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Eva Maria Huber

Structural and Functional Characterization of the Immunoproteasome

Doctoral Thesis accepted by
the Technical University Munich, Germany

Springer

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Inhibitors for the immuno- and constitutive proteasome: current and future trends in drug development.
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To my family

Supervisor's Foreword

Curing diseases is the primary long-term goal of contemporary scientific research. However, developing new drugs and bringing them to application demands for enormous efforts and staying power of both academia and pharmaceutical industry. Often the structural analysis of a target protein and its understanding at the atomic level form the foundation of such a long-lasting process. This proved true also for the 20S proteasome core particle (CP), a protease of 720 kDa and 28 single subunits.

In 1995, the first X-ray structure of a 20S proteasome, namely that of the archaeon *Thermoplasma acidophilum* has been elucidated by Löwe and coworkers [1]. Only, 2 years later, the structure of the proteasome from baker's yeast—the first eukaryotic proteasome—was solved [2]. This milestone in proteasome research stimulated the development of the multitude of inhibitory compounds that is known today. Many of these drugs have been structurally analyzed in complex with the yeast 20S proteasome and the obtained X-ray data served as intermediate and validation steps in the drug design development process. Up to now, two proteasome inhibitors have made their way from bench to bedside: Velcade® and Kyprolis®. Since the proteasome is essential for many cellular processes including cell cycle progression, both compounds are applied to patients suffering from blood cancer. However, recently, a novel therapeutic application of proteasome inhibition has been discovered. The compound ONX 0914 (formerly PR-957)—identified in high-throughput screenings—was shown to be of therapeutic benefit in animal models of autoimmune diseases such as rheumatoid arthritis and lupus erythematodes [3, 4]. Remarkably, despite its pronounced structural similarity to other proteasome inhibitors, ONX 0914 selectively targets only the 20S immunoproteasome, a specialized version of the proteasome known in vertebrates.

The immunoproteasome selectivity of ONX 0914 fascinated me as a chemist and formed the starting point for the Ph.D. thesis of Dr. Huber. Her work culminated in the first X-ray structure of an immunoproteasome. Finally, apo and ligand complex structures of the immunoproteasome with ONX 0914 provided an explanation for its selectivity. The structural results described herein represent a valuable contribution for modeling and designing novel proteasome-type selective and subunit-specific inhibitory compounds. Apart from the immunoproteasome structure described herein, Dr. Huber conducted yeast mutagenesis experiments which

aimed at imitating all three 20S proteasomes types of vertebrates in yeast: the constitutive proteasome, the immunoproteasome and the thymoproteasome. Together, the topic and the results of this thesis will definitely inspire further efforts in academic research as well as in medicinal chemistry.

Munich, June 2013 Prof. Michael Groll

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Finally, I thank my family for having enabled my studies, for their support, their confidence in me, and their interest in my work.

Contents

Abbreviations

Å	Ångström
aa	Amino acid
Ac	Acetate/acetyl
AMC	7-Amino-4-methylcoumarin
AMP	Adenosine monophosphate
Amp	Ampicillin
APS	Ammonium persulfate
ATP	Adenosine triphosphate
B. taurus	*Bos taurus*
bp	Base pairs
Braap	Branched chain amino acid preferring
BSA	Bovine serum albumin
°C	Degree Celsius
Cbz	Carboxybenzyl
cCP	20S Constitutive proteasome
CD8	Cluster of differentiation 8
ChTL	Chymotrypsin-like
CL	Caspase-like
CM	Complete medium
CP	Core particle, 20S proteasome
cTEC	Cortical thymic epithelial cells
CTL	Cytotoxic T lymphocyte
2D	Two-dimensional
3D	Three-dimensional
Da	Dalton
ddH_2O	Double distilled water
DMSO	Dimethylsulfoxid
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate

DTT	Dithiothreitol
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
EtOH	Ethanol
FDA	U. S. food and drug administration
5-FOA	5-fluoroorotic acid
HCl	Hydrochloric acid
Hsp	Heat shock protein
IC_{50}	Half maximal inhibitory concentration
iCP	20S Immunoproteasome
IFN	Interferon
IL	Interleukin
K	Kelvin
kbp	Kilo base pairs
kDa	Kilo Dalton
LB	Luria Bertani
LCMV-WE	Lymphocytic choriomeningitis virus strain WE
LiAc	Lithium acetate
mA	Milliampere
MDa	Mega Dalton
MES	2-(*N*-morpholino) ethanesulfonic acid
MHC	Major histocompatibility complex class
MPD	2-Methyl-2,4-Pentanediol
NCS	Non-crystallographic symmetry
NEPHGE	Non-equilibrium pH gradient gel electrophoresis
NF-κB	Nuclear factor-κB
Ntn	N-terminal nucleophile
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PDB	Protein Data Bank
PEG	Polyethylene glycol
pNA	Para-nitroaniline
R_{free}	Free R-factor
r.m.s.d.	Root-mean-square deviation
rpm	Rounds per minute
R_{work}	Crystallographic R-factor
S	Svedberg
S.cerevisiae	*Saccharomyces cerevisiae*
SDS	Sodiumdodecylsulfate
SLS	Swiss Light Source
Snaap	Small neutral amino acid preferring
SOC	Super optimal broth with catabolite repression
Suc	Succinyl

T. acidophilum	*Thermoplasma acidophilum*
TAE	Tris-Acetate-EDTA
TAP	Transporter associated with antigen processing
tCP	20S thymoproteasome
TCR	T cell receptor
TE	Tris-EDTA
TEMED	*N,N,N′,N′*-tetramethylethylenediamine
TL	Trypsin-like
TLS	Translation, Libration, Screw
T_m	Melting temperature
TNF	Tumour necrosis factor
Tris	Tris (hydroxymethyl-) aminomethane
Tris-HCl	Tris (hydroxymethyl-) aminomethane hydrochloride
U	Unit
UV	Ultraviolet
V	Volt
VIS	Visible
v/v	Volume per volume
wt	Wildtype
w/v	Weight per volume
yCP	Yeast 20S proteasome
YPD	Yeast extract peptone dextrose