Proteasomes: the changing face of proteolysis

The proteasome, the multimeric, multicatalytic complex that is responsible for much of the proteolysis within cells, catalyzes several different types of peptide cleavage. Recently, it has been found to increase its repertoire of catalytic activities even further, by varying its catalytic subunits and by associating with regulatory complexes.

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Proteasomes are large (≈700 kDa) multimeric proteinase complexes which are present in all eukaryotic cells and in archaebacteria of the genus *Thermoplasma* [1,2]. They have a major role in the intracellular, nonlysosomal degradation of short-lived proteins by both ubiquitin-dependent and ubiquitin-independent pathways. The ubiquitin-dependent pathway targets proteins for rapid degradation by the covalent attachment of multiple ubiquitin molecules to the protein [3]. Proteasomes are found in the cytoplasm and nuclei of cells, but do not show an even distribution throughout these compartments. Indeed, their distribution changes during the cell cycle [4].

Proteasomes are cylindrical in shape, being composed of four stacked rings of seven subunits. In the simple archaebacterial proteasome there are only two types of subunits: α subunits, which form the outer rings, and β subunits, which form the inner rings. The β subunits are expressed as precursor polypeptides that require processing. Studies of expressed archaebacterial subunits in *E. coli* indicate that the α subunits form seven-membered rings to which the β subunits are then located. The necessary processing of the β subunits appears to occur during their insertion into the complex [5].

In eukaryotes the structure of the proteasome is thought to be similar to that in bacteria, although the rings are each composed of non-identical subunits which are classed as either α- or β-type depending on their sequence similarity to the archaebacterial subunits (Fig 1). The α-type subunits share a highly conserved N-terminal region and appear to be structurally important, whereas the β-type subunits, which are more divergent, appear to be catalytically important. In yeast, the genes encoding all seven α and seven β subunits have been cloned (Table 1), and, with the exception of Y13, have been shown to be essential [6]. Sequence analysis of proteasome subunits shows that they represent a novel family of proteolytic enzymes.

Studies on the kinetics of cleavage of model peptide substrates and using specific inhibitors and activators have demonstrated the presence of at least five distinct catalytic activities in the complex, and have shown that the proteasome can cleave peptides on the carboxyl side of almost any amino acid [1]. Three broad activities, cleavage of synthetic peptide substrates on the carboxyl side of basic, hydrophobic and acidic amino acids, have been widely used as model activities. Mutation studies in yeast have revealed a number of β-type subunits to be associated with catalytic activity [6]. But no one subunit has been shown to have catalytic activity *per se*, and at least two subunits seem to be required for any given activity, raising the intriguing possibility that two or more subunits may associate to form each catalytic site.

A number of different proteins have been reported to associate with the proteasome, leading to activation or inhibition of catalytic activities [2]. The association of a multimeric regulatory ATPase complex with the ends of the proteasome gives rise to a structure referred to as the 26S proteinase (Fig. 1). It is the 26S proteinase which is believed to be responsible for ubiquitin-dependent protein degradation [2,7]. The extra subunits of the 26S

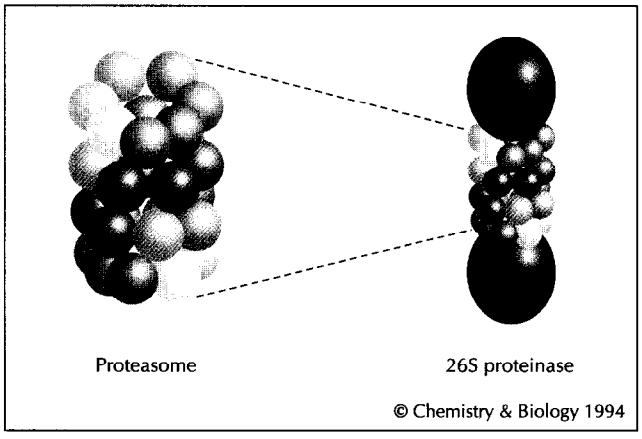

Fig. 1. A model of the structure of eukaryotic proteasomes and 26S proteinase. The cylindrical structure of the proteasome is composed of four stacked rings of seven non-identical subunits in a pseudo-helical arrangement. The two inner rings are composed of β-type subunits (green), believed to harbour the catalytic sites, and the outer rings are composed of α-type subunits (orange), which are structurally important and contain putative nuclear localization signals and phosphorylation sites [1,5,15,16]. Association with two copies of the regulatory ATPase complex (purple) produces the 26S proteinase.

Table 1. Yeast proteasome subunits and their human homologs.

Yeast subunit	Mammalian homolog	Subunit type
Y7	C3	α
Y13	C9	α
PRS1	C8	α
PRS2	Iota	α
PRE5	C2	α
PRE6	*	α
PUP2	Zeta	α
PRE1[a]	C7	β
PRE2[a]	MB1 (X) / LMP7	β
PRE3[b]	δ (Y) / LMP2	β
PRE4[b]	RN3	β
PRS3	C5	β
PUP1	MECL-1	β
PUP3	C10	β

*Sequence for mammalian subunit not yet available. Mutations in yeast subunit genes have been shown to affect cleavage after [a]hydrophobic and [b]acidic residues; data taken from [6,9,10].

proteinase confer a different substrate specificity on the proteasome, allowing it to degrade ubiquitinated, and possibly also other, proteins in an ATP-dependent process. The precise ratio of 26S proteinase to any other forms of the proteasome is not yet clear.

Proteasomes and 26S proteinase complexes are involved in the degradation of short-lived proteins and have been shown, as part of the ubiquitin pathway, to be involved in the degradation of a number of nuclear regulatory proteins such as cyclins and transcription factors [8]. In mammalian cells a significant proportion of proteasomes in the cytoplasm appear to be associated with the endoplasmic reticulum which may, in part, reflect their role in antigen presentation [1]. Peptide fragments of antigens are believed to be produced in the cytoplasm, then transported into the endoplasmic reticulum via a specialized transporter, the TAP1/TAP2 heterodimer. In the endoplasmic reticulum, they bind to newly-synthesized major histocompatibility complex (MHC) class I proteins and are transported to the cell surface for display and recognition by T cells. The notion that proteasomes might be important in the formation of these antigenic peptides arose when two non-essential genes coding for proteasome subunits (LMP2 and LMP7) were found to lie within the MHC class II region, in close proximity to the genes coding for the TAP transporter proteins (see [9]). Furthermore, induction of transcription of the LMP2 and LMP7 genes was shown to be part of the pleiotropic response mediated by γ-interferon on immunological challenge, resulting in an increased expression of these subunits in proteasomes.

The idea that LMP2 and LMP7 were important in antigen presentation was called into question, however, when it was shown that peptide formation and stable class I MHC expression (which requires peptide binding) occurred in human lymphoblastoid cell mutants lacking the genes for LMP2 and LMP7. But subsequent studies by a number of different groups comparing these mutant lymphoblastoid cells or γ-interferon-induced cells with normal, untreated cells showed that, although LMP2 and LMP7 were not absolutely required for peptide generation, their presence in proteasomes both increased the variety of peptides formed and caused a shift in the type of peptide produced, increasing the relative amount of peptides that could be efficiently bound by class I molecules [10–13]. The presence of these two subunits in proteasomes resulted in increased endopeptidase activity for cleavage following hydrophobic and basic residues, with little effect on the cleavage following acidic residues [11]. The presence of LMP2 and LMP7 therefore results in a population of peptides in which those with a basic or hydrophobic C-terminal residue are over-represented. The C-terminal residue is crucial in the selective binding of a peptide by MHC class I molecules; surface-expressed peptides, the peptides that bind to MHC class I molecules tightly enough to be carried to and retained at the cell surface, terminate almost exclusively in basic or hydrophobic residues [14]. Thus, although LMP2 and LMP7 are not essential for antigen presentation, their increased expression and incorporation into proteasomes in response to γ-interferon leads to an increased production of peptides suitable for presentation at the cell surface. Increasing the levels of LMP2 and LMP7 enhances antigen presentation over basal levels without changing the overall rate of protein degradation [10]. Gene knockout experiments have also confirmed the role of LMP7 in efficient antigen presentation [13].

The γ-interferon-induced expression of LMP2 and LMP7 subunit proteins in proteasome complexes may be explained either by the *de novo* synthesis of new proteasomes incorporating the MHC-encoded subunits, or by subunit exchange in pre-formed complexes. Studies with [^{35}S]methionine-labelled cells showed that γ-interferon did not alter the turnover rate of any incorporated proteasome subunits, indicating that proteasomes incorporating the LMP subunits are newly synthesized and that the LMP-encoded subunits are not exchanged for other subunits in pre-existing complexes [12].

The 'housekeeping' proteasome subunits that are replaced by the MHC-encoded subunits have only recently been identified. A number of studies have now shown that when the expression of LMP2 and LMP7 and their incorporation into mature proteasomes are increased, the incorporation of two other subunits is decreased [9,10,12]. One of these subunits is δ, which is closely related in primary sequence to LMP2. The other is MB1, which is related to LMP7. Free δ can be found in immunoprecipitates as the precursor form, but is only found in proteasomes as the mature processed form [12]. No free mature form can be detected, indicating that processing of β-type subunits occurs at or subsequent to

their assembly into proteasomes. It thus appears that the housekeeping subunits, δ and MB1, and the LMP subunits compete with each other for sites within newly forming complexes.

The replacement of δ with LMP2 and of MB1 with LMP7 appears to be specific. In cell lines expressing only LMP2 and not LMP7, only the incorporation of δ is reduced. When LMP2/LMP7 levels increase, on treatment of cells with γ-interferon, the expression level of δ precursor does not decrease, but incorporation of δ and MB1 into the complex decreases as incorporation of LMP2 and LMP7 increases. Transfecting cells with LMP2 and LMP7 genes controlled by promoters allowing high expression indicates that it is the high levels of LMP2 and LMP7 protein, and not γ-interferon or other γ-interferon-induced proteins, that are responsible for the decreased incorporation of δ or MB1 into proteasomes. There is no down-regulation of the mRNAs coding for δ and MB1 or any other proteasome subunit by γ-interferon [12]. Thus, the reduced presence of δ and MB1 protein on γ-interferon induction is most likely to be due to the degradation of the unincorporated subunits.

In yeast all the proteasome subunits known to be involved in catalysis are of the β type (Table 1). Studies have shown that mutations in the genes coding for the PRE1/2 and PRE3/4 subunits affect the chymotrypsin-like and peptidyl-glutamyl-peptide hydrolase activites, respectively [6]. LMP2 and LMP7 are closely related to the essential β-type yeast subunits PRE3 and PRE2, respectively. The LMP7 homolog, PRE2, is required in yeast for efficient cleavage after hydrophobic amino acids. The transfection of high levels of LMP7 into cells resulted in a greatly increased capacity to cleave substrates after hydrophobic and basic residues without change in the ability of the proteasome to cleave after acidic residues [11]. Cells transfected with LMP2 showed an increased ability to cleave substrates following basic amino acids, a decreased ability to cleave after acidic residues and no change in ability to cleave following hydrophobic amino acids. These data suggest that LMP2 promotes cleavage after basic residues but replaces a subunit important for cleavage after acidic residues. Indeed, the yeast homolog of LMP2, PRE3, is required for efficient cleavage following acidic residues.

Despite their high sequence similarity, the charges of LMP7 and MB1 are different; the calculated pI value for LMP7 is near neutral, whereas MB1 is a basic protein. The exchange of LMP7 for MB1 may thus affect the conformation and hence the activities of the proteasome. Small conformational changes in proteasomes are known to have profound effects on catalytic activity [15]. LMP2 and δ are both acidic with pI values of ≈5. Presumably, there are structural constraints placed on which subunits can be included in a specific position within the proteasome complex, and these are reflected in the very close similarities between LMP2 and δ, and LMP7 and MB1. As proteasomes can have various combinations of subunits, they can produce a much wider range of peptides from a given protein substrate than if only one combination of subunits were possible, an obvious advantage for the immune system.

Evolution has thus given the proteasome a novel method of extending its potential substrates and products. The proteasome incorporates non-essential subunits in place of normal constitutive subunits, thus changing its catalytic profile. It can also associate with other, regulatory proteins to form the much larger 26S proteinase, the ATP-dependent proteolytic machinery of the ubiquitin-dependent degradation pathway. This ability to use the same core complex for a wide variety of functions is an obvious asset to the cell.

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