Optimal Length Transportation Hypothesis to Model a Proteasome Product Size Distribution

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Abstract. We discuss translocation features of the 20S proteasome in order to explain typically observed proteasome length distribution. We assume that a protein transport depends significantly on the fragment length with one optimal length which can be transported the most efficiently. Suggesting a simple one-channel model we show that this hypothesis can explain one or three peak length distributions found in the experiment. The possible mechanism of such a translocation can be based on fluctuationally driven transport.

Keywords: Proteasome, protein translocation, stochastic process, ratchets

1. Introduction

Proteasomes are multicatalytic cellular protease complexes that degrade intracellular proteins into smaller peptides. They are present in all eukaryotic cells, archaea, and certain bacteria (Coux et al., 1996; Tamura et al., 1995; Kloetzel, 2001) In the experiment (Hendill, 1988) about 5×10^5 proteasomes have been found in the nucleus and the cytoplasm of one eukaryotic cell. It means that estimating the total cell number in a human body as 6×10^{13} and the degradation time of an average 400aa long protein as 3.5min (Kisselev et al., 1998) we came to the very approximate conclusion that 8.5×10^{18} proteins may be destroyed in our body by the proteasomes in 1 minute. Proteasomes are absolutely essential for the homeostasis because the removal of proteasome genes in eukaryotes is lethal (Hilt and Wolf, 1995). Many roles in the cell's metabolism are played by proteasomes: they destroy abnormal and misfolded proteins tagged with Ubiquitin and are an essential component of the ATP-Ubiquitin-dependent pathway for protein degradation (Ciechanover, 1994; Hochstrasser, 1996). Proteasomes play an important role in the immune system by generating antigenic peptides of 8-12 residues to be presented by the MHC class I molecules and hence are the main supplier of peptides for its recognition by killer T-cells (Rock and Goldberg, 1999; Kloetzel, 2001; Kloetzel, 2004a; Lankat-Buttgereit and Tampe, 2002; Shastri and Schwab, 2002; Goldberg et al., 2002). As a part of the Ubiquitin system proteasomes are involved in the regulation of the cell cycle and the cell stress response. Recently the

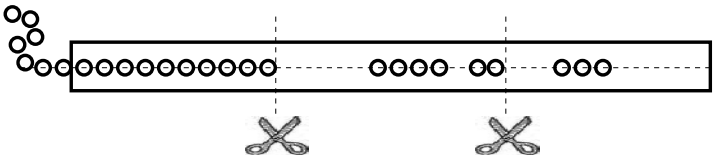

Figure 1. The model of the proteasome with two cleavage centers. The protein with not specified sequence (denoted as "0-0-0-0") enters the single proteasome channel from the left. It can be cleaved at any point by cleavage sites denoted by scissors. The cleavage rate can depend on the position. The cleaved fragments move with different velocities depending on the length. After a waiting time computed with the Gillespie algorithm either the cleavage or translocation may occur.

proteasome inhibition has been suggested as a new successful target for the cancer treatment (Orlowski, 1999; Adams et al., 2000; Dou et al., 2003). The proteasome's function has been directly linked to the pathophysiology of malignancies, neurodegenerative disorders, type I dyabetes, cachexia (Sakamoto, 2002; Glickman and Ciechanover, 2002) and to ageing (Zeng et al., 2005).

Proteasomes have been found in the form of different similar molecular complexes which consist of the central part, the 20S proteasome, and regulating caps, the 19S (Coux et al., 1996) and PA28 particles (Rechsteiner et al., 2000; Kloetzel, 2004b). The most important 26S complex, which degrades ubiquitinated proteins, contains in addition to the 20S proteasome a 19S regulatory complex composed of multiple ATPases and components necessary for binding protein substrates (Coux et al., 1996). The 20S proteasome is a barrel-shaped structure composed of four stacked rings of 28 subunits(Peters et al., 1993; Coux et al., 1996) The active cleavage sites are located within the central chamber of the 20S proteasome, into which protein substrates must enter through narrow openings of outer rings. The 20S proteasome degrades proteins by a highly processive mechanism (Akopian et al., 1997), making many cleavages of the protein and digesting it to small products. This is important for the intracellular proteolytic system because the release of large protein fragments could interfere with the cell function and regulation (Kisselev et al., 1998). Proteasomes can be found in its usual form as a constitutive proteasome, or as an immunoproteasome with modified cleavage centers (Kloetzel, 2001; Kloetzel, 2004a).

Due to its significance in the cellular metabolism the simulation of the proteasome function is the central task in the building of a virtual immune system (Lund and Brunak, 2006). In the long road from the

Table I. Parameter values

Parameter	Description	Dimension	Default value
l	Protein length	Amino acids	300
L	Proteasome length	Amino acids	80
D	Distance between a cleavage center and the proteasome end	Amino acids	15
N	Number of degraded proteins	-	10^4

theoretical idea to the pharmacy to create a new drug the simulation and prediction of the proteasome function seem to be possible now only on its early stages, namely, for the experiments *in vitro*. These experiments study the digestion of different substrates by the proteasome and timely dynamics of the fragment concentration in the course of time by mixing of purified proteasomes and different substrates. The experimental results are analyzed e.g. by Mass Spectroscopy methods and supply us with information about the substrate cleavage pattern and the quantity of different fragments cut off from the initial substrate. But even on these first stages of drug design the simulation of the proteasome could significantly decrease the experiment costs through the identification and prediction of proper parameter ranges.

One of the important experimental result that describes the proteasome function is a length distribution of the fragments obtained in vitro experiments by the analysis of generated cleavage products. It was found that for long substrates this dependence typically is a nonmonotonous one and has one peak around the length of 7-12 aa for practically all types of the proteasome (Nussbaum et al., 1998; Nussbaum, 2001; Kisselev et al., 1998; Cascio et al., 2001). It is important to note that namely this length of peptides is the most requested one for a normal functioning of the immune system. The proteasome products can be also a little bit longer because they can be cut further by proteases. The mechanism behind such a length distribution is not completely clear. It was widely believed that the proteasome degrades proteins according to the "molecular ruler" to yield products of rather uniform size, as first proposed by (Wenzel et al., 1994). It was proposed that peptides of 7–9 residues were generated as a result of coordinated cleavages by neighboring active sites. However, evidence for the molecular ruler is quite limited because the maximum in the length distribution is smoothed and not pronounced as a delta peak (Kisselev et al., 1998). Important to note that in some experiments three peak length distribution has been found (Köhler et al., 2001).

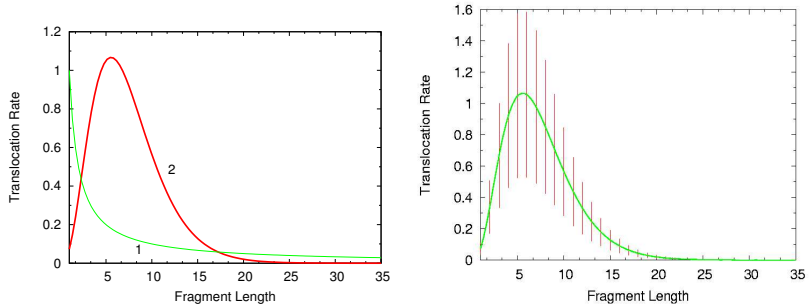

Figure 2. Left: Two qualitatively different forms of translocation rates, a monotonously decreasing function $R1(x)$ (curve 1) and a nonmonotonous function $R2(x)$ with one maximum at the most optimal for translocation length. For exact expressions see the text; Right: Translocation rate function $R2(x)$ and the intervals of its random variation (vertical bars).

To model the proteasome mechanism one should adequately describe three essential processes involved in the proteasome function: selection of cleavage sites, kinetics of generated fragments, and a peptide translocation inside the proteasome. Three algorithms are available for the prediction of cleavage sites, PAProC (Kuttler et al., 2000), Netchop (Kesmir et al., 2002), and ProteaSMM (Tenzer et al., 2005). Several theoretical models for the kinetic of proteasome degradation have been published before. Some of them describe the degradation of short peptides with qualitatively different kinetics (Stein et al., 1996; Stohwasser et al., 2000; Schmidtke et al., 2000) or small number of cleavage positions (Holzhütter and Kloetzel, 2000). The theoretical model (Holzhütter and Kloetzel, 2000; Peters et al., 2002) for the degradation of long substrates is applied to specific proteins with predefined cleavage sites and is fitted to experimental data describing the fragment quantity after proteasomal degradation. Much less attention has been paid in the literature to the description of peptide translocation inside the proteasome chamber. To fill this gap in this paper we address solely the protein translocation and show that the differences in the length dependent velocity rates can be of crucial importance for the length distribution.

2. Model

The model assumes that the proteasome has only one channel and two cleavage centers, thus a symmetric structure as in reality. Two cleavage centers represent two internal rings, which in reality can have up to 6

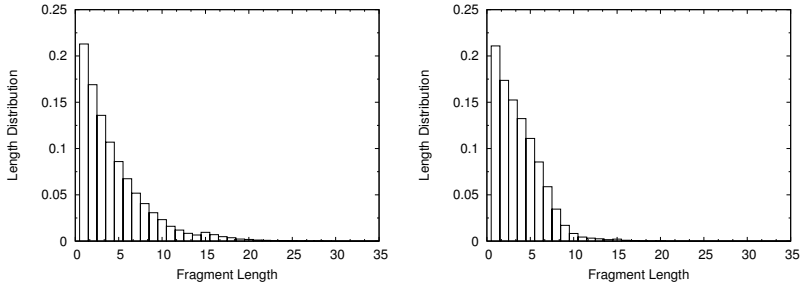

Figure 3. Length distribution for $R1(x)$ and $R_c(p) = 0.01$ (left) and for $R2(x)$ and $R_c(p) = 0.01$ (right).

cleavage centers but distributed in the 3D structure. The substrate enters this channel, it can be cleaved or translocated as well as generated fragments until they leave the channel (see Fig.1). The translocation and cleavage are modelled by the Gillespie algorithm according to the translocation and cleavage rates (Gillespie, 1976). The peptide or its part inside the proteasome can either be shifted by one amino acid or can be cleaved if it is located near the cleavage center. In this version of the model the outrunning of fragments is forbidden, as well as the peptide cannot be translocated to the position already occupied by another fragment. The translocation rates of the substrate or fragments depend only on its length inside the proteasome and are described by the function $R_t(x)$, where x is the length of the substrate or fragment part which is inside the proteasome. Hence if the initial substrate enters the proteasome, this length will be increased. The probability of cleavage is described by the function $R_c(p)$, where p is the position in the substrate sequence. For generated fragments the cleavage rates remain the same as they were in the initial substrate for the corresponding positions. We assume that the substrate is degraded by a highly processive mechanism and that the protein cannot leave the proteasome from the other side until completely processed (see for experimental argumentation (Akopian et al., 1997)). When the protein is degraded, its fragments lengths are counted in the length distribution. To obtain reliable statistics the length distribution is averaged over large number of proteins N, what also corresponds to the usual experimental set up. The standard parameters that are used in the simulations are in given in Tabel I together with its default values.

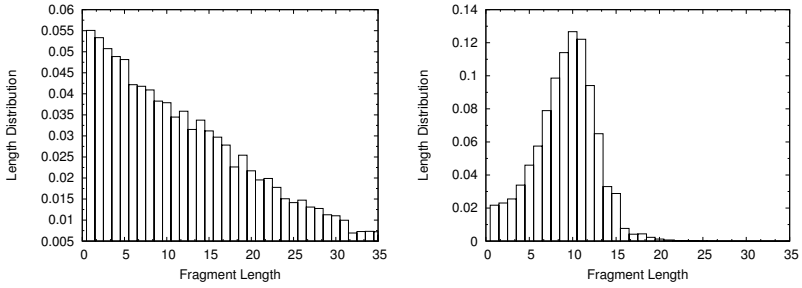

Figure 4. Length distribution for $R1(x)$, $R_c(p) = 0.001$ (left) and for $R2(x)$, $R_c(p) = 0.001$ (right). It can be clearly seen that nonmonotonous translocation rate can result in one peak distributions.

3. Results

First let fix cleavage rates $R_c(p)$ to a constant and show that different translocation rates can result in qualitatively different forms of length distribution. To check different translocation rates functions we have used a decreasing function $R_t(x) = R1(x) = 1/x$ and a nonmonotonous function $R_t(x) = R2(x) = (0.5x)^3 e^{-\alpha x}$ with $\alpha = 0.54$ (Fig. 2 left). If cleavage rates are relatively high $R_c(p) = 0.01$, the difference in the length distribution will be not so much pronounced because the probability of cleavage will dominate over the probability of translocation, and, as a result, short fragments will dominate in the length distribution. Comparing Fig. 3, left and right, we see that for both translocation rate functions the length distribution is a monotonuosly decreasing function.

The situation qualitatively changes if a cleavage rates are not so high, e.g. $R_c(p) = 0.001$ (see Fig 4). For monotonously decreasing function the length distribution is also monotonously decreasing (left), however for the translocation rate function with the optimal length of transportation, one can clearly see the peak in the length distribution (right). Hence we have shown that translocation rate dependencies can be of a crucial importance for the length distributions. If this function is nonmonotonuos and has a clearly defined optimal transportation length, this can result in one peak length distribution observed in numerous experiments (Nussbaum et al., 1998; Nussbaum, 2001; Kisselev et al., 1998; Cascio et al., 2001). Since namely the length corresponding to this peak is the most important length for the immune system, we conclude that translocation properties of the protein should be certainly taken into account in the creation of the virtual proteasome system. Interesting, that tuning the parameters, namely setting $R_t(x) = R2(x)$,

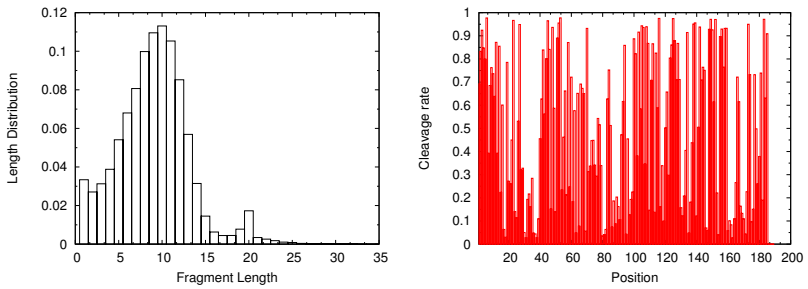

Figure 5. Left: Three peak length distribution for $R2(x)$, $R_c(p) = 0.001$, $\alpha = 0.47$, $D = 20$. Right: Casein cleavage strength pattern computed with Netchop.

$\alpha = 0.47$, $D = 20$ one can also obtain three peak length distribution (see Fig. 5, left) found in the experiment by the degradation of the casein with size exclusion chromatography (Köhler et al., 2001).

4. Discussion

We have presented here an optimal length transportation hypothesis, namely, discussed that nonmonotonuos translocation rate functions can play an important role in the production nonmonotonous length distribution found in the experiment. A question naturally arises: what is a possible mechanism behind such translocation rates? In (Zaikin and Pöschel, 2005) we have assumed that the proteasome has a fluctuationally driven transport mechanism and have shown that generally such a mechanism results in a nonmonotonous translocation rate. Since the proteasome has a symmetric structure three ingredients are required for fluctuationally-driven translocation: the anisotropy of the proteasome-protein interaction potential, thermal noise in the interaction centers and the energy input. Under assumption that the protein potential is asymmetric and periodic, and that the energy input is modeled with a periodic force or colored noise one can even obtain nonmonotonous translocation rates analytically (Zaikin and Pöschel, 2005). Proteins and especially unfolded synthetic peptides have indeed a periodic constituent in the potential due to a peptide bond, however this periodicity can be hidden by unperiodic, sequence specific potential. In this case the real translocation rates for different fragments are varying around the function computed for a not sequence specific case. Let us analyze how much this variation can change the length distribution. For this we perform simulations with all parameters as in Fig. 4 right, but on all simulation step we change the translocation rates randomly up to

50% of its initial value determined by the function $R2(x)$ (see Fig. 2, right). It means that at any step i the translocation rate for the fragment of the length X is equal to $R2(X)(1 + R_i)$ where R_i are random numbers uniformly distributed in the interval $[-0.5 : 0.5]$. Surprisingly, we have found that in this case the length distribution is exactly the same as in Fig. 4 right (not shown here). Hence, despite the real nonperiodicity of the potential the nonperiodic constituent is not so important for the length distribution as the periodic one, which define the nonmonotonous rate dependence. Surely, this is true for rather long proteins ($l > 150$) and large statistics $N > 10^4$.

Another open question is the energy input which supplies the protein translocation. Here it is important to note that a translocation in the 20S proteasome can be ATP independent, hence the ATP molecules are surely not the single energy source if we assume the active transport and not a diffusion as a driving mechanism. One can guess that the energy released in the peptide cleavage and filtered by peristaltic proteasome motions provides the energy for the transport, hence motivating the periodic force or colored noise used in (Zaikin and Pöschel, 2005). Experimentally observed mechanical transformations of the proteasome in the course of time (Osmulski and Gaczynska, 2000; Osmulski and Gaczynska, 2002; Gaczynska et al., 2003) can be the facts in favor of this hypothesis.

Next important question is the influence of the sequence specific cleavage strength. Indeed different proteins have different cleavage patterns. Let us simulate the degradation of the casein as in (Köhler et al., 2001) with constant cleavage rates and with sequence specific cleavage rates computed with Netchop algorithms. Casein has a length of $l = 188$aa and the cleavage pattern as in in Fig.5 right. All other parameters are the same as in the case of three peak length distribution, see Fig. 5 left. We have rescaled the cleavage strength to have the same mean value of 0.001. To our surprise the length distributions of not sequence specific case (Fig.6 left) and sequence specific case (Fig.6 right) are practically identical. Hence to model the length distribution as a result of different translocation rates it is not so important to consider the cleavage pattern of the substrate. Of course, the cleavage pattern should be by no mean taken into account if the substrate is not so long or there are relatively small number of cleavage sites. In this case the cleavage pattern can significantly change the length distribution. Also if we consider not the length distribution but the generation of some specific fragments, the cleavage pattern is of crucial importance even for the degradation of long proteins (e.g. (Peters et al., 2002)).

At the present stage the transport model discussed cannot be used for quantitative predictions and serves as an illustration for an optimal

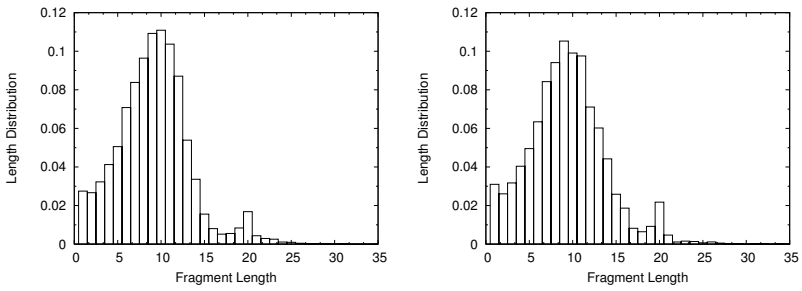

Figure 6. Length distribution as a result of Casein degradation, with non sequence specific cleavage rates (left) and with the cleavage pattern computed with the Netchop algorithm (right).

length transportation hypothesis. We identify two possible directions which can be taken in order to develop the proteasome model able to perform quantitative predictions and take into account translocation properties. A first possibility would be to simulate a population of proteasomes, each of them modeled with a Gillespie algorithms, as in this paper. A second option would be to take into account the length dependent protein transport correction in kinetic models of the proteasome function, as in (Peters et al., 2002; Luciani et al., 2005). In both cases, one should implement a specific protein sequence, which certainly influences a cleavage pattern and the proteasome-protein interaction potential, and description of in- and outfluxes, taking into account the possibility of gate opening and closing (Köhler et al., 2001). Of course, more precise model would be based on molecular dynamics simulations, but taking into account the large quantity of atoms in this system and long degradation time, up to 5 minutes, this seems to be impossible with current computational facilities. Also this would be very interesting to solve an inverse task, namely to identify the translocation rates from the experiments where the substrate length was an important parameter as e.g. in (Dolenc et al., 1998). In particular, from the ratchet model of translocation rates (Zaikin and Pöschel, 2005) and the model presented here it follows that the temperature decrease can result in the qualitative change of the length distribution.

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