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Enhanced rate of degradation of basic proteins by 26S immunoproteasomes

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ABSTRACT

Immunoproteasomes are alternative forms of proteasomes specialized in the generation of MHC class I antigenic peptides and important for efficient cytokine production. We have identified a new biochemical property of 26S immunoproteasomes, namely the ability to hydrolyze basic proteins at greatly increased rates compared to constitutive proteasomes. This enhanced degradative capacity is specific for basic polypeptides, since substrates with a lower content in lysine and arginine residues are hydrolyzed at comparable rates by constitutive and immunoproteasomes. Crucially, selective inhibition of the immunoproteasome tryptic subunit β2i strongly reduces degradation of basic proteins. Therefore, our data demonstrate the rate limiting function of the proteasomal trypsin-like activity in controlling turnover rates of basic protein substrates and suggest new biological roles for immunoproteasomes in maintaining cellular homeostasis by rapidly removing a potentially harmful excess of free histones that can build up under different pathophysiological conditions.

1. Introduction

The 26S proteasome is an ATP-dependent protease that is responsible for the degradation of the majority of cellular proteins in eukaryotic cells. This multi-subunit complex consists of the 20S proteasome, in which proteins are degraded, and one or two 19S regulatory particles, which are responsible for recognizing, unfolding, and translocating polyubiquitinated substrates into the 20S internal proteolytic cavity [1]. The 20S proteasome is a barrel-shaped structure composed of four stacked heptameric rings. The two outer rings consist of α-subunits, while the two central rings are made up of β-subunits. Three of the subunits in the β rings (β1, β2, and β5) contain the proteolytic active sites that are positioned on the interior face of the cylinder. Proteolytic activities of proteasomes measured using short fluorogenic substrates have defined three distinct cleavage preferences: β1 has caspase activity (i.e. cleaving after acidic residues); β2 possesses tryptic activity (i.e. cleaving after basic residues); and β5 displays chymotryptic activity (i.e. cleaving after hydrophobic residues). Lymphoid cells and cells exposed to cytokines such as interferon-γ (IFN-γ) or tumor necrosis factor (TNF)-α express three homologous subunits (β1i/LMP2, β2i/MECL-1, β5i/LMP7) that replace the constitutive ones in newly assembled, so-called immunoproteasome particles [2].

Experiments with small fluorogenic substrates have shown that immunoproteasomes have a greater capacity to cleave after hydrophobic and basic residues, and a lower capacity to cleave after acidic residues. Consequently, peptides generated by immunoproteasomes should have a higher percentage of hydrophobic and basic C-termini, both of which favor uptake by TAP transporters and which are essential for tight binding to MHC class I molecules [3]. Furthermore, this altered cleavage specificity may also enhance the production of longer precursors to the MHC-presented peptide without affecting the overall size distribution of proteasomal products [4]. Although there are examples of epitopes that are generated with lower efficiency, or which are not released by immunoproteasomes, the pivotal role of immunoproteasomes in the generation of the vast majority of MHC class I ligands was definitively demonstrated in transgenic mice lacking all three proteasomal catalytic β-immune subunits [5]. Additionally, immunoproteasomes have been shown to be important for efficient cytokine production [6] and have been implicated in a number of pathological disorders such as cancer and neurodegenerative and autoimmune diseases [7–9]. Recently, immunoproteasomes were reported to play a major role in protecting cell viability under cytokine-induced oxidative stress due to their enhanced capacity to degrade nascent, oxidant-damaged polyubiquitinated proteins [10], although subsequent studies failed to confirm these data [11].

Our previous studies have shown that oxidized ovalbumin is degraded in vitro with comparable efficiency by both constitutive and immuno 20S and 26S proteasomes [4]. However, additional data concerning the effects of the INF-γ-induced subunits on the hydrolysis rates of non-ubiquitinated proteins are not available. To address this, we investigated

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the effect of INF-γ-induced β-subunits on the degradation of several loosely folded proteins that are hydrolyzed in vitro by 26S proteasomes in a linear, ATP-dependent manner, without ubiquitination [12]. In this way, we discovered that compared to constitutive proteasomes, 26S immunoproteasomes exclusively degrade at greatly enhanced rates proteins that are characterized by an exceptional high content in basic residues. We further demonstrated that the proteasomal tryptic site has a rate limiting function in controlling turnover rates of basic proteins and suggested potentially new roles of immunoproteasomes in catalyzing the rapid removal of histones.

2. Methods

2.1. Proteasome purification

26S proteasomes and immunoproteasomes were purified from rabbit muscle and spleen, respectively (Pel Freez Biologicals, Rogers, AR, USA), as described previously [4,13] and are free of aminopeptidases that may act on proteasome products.

2.2. Protein degradation and peptide analysis

Protein degradation, analysis of new amino groups using fluorescamine, and HP-SEC analysis were performed as previously described [4,12,14,15]. More details are provided in the Supplementary Materials and Methods.

3. Results

3.1. Enhanced rates of breakdown of basic proteins by the 26S immunoproteasome

Incorporation of INF-γ-induced β subunits significantly modifies proteasome peptidase activities [2]. Accordingly, 26S immunoproteasomes show an enhanced capacity to cleave short fluorogenic peptides on the carboxyl side of both basic (Fig. S1A) and hydrophobic (Fig. S1B) residues and a reduced ability to cleave after acidic amino acids (Fig. S1C). Specifically, the incorporation of INF-γ-induced subunits increases the maximal rate (V_{max}) at which proteasomes hydrolyze the basic substrates Z-ARR-amc, Boc-LRR-amc, and Bz-VGR-amc by two to threefold, and the hydrophobic substrate AFF-amc by more than sevenfold, while it reduces the V_{max} of the degradation of the acidic peptide Suc-YVAD-amc by about one-half (Table 1). Notably, in the case of the caspase site of immunoproteasomes, at a reduced maximum velocity the K_m value increases by nearly fourfold (Table 1). In contrast, the difference in K_m between constitutive and immunoproteasomes is much lower for the chymotrypsin-like activity, while for the trypsin-like activity it seems to mainly depend on the substrate utilized (Table 1).

Table 1
Kinetics parameters for the degradation of different fluorogenic peptides by 26S proteasomes and immunoproteasomes.

Substrate	26S proteasomes			
	Immuno		Constitutive	
	V_{max} (nmol/mg·min)	K_m (μM)	V_{max} (nmol/mg·min)	K_m (μM)
Bz-VGR-amc	379 ± 33	1801 ± 319	120 ± 11	665 ± 171
Z-ARR-amc	115 ± 3	668 ± 33	51 ± 3	493 ± 61
Boc-LRR-amc	247 ± 17	573 ± 65	127 ± 10	697 ± 82
AAF-amc	116 ± 25	170 ± 53	15 ± 4	139 ± 57
Suc-YVAD-amc	12 ± 3	503 ± 167	23 ± 2	127 ± 33

Maximum velocity (V_{max}) and Michaelis–Menten constant (K_m) were calculated as described in Materials and Methods from the data shown in Supplementary Fig. S1. Values are mean ± SE.Maximum velocity (V_{max}) and Michaelis–Menten constant (K_m) were calculated as described in Materials and Methods from the data shown in Supplementary Fig. S1. Values are mean ± SE.

A more relevant question, however, is to understand how these differences in peptidase activity, unveiled by the use of short fluorogenic peptides, relate to the true process of protein degradation and, specifically, whether they influence the overall rates of protein breakdown by proteasomes. To address this point, we studied in vitro degradation by 26S constitutive and immunoproteasomes of IGF-1, casein and histones by measuring the appearance of new amino groups generated as a consequence of hydrolysis of the substrate with fluorescamine. Casein and histones have little tertiary structure and are degraded by purified 26S proteasomes without ubiquitylation at linear rates for several hours in the presence of ATP [12,15]. On the contrary, IGF-1 requires preliminary denaturation by reduction of disulfide bonds and carboxymethylation of the cysteins in order to be hydrolyzed in vitro by 26S proteasomes in an ATP-dependent but ubiquitin-independent manner [15]. As shown in Fig. 1a, IGF-1 and casein were hydrolyzed by 26S constitutive and immunoparticles at linear, identical rates, thus confirming findings previously reported for ovalbumin with other two model proteasome substrates [4]. In contrast, the results obtained for the hydrolysis of H1, the linker histone in chromatin protects internucleosomal DNA, were unexpected. In fact, this extremely basic substrate was degraded six times faster by immuno- than by constitutive 26S proteasomes (Fig. 1b). Importantly, a four-fold increase in histone concentration did not modify the rates of H1 degradation, thus demonstrating that in these experiments both proteasomal species were catalyzing the hydrolysis reaction at maximum velocity (i.e. in conditions of substrate saturation) (Fig. S2).

These results were subsequently confirmed by directly comparing the rates of substrate consumption. Towards this end, histone H1 was incubated with 26S constitutive or immunoproteasomes and the amount of undegraded protein present at different time points was quantified. In agreement with the fluorescamine data, these experiments clearly revealed the greatly increased rates of histone H1 hydrolysis by 26S proteasomes containing INF-γ-induced β-subunits (Fig. 1c). The enhanced capacity of immunoproteasomes to hydrolyze basic proteins was subsequently confirmed by assessing the rates of degradation of the core histones H2A, H2B, and H3. Similarly to histone H1, these substrates were also degraded at rates that were about four-fold higher by proteasome containing immune β-subunits compared to regular 26S particles (Figs. 2a, b and S3). This enhanced capacity of immunoproteasomes to hydrolyze proteins rich in lysine and arginine was subsequently confirmed by assessing degradation of another completely unrelated basic substrate, namely myelin basic protein (MBP). Similar to histones and casein, MBP has very little tertiary structures and therefore can be degraded by proteasomes without the need for ubiquitination [16,17]. As shown in Figs. 3 and S4, MBP was also degraded about four-fold faster by 26S immuno- than by 26S constitutive proteasomes. Taken together, these results clearly demonstrate that highly basic proteins are hydrolyzed with higher efficiency by immunoproteosomes than by constitutive proteasomes.

The rate limiting role of immunoproteasome tryptic activity in determining the rate of hydrolysis of basic proteins was subsequently investigated using leupeptin, a competitive inhibitor that was reported to specifically inactivate the β2 subunit of constitutive proteasomes [12]. Preliminary experiments using fluorogenic peptides indeed showed that leupeptin is able to block the β2i subunit of 26S immunoproteasome with high efficiency without effecting β1i and β5i sites (Table 2). Having established conditions that allow selective inhibition of the tryptic site of the immunoproteasome in the absence of any detectable effects on the other two peptidase activities, we analyzed the effect of leupeptin on hydrolysis of histones and MBP. As shown in Fig. 4, leupeptin consistently decreased the degradation of these basic proteins by about 40%. Although incomplete, the inhibition obtained with a competitive inhibitor that specifically targets only the β2i subunit unambiguously demonstrates the rate limiting role of immunoproteasome tryptic activity in controlling the turnover rates of basic proteins.

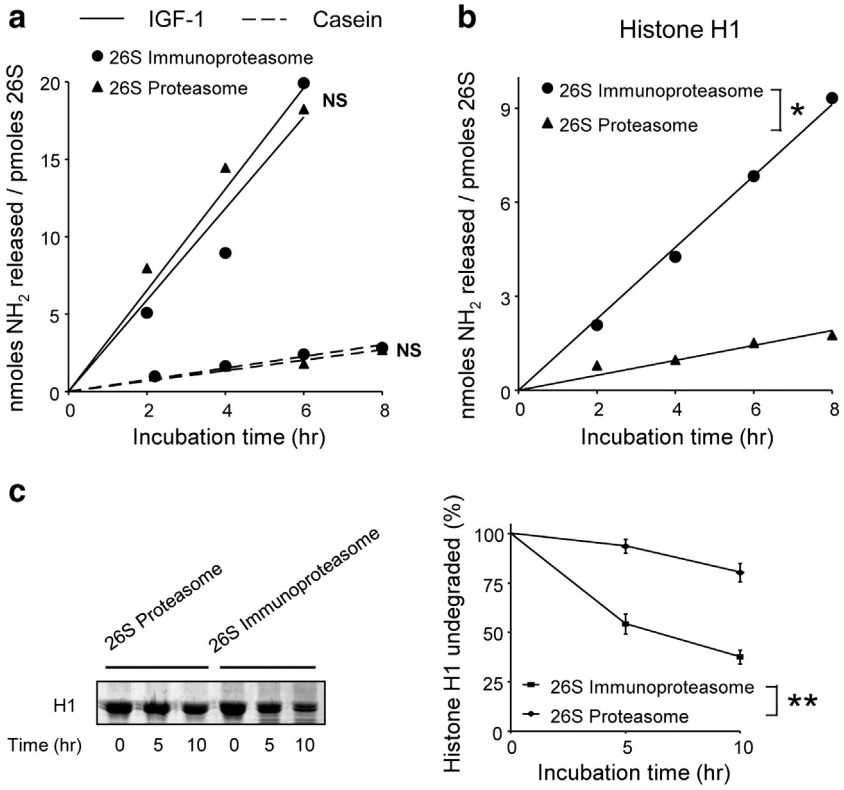


Fig. 1. Enhanced rates of histone H1 hydrolysis, but not of IGF-1 and casein, by 26S immunoproteasomes. IGF-1 and casein (a) and histone H1 (b) were incubated with 26S proteasomes and immunoproteasomes and the amino groups released were measured with fluorescamine at the indicated time points. Data are representative of three independent experiments. NS, not significant. * $P < 0.05$. (c) Undegraded H1 present at different time points was separated by SDS-PAGE and quantified by densitometric analysis. Data are the average of three independent experiments ± SEM. ** $P < 0.005$.

3.2. Different rates of substrate hydrolysis do not affect the size distribution of proteasomal products

In principle, the faster rate of degradation of basic proteins by 26S immunoproteasomes should result in enhanced fragmentation of the substrate into smaller products. To test this hypothesis, we analyzed the size distribution of products generated by hydrolysis of H1 on a HP-size exclusion chromatographic method recently developed by our group that allows linear separation and accurate quantification of peptides in the range of 1 to 40 residues [13,14]. In particular, the protein was degraded under conditions ensuring that peptides released by 26S proteasomes do not re-enter the degradative particle and therefore are not subjected to a second round of hydrolysis (i.e. the substrate was present in large excess and not more than 10% was degraded at the end of the incubation, Fig. S5). The analysis of the size distribution of

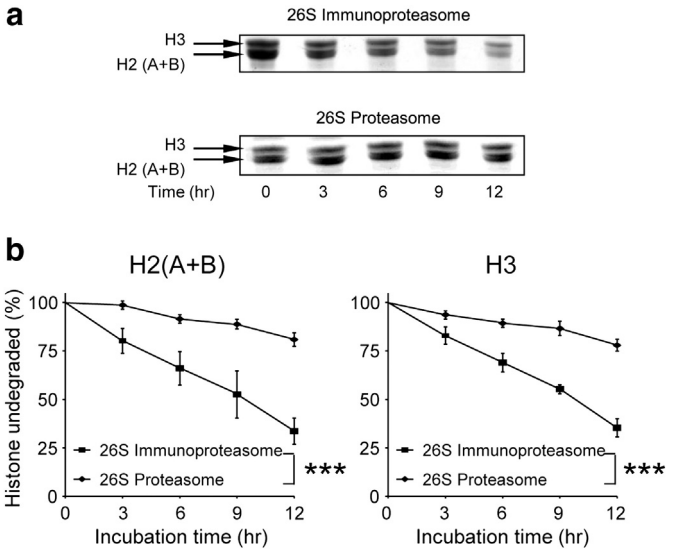


Fig. 2. Enhanced rates of core histone degradation by 26S immunoproteasomes. (a) Core histones H2A, H2B, and H3 were incubated with 26S proteasomes and immunoproteasomes and the undegraded proteins separated by SDS-PAGE. (b) Densitometric quantification of residual proteins. Data are the average of three independent experiments ± SEM. *** $P < 0.0005$.

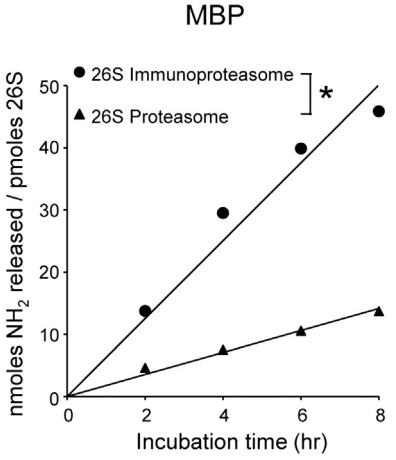


Fig. 3. Enhanced rates of MBP hydrolysis by 26S immunoproteasomes. MBP was incubated with 26S proteasomes and immunoproteasomes, and the amino groups released were measured using fluorescamine at the indicated time points. Data are representative of three independent experiments. * $P < 0.05$.

Table 2
Effect of leupeptin on peptidase activities of 26S immunoproteasomes.

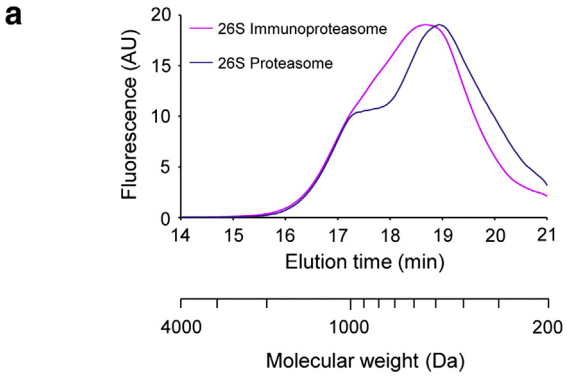
Activity	100 μM leupeptin (% inhibition)
Chymotrypsin-like	0
Caspase-like	0
Tryptic-like	97.6 ± 0.4

Chymotrypsin-like, caspase-like and trypsin-like of 26S immunoproteasomes were assessed using Z-GGL-amc, Suc-YVAD-amc and Bz-VGR-amc (100 μM each) in the presence or absence of leupeptin. Data are the means of three independent measurements ± SE.

peptides released during degradation of H1 unambiguously demonstrated that 26S immunoproteasomes do not generate increased amounts of shorter products (Fig. 5a and b). This disproves the hypothesis that proteins rich in basic residues are fragmentized into smaller pieces by proteasome variants displaying enhanced trypsin-like peptidase activity.

4. Discussion

Chymotrypsin-like activity of proteasomes has been generally viewed as the most important and rate-limiting step in protein breakdown in vivo [18]. This notion, however, was challenged in a study by Kisselev demonstrating that the relative contributions of the three proteasomal proteolytic sites depend on the protein being degraded and its amino acid composition [12]. Accordingly, in this study, the importance of trypsin-like activity was found to strongly correlate with the content in basic amino acids of the substrate. It is unclear, however, whether and to what extent the different susceptibility of



b

	Mean	Median
26S Immunoproteasome	3.86 ± 0.02	3.06 ± 0.02
26S Proteasome	3.60 ± 0.05	2.63 ± 0.02
	NS	NS

Fig. 5. Same size distribution of peptides generated during hydrolysis of H1 by 26S proteasomes and immunoproteasomes. (a) Size distribution of peptides generated from H1 by 26S proteasomes and immunoproteasomes. Equal amounts of peptides generated during degradation of H1 were reacted with fluorescamine and immediately fractionated by HP-SEC. Similar data were obtained in four independent experiments. (b) Mean sizes and medians were calculated from the distributions of products obtained by HP-SEC. Values are averages from four experiments ± SEM. NS, not significant.

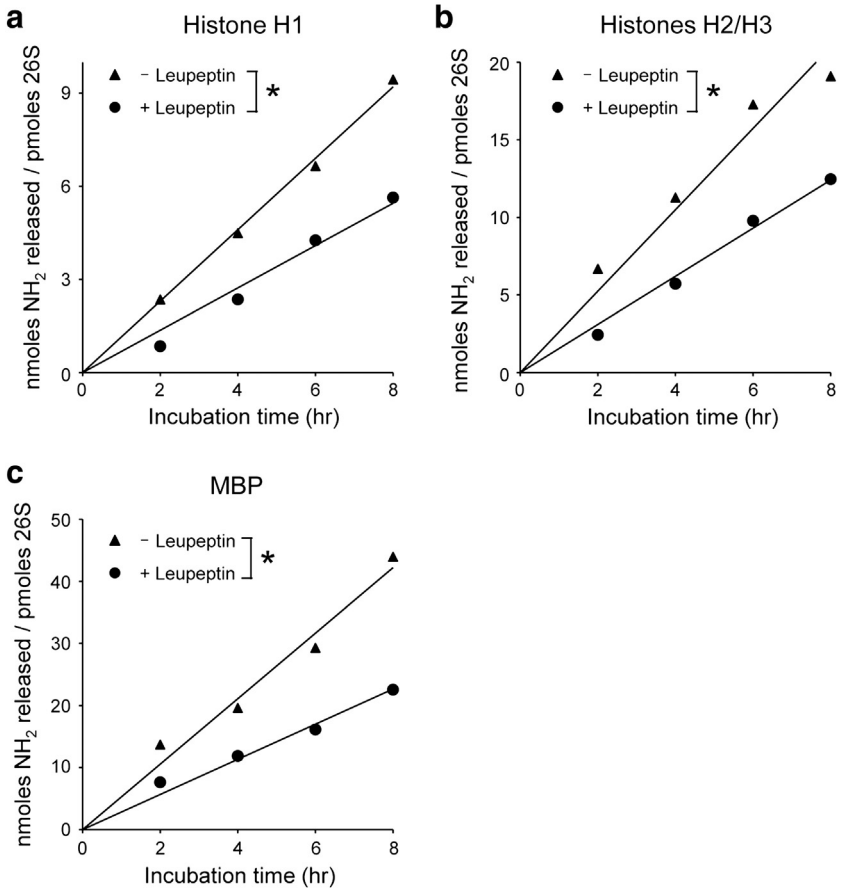


Fig. 4. Selective inhibition of the trypsin-like activity of 26S immunoproteasomes slows the hydrolysis of basic proteins. Histones H1 (a), H2/H3 (b) and MBP (c) were incubated with 26S immunoproteasomes in the presence or absence of 100 μM leupeptin and the amino groups released were measured with fluorescamine at the indicated time points. Data are representative of three independent experiments. * $P < 0.05$.

the peptide bonds of a polypeptide to hydrolysis at the three proteolytic sites affects the overall rate of substrate degradation by proteasomes. This question is especially relevant in the case of immunoproteasomes, where the constitutive catalytic β-subunits are replaced by new ones that display highly modified peptidase specificities. To address this point, we studied degradation of several naturally (casein, histones and MBP) or artificially (carboxymethylated IGF-1) loosely folded proteins that are hydrolyzed in an ATP-dependent linear manner in the absence of ubiquitination by 26S constitutive and immunoproteasomes. In this way, we were able to demonstrate that despite the clear differences in peptidase activities seen with short fluorogenic peptides, 26S constitutive and immunoproteasomes degrade neutral IGF-1 (pI 7.4) and moderately acidic casein (pI 5.2) at the same rates. These results obtained with two widely used proteasomal model substrates confirm our previous data showing that oxidized ovalbumin is degraded in vitro with comparable efficiency by 26S constitutive and immunoproteasomes [4]. For these substrates, therefore, it seems likely that the rates of hydrolysis do not simply depend on the efficiency of peptide bonds cleavage at proteasomal active sites, while other factors (e.g. the affinity of the protein for the regulatory 19S particle and/or the velocity of its translocation into the proteolytic internal chamber) are crucial and probably represent rate-limiting steps of the degradation process. In this respect, the observation that breakdown rates by 26S proteasomes of several unfolded proteins inversely correlate with their molecular weights (Fig. 1 and [15]) is of interest, as it suggests that shorter polypeptides might interact with higher affinity with the 19S cap and/or diffuse faster through the α-pore of the 20S core particle. At present, we cannot exclude the possibility that proteins extraordinarily rich in hydrophobic residues are hydrolyzed at enhanced rates by 26S immunoproteasomes. However, the elevated tendency to aggregate and precipitate, together with their tightly folded structure, precludes the use of such substrates in degradation experiments such as those performed herein. In accordance with our conclusions, Kisselev was unable to establish any correlation between the content of hydrophobic residues and the degree of proteolytic inhibition seen upon inactivation of the chymotrypsin-like site for four proteins investigated, including ovalbumin and casein [12].

Completely different results were obtained when we assessed the degradation of substrates characterized by an unusually high content in lysine and arginine such as histones and MBP. In fact, these extremely basic proteins (H1 pI 11.4, H2A pI 11.3, H2B pI 10.8, H3 pI 11.5, MBP pI 11.1) were hydrolyzed at 4–6 fold higher rates by 26S immunoproteosomes than by constitutive proteasomes. Notably, this unexpected difference in breakdown rates cannot be ascribed merely to a difference in the affinity of substrates for the two variants of proteasomes (i.e. to a difference in their K_m), since an increase in the concentrations of substrates does not further enhance their degradation. This demonstrates that under the experimental conditions used both 26S proteasomes and immunoproteasomes are acting at maximum velocity. In contrast, the higher rates of basic protein degradation are likely to directly depend on the enhanced trypsin-like activity of immunoproteasomes, as suggested by the observation that the lower rates of breakdown of histone H1 by constitutive 26S proteasomes are comparable to those measured for a substrate of similar molecular mass such as casein (panels A and B in Fig. 1). It seems likely, therefore, that in the case of extremely basic protein substrates the overall rates of proteasomal hydrolysis are mainly determined by the efficiency of peptide bond cleavage at the tryptic site. Accordingly, the enhanced trypsin-like activity caused by replacement of the constitutive β2 subunit with the INF-γ-induced variant β2i could account for the higher hydrolytic capacity of 26S immunoproteasomes towards histones and MBP.

This hypothesis was directly tested using leupeptin, a competitive inhibitor that has been reported to selectively suppress the trypsin-like activity of proteasomes [12], and which we showed is effective in inactivating the β2i subunit without effecting β1i and β5i. Crucially, in our experiments leupeptin was capable of effectively slowing the hydrolysis by 26S immunoproteasomes of all basic proteins tested, thus demonstrating the rate limiting function of the β2i subunit in determining turnover rates of basic substrates. In this regard, it should be stressed that the incomplete inhibition of basic protein degradation seen with leupeptin was somewhat expected considering its competitive mechanism of action. In fact, it is not surprising that leupeptin cannot completely prevent binding at the β2i active site of protein stretches rich in lysine and arginine, which are likely to present a strong affinity to the tryptic site and reach a high concentration in the small volume of the internal proteasomal cavity. Interestingly, the increased rate of peptide bond cleavage at the β2i subunit does not alter the size distribution of products generated from histones. Therefore, it is likely that the number of cleavages made by proteasomes in a polypeptide depends on the intrinsic properties of the particle (rather than on the catalytic efficiency of active sites). This is also demonstrated by the lack of difference in the size distribution of peptides generated when an active site is inhibited [15,19].

It was recently reported that during somatic DNA damage response and spermatogenesis core histones (but not the linker histone H1) are preferentially degraded by special forms of proteasomes containing the activator PA200 in an acetylation, but not polyubiquitination-dependent, process [20]. Furthermore, testes were found to express high levels of INF-γ-induced β-subunits, thus indicating a specific role of immunoproteasomes in the hydrolysis of histones during spermatogenesis. Moreover, during transcription, histones are removed from DNA at promoter regions or active gene bodies in somatic cells [21,22], and several lines of evidence suggest that the released histones are rapidly degraded by proteasomes even in the absence of ubiquitination [23]. Our data showing that in vitro histones are hydrolyzed by 26S proteasomes with no need for polyubiquitination, in a process that is strongly accelerated when the INF-γ-induced β-subunits are incorporated, have important implications in fully understanding all the possible biological functions of immunoproteasomes. Following stimulation of mammalian cells with pro-inflammatory cytokines such as interferon-γ or TNF-α, several regulatory pathways are activated that rely on the rapid transcription of hundreds of different genes [24]. In this situation, the accumulation of histones released from sites of active transcription might be harmful for the cell [25]. The rapid formation of immunoproteasomes induced by several cytokines may, therefore, be useful to efficiently remove non-chromatin bound histones, thus preventing genomic instability, hypersensitivity to DNA damaging agents, and blocking transcription caused by accumulation of these basic proteins [25]. In agreement with this hypothesis, immunoproteasomes were recently shown to play a specific role in the control of cytokine production and T cell differentiation [26]. Moreover, it appears unlikely that immunoproteasomes evolved exclusively to improve generation of class I epitopes since MHC-I molecules accommodate peptides with basic residues at their C-terminus only occasionally in humans and never in mice [6]. Accordingly, no decrease in cell surface expression of MHC class I molecules was observed in β2i-deficient mice [27]. Intriguingly, β2i is the only cytokine-induced proteasomal subunit to be encoded outside the MHC region [2,3]. Although recently generated mice lacking all three immunoproteasomal catalytic subunits are viable and apparently healthy [5], previous studies detected a 20–30% decrease in the number of $CD8^+$ T in the thymus, blood, and spleen of β2i-deficient mice [27]. Importantly, this decrease does not correlate with MHC class I expression but, rather, it seems that $CD8^+$ T cells $\beta2i^{-/-}$ expand less readily than wild-type $CD8^+$ [6]. This highlights that there is a requirement for immunoproteasomes (and specifically the β2i subunit) for the survival of T cells in a pro-inflammatory environment.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamcr.2014.05.005.

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References

[1] D. Voges, P. Zwickl, W. Baumeister, The 26S proteasome: a molecular machine designed for controlled proteolysis, Annu. Rev. Biochem. 68 (1999) 1015–1068.
[2] A.L. Goldberg, P. Cascio, T. Saric, K.L. Rock, The importance of the proteasome and subsequent proteolytic steps in the generation of antigenic peptides, Mol. Immunol. 39 (2002) 147–164.
[3] P.M. Kloetzel, Antigen processing by the proteasome, Nat. Rev. Mol. Cell Biol. 2 (2001) 179–187.
[4] P. Cascio, C. Hilton, A.F. Kisselev, K.L. Rock, A.L. Goldberg, 26S proteasomes and immunoproteasomes produce mainly N-extended versions of an antigenic peptide, EMBO J. 20 (2001) 2357–2366.
[5] E.Z. Kincaid, J.W. Che, I. York, H. Escobar, E. Reyes-Vargas, J.C. Delgado, R.M. Welsh, M.L. Karow, A.J. Murphy, D.M. Valenzuela, G.D. Yancopoulos, K.L. Rock, Mice completely lacking immunoproteasomes show major changes in antigen presentation, Nat. Immunol. 13 (2012) 129–135.
[6] M. Groettrup, C.J. Kirk, M. Basler, Proteasomes in immune cells: more than peptide producers? Nat. Rev. Immunol. 10 (2010) 73–78.
[7] F. Cerruti, M. Martano, C. Petterino, E. Bollo, E. Morello, R. Bruno, P. Buracco, P. Cascio, Enhanced expression of interferon-gamma-induced antigen-processing machinery components in a spontaneously occurring cancer, Neoplasia 9 (2007) 960–969.
[8] T. Egerer, L. Martinez-Gamboa, A. Dankof, B. Stuhlmuller, T. Dorner, V. Krenn, K. Egerer, P.E. Rudolph, G.R. Burmester, E. Feist, Tissue-specific up-regulation of the proteasome subunit beta5i (LMP7) in Sjogren's syndrome, Arthritis Rheum. 54 (2006) 1501–1508.
[9] M. Mishto, E. Bellavista, A. Santoro, A. Stolzing, C. Ligorio, B. Nacmias, L. Spazzafumo, M. Chiappelli, F. Licastro, S. Sorbi, A. Pession, T. Ohm, T. Grune, C. Franceschi, Immunoproteasome and LMP2 polymorphism in aged and Alzheimer's disease brains, Neurobiol. Aging 27 (2006) 54–66.
[10] U. Seifert, L.P. Bialy, F. Ebstein, D. Bech-Otschir, A. Voigt, F. Schroter, T. Prozorovski, N. Lange, J. Steffen, M. Rieger, U. Kuckelkorn, O. Aktas, P.M. Kloetzel, E. Kruger, Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress, Cell 142 (2010) 613–624.
[11] J.A. Nathan, V. Spinnenhirn, G. Schmidtke, M. Basler, M. Groettrup, A.L. Goldberg, Immuno- and constitutive proteasomes do not differ in their abilities to degrade ubiquitinated proteins, Cell 152 (2013) 1184–1194.
[12] A.F. Kisselev, A. Callard, A.L. Goldberg, Importance of the different proteolytic sites of the proteasome and the efficacy of inhibitors varies with the protein substrate, J. Biol. Chem. 281 (2006) 8582–8590.
[13] M. Raule, F. Cerruti, N. Benaroudj, R. Migotti, J. Kikuchi, A. Bachi, A. Navon, G. Dittmar, P. Cascio, PA28alphabeta reduces size and increases hydrophilicity of 20S immunoproteasome peptide products, Chem. Biol. 21 (2014) 470–480.
[14] D. Berko, S. Tabachnick-Cherny, D. Shental-Bechor, P. Cascio, S. Mioletti, Y. Levy, A. Admon, T. Ziv, B. Tirosh, A.L. Goldberg, A. Navon, The direction of protein entry into the proteasome determines the variety of products and depends on the force needed to unfold its two termini, Mol. Cell 48 (2012) 601–611.
[15] A.F. Kisselev, T.N. Akopian, K.M. Woo, A.L. Goldberg, The sizes of peptides generated from protein by mammalian 26 and 20 S proteasomes. Implications for understanding the degradative mechanism and antigen presentation, J. Biol. Chem. 274 (1999) 3363–3371.
[16] J. Lucas, D. Lobo, E. Terry, E.L. Hogan, N.L. Banik, Susceptibility of myelin proteins to a neutral endoproteinase: the degradation of myelin basic protein (MBP) and P2 protein by purified bovine brain multicatalytic proteinase complex (MPC), Neurochem. Res. 17 (1992) 1261–1266.
[17] A. Belogurov Jr., A. Kudriaeva, E. Kuzina, I. Smirnov, T. Bobik, N. Ponomarenko, Y. Kravtsova-Ivantsiv, A. Ciechanover, A. Gabibov, Multiple sclerosis autoantigen myelin basic protein escapes control by ubiquitination during proteasomal degradation, J. Biol. Chem. (2014), http://dx.doi.org/10.1074/jbc.M113.544247.
[18] W. Heinemeyer, M. Fischer, T. Krimmer, U. Stachon, D.H. Wolf, The active sites of the eukaryotic 20S proteasome and their involvement in subunit precursor processing, J. Biol. Chem. 272 (1997) 25200–25209.
[19] A.K. Nussbaum, T.P. Dick, W. Keilholz, M. Schirle, S. Stevanovic, K. Dietz, W. Heinemeyer, M. Groll, D.H. Wolf, R. Huber, H.G. Rammensee, H. Schild, Cleavage motifs of the yeast 20S proteasome beta subunits deduced from digests of enolase 1, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 12504–12509.
[20] M.X. Qian, Y. Pang, C.H. Liu, K. Haratake, B.Y. Du, D.Y. Ji, G.F. Wang, Q.Q. Zhu, W. Song, Y. Yu, X.X. Zhang, H.T. Huang, S. Miao, L.B. Chen, Z.H. Zhang, Y.N. Liang, S. Liu, H. Cha, D. Yang, Y. Zhai, T. Komatsu, F. Tsuruta, H. Li, C. Cao, W. Li, G.H. Li, Y. Cheng, T. Chiba, L. Wang, A.L. Goldberg, Y. Shen, X.B. Qiu, Acetylation-mediated proteasomal degradation of core histones during DNA repair and spermatogenesis, Cell 153 (2013) 1012–1024.
[21] R.B. Deal, J.G. Henikoff, S. Henikoff, Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones, Science 328 (2010) 1161–1164.
[22] M.F. Dion, T. Kaplan, M. Kim, S. Buratowski, N. Friedman, O.J. Rando, Dynamics of replication-independent histone turnover in budding yeast, Science 315 (2007) 1405–1408.
[23] Y.S. Chen, X.B. Qiu, Transcription-coupled replacement of histones: degradation or recycling? J. Genet. Genomics 39 (2012) 575–580.
[24] U. Boehm, T. Klamp, M. Groot, J.C. Howard, Cellular responses to interferon-gamma, Annu. Rev. Immunol. 15 (1997) 749–795.
[25] R.K. Singh, M.H. Kabbaj, J. Paik, A. Gunjan, Histone levels are regulated by phosphorylation and ubiquitylation-dependent proteolysis, Nat. Cell Biol. 11 (2009) 925–933.
[26] T. Muchamuel, M. Basler, M.A. Aujay, E. Suzuki, K.W. Kalim, C. Lauer, C. Sylvain, E.R. Ring, J. Shields, J. Jiang, P. Shwonek, F. Parlati, S.D. Demo, M.K. Bennett, C.J. Kirk, M. Groettrup, A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis, Nat. Med. 15 (2009) 781–787.
[27] M. Basler, J. Moebius, L. Elenich, M. Groettrup, J.J. Monaco, An altered T cell repertoire in MECL-1-deficient mice, J. Immunol. 176 (2006) 6665–6672.