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Structure and assembly of the 20S proteasome

W. L. H. Gerards, W. W. de Jong, W. Boelens and H. Bloemendal*

Department of Biochemistry, University of Nijmegen, P.O. Box 9101, NL-6500 HB Nijmegen (The Netherlands), Fax +31 24 3540525, e-mail: H.Bloemendal@bioch.kun.nl

Abstract. The barrel-shaped 20S proteasome is one of the two components of a larger 26S particle, the multicatalytic 2000-kDa protease complex. The proteolytic sites are located in the inner chamber of the 20S particle and are only accessible via narrow entrances. This paper reviews the current knowledge concerning proteasome formation, proteolytic activities, structural aspects and assembly. Eukaryotic proteasomes are made up by four rings each of which contains seven different subunits occurring at fixed positions. While the outer rings contain α-type subunits, the inner ones comprise β-type subunits. The current assembly model for eukaryotic 20S proteasomes is based upon the detection of 13S and 16S intermediates, respect-ively, in addition to previous findings with archaebacterial and eubacterial proteasome assembly. The available data suggest a cooperative assembly of the α-type and β-type subunits into half proteasome-like complexes followed by dimerization into proteasomes. During or after dimerization of half proteasomes, the β-type subunits are processed. The prosequence of the β-type subunits is essential for the assembly process and prevents protease activity of immature proteasomes.

Key words. 20S proteasome; proteasomal subunits; assembly; circular structure; proteolytic activity.

Introduction

The 20S proteasome is a component of the ubiquitin-proteasome-dependent proteolysis system. This system plays a major role in the turnover of long-lived proteins [1], misfolded proteins [2] and in the selective degradation of key proteins [3]. It has become clear that controlled protein degradation is an important and efficient way to remove nonfunctional proteins or to regulate the activity of key proteins. Target proteins are selectively recognized by the ubiquitin system and subsequently marked by covalent linkage of multiple molecules of ubiquitin, a small conserved protein [4, 5]. The polyubiquitinated proteins are degraded by the 26S proteasome. This complex is composed of two large subcomplexes: the 20S proteasome constituting the proteolytic core and the 19S regulatory complex (also called PA700), which confers polyubiquitin binding and energy dependence. A simplified scheme of the ubiquitin pathway is depicted in Figure 1.

* Corresponding author.

The proteasome is involved in many different important cell processes, ranging from cell-cycle control to antigen processing, which makes the complex a major subject for research. This has resulted in enormous progress in understanding the structure and function of the complex. Here we provide an overview of the structure and assembly of the 20S proteasome. For information about the function of the proteasome complex, we refer to recently published reviews [3, 6–8].

The 20S proteasome

As mentioned before, the degrading component in ubiquitin-dependent proteolysis is the 26S proteasome. The catalytic core of this complex is the 20S proteasome, which is highly conserved and can be in found in eukaryotes, archaebacteria [9] and some eubacteria [10]. In eukaryotes, the amount of proteasomes can constitute up to 1% of the cell protein content, depending on the average protein breakdown rates of the organ [11]. Proteasomes are localized in nucleus and cytosol [12], sometimes colocalizing or associating with the cy-

toskeleton [13]. Despite the abundance and characteristic properties of the proteasome, progress in understanding the function and mode of action of the particle has, until recently, been slow.

The 20S proteasome was independently discovered by groups working in different fields, and hence was given different names. Scherrer and colleagues [14] observed ring-shaped particles in ribosome-free messenger RNA (mRNA) preparations. In 1984 this particle was called prosome, referring to its presumed role in programming mRNA translation [15]. DeMartino and Goldberg [16] isolated a 700-kDa 'neutral protease' from rat liver. In 1980 Wilk and Orlowski isolated a large protease complex from the pituitary that possessed three different catalytic activities. They called it multicatalytic protease [17, 18]. Monaco and McDevitt immunoprecipitated complexes consisting of 'low molecular weight proteins (LMPs)' with a possible role in antigen presentation [19]. Altogether, this complex has been given 21 different names in the literature. Since all particles were shown to be identical [20–22], the name 'proteasome', which is now generally accepted, was proposed, referring to its proteolytic and particulate nature [20].

Structure and components of the 20S proteasome

The 20S proteasome has a cylindrical structure, 14.8 nm in length and 11.3 nm in diameter. It is composed of 28 subunits, arranged in four stacked rings, resulting in a molecular mass of about 700 kDa. This overall architecture is conserved from bacteria to man [9, 23, 24]. The proteasome isolated from the archaebacterium *Thermoplasma acidophilum* contains only two different but related subunits, α and β, located in the outer rings and the inner rings, respectively [9, 25, 26]. In eukaryotes, 14 different subunits, ranging from 21 kDa to 32 kDa, are present in the complex. Based on the sequence homology with the *T. acidophilum* α- or β-subunit, the eukaryotic subunits can be divided into α-type and β-type, respectively [27–29]. Table 1 shows some characteristics and alternative names of the subunits of the human and yeast 20S proteasome. The new nomenclature as proposed by Groll and coworkers [30] is also included. As in *T. acidophilum*, immuno-electron microscopy (EM) studies revealed that the eukaryotic α-type subunits reside in the outer rings and the β-type subunits in the inner rings. Furthermore, these studies indicated that in the eukaryotic 20S proteasome seven different subunits constitute a ring, each subunit located at a defined position [30–34]. Therefore, the archaebacterial proteasome forms an $\alpha_7\beta_7\beta_7\alpha_7$ complex, whereas the eukaryotic proteasome assembles as an $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$ particle (fig. 2).

In yeast not more than seven different α- and β-type subunits have been found [28], which are, except for subunit Y13, all essential for life [3]. Higher eukaryotes have three extra genes coding for the β-type subunits LMP7, LMP2 and MECL1. These genes are γ-interferon-inducible and group together in a phylogenetic tree with the γ-interferon-downregulated β-type subunit genes MB1/X, Delta/Y and Z, respectively [3, 8]. The γ-interferon-inducible gene products replace their counterparts, resulting in proteasomes with altered proteolytic activities. The γ-interferon-inducible subunits probably have a function in the generation of antigenic peptides (reviewed in refs 8, 35, 36).

Two years ago the crystal structure of the proteasome of *T. acidophilum* was solved, which provided the first

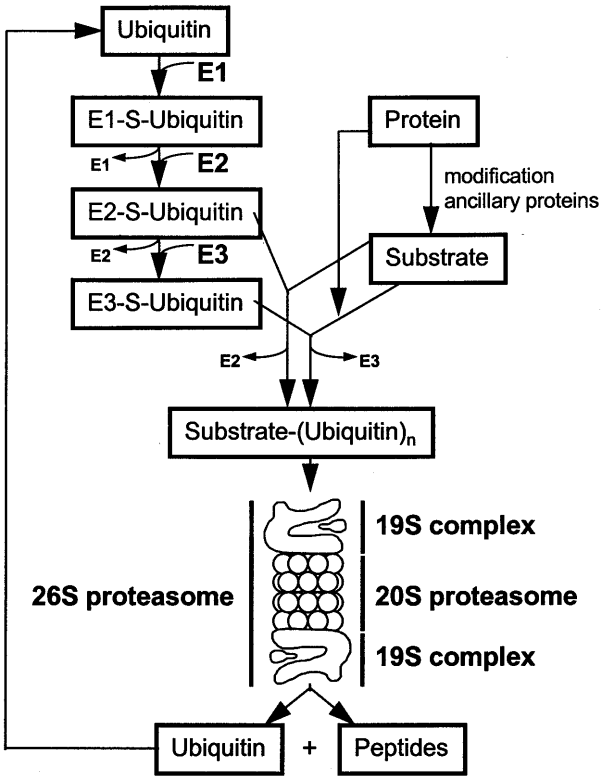

Figure 1. Schematic representation of the proteasome–ubiquitin pathway. Ubiquitin is first activated by a ubiquitin-activating enzyme (UBA or E1) and passed on to a ubiquitin-conjugating protein (UBC or E2). Ubiquitin is then linked directly, or with the help of ubiquitin ligases (E3), via an isopeptide bond to a lysine residue of the substrate protein. Polyubiquitinated proteins are recognized and selectively degraded by the 26S proteasome, yielding reusable ubiquitin molecules and peptides of 5 to 15 amino acids. Conversion of a protein into a substrate for ubiquitination can in certain cases occur after posttranslational modification or association with ancillary factors. Proteins can also be recognized by an E3 ubiquitin ligase without prior modification or association (adapted from Ciechanover et al. [78]).

Table 1. Nomenclature and molecular masses of proteasomal subunits.

Systematic name	Human gene	Yeast gene	Molecular mass of human subunit (kDa)
$\alpha 1$	HsPROS27/HsIota	C7/PRS2	27.4
$\alpha 2$	HsC3	Y7	25.9
$\alpha 3$	HsC9	Y13	29.5
$\alpha 4$	XAPC7/HsC6	PRE6	27.9
$\alpha 5$	HsZeta	PUP2	26.4
$\alpha 6$	HsPROS30/HsC2	PRE5	30.2
$\alpha 7$	HsC8	C1/PRS1	28.4
$\beta 1$	HsDelta/Y	PRE3	25.3 (21.9)
$\beta 1$i	LMP2		23.2 (20.9)
$\beta 2$	Z	PUP1	30.0 (24.5)
$\beta 2$i	MECL1		28.9 (23.8)
$\beta 3$	HsC10-II	PUP3	22.9
$\beta 4$	HsC7-I	PRE1/C11	22.8
$\beta 5$	MB1/X	PRE2	nd (22.4)
$\beta 5$i	LMP7		30.4 (21.2)
$\beta 6$	HsC5	C5/PRS3	26.5 (23.3)
$\beta 7$	HsBPROS26/HsN3	Pre4	29.2 (24.4)

The systematic nomenclature is based on the position of the subunits in the yeast proteasome as recently proposed by Groll and coworkers [30]. The molecular masses of the primary gene products were obtained from [8]. Values between parentheses correspond to the molecular mass of the processed (mature) subunit. The three γ-interferon-inducible subunits are indicated with an 'i'.

detailed insight into proteasome structure and function [26]. Although the archaebacterial α- and β-subunits have only 26% sequence identity, the crystal structure revealed that both subunits have a very similar fold. The core of the proteins is formed by two five-stranded antiparallel β-sheets, flanked by three α-helixes on top and two at the bottom [26]. In the last 3 years several multimeric enzymes, which share no significant homology with proteasomal subunits, have been shown to contain the same folding pattern and catalytic mechanism [37]. They were designated N-terminal-nucleophile (Ntn-) hydrolases [30] according to their putative catalytic mechanism (see below), which confers either amidohydrolase or amidotransferase activity [38].

The crystal structure of the archaebacterial proteasome further revealed that the rings and the subunits within the rings are very closely packed, leaving no holes in the wall of the cylindrical structure. A small 13-Å-diameter gate, at both ends of the particle, is the only connection between the outside and the three inner cavities of the proteasome. Since the catalytic sites reside in the largest compartment, in the center of the complex, the small openings are a physical barrier protecting native cellular proteins from uncontrolled degradation.

A second breakthrough in proteasome research is the recent solution of the crystal structure of the yeast proteasome [30]. It clearly shows that each of the identical halves of the proteasome consists of 14 different subunits located at defined positions (fig. 2). The stacked α, β, β, α rings are each $\sim 10°$ out of register. A recently constructed model of the subunit topology of the human 20S proteasome, based upon data obtained from chemical cross-linking and immuno-EM studies, is very similar to the subunit topology of the yeast proteasome but may differ in the position of four β-type subunits (see legends of fig. 2 [34]).

Like the archaebacterial α- and β-subunits, all yeast subunits have a β-sandwich structure, flanked at the top and bottom by α-helices, typical for Ntn-hydrolases [30, 37]. Differences between subunits appear in the length of turns, in long insertions connecting and/or extending structural elements, and in the N-terminal and especially the C-terminal regions. Many of these subunit-specific elements are involved in intersubunit contacts [30].

The overall architecture of the yeast particle is very similar to the archaebacterial protease complex. The yeast proteasome is also a barrel-shaped complex containing three inner cavities, but it lacks the 13-Å-wide entry ports at the ends, as are present in the *T. acidophilum* particle. While the yeast α-discs seem firmly closed, there are some narrow openings present in the side wall at the interface between the α- and β-rings of the complex. They have a diameter of about 10 Å, which may allow passage of unfolded, extended polypeptides. Binding of the 19S regulatory complex or the proteasome regulator PA28 to the particle poles, which activates peptide hydrolysis in both cases [35, 39], may cause a rearrangement of the α-rings, and open the entry ports of the 20S proteasome [30].

Figure 2. Schematic representation of the human 20S proteasome. The three-dimensional representation (left) and rolled-off cylinder envelope show the subunit topology of the human particle, based upon the yeast crystal structure [30]. The model of the human 20S proteasome as proposed by Kopp and coworkers [34] differs from the one shown here in the position of four β-type subunits: C10-II and C5, and C7-I and MB1 are exchanged. HSB = HsBPROS26; 30 = HsPROS30; 27 = HsPROS27.

Proteolytic activity

The first report on the multicatalytic properties of the proteasome stems from 1983, when Wilk and Orlowski distinguished three different proteolytic activities: 'trypsin-like', 'chymotrypsin-like' and 'peptidylglutamyl-peptide hydrolase' activity (PGPH) [18]. These three proteasomal activities refer to peptide bond cleavage at the carboxyl side of basic, hydrophobic and acidic amino acid residues, respectively. They were identified using short synthetic peptide substrates and believed to be catalysed at independent sites, because the different proteolytic activities respond differentially to various activators and inhibitors. With similar approaches, at least two additional proteolytic activities have been described [40–42]. In contrast, the much simpler archaebacterial particle has mainly chymotrypsin-like activity [9, 43].

The proteolytic mechanism has been a matter of debate for a long time. Proteasomal subunits show no sequence homology to members of the four classical proteases: the serine, cysteine, aspartic acid or metallopeptidases [44]. Studies with standard inhibitors revealed that the proteasome might be an unusual type of serine endopeptidase. However, unlike typical serine peptidases, it is insensitive to diisopropyl fluorophosphate and peptide chloromethyl ketones [41, 42]. Major contributions to understanding the structure of the proteolytic center were the solution of the crystal structures of proteasomes of the archaebacterium *T. acidophilum* [26] and yeast [30], and the mutational analysis of their subunits [45–47]. Mutagenesis studies revealed that neither serine nor histidine residues of the β-subunit are essential for the activity of the *T. acidophilum* enzyme [45]. Several lines of evidence demonstrate that the hydroxyl group of the N-terminal threonine residue of the β-subunit (Thr-1, see fig. 3) is the active-site nucleophile:

1) The replacement of the Thr-1 β-subunit by an alanine residue abolished the proteolytic activity, suggesting that this threonine residue is near or in the catalytic center [46].

2) Mutation of this threonine to serine, which contains a similar side chain, does not impair proteolytic activity [46].

3) In the crystal structures, the hydroxyl group of this threonine is in close proximity or bound to the aldehyde group of the proteasome inhibitor *N*-acetyl-Leu-Leu-nor-leucinal [26, 30].

4) Lactacystin, a natural covalent inhibitor of eukaryotic proteasomes, binds to the N-terminal threonine of subunit MB1/X [48].

5) The Thr-1 residue is conserved in all bacterial β-subunits and in 6 out of 10 mature human β-type subunits (fig. 3 [46]).

Besides a nucleophile, a basic group which can function as a proton acceptor/donor is essential to catalyse peptide bond hydrolysis. In the major subunit the two most likely proton acceptors/donors are the free α-amino group of Thr-1 and the side chain amino group of Lys-33. Both are conserved in the potentially active β-type subunits and in close proximity to the active site. Furthermore, mutation of Lys-33 in the *T. acidophilum* β-subunit impaired proteolysis [46]. The fact that not all β-type subunits have the Thr-1 residue indicates that not all of these subunits are proteolytically active. Potentially active are the three γ-interferon-inducible subunits and their constitutive counterparts. Mature HsBPROS26 is probably inactive despite its N-terminal threonine (fig. 3), since this subunit has an extended N-terminus compared to the active subunits and lacks other conserved amino acid residues near the catalytic center (e.g. Lys-33) [46].

The crystal structure of yeast proteasomes, saturated with the peptide-aldehyde inhibitor, revealed that indeed only the three subunits PUP1/Z, PRE3/Delta and PRE2/MB1, with an N-terminal threonine, bind the inhibitor. From the size and charge distribution of each

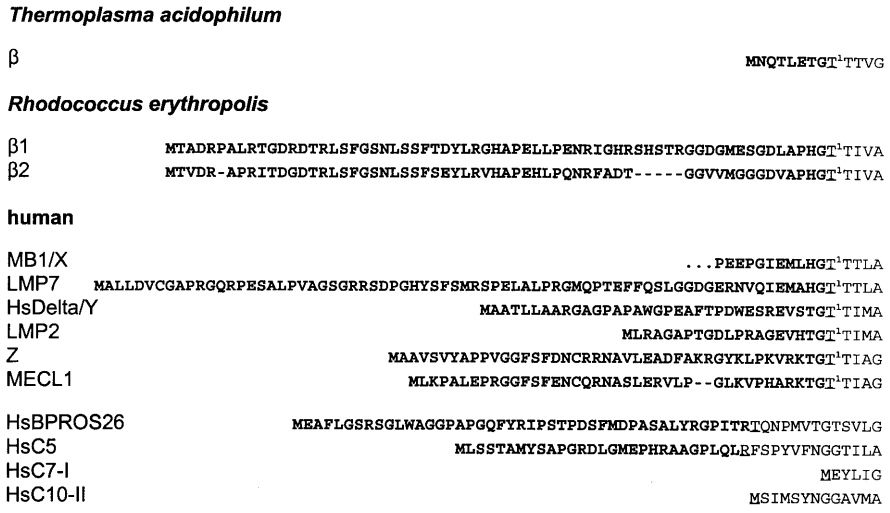

Figure 3. N-terminal amino acid sequences of bacterial and human β-type subunits. The aligned N- terminal β-type subunit sequences are obtained from Lupas and coworkers [66]. Prosequences are depicted in bold, the mature sequences in regular font and the N-terminal amino acid of the mature protein is underlined. T^1 is the conserved Thr-1, which is most likely acting as the nucleophile in the proteolysis reaction.

peptide-binding pocket it has been suggested that subunit PRE2/MB1 may confer trypsin-like and chymotrypsin-like activity, that PRE3/Delta is essential for PGPH activity and that Pup1/Z may cleave behind large amino acid residues [30]. This active-site assignment is in line with previous mutagenesis [47, 49–51] and inhibitor studies [13]. From the crystallographic structure of the yeast proteasome a potentially new type of active centre has been postulated, which is formed by three to four main-chain carbonyl oxygens at the ends of helix H2 of all seven subunits, together with some water molecules [30].

The contribution of each individual active centre and proteolytic activity, assayed with short test peptides, to the degradation of longer peptides and complete proteins is unknown. *Thermoplasma* proteasomes that possess just only one type of active site, nevertheless do not only have chymotryptic-like activity but in addition some tryptic and PGPH-like activities [43]. Furthermore, they are able to cleave behind most amino acids in a protein. This indicates that the 20S proteasome is in fact a nonspecific endopeptidase [52]. However, the generated peptides fall into a rather narrow size range of 6 to 10 amino acids, suggesting the existence of some kind of 'molecular ruler' [52]. The average length of the degradation products being 7 to 8 amino acids is in agreement with the distance between the active sites in the archaebacterial proteasome [26]. A similar nonspecific endopeptidase activity and size distribution of degration products from whole proteins was observed for eukaryotic proteasomes [53–56]. Unlike conventional proteases, the *Thermoplasma* proteasome degrades proteins 'processively'. This means that a protein is completely degraded into peptides without release of intermediates [43].

While unfolded peptides are usually digested, most native proteins are resistant to proteolytic degradation by the 20S proteasome in vitro [42, 57]. Denaturation of the substrate protein by oxidation [58] or reduction of disulphide bridges [59] can render it accessible to degradation by proteasomes. Also, small gold particles with a diameter of 2 nm, containing unfolded substrate, cannot enter the proteasome [59]. These results suggest that a relatively narrow opening controls acces to the inner proteolytic compartment of the proteasome. This hypothesis was confirmed by the crystal structure of the archaebacterial proteasome, which shows that the sole openings in the archaebacterial proteasome are two 13-Å-wide holes surrounded by hydrophobic amino acid residues in the center of the α-rings at each side [26]. The hole in the middle of the α-rings of the yeast proteasome seems to be closed [30] and is probably only open when bound byregulatory complexes. Such a size exclusion mechanism implies that energy-dependent unfolding of substrates is essential for proteolysis by the proteasome. In eukaryotes, protein unfolding might be a function of the various ATPases which are components of the 19S regulatory complex. 20S proteasomes may also act in cooperation with molecular chaperones capable of recognizing and stabilizing unfolded polypeptides [59, 60].

Assembly

Throughout evolution the number of different proteins contributing to the 20S proteasome complex has apparently increased: the 20S proteasome of the archaebacteria *T. acidophilum* and *Methanosarcina thermophila* consists of one α- and one β-subunit [9, 61], two α-type and two β-type subunits form the complex of the eubacterial actinomycete *Rhodococcus erythropolis* [10] and the eukaryotic 20S proteasomes is composed of seven different α-type and seven different β-type subunits. In higher eukaryotes there is a further diversification of the β-type subunits. Yeast contains seven genes encoding β-type subunits [28], whereas mammals have ten β-type subunit-encoding genes (see above [8, 24]). Hence, the assembly mechanisms are more complex in higher-order species. Considerable knowledge is available about the assembly of the archaebacterial 20S proteasome. Expression of the *T. acidophilum* α- (Ta-α) and β-subunit (Ta-β) in *Escherichia coli* resulted in fully assembled and functional proteasome complexes [62]. The prosequence of Ta-β is dispensable for correct assembly. Separate expression of Ta-α resulted in the formation of double seven-membered α-rings, whereas Ta-β alone was monomeric, and its prosequence was not split off [63]. Therefore, in *T. acidophilum* the formation of α-rings might be the first step in proteasome assembly, and subsequently, α-rings function as a matrix which chaperones the folding, assembly and proteolytic processing of the β-subunits [63, 64]. The 35 amino acid residues at the N-terminus of the α-subunit, lacking in the β-subunit, are important for ring formation [63]. This part contains the α-helix H0 (residues 21–32) which indeed contacts the H0-preceding loop of the neighboring α-subunit in rings [26].

Proteolytic processing of the inactive precursor to activate the β-subunit probably involves Ntn-hydrolases [37]. In vitro denaturation/reconstitution experiments with purified Ta-α and precursor- Ta-β resulted in processing of the β-subunit, showing that proteolytic Ta-β processing is indeed autocatalytic. Both processed and unprocessed Ta-β were efficiently incorporated into proteasome particles. Since no processed Ta-β remained unassembled, in vitro processing appears to be connected to assembly [64]. Active β-subunits are needed for the processing of Ta-β, suggesting that the residues important for protease activity are also essential for processing. However, the mutant Ta-β–Thr1Cys can efficiently be processed, but is proteolytically inactive, indicating that in the major complex the geometry of the active sites for processing and catalysis is slightly different [64].

The autocatalytic processing pathway of the β-subunits of *T. acidophilum* has been shown to occur via intermolecular cleavages [64].

The *Rhodococcus* proteasome consists of two different α-type and two β-type subunits [10]. Recombinant expression of all four combinations of one α-type and one β-type subunit yielded fully assembled and proteolytically active native-like particles [65, 66]. Remarkably, in vitro studies indicate that the outer and inner rings of the particle most likely have a random distribution of the α- and β-type subunits, respectively [67].

As in *T. acidophilum*, inactive β-type precursors of Rhodococcus are proteolytically activated only in the presence of an α-type subunit. Contrary to Ta-α, which assembles into seven-membered rings, the two α-type subunits of *Rhodococcus* cannot assemble into ring complexes upon expression or coexpression [66]. This may indicate that initial formation of a complete α-ring is not a prerequisite for proteasome assembly. The folding of the α- and β-type subunits of *Rhodococcus* occurs very rapidly; the rate-limiting step seems to be the dimerization of two half proteasomes and the subsequent processing of the β-type subunits. Although proteolytically active holoproteasomes are formed upon coexpression of an α-type subunit and the mature part of a β-type subunit, the β-prosequence greatly facilitates folding of the β-type subunits and their processing. This self-chaperoning activity also functions in *trans*, since separate addition of the propeptide also stimulates β-subunit folding and processing [67].

Up to now, the assembly of the complex eukaryotic proteasome has not been fully understood. It is most likely a coordinated process, since each of the 14 different subunits resides at a fixed position (see above). An assembly model of eukaryotic proteasomes is depicted in figure 4. Several groups have identified complexes of 13S–16S containing most of the α- and several β-type subunits [68–71]. The 13S–16S complexes probably are assembly intermediates which can be converted into mature proteasomes. Completion of the maturation process was inhibited by cyclohexamide [68, 70] and did not occur in purified 16S complexes [71]. This suggests that additional factors are necessary for the assembly process. Interestingly, the chaperonin Hsc73 was found to be associated to the 16S complexes [71, 72]. This chaperone is possibly involved in keeping the β-type subunits at the interface of two half-proteasomes in the proper conformation to enable maturation processes to occur.

In an alternative attempt to get insight into early steps in proteasome assembly, the assembly properties of some single recombinant human subunits was

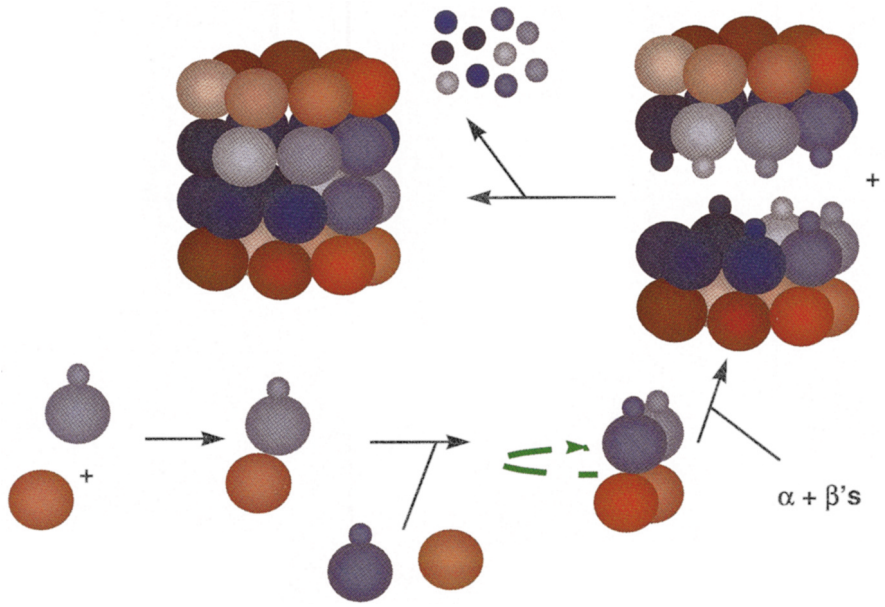

Figure 4. Proposed schematic model of the eukaryotic proteasome assembly pathway. The α-β double ring is formed in a coordinated fashion by coassembly of α- and β-type subunits. Two half-proteasomes assemble into mature proteasomes. Red spheres represent the α-type subunits and the β-type subunits are depicted in blue.

studied in vitro. Like the α-sununit of *Thermoplasma*, the human α-type subunit C8 (HsC8) formed double seven-membered rings by itself [73]. In contrast, HsPROS27 and HsPROS30, two other human α-type subunits only formed double-ring complexes upon coexpression with HsC8 [74], indicating that HsC8 might have a function in early steps of the proteasome assembly pathway. The HsC8:HsPROS30 or HsC8:HsPROS-27 ratio of the mixed complexes was surprisingly heterogeneous, varying from 6:1 to 1:6 [74]. Apparently, the three subunits tested lack binding sites that selectively interact with a specific neighboring subunit. This probably means that for correct positioning of the specific interaction of α-type subunits with β-type subunits is needed (see fig. 4).

As mentioned earlier, an important maturation event to activate the proteasome is the proteolytic processing of some β-type subunits (fig. 3). Apparently, this is a late assembly event, since the proteolytically inactive 13S–16S complexes contain some pro-β-subunits, whereas the active 20S proteasomes only contain mature β-type subunits [68, 75]. It has very recently been shown that prosubunit processing of LMP2 and Delta/Y takes place in the 16S precursors and not in the 13S complexes [71]. Furthermore, correct processing seems to require proper interaction of two half-proteasomes [47]. For efficient processing of β-type subunits and integration into proteasomes of eukaryotes, a correct propeptide is essential. The propeptide most likely prevents preliminary activation of the β-type subunits and in some cases can act as an intramolecular chaperone, important for correct folding of a subunit [47]. A similar function for prosequences of various other proteases has been reported [76]. Whether this property is common to all proteasomal propeptides is not clear. Exchange of the prosequence of LMP2 with the propeptide of LMP7 (P7LMP2) resulted in partially processed chimeric β-type subunits [72, 75]. These polypeptides were correctly incorporated into the proteasome particle, albeit with low efficiency, indicating that the prosequence probably is not involved in the correct positioning.

Several mutated pro-β-subunits are partially processed, resulting in proteins which are 8 to 10 amino acids longer than the mature proteins [75]. Interestingly, three of the predicted inactive human β-type subunits also contain N-terminal extensions of 7 to 9 amino acids (fig. 3) [46, 77]. Probably, β-type subunit processing is a two-step mechanism: at first the propeptide is trimmed, leaving 7 to 10 amino acid residues, whereafter active subunits are obtained by cleavage at the consensus site (prior to Thr-1) [72, 75].

Conclusions

The eukaryotic 20S proteasome is a complex particle consisting of 14 different subunits, all located at a

specific position. As far as the complete assembly pathway of the 20S proteasome is concerned, it is clear that this must be tightly coordinated. We are only beginning to understand this extremely complicated process. The solution of the crystal structures of the *Thermoplasma* and yeast proteasome are of great help in this respect and enables the design of detailed mutational analysis of the binding sites of neighbouring subunits. These experiments will certainly give more insight into the steps of the assembly process. Understanding the proteasome assembly mechanism is not only a challenge by itself but may also help to unravel the functional aspects of the proteasome, notably the binding, unfolding and degradation of polypeptide substrates.

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