Differences in Catalytic Activities and Subunit Pattern of Multicatalytic Proteinase Complexes (Proteasomes) Isolated from Bovine Pituitary, Lung, and Liver

CHANGES IN LMP7 AND THE COMPONENT NECESSARY FOR EXPRESSION OF THE CHYMOTRYPSIN-LIKE ACTIVITY*

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All eukaryotic cells contain a high molecular mass (700 kDa, 19 S) multisubunit, multicatalytic proteinase complex (MPC, proteasome), constituting up to 0.5-1% of the soluble protein fraction of tissue homogenates (1–3). The complex contains a total of 28 subunits with molecular masses of 21–32 kDa, of which 14 are nonidentical. It is organized in four stacked rings each containing seven subunits. A similarly organized particle containing only two nonidentical subunits designated as α and β, and having the general structure α2β2δ2ε2γ2 has been found in the archaebacterium Thermoplasma acidophilum (4). Unlike the archaebacterial proteasome which primarily exhibits only chymotrypsin-like (ChT-L) activity (4–6), the mammalian proteasome exhibits five distinct endopeptidase activities (7). Initial work on the specificity of the MPC from bovine pituitaries (8–10) led to the identification of three distinct catalytic activities, cleaving bonds on the carboxyl side of hydrophobic, basic, and acidic amino acids, each associated with a different component of the complex. The three activities have been designated as ChT-L, trypsin-like (T-L), and peptidylglutamyl-peptide hydrolyzing (PGPH), based on the nature of the amino acid residue in the R1 position (10). Subsequent work led to identification of two additional catalytic components, one showing a preference toward cleavage of peptide bonds on the carboxyl side of branched chain amino acids (BrAAP) in natural and synthetic peptides and proteins, the other showing preference toward bonds between small neutral amino acids (SNAAP) (7, 11). That the BrAAP activity is an important factor in the protein degrading activity of the MPC was shown in experiments in which inactivation of the other four activities by exposure of the MPC to 3,4-dichloroisocoumarin (DCI) did not decrease, but indeed increased degradation of many natural peptides and such proteins as caspase and proinsulin.

It is now well established that the MPC (19 S) constitutes a major extralysosomal proteolytic system and that it is responsible for the "catalytic core" of a larger 26 S complex (12–15) for ubiquitin-dependent and ubiquitin-independent pathways of intracellular proteolysis. These pathways are involved in diverse cellular functions such as cell growth and mitosis, antigen processing, and degradation of short-lived regulatory proteins such as oncogene products, transcription factors, and cyclins (16–27).

The possibility that the MPC might be involved in antigen processing was suggested by observations that two genes, LMP7 and LMP 2, located in the MHC class II region code for low molecular weight proteins having amino acid sequences closely similar to some MPC subunits (17, 22, 25, 26, 28, 29). Furthermore, interferon-γ (IFN-γ) stimulation of human cells was shown to lead to an increased expression of the LMP7 and LMP2 genes, with the products apparently replacing two highly homologous subunits X and Y (the latter also referred to as subunit δ) (29–32), respectively. Incorporation of these subunits into the MPC was suggested to generate so called "immunoproteasomes" (33) that could represent a proteasomal
subpopulation capable of more efficiently processing protein antigens into the short 8 to 9 amino acid-containing peptides that are transferred to class I molecules encoded by the MHC for presentation on the cell surface to cytotoxic lymphocytes. Since the majority of peptides generated during antigen processing contain hydrophobic or basic amino acid residues at the COOH terminus, support for the above hypothesis could have been anticipated to come from experiments showing that cells stimulated by IFN-γ would show an increase in expression of the ChT-L and T-L activities of the MPC. Indeed, reports that stimulation of lymphoblastic and mononuclear cell lines in culture by IFN-γ leads to an increase in the rate of cleavage of bonds after hydrophobic and basic residues (34) in synthetic peptide substrates, and to similar activity increases in LMP7 gene transfection experiments (35), seemed to support this possibility. However, a subsequent study of the effect of exposure of the same cell lines to IFN-γ failed to detect any effect on the ChT-L and T-L activities of the MPC (36). Moreover, exposure of mouse fibroblast cells to IFN-γ was reported to decrease rather than increase the ChT-L activity and to have no effect on the T-L activity (32).

We report here that the contents of LMP7 and BO2 subunits (the latter identical with X) differs markedly in MPCs isolated from bovine lung, pituitary, and liver and that marked changes are also detected in the catalytic profile of complexes isolated from the three organs. Thus, the concentration of LMP7 was found to be greatly increased in the lung compared with that in the liver and pituitary, and this increase was associated with a decrease in the concentration of subunit BO2. Changes in the catalytic profile of MPCs isolated from lung and liver involved a moderate depression of the ChT-L, T-L, PGPH, and SNAAP activities, and a prominent activation of the BrAAP activity with an increase in affinity (lowering of $K_m$) toward substrates containing branched chain amino acid residues in the P$_1$ position. The possible significance of these changes for antigen processing is discussed.

**MATERIALS AND METHODS**

Purification of the MPC—Fresh frozen bovine pituitaries, bovine lungs, and bovine livers were obtained from Pel Freeze Inc. (Rogers, AR). Isolation of the MPC from the three tissues was carried out according to a previously described procedure (37). After the last purification step the enzyme was stored frozen at −20°C in 0.5-mM aliquots, containing 0.5–1.0 mg protein/ml, and thawed before being used in experiments. MPCs were synthesized as described previously (4–6,8). The ChT-L and T-L activities were measured with synthetic substrates Z-GGL-pNA (D)ALR-2NA and Z-GLY-pAB, respectively, as the substrates. Determination of the activities of the BrAAP and SNAAP components of the MPC separated by HPLC were manually collected and concentrated to 150 mm, 300 Å) was treated with 1.0 µl of 10% DCI in Me$_2$SO. The reaction was terminated after 10 min by addition of 5% 2-mercaptoethanol and 1% SDS. Equal amounts of the DCI-treated and native MPC were subjected to SDS-PAGE in 10% polyacrylamide gel electrophoresis (PAGE) under non-dissociating and dissociating conditions showed about a 95% purity of all three preparations.

**RESULTS**

SDS-PAGE electrophoresis of equal amounts of protein from MPC preparations obtained from pituitary, lung, and liver are shown in Fig. 1. About 14 different protein bands are clearly visible with the overall subunit pattern being similar in all three complexes. Nevertheless, closer inspection shows marked differences, both quantitative and qualitative, in the band patterns of the three complexes. The intensity of some bands is greater in the lung MPC (for example the slowest moving band), whereas some other bands show higher density either in the liver or in the pituitary. The structural and functional significance of these differences, with the exception of those discussed below, remains to be established. The fastest moving, somewhat diffuse band in the pituitary preparation was subjected to NH$_2$-terminal amino acid sequencing. The obtained 23 amino acid sequence RFSYPAFNGTVLAIAGEDFSIV showed complete identity with the amino acid sequence of component C5 of the rat hepatoma proteasome (42). The second fastest moving band, more intense in the pituitary than in the lung and appearing in the liver as a doublet, was shown to correspond to the slow peak eluting during HPLC of the MPC isolated from each of the three organs (Fig. 2). Because of its mobility in PAGE and HPLC, the component was designated as BO2 (Bo referring to bovine). Identical NH$_2$-terminal amino acid sequences were
Equal amounts of proteins were subjected to SDS-PAGE as described under “Materials and Methods.” The molecular mass markers are derived from the known amino acid sequence of the subunits represented by the respective protein bands.

found for this component both when BO2 was isolated by HPLC and subjected to amino acid sequencing, or when the second fastest moving band in PAGE was blotted to polyvinylidene difluoride membranes and then subjected to sequencing (see below). A faint band, clearly visible in the lung but not in the pituitary complex, was identified by immunoblotting as corresponding to LMP7. Its identity with LMP7 was also confirmed by NH2-terminal amino acid sequencing (see below and Table I). This band was also visible on direct inspection in PAGE of the liver complex, but its intensity was fainter than that in the lung and is not visible in the photographic prints (Fig. 1). The quantitative differences in the amounts of LMP7 in the three preparations are clearly visible in immunoblots shown in Fig. 3. The intensity of the band in the lung is many times greater than that in the pituitary and also greater than in the liver. Densitometric scanning of the bands in immunoblots showed that the amount of LMP7 in the lung and liver was respectively 10 and 5 times greater than that in the pituitary MPC (for details see legend to Fig. 3, and “Material and Methods”).

HPLC of MPC preparations results in dissociation of subunits of the complex and a reproducible separation of 13 peaks. When equal amounts of protein (25 μg) from each of the pituitary, lung, and liver MPCs were subjected to HPLC, the subunit patterns shown in Fig. 2 were obtained. Several quantitative differences in the patterns are clearly visible in the three preparations. The second well-separated peak (designated as BO2) was markedly higher in the pituitary than in either the lung or the liver. Integration of the areas under the peak in several preparations showed that the BO2 subunit in lung (panel B, in Fig. 2) amounted on average to only 60% of that present in the pituitary (panel A, Fig. 2), and that the corresponding amount in the liver (panel C, Fig. 2) constituted about 80% of that in the pituitary. When 0.5 mg of protein of pituitary MPC was separated by HPLC and the effluent containing the BO2 component was concentrated and subjected to amino-terminal sequencing, the sequence shown in Table I was obtained. An identical NH2-terminal sequence was obtained from the second fastest moving band in PAGE of the bovine lung MPC (Fig. 1). The NH2-terminal amino acid sequences of the first 10 amino acids in both bands of the doublet visible in PAGE of the liver MPC were also identical with those of the lung, and the separation of the two bands could have resulted from small differences arising from post-translational modifications in the part of the protein, COOH-terminal to the sequences shown in Fig. 1.

The NH2-terminal sequence of BO2 (Table I) shows complete identity with sequences reported previously for human subunit X (43), bovine pituitary 21-kDa subunit (44), bovine lens L2 (45), the ε subunit of human reticulocytes (46), and also close similarity to the NH2-terminal sequence of LMP7 from bovine lung, the sequence of a rat liver proteasome subunit (47), the internal stretch of amino acid sequence encoded by the LMP7 gene from a human T cell line (22), and the subunit PRE2 from yeast (48). Examination of the complete amino acid sequence of BO2 and LMP7 showed that within a stretch of 204 amino acids 135 (66%) showed identity, and a considerable number of the remaining amino acids represented conservative replacements.

Several lines of evidence indicate that BO2 is necessary for expression of the ChT-L activity of the MPC. Thus, the ChT-L activity is the only catalytic activity of the complex that was reported to be sensitive to inactivation by diisopropylfluorophosphate, and indeed two separate studies have reported incorporation of labeled DFP into this subunit (49, 50). Furthermore, the PRE2 gene encoding a subunit showing high homology to the BO2 and LMP7 sequences was shown to be necessary for expression of the ChT-L activity, since yeast pre2 mutants show very low ChT-L activity and accumulation in cells of ubiquitin-protein conjugates (48).

To gain additional evidence that BO2 is indeed involved in expressing ChT-L activity, we took advantage of the previously described high susceptibility of this activity to DCI inactivation (7, 37, 51). Treatment of the MPC with DCI is associated with some proteolytic degradation of BO2, as evidenced by the disappearance of the peak in HPLC, and a decrease in the intensity of the BO2 bands in PAGE (Fig. 4). In addition chemical modification of this subunit by covalent attachment of one or more DCI molecules could be likely the cause of the appearance of a slower migrating BO2-derived band having the same NH2-terminal sequence as native BO2. All the other components remain unchanged under these conditions, a conclusion confirmed by scanning densitometry carried out by the procedure described in the legend to Fig. 3. The possibility was therefore considered that the inactivation of the ChT-L activity and changes in the BO2 component observed by HPLC and SDS-PAGE are an expression of the same event. We proceeded therefore to determine the pseudo-first-order rate constant of inactivation of the ChT-L activity and to compare it with the rate constant of disappearance of the BO2 peak in HPLC. A summary of the data obtained for pituitary MPC is given in Table II. Almost identical rate constants were obtained for the two processes, further adding to the evidence that BO2 is necessary for expression of the ChT-L activity, and that changes in BO2 after DCI treatment and inactivation of the ChT-L activity represent two linked processes. It is notable that the rate constants of inactivation (kobs/l) of the other components of the pituitary MPC greatly differ from those of the ChT-L activity. Previous studies of the kinetics of inactivation by DCI of the activities of the bovine pituitary MPC showed that inactivation rate constants for the PGPH, T-L, and SNAAP activities are, respectively, 3, 7.5, and 9 times lower than for the ChT-L activity, and that the BrAAP activity is resistant to inactivation by DCI (7). Additional confirming evidence for the conclusion that BO2 is necessary for expression of the ChT-L activity was obtained by showing that treatment of the MPC with DCI in the presence of the substrate of the ChT-L activity Cbz-Gly-Gly-Phe-pAB (5 mM), prevented disappearance of the BO2 peak in HPLC and that this substrate also prevented incorporation of 3H-labeled DCI into this peak (data not shown). The association of a decreased ChT-L activity and a decreased amount of the BO2 subunit in MPC preparations from bovine lung compared with those in bovine pituitary also supports the same conclusion. Collectively, all these findings firmly establish the conclusion that the BO2 com-
ponent is necessary for the expression of the ChT-L activity.

The possibility that incorporation of the LMP7 subunit into the MPC is related to the antigen-processing function, and the finding that antigen processing of cytoplasmic proteins for presentation by class I molecules involves in the majority of cases the generation of peptide fragments having a hydrophobic residue at the carboxyl terminus, a reaction consistent with the involvement of an activity with ChT-L specificity, prompted examination of whether changes in the contents of BO2 and LMP7 subunits of the three preparations are reflected in changes of the ChT-L activity. Furthermore, the finding in both PAGE and HPLC of quantitative differences in the pattern of subunits of the three preparations beyond those related to LMP7 and BO2, induced us to extend such study to examination of differences between the activities of all five known catalytic components. A summary of the catalytic activities measured at a single substrate concentration is given in Table III. The ChT-L activities measured with two different substrates (Cbz-GGF-pAB and Suc-LLVY-MCA) were moderately but statistically significantly decreased both in the lung and the liver when compared with those in the pituitary. Although the ChT-L activities appeared to be higher in the liver MPC than those in the lung, the differences were not significant. The lung and liver MPCs showed also significantly lower T-L and PGPH activities compared with those in the pituitary. No differences were found between the T-L activity in the lung and liver, but the PGPH activity of the liver MPC was significantly higher than that in the lung. The SNAAP activities were somewhat higher in the pituitary MPC than those in the lung and liver complexes.

The most conspicuous differences between the three MPC preparations were found in the BrAAP activity. The highest activity of this component was found in the lung, whereas the pituitary showed the lowest activity. These differences were found in three consecutive preparations. The activity in the liver, although lower than in the lung, was still significantly higher than in the pituitary. Thus, when measured with Z-GPALA-pAB as the substrate the activities in the lung and

| Source                  | NH2-terminal amino acid sequence | Refs.  |
|-------------------------|----------------------------------|--------|
| BO2 pituitary           | XTTLAFKFRHGVI                   | This manuscript |
| BO2 lung                | TTTLAGFRHGIVAD                  | This manuscript |
| Human subunit X         | TTTLAFKFRHGIVAD                 | 43     |
| Bovine lung LMP7        | TTTLAFKFRHQVAD                  | This manuscript |
| Human T cell line LMP7  | TTTLAFKFRHQVAD                  | 22     |
| Yeast PRE2              | TTTLAFRFGGVAVD                  | 48     |

Fig. 2. Subunit patterns obtained by HPLC from MPC preparations isolated from bovine pituitaries (panel A), lung (panel B), and liver (panel C). The conditions of HPLC are given under "Materials and Methods." Arrows indicate the position of the BO2 peak.

Fig. 3. Visualization of LMP7 in preparations of the MPC from bovine pituitaries (1), lung (2), and liver (3) by immunoblotting with a specific antibody (for details see "Materials and Methods"). Densitometric scanning of immunoblots was carried out in order to determine the relative amounts of LMP7 in the three MPC preparations. The photographic negative obtained during the chemiluminescence procedure was transilluminated with a light box and photographed with a Cohu video camera. The video image was captured on a Macintosh computer as a digitized black and white image. Intensity of the bands on immunoblots was quantitated using the NIH Image program (version 1.53b), according to the recommendations of the manufacturer. This procedure showed that the amounts of LMP7 in the lung and liver were, respectively, 10 and 5 times greater than in the pituitary. The molecular mass of the LMP7 band is about 23 kDa as derived from the amino acid sequence of this subunit and its position in relation to the other MPC bands.

Fig. 4. Effect of exposure of the lung MPC to 3,4-dichloroisocoumarin. The lung enzyme was exposed to 20 µM DCI as described under "Materials and Methods." A, SDS-PAGE of the native lung enzyme. B, SDS-PAGE of lung DCI-treated enzyme. Molecular markers are derived from the molecular mass of subunits subjected to amino acid sequencing.
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Inactivation rate constants of the chymotrypsin-like activity and the rate constants of disappearance of subunit BO2 after exposure of the MPC to 3,4-dichlorosocoumarin and HPLC separation of subunits

Data represent mean values from the number of measurements indicated in column N. Activity was measured with Z-Gly-Gly-Leu-pNA at a concentration of 0.4 mM. Incubation mixtures containing pituitary MPC (0.5 mg protein/ml) and DCI were incubated at 26 °C for up to 15 min. Aliquots of the enzyme (5 μg) were transferred at different time intervals to tubes containing substrate at 37 °C for determination of activity, as described in "Materials and Methods." Measurements of rate constants of degradation were carried out after exposure of the MPC to 20 μM 3,4-dichlorosocoumarin. Aliquots of the enzyme containing 25 μg of protein were withdrawn at different time intervals of up to 5 min and subjected to HPLC separation of the subunits.

| Measured parameter | Inhibitor conc. | k | t½ | kmax/μM | N |
|---------------------|----------------|---|-----|---------|---|
| Inactivation rate constant of chymotrypsin-like activity | 0.020 | 0.25 | 2.83 | 207 | 3 |
| Rate constants of degradation of the BO2 component | 0.020 | 0.24 | 2.85 | 202 | 2 |

Table III

Activities of the five catalytic components in the multicatalytic proteinase complexes isolated from bovine pituitary, lung, and liver

Data are mean values ± standard error of the mean (S.E.) from four separate determinations. The test gave the indicated p values for the MPC activities from lung and liver versus those from the pituitary. The differences between the activities in the liver and lung were found to be insignificant for the ChT-L and T-L activities, but significant for the PGPH (p < 0.01), BrAAP (p < 0.0005), and SNAAP (p < 0.005) activities.

| Component | Substrate | [S] | Activity (μmol/mg/h) |
|-----------|-----------|-----|---------------------|
|           |           |     | Pituitary | Lung | Liver |
| ChT-L     | Cbz-GGF-pAB | 1.0 | 4.28 ± 0.34 | 2.95 ± 0.25 | 3.2 ± 0.090 |
|           | Suc-LLVY-MCA | 0.1 | 0.857 ± 0.12 | 0.565 ± 0.01 | 0.586 ± 0.016 |
| T-L       | Bz-GFKR-2NA | 1.0 | 5.35 ± 0.46 | 3.59 ± 0.31 | 3.33 ± 0.122 |
| PGPH      | Cbz-LFE-2NA | 0.6 | 25.4 ± 1.0 | 11.7 ± 0.36 | 15.1 ± 0.81 |
| BrAAP     | Cbz-GPALA-pAB | 1.0 | 3.83 ± 0.36 | 23.9 ± 1.5 | 13.2 ± 0.136 |
| SNAAP     | Cbz-GPAGG-pAB | 2.0 | 4.83 ± 0.103 | 3.22 ± 0.069 | 3.74 ± 0.032 |

* p < 0.01.  
** p < 0.025.  
*** p < 0.0005.  
**** p < 0.005.

liver were more than six and three times higher, respectively, than in the pituitary.

To gain insight into the kinetic basis of the differences between the activities of the five catalytic components, we determined the maximal velocity (Vmax) as a measure of catalytic efficiency, the affinity toward substrates (Km), and the specificity constants (Vmax/Km). A summary of these kinetic parameters is given in Table IV. Neither the ChT-L nor the PGPH component exhibited Michaelis-Menten kinetics when Suc-LLVY-MCA and Z-LFE-2NA, respectively, were used as substrates for activity measurements. Plots of velocity versus substrate concentration yielded sigmoidal curves for both substrates, and as a result both the Km and Vmax values were dependent on the range of substrate concentrations used, which in turn were limited by the solubility of the substrates. This kinetic behavior apparently results from the presence of more than one binding site for these substrates, and/or from allosteric interactions between subunits expressing the activities, as reported previously from several laboratories (49–51). However, when the ChT-L activity was measured with Cbz-GGF-pAB, the reaction appeared nevertheless to follow Michaelis-Menten kinetics. These measurements have shown that depression of the ChT-L activity in the lung was accompanied by a decrease of both the Vmax and Km, with the Vmax/Km ratios remaining the same. Vmax decreases were also mainly responsible for the decrease of the T-L and SNAAP activities in the lung with small changes in the specificity constants.

Of particular interest were the findings for the BrAAP component. Thus, whereas the Vmax for this component was only moderately increased in the lung and decreased in the liver, the biggest changes were observed for the Km values in preparations from both organs. Indeed, the Km values were as much as 20 times lower in the lung and liver MPC than those for the pituitary enzyme. Thus, the high activities observed for the BrAAP component in the liver and lung MPC preparations shown in Table II are accounted for by the fact that the 1 mM substrate concentrations used in the assays were more than 2-fold higher than the Km for the lung and liver preparations, but several times lower than the Km for the pituitary MPC. Because of the low Km values, the Vmax/Km ratios expressing the specificity constants for substrates containing a branched chain amino acid in the P1 position were about 20 times higher for the lung and liver MPC than those for the pituitary. By contrast, only minor changes of the Vmax/Km ratios were observed for the other activities of the MPC, indicating that unlike the specificity of the BrAAP component, the specificities of the other catalytic components are similar in all three preparations.

DISCUSSION

Polyacrylamide gel electrophoresis under dissociating conditions of MPCs isolated from bovine pituitaries, lung, and liver clearly demonstrate qualitative as well as quantitative differences between the subunit composition of the complexes from the three organs. These differences were manifested by virtually complete absence of single subunits in some of the preparations, as well as by the different intensities of protein bands representing different subunit concentrations. The functional significance of these differences is not clear, since the identity of the subunits expressing the known catalytic components of the complex, with the exception of the ChT-L activity, is not known. The identification therefore of BO2 as the component that is necessary for the expression of the ChT-L activity, and the finding that its amount is decreased in the lung and liver compared with that in the pituitary, provided an opportunity to examine the relationship between the expression of ChT-L
activity and the quantitative contents of the BO2 component in the three organs. The high ChT-L activities in the pituitary and the decreased activities in lung and liver are therefore consistent with the finding of lower amounts of the BO2 subunit in preparations from the latter two organs.

Recent reports have indicated that stimulation of cells with IFN-γ is associated with increased expression of LMP7 and LMP2 genes and down-regulation of expression of the X and Y subunits of the MPC and their partial replacement by products of the LMP7 and LMP2 genes, respectively. This replacement was suggested to change the specificity of the MPC in a way that favors the generation of the eight to nine amino acid hydrophobic and basic residues in the P1 position. However, in a similar study (36) exposure of lymphoblastoid cells to IFN-γ failed to produce an increase in the ChT-L or T-L activity of MPC preparations purified from these cells. Furthermore, stimulation with IFN-γ of mouse fibroblast cells was reported to decrease rather than increase the ChT-L activity and to have no effect on the T-L activity (32). It is of interest that in the latter study IFN-γ induced a change in the specificity of the MPC toward a 25-amino acid residue peptide whose internal sequence contained a known nine amino acid antigenic peptide. It remains to be determined whether the increased cleavage frequency in this study of a bond on the carboxyl side of a leucine residue is solely incidental or an expression of an altered ChT-L activity induced by IFN-γ stimulation. It should be noted that these groups have used for determination of the ChT-L activity Suc-Leu-Leu-Val-Tyr-7-MCA, a substrate that has limited solubility, and yields with bovine and human red blood cell MPC preparations sigmoidal velocity versus substrate concentration plots, and therefore different \( V_{\text{max}} \) and \( K_m \) values, depending on the range of substrate concentrations used. It would be therefore important to reexamine the kinetics toward this substrate of MPC preparations from cells used in some of the above studies, in order to determine whether the kinetic behavior of the ChT-L activity is the same in the different cell lines.

The results reported here indicate that the increased amounts of LMP7 in MPC preparations isolated from the lung and liver, while being associated with a decrease in the concentrations of the BO2 subunit, are not associated with increases in the ChT-L or T-L activities. Indeed, small but significantly decreased activities were found not only for these two activities, but also for

| Component | Substrate | Range of conc. (mM) | Kinetic parameters | Pituitary | Lung | Liver |
|-----------|-----------|---------------------|-------------------|-----------|------|-------|
| Chymotrypsin-like | Z-GGF-pAB | 1.0–8.0 | \( V_{\text{max}} \) 75.7 ± 17 | 36.5 ± 3.0<sup>a</sup> | 69.1 ± 9.1<sup>b</sup> | 28.3 ± 4.7<sup>b</sup> |
| | | | \( K_m \) 31 ± 3.1 | 15.3 ± 1.6<sup>a</sup> | 7.6 | 2.4 |
| Trypsin-like | Bz-GGKR-2NA | 0.2–2.0 | \( V_{\text{max}} \) 9.5 ± 0.63 | 5.86 ± 0.37<sup>c</sup> | 6.29 ± 0.35<sup>b</sup> | 0.77 ± 0.09<sup>c</sup> |
| | | | \( K_m \) 0.77 ± 0.07 | 0.775 ± 0.09<sup>d</sup> | 7.6 | 8.2 |
| BrAAP | Z-GPALA-pAB | (1.0–8.0) | \( V_{\text{max}} \) 20.9 ± 1.1 | 33 ± 2.7<sup>d</sup> | 15.5 ± 0.23<sup>d</sup> | 91 |
| | | Lung and liver (0.2–1.6) | \( K_m \) 4.8 ± 0.32 | 0.41 ± 0.08<sup>d</sup> | 0.17 ± 0.012<sup>d</sup> | 91 |
| SNAAP | Z-GPAGG-pAB | (1.0–10.0) | \( V_{\text{max}} \) 12.6 ± 1.2 | 6.6 ± 0.17<sup>d</sup> | 7.77 ± 0.087<sup>d</sup> | 2.14 ± 0.063<sup>d</sup> |

<sup>a</sup> \( p < 0.01. \)
<sup>b</sup> ns not significant.
<sup>c</sup> \( p < 0.005. \)
<sup>d</sup> \( p < 0.0005. \)
<sup>e</sup> \( p < 0.05. \)
<sup>f</sup> \( p < 0.025. \)
those of the PGPH and SNAAP components. It is notable that these activity changes were associated only with minor changes of the $V_{\text{max}}/K_m$ ratios, parameters that are generally considered as reliable indicators of specificity. In view of these results it would seem important to extend studies on the effect of IFN-γ on a wider range of cells in culture, since the possibility must be considered that differences might exist in response of different cell strains to IFN-γ stimulation.

The most remarkable change accompanying the increases of LMP7 in the lung and liver and the commensurate decrease in the contents of the BO2 subunit was the great decrease of the $K_m$ value of the BrAAP component toward the substrate with a branched chain amino acid in the P$_1$ position. This was associated with a 20-fold increase in the specificity constant $V_{\text{max}}/K_m$. The BrAAP component was shown to be characterized by selectivity toward peptide bonds in which the carbonyl group is provided by any one of the branched chain amino acids (7, 51), lectivity toward peptide bonds in which the carbonyl group is $\text{CH}_3$, and selectivity toward peptide bonds in which the carbonyl group is $\text{CH}_3$.

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The most remarkable change accompanying the increases of LMP7 in the lung and liver and the commensurate decrease in the contents of the BO2 subunit was the great decrease of the $K_m$ value of the BrAAP component toward the substrate with a branched chain amino acid in the P$_1$ position. This was associated with a 20-fold increase in the specificity constant $V_{\text{max}}/K_m$. The BrAAP component was shown to be characterized by selectivity toward peptide bonds in which the carbonyl group is provided by any one of the branched chain amino acids (7, 51), lectivity toward peptide bonds in which the carbonyl group is $\text{CH}_3$, and selectivity toward peptide bonds in which the carbonyl group is $\text{CH}_3$.

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