

- Groll M., Ditzel L., Lowe J., Stock D., Bochtler M., Bartunik H.D., Huber R. 1997. Structure of 20S proteasome from yeast at 2.4 Å resolution. *Nature*. 386 : 463–471.
- Kirk R., Laman H., Knowles P.P., Murray-Rust J., Lomonosov M., Meziane el K., McDonald N.Q. 2008. Structure of a conserved dimerization domain within the F-box protein Fbxo7 and the PI31 proteasome inhibitor. *J. Biol. Chem.* 283 : 22325–22335.
- Konstantinova I.M., Tsimokha A.S., Mittenberg A.G. 2008. Role of proteasomes in cellular regulation. *Int. Rev. Cell Mol. Biol.* 267 : 59–124.
- Kulichkova V.A., Artamonova T.O., Lyublinskaya O.G., Khodorkovskii M.A., Tomilin A.N., Tsimokha A.S. 2017. Proteomic analysis of affinity-purified extracellular proteasomes reveals exclusively 20S complexes. *Oncotarget*. 8 : 102134–102149.
- Leggett D.S., Glickman M.H., Finley D. 2005. Purification of proteasomes, proteasome subcomplexes, and proteasome-associated proteins from budding yeast. *Methods Mol. Biol.* 301 : 57–70.
- Li X., Thompson D., Kumar B., DeMartino G.N. 2014. Molecular and cellular roles of PI31 (PSMF1) protein in regulation of proteasome function. *J. Biol. Chem.* 289 : 17392–17405.
- McCutchen-Maloney S.L., Matsuda K., Shimbara N., Binns D.D., Tanaka K., Slaughter C.A., DeMartino G.N. 2000. cDNA cloning, expression, and functional characterization of PI31, a proline-rich inhibitor of the proteasome. *J. Biol. Chem.* 275 : 18557–18565.
- Stadtmauer B.M., Hill C.P. 2011. Proteasome activators. *Mol. Cell.* 41 : 8–19.
- Wang X., Chen C.F., Baker P.R., Chen P.L., Kaiser P., Huang L. 2007. Mass spectrometric characterization of the affinity-purified human 26S proteasome complex. *Biochem.* 46 : 3553–3565.
- Zaiss D.M., Standera S., Holzhutter H., Kloetzel P., Sijts A.J. 1999. The proteasome inhibitor PI31 competes with PA28 for binding to 20S proteasomes. *FEBS Lett.* 457 : 333–338.
- Zaiss D.M., Standera S., Kloetzel P.M., Sijts A.J. 2002. PI31 is a modulator of proteasome formation and antigen processing. *Proc. Natl. Acad. Sci. USA*. 99 : 14344–14349.

EXPRESSION AND PURIFICATION OF C-TERMINAL REGION OF PI31 PROTEIN TO INHIBIT 20S PROTEASOME ACTIVITY

E. E. Diakonov^a, E. A. Malkina^{a, b}, V. A. Kulichkova^a, A. N. Tomilin^{a, c}, and A. S. Tsimokha^{a, *}

^aInstitute of Cytology, RAS, St. Petersburg, 194064 Russia

^bThe Herzen State Pedagogical University of Russia, St. Petersburg, 191186 Russia

^cInstitute of Translational Biomedicine, St. Petersburg State University, St. Petersburg, 199034 Russia

*e-mail: atsimokha@incras.ru

The proteasome inhibitor PI31 protein is 271 amino acid residues long and contains N-terminal globular domain and C-terminal proline-rich domain. The proteasome inhibition is conferred by the C-terminal proline-rich domain of PI31. *E. coli* recombinant expression construct of a fusion protein 6His-cPI31 consisting of C-terminal domain of PI31 (151–271 a. a.) and the N-terminal His-tag was made. The expressed protein was purified using nickel-agarose affinity chromatography. The recombinant protein 6His-cPI31 inhibited chymotrypsin-like activity of 20S proteasome, but not 26S-proteasome *in vitro*.

Keywords: proteasome inhibitor PI31, proteasome, proteolysis