Crystal structure of the boronic acid based proteasome inhibitor bortezomib in complex with the yeast 20S proteasome

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Proteasomes are responsible for the cytoplasmic turnover of the vast majority of proteins, and are hence central to many cellular processes. Thus, manipulation of proteasomal activity is a key goal to control the stability of regulatory proteins (Groll and Huber, 2004, Kisselev and Goldberg, 2001). While proteasome inhibitors have been used extensively as investigative tools, the observation that they cause apoptosis in certain tumor-derived cell lines has led to their application as potential cancer therapeutics (Adams et al., 1998). The proteasome inhibitor bortezomib has been approved for the treatment of multiple myeloma patients (Ludwig et al., 2005, Teicher et al., 1999). Although crystal structures of the proteasome core particle, complexed with peptide aldehyde based inhibitors are known, the X-ray structure of bortezomib with the 20S proteasome so far has not been reported. We detrmined the crystal structure of the yeast 20S proteasome in complex with bortezomib at 2.8 Å resolution (Groll et al., 2005). The structural data for the bortezomib-proteasome complex presented here explain the different *in vivo* binding affinities of bortezomib for the individual subunits at atomic resolution. Subunit specificities can roughly be attributed to interactions of the leucine, pyrazyl and boronate moieties.

Since bortezomib is now used for the treatment of the universally fatal hematologic malignancy multiple myeloma, with clinical trials for other malignancies ongoing, it is imperative to establish its mode of action at the molecular level. Our data immediately suggest possibilities for the design of bortezomib-derivatives with superior inhibition characteristics and for the design of inhibitors that will specifically bind to single proteolytically active sites. Thus, the crystal structure of this 20S-bortezomib complex may serve as a new lead for rational design of selective boronic acid-based proteasome inhibitors.

References

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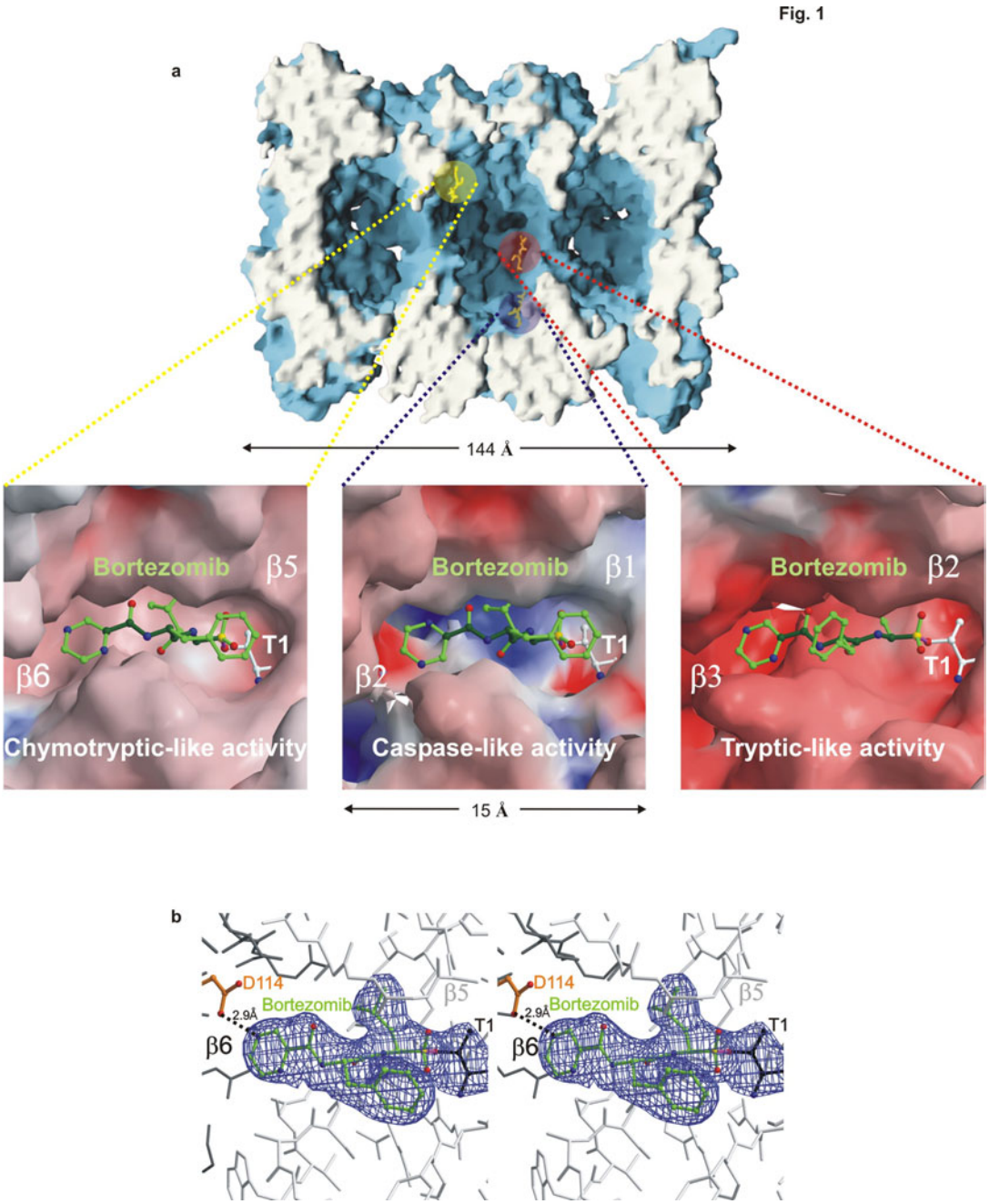

Figure 1

a) Surface representation of the yeast 20S proteasome (coloured in blue) crystallized in the presence of bortezomib, clipped along the cylindrical pseudo-sevenfold symmetry axis (coloured in white). The various proteolytic surfaces are marked by a specific colour coding: red = subunit β1, blue = subunit β2 and green = subunit β5. Cleavage preferences, termed chymotryptic-like, caspase-like and tryptic-like activity are zoomed and illustrated in surface representations; the nucleophilic threonine and bortezomib are presented as a ball and stick model. Basic residues are coloured in blue, acidic residues in red and hydrophobic residues in white. **b)** Stereorepresentation of the chymotryptic-like active site of the yeast 20S proteasome (coloured in white and grey) and bortezomib (coloured in green). Covalent linkage of the inhibitor with the active site Thr1 of subunit β5 is drawn in pink. The electron density map (coloured in blue) is contoured from 1.2 with $2F_O$-F_C coefficients after two fold averaging. Apart from the bound inhibitor, structural changes only were noted in the specificity pockets. Temperature factor refinement indicates full occupancy of bortezomib bound to the chymotryptic-like active site. Bortezomib has been omitted for phasing.