Affinity purification strategy to capture human endogeneous proteasome complexes diversity and to identify Proteasome Interacting Proteins

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INTRODUCTION

The 26S proteasome is the proteolytic machine of the ubiquitin-proteasome system. This pathway is of particular importance since it is involved in the degradation of most intracellular proteins. Major biological processes, such as cell cycle progression, apoptosis, DNA repair, epitope generation and cell quality control are tightly regulated by this system. Many studies have demonstrated that a dysregulation of this machinery is related to various pathologies such as cancers and the proteasome has recently been identified as a pharmacological target for their treatment.

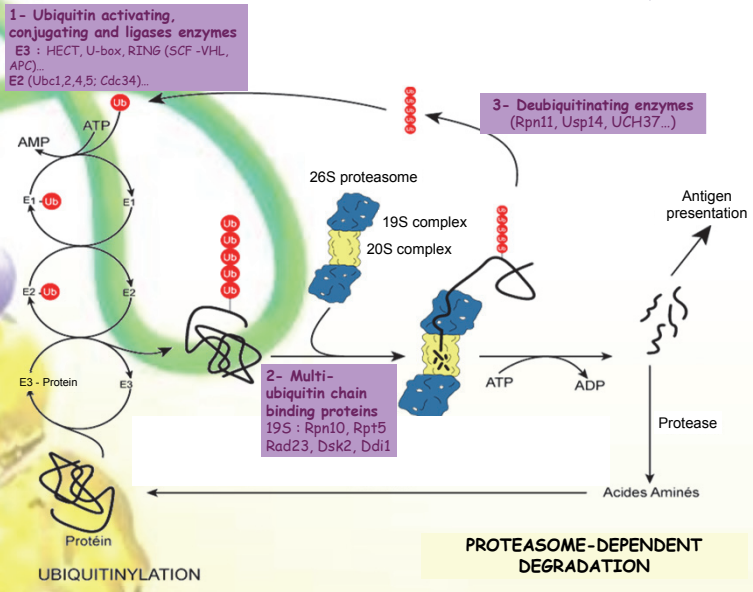
The 26S proteasome is a 2.4 MDa complex composed of multisubunit subcomplexes : a core protease, the 20S proteasome, and two regulatory elements, the 19S particles. A variety of proteins, named "Proteasome Interacting Proteins" (PIPs), interact with the proteasome.

Despite its physiological importance, many aspects of the mammalian proteasome structural organization and regulation remain to be understood. It is known however that its subunit composition and dynamic association to various proteins regulate its stability and activity upon diverse stimuli.

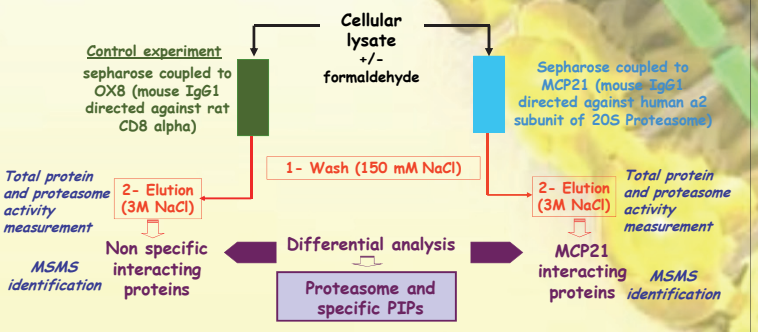
Therefore, we developed a new affinity purification strategy to characterize all proteasome complexes and PIPs in human erythrocytes (Bousquet-Dubouch *et al.*, 2009). This new single-step procedure, based on the high-affinity binding of a subunit of the 20S core particle to a monoclonal antibody, permits to detect endogenous interactions without relying on over-expression or tagging strategies and can be used with whatever human sample as starting material.

Subsequent proteomic analyses identified all proteasomal subunits, known regulators and recently assigned partners. Moreover, other proteins implicated at different levels of the ubiquitin-proteasome system were also identified for the first time.

This novel approach, through the identification of partners affecting proteasomal function, will help to better understand the function of this complex proteolytic machine in different cell physiological states.

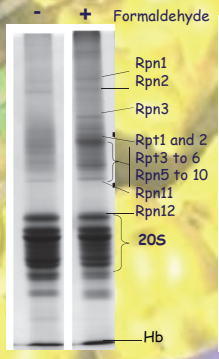


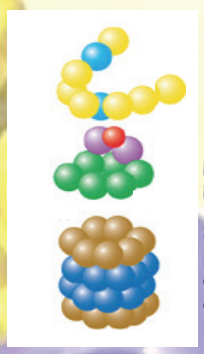
Strategy



Identified proteins in purified proteasome complexes

20S core complex subunits and 19S regulatory subunits :





Sequence coverages

19S Lid
Rpn 3, 5 to 9, 11, 12, 14

19S Base
Rpn 1, 2, 10 and 13
Rpt 1 to 6

17% - 86 %

20S core particle
β 1 to 7
α 1 to 7
α 1 to 7
β 1 to 7

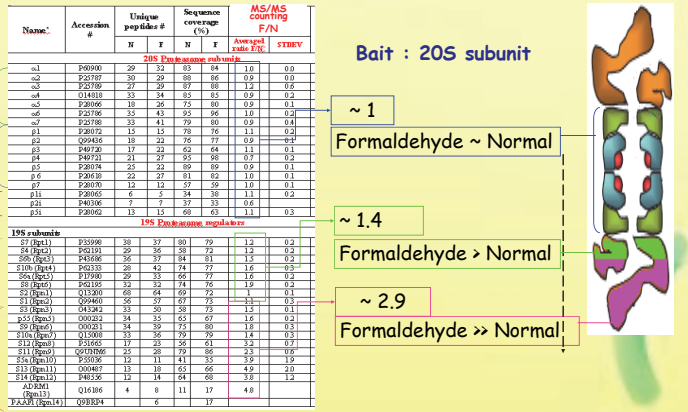
35% - 96 %

Other regulatory complex subunits : PA28 (α/β) and PA200 16% - 88%

☑ **Our strategy enables to purify 20S proteasome and its known regulators with high recoveries**

Benefit of using Formaldehyde crosslink

➢ **The spectral counting approach** (Liu *et al*, 2004) **was used to estimate the relative abundance of each proteasome subunit in the formaldehyde-treated sample versus in the non-treated sample (using MFPaQ software -** Bouyssié *et al* , 2007)



☑ **Our purification strategy is based on the use of a 20S subunit as a bait. This can explain why *in-vivo* crosslinking with formaldehyde permits to maintain the most remote subunits from the 20S core. Formaldehyde therefore helps to preserve the weak association between the 20S core particle and its regulators.**

Specific human erythrocytes Proteasome Interacting Proteins (PIPs) belonging to the Ubiquitin Proteasome System

Description	Fonction
Usp14, UCH37, USP7[2,3] (HAUSP)	Deubiquitinating enzymes
SCF [Skp1, Cul1[2], Fbox7, Rbx1[2]], E3C[2], Cullin- associated Nedd8-dissociating protein[1,3]	E3 ligases or related proteins
PACs 1, 2 and 3[1,3]	Chaperones involved in proteasome assembly
VCP protein[3], HSPs (70, 90[1]), HSC71, HSPA5[1]	Other chaperones
Ecm29[1], HSP90[1]	Proteins involved in proteasome stability
PI31	Proteasome inhibitor
hHR23B[1] (UBA/UBL), eEF1α1[1], Ddi1[1]	Substrate delivery to the proteasome
Poly-Ubiquitin (Lys48-linked)	Major signal for protein degradation by the proteasome

[1] : Identified only after formaldehyde cross-linking
[2] : Identified only without formaldehyde cross-linking
[3] : New PIPs

☑ **Hausp, a deubiquitinase involved in the stability of p53 was further validated using a reverse immuno-purification experiment**

CONCLUSIONS

➢ The immuno-purification of proteasome complexes is possible in a single-step procedure without relying on overexpression or tagging strategies and with whatever human sample as starting material.
➢ *In-vivo* formaldehyde cross-linking stabilizes weak interactions between the 20S core particle and its activators.
➢ Subsequent proteomic analyses identified all proteasomal subunits, known regulators and recently assigned partners. Moreover, other proteins implicated at different levels of the ubiquitin-proteasome system were also identified. Among these, Hausp, a deubiquitinase involved in the stability of p53, was further validated as a new PIP by a reverse immuno-precipitation experiment. The presence of Hausp within proteasome complexes might be a mean to regulate its activity.

REFERENCES

- Bousquet-Dubouch *et al.* 2009 MCP 8, 1150-1164
- Liu *et al.* 2004 *Anal Chem* 76, 4193-201
- Bouyssié et al 2007 *MCP* 6, 1621-1637

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