h prior to harvest by trypsinization. The cells were dissolved in focusing buffer and isoelectric focusing followed by SDS-gel electrophoresis (11). After fixation, the gels were dried onto Whatman 3MM paper, and the dried gels were exposed to Kodak X-Omat AR film for 6 days. Individual radiolabeled proteins were quantified with a PhosphorImager (Molecular Dynamics).

#### RESULTS

### Isolation of Full-length cDNA for Human MCP Activator

The amino acid sequences from five peptides produced by CNBr and V8 protease cleavage of gel-purified activator were used to design degenerate oligonucleotides for amplification by PCR. The sequenced PCR products were used as probes for high stringency screening of cDNA libraries from HeLa cells and human tonsils (12). The longest clone contained an open reading frame for a 249-residue polypeptide with a calculated molecular mass of 27,350 Da in reasonable agreement with an apparent molecular mass of 29 kDa for the smaller subunit of human red blood cell activator (see Fig. 1). Each of the five sequenced peptides is present in the open reading frame, which is terminated by the stop codon TGA, followed by a poly(A) tail (not shown).

A search of several gene banks using the entire sequence revealed an exact match in Protein Identification Resource library to a recently submitted γIFN-induced protein of unknown function (13). And, as shown below, we observe that synthesis of the 29-kDa activator is increased 6-fold by γIFN. Since extensive homology with other known sequences was not found, the activator appears to be a novel protein. Several potential phosphorylation sites are present in the deduced sequence for the activator. Serine 38 is a candidate CAMP/cGMP-dependent kinase phosphorylation site; serine 179 conforms to protein kinase C consensus sequences, whereas threonine 23 and 165 are potential casein kinase II sites.

On two-dimensional PAGE, the recombinant activator forms three isoelectric species (see below). This raises the possibility of phosphorylation at one or more of these sites. To study the relevance of the putative phosphorylation sites of the activator, recombinant BL21(DE3) cells were induced with IPTG (0.4 mM) in the presence of 50 μCi/ml carrier-free phosphorus-32. Following induction, the cells were harvested by centrifugation and lysed in 1% SDS. Proteins were separated on a 12% SDS-polyacrylamide gel, and the dried gel was exposed to x-ray film. Although several labeled E. coli proteins were evident, the recombinant activator was not phosphorylated by purified casein kinase II despite the fact that a 30 kDa band in MCP was labeled under identical circumstances as
observed previously by Ludemann et al. (14). Thus, the isoelectric series does not appear to be generated by phosphorylation.

**A KEKE Motif in Human Activator**—An unusual and striking feature of the activator sequence is the lysine-glutamate rich region extending from lysine 70 to lysine 97. This "KEKE motif" is particularly interesting because similar stretches of "alternating" glutamic acids and lysines, though reasonably rare among known human proteins, are present in MCP subunits C9 and in pros 28.1, in subunit 12 of the 28 S protease, as well as in certain chaperonins (Fig. 2). Computer analysis of the KEKE motif predicts this region to form a very hydrophilic α-helix. The α-helix destabilizing prolines are absent from the activator KEKE motif, but enriched in both flanking regions, e.g., prolines 60, 64, 66, and 68 occupy the amino-terminal edge, and prolines 95, 100, and 103 are present at the carboxy-terminal boundary.

**Expression and Characterization of Recombinant Activator**—To determine if the activator cDNA was full-length and to initiate biochemical studies on the protein, we subcloned the longest cDNA into a pAED4 expression system. High levels of a soluble protein fraction derived from noninduced recombinant cells; Human, partially purified human red blood cell activator; Mix, recombinant and purified human activator preparations were mixed prior to electrophoresis; STD, molecular mass standards.

**Fig. 1.** Nucleotide sequence of a cDNA and deduced amino acid sequence for the human MCP activator. Amino acids are shown in the single-letter code. V8 protease and CNBr-derived peptides obtained by direct sequencing are shown on white. The KEKE motif is boxed. *; stop codon TAG.

**Fig. 2.** KEKE Motifs. Sequences for protease subunits C9 and pros 28.1, 28 S protease subunit 12, regulator plus several chaperonins, and one protein kinase are presented in the single-letter amino acid code. The sequences shown above were selected from among the 100 KEKE-plus proteins in the Protein Identification Resource library because of their likely involvement in antigen presentation. To identify proteins containing KEKE motifs, the sequences shown above were used to search the Protein Identification Resource library using FAST A. In addition, the Protein Identification Resource library was challenged with the artificial sequences: (KE)..<sub>n</sub>, (KKEE)..<sub>n</sub>, and (KKKEE)<sub>n</sub>. To the 50 sequences most similar to each query sequence, we applied the following criteria to determine whether they conformed to our admittedly arbitrary definition of a KEKE motif. The sequence was 13 amino acids or longer; it started and ended with a Lys or Glu; greater than 60% of the residues were Lys or Glu/Asp; no more than 4 consecutive negative or positive residues were present, and the used to search the Protein Identification Resource library (release 39) only 98 proteins other than those listed above fulfilled these requirements.

**Fig. 3.** In vivo expression of the activator cDNA. A, relative migration of recombinant and human red blood cell activator separated on a 12% SDS-polyacrylamide gel. Soluble protein fractions obtained from either induced or noninduced recombinant E. coli were electrophoresed on a 12% SDS-polyacrylamide gel and stained with Coomassie Brilliant Blue. B, Western blots of an equivalent gel using mouse antibodies against purified human red blood cell activator. IPTG, soluble protein fraction from recombinant cells induced with IPTG; ~, protein fraction derived from noninduced recombinant cells; Human, partially purified human red blood cell activator; Mix, recombinant and purified human activator preparations were mixed prior to electrophoresis; STD, molecular mass standards.
Progressive activation of Suc-Leu-Leu-Val-Tyr-MCA cleavage was readily detected upon peptide overlay of native gels (see Fig. 4A). Quantitation using a fluorometer revealed about 25-fold stimulation of Suc-Leu-Leu-Val-Tyr-MCA hydrolysis in the presence of recombinant activator and the molecule purified from human red blood cells. Cleavage of these peptides is enhanced to a greater extent than cleavage of Pro-Phe-Arg-MCA (Fig. 4B). This pattern is identical to that previously demonstrated for red blood cell activator (11). Prolonged incubation of [35S]methionine-labeled activator with the MCP followed by SDS-PAGE and autoradiography produced no evidence for cleavage of the recombinant activator (data not shown). This eliminates the possibility that the recombinant protein is a coactivating substrate. We conclude from these physical and enzymatic tests that recombinant activator is very similar, if not identical, to the protein obtained from human red blood cells.

**γIFN-induced Synthesis of Activator in HeLa Cells—**The cDNA for activator matches exactly the sequence of a γIFN-induced protein, and MCP has previously been implicated in antigen presentation (15-19). For these reasons, we examined the effects of γIFN on activator synthesis. HeLa cells were grown in the presence or absence of γIFN for 3 days and then pulse-labeled with [35S]methionine. Cellular proteins were separated by two-dimensional electrophoresis, and newly synthesized proteins were detected autoradiographically. It is evident from the autoradiograms in Fig. 6 that synthesis of the three 29-kDa species comprising the activator is markedly stimulated by γIFN. Phosphorimage analysis indicates, at minimum, a 5-fold increase in the rate of activator synthesis following exposure to γIFN. Thus, in addition to subunits encoded by LMP2 and LMP7 (20), the 29-kDa activator represents another γIFN-inducible component of the MCP degradative pathway.

The results in Fig. 6 bear on two recent reports that γIFN alters MCP's peptidase activities. Both Driscoll et al. (21) and Gaçynska et al. (22) report differences in the cleavage of specific fluorogenic peptides by MCP isolated from γIFN-treated hepatoma or lymphoblastoid cell lines. They attribute the observed differences to the presence or absence of MCP subunits encoded by genes in the MHC class II region. γIFN-induced
Fig. 6. **Induction of activator synthesis by γIFN.** Proteins synthesized in HeLa cells grown in the presence or absence of γIFN were analyzed by two-dimensional PAGE as described under "Experimental Procedures." A, Coomassie stain of two-dimensional gel showing the position of human red blood cell activator. B, autoradiogram of proteins synthesized in HeLa cells grown 3 days in McCoy's medium. C, autoradiogram of proteins synthesized in HeLa cells cultured 3 days in McCoy's medium plus 200 units/ml γIFN. Phosphorimage analysis revealed that in cells exposed to γIFN, 5.7-fold more [35S]methionine was incorporated into the activator; incorporation ratios (γ IFN) for seven reference proteins were 0.95, 2.0, 1.2, 1.0, 0.5, 0.9, and 1.1 indicating that the two gels were equally loaded.

**Table I**

<table>
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<tr>
<th>Source of MCP</th>
<th>Activator</th>
<th>sLLVY-MCA</th>
<th>gGFF-MCA</th>
<th>PFR-MCA</th>
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<tr>
<td></td>
<td>Fluorescence</td>
<td>Stimulation</td>
<td>Fluorescence</td>
<td>Stimulation</td>
</tr>
<tr>
<td>721.45</td>
<td>-</td>
<td>41</td>
<td>-</td>
<td>56</td>
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<tr>
<td></td>
<td>+</td>
<td>1309</td>
<td>32</td>
<td>398</td>
</tr>
<tr>
<td>721.174</td>
<td>-</td>
<td>19</td>
<td>-</td>
<td>11</td>
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<tr>
<td></td>
<td>+</td>
<td>729</td>
<td>38</td>
<td>26</td>
</tr>
<tr>
<td>Human red blood cells</td>
<td>-</td>
<td>382</td>
<td>11</td>
<td>17</td>
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<td></td>
<td>+</td>
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<td>39</td>
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**DISCUSSION**

We have cloned and expressed a cDNA that encodes a MCP activator. The immunological, physical, and enzymatic tests presented in Figs. 3–5 provide considerable evidence that the expressed protein is equivalent to activator purified directly from red blood cells. There is, however, a significant difference between the two preparations. Activator from human red blood cells migrates on SDS-PAGE as a close doublet of 31- and 29-kDa proteins (11). DeMartino and co-workers (10) report that bovine red blood cell activator is a single 28-kDa protein, and we find a single 30-kDa activator in rabbit reticulocytes. These differences raise obvious questions. Is the doublet generated by modification of a single polypeptide, or are there two distinct proteins? The following observations demonstrate the existence of two related proteins. First, the five peptides obtained from the 29-kDA subunit are similar, but not identical, to four peptides directly obtained from the 31-kDa species. Second, we have obtained a partial cDNA with an open reading frame.
encoding a 235-residue protein that is 48% identical to the activator sequence shown in Fig. 1. Thus, it is virtually certain that human cells can express two distinct, though closely related, proteins. The molecule described here corresponds to the faster migrating protein in the SDS-PAGE doublet. By itself, it is capable of activating the MCP, and it is induced by γIFN treatment of HeLa cells (Fig. 6). The 31-kDa species is not induced by γIFN treatment of HeLa cells. And at present, it is not known whether this larger protein activates peptide hydrolysis by the MCP.

The alternating lysines and glutamic acids in the activator represent an unusual arrangement of amino acids. Only 106 proteins in the Protein Identification Resource library (release 39) contain KEKE motifs. Four of these mammalian proteins are MCP subunits or are found in protein complexes that bind MCP. Five chaperonins constitute another set of proteins enriched in KEKE motifs, and nine “KEKE-plus” proteins are expressed by the malarial parasite, Plasmodium. In view of the proposed role of the MCP in antigen presentation (15–19), it is interesting that one of these malarial proteins, ABRA (23), has been proposed to function as an immunological “smokescreen.” Although one might dismiss the KEKE motifs presented in Fig. 2 as curiosities, equivalent regions containing the equally abundant amino acids, arginine and aspartate, are virtually absent from proteins in the Protein Identification Resource library. Hence, KEKE motifs are not simply statistically expected arrangements of amino acids in a large collection of proteins. Their occurrence in three interacting protein complexes suggests that they promote binding of the activator or 26 S regulatory complexes to MCP. In fact, using Ub-peptide fusions (24), we have obtained direct evidence that a KEKE region promotes association of Ub with activator. KEKE motifs may not only serve to assemble protease complexes, they may also channel peptide substrates to MCP active sites by interacting with “KEKE-like” regions in polypeptide substrates. In this regard, we have surveyed 54 peptides presented by MHC class I receptors, and we found that nine are derived from precursors that contain KEKE regions. By comparison, less than 0.1% of the more than 100,000 sequences in the Protein Identification Resource library contain KEKE motifs.

Stimulation of activator synthesis by γIFN (Fig. 6) adds further circumstantial evidence for involvement of MCP in antigen presentation. The activator greatly stimulates cleavage of Tyr-MCA and also increases cleavage of Arg-MCA bonds (Table I). This specificity is consistent with the general observation that MHC bound peptides are anchored at their carboxyl terminus by positively charged or hydrophobic residues (25). Although the greater stimulation of Glu-MCA bonds by activator (Fig. 5B) would appear inconsistent with playing a role in antigen presentation, we note that a 12-residue precursor to an MHC I ligand (26) requires cleavage after a glutamic acid residue for its formation. In fact, it has not been shown that the MCP directly generates either the amino or carboxyl terminus of presented peptides, and controversy surrounds proposed mechanisms of MHC class I restricted antigen presentation (25). Still, it seems reasonable to suggest that both activator and MCP subunits LMP2 and LMP7 serve to optimize antigen presentation in concert with other γIFN-up-regulated components of the antigen processing system, such as TAP1/TAP2, MHC class I and MHC class II molecules, β2-microglobulin, and Jak 1 kinase (27). In summary, the experiments described here provide important information on a MCP activator and focus attention on its likely involvement in antigen presentation. They also point to a role for KEKE motifs in protein association.

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