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Heat-Shock Protein HSP70 Decreases Activity of Proteasomes in Human Neuroblastoma Cells Treated by Amyloid-Beta 1-42 with Isomerized Asp7

A. V. Morozov[a, *], M. M. Yurinskaya[a, b], V. A. Mitkevich[a], D. G. Garbuz[a], O. V. Preobrazhenskaia[a], M. G. Vinokurov[b], M. B. Evgen'ev[a], V. L. Karpov[a], and A. A. Makarov[a]

[a] *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russia*

[b] *Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow oblast, 142290 Russia*

**e-mail: Runkel@inbox.ru*

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Abstract—Experimental evidences indicate that heat-shock protein 70 (HSP70) can serve as a prospective therapeutic agent to treat Alzheimer's disease (AD). It has demonstrated a neuroprotective effect in vivo on mice models of AD. Moreover, HSP70 decreases oxidative stress in neurons induced by amyloid-β (Aβ_{42}) and its more toxic form with isomerized Asp7 (isoAβ_{42}). The dysfunction of Ubiquitin-proteasome system (UPS) is observed in AD. UPS is responsible for the degradation of the majority of cellular proteins and plays an important role in protecting cells from oxidative stress. Here, we have shown that the incubation of human neuroblastoma cells SK-N-SH with isoAβ_{42} increases the activity of intracellular proteasomes, which are the principal elements of the UPS. On the contrary, the proteasomal activity was decreased in isoAβ_{42}-treated cells in the presence of exogenous HSP70. These results highlight the existence of an interplay between Aβ peptides, proteasomes, and HSP70.

Keywords: HSP70, proteasome, amyloid-β peptide (Aβ_{42}) isoA$\beta_{42,}$ neuroblastoma SK-N-SH

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Alzheimer's disease (AD) is a severe neurodegenerative disorder associated with accumulation of amyloid beta peptide (Aβ) plaques in neurons and intercellular space in brain [1]. Posttranslational modifications contribute to aggregation and toxic properties of amyloid peptides, as well as to their degradation [2–7]. Isomerization of Asp7 in Aβ_{42} (isoAβ_{42}) is a very frequent modification [2, 8]. Compared to Aβ_{42}, isoAβ_{42} aggregates better and exhibits more prominent cytotoxic and amyloidogenic properties [9–11].

AD is accompanied by the oxidative stress in neurons [12], which might be caused by both Aβ_{42} and isoAβ_{42} [13, 14]. An essential tool for protecting cells from oxidative stress and for maintaining homeostasis is represented by chaperone proteins, in particular by the heat-shock protein 70 (HSP70) [15–17]. HSP70 accumulates in the brains of AD patients, which is considered to be a sign of organism's adaptive reactions [18]. Exogenous HSP70 is thought to be useful in AD therapy [19]. A prominent neuroprotective effect of the intranasal recombinant HSP70 was observed in the murine model of AD [19]. Furthermore, exogenous HSP70 decreases the cytotoxic effect of Aβ_{42} and isoAβ_{42} in vitro [13, 14]. The effect of HSP70 is associated with the protection of cells from oxidative stress and the inhibition of apoptosis [13, 14]. However, the effect may be broader. Indeed, HSP70 slows down the aggregation of amyloid peptides [20] and regulates the rate of protein degradation, as well as functional status of cellular proteasomes [21, 22].

Another typical feature of AD is the disturbed protein degradation and malfunction of the ubiquitin-proteasome system (UPS) [23, 24]. The experimental data show that intracellular amyloid peptides are the most toxic and therefore the most important in pathogenesis of AD [25]. Aβ_{42} is synthesized and accumulated inside the cell and, as a substrate of UPS, it is degraded by the 20S proteasome [26]. 20S proteasome, the major UPS component, is a multisubunit protein complex that hydrolyses most intracellular proteins [27]. The free 20S proteasome particles as well as 20S proteasomes associated with the 11S regulatory particles hydrolyze damaged and oxidized proteins [28]. Furthermore, upon binding to the 19S regulatory particle the 20S proteasome forms 26S proteasome, which selectively degrades ubiquitinated substrates [27]. All proteasomes exhibit three catalytic activities, i.e. chymotrypsin-like, trypsin-like and caspase-like activities [27]. Recently we demonstrated that Aβ_{42} and isoAβ_{42} decrease activity of free 20S pro-

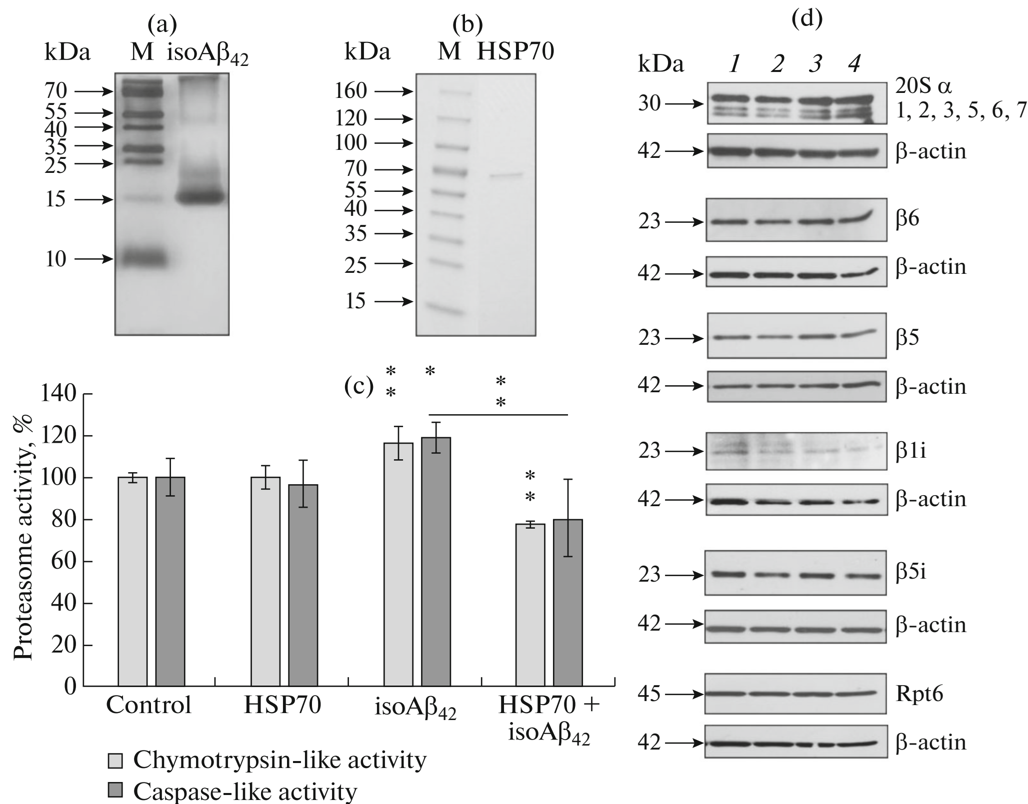

Fig. 1. Changes in the proteasome activity in SK-N-SH cells induced by isoAβ_{42} and HSP70. (a) Analysis of aggregation status of isoAβ_{42} by native 15% PAGE in Tris/glycine buffer. Loading: 2 μg of the peptide. Gel was stained with colloidal silver. M is Molecular weight marker Prestained Protein Ladder (Fermentas, Lithuania). After reconstitution isoAβ_{42} is represented mostly by the trimers and other low-molecular oligomers. (b) Analysis of HSP70 purity by 4–20% gradient PAGE. Loading: 0.4 μg of protein. Gel was stained with Roti-blue quick protein stain (Roth, Germany). M—Molecular weight marker Prestained Protein Ladder (Fermentas). Sample contains intact HSP70 and no protein contaminants. (c) Analysis of chymotrypsin-like (light bars) and caspase-like (dark bars) proteolytic activities of the proteasomes in lysates of SK-N-SH cells treated with isoAβ_{42} and/or HSP70. Mean ± SD of five independent experiments is shown. Significance of difference was estimated by Student t-test and indicated with asterisks: *$p < 0.05$; **$p < 0.01$. (d) The effect of isoAβ_{42} and/or HSP70 on the level of 20S proteasome subunits and Rpt6 subunit of 19S regulator. The results of immunoblotting with lysates of SK-N-SH cells, untreated and treated with isoAβ_{42} and/or HSP70 for 24 h are shown. A panel of antibodies against proteasome subunits and 19S regulator was used (see table). Control (*1*); cells treated with HSP70 (*2*); isoAβ_{42} (*3*); isoAβ_{42} after preliminary incubation with (*4*) HSP70 for 2 h.

teasomes, but increase activity of the 20S proteasomes capped with regulatory particles [29]. Despite the importance of UPS for a cell's adaptation to oxidative stress and the impact of individual HSP70 and amyloid peptides of functioning of proteasomes, their combined effect has not yet been studied.

We have determined the effect of isoAβ_{42} and exogenous HSP70 on the activity and subunit content of proteasomes in human neuroblastoma cells SK-N-SH. HSP70 has been shown to have no effect on the activity of proteasomes, whereas the treatment of neuroblastoma cells with isoAβ_{42} results in the increased proteasome activity. However, in HSP70-treated SK-N-SH cells isoAβ_{42} reduced the proteasome activity. The putative molecular mechanism of interaction between HSP70, proteasomes, and isoAβ_{42} has been considered.

MATERIALS AND METHODS

Human neuroblastoma cells SK-N-SH (ATCC® HTB-11) were cultured in RPMI 1640 (Sigma Aldrich, Germany) supplemented with 10% FCS (fetal calf serum) (Sigma Aldrich), 2 mM L-glutamine, antibiotics (penicillin/streptomycin) at 37°C, 5% CO_2, and 95% humidity.

The synthetic peptide isoAβ_{42}: [H2N]-DAEFRH-[isoD]SGYEVHHQKLVFFAEDVGSNKGAIIGL-MVGGVVIA-[COOH] was obtained from Biopeptide (United States). The peptide was processed according to [30]. Cold hexafluoropropanol (HFIP) (Fluka, United States) was added to dry isoAβ_{42} at final concentration of 1 mM and incubated for 60 min at room temperature. Then the solution was placed on ice for 10 min and transferred into non-siliconized microcentrifuge tubes (0.56 mg of peptide per tube). The tubes were dried in a vacuum using Eppendorf Con-

centrator 5301 (Eppendorf, Germany). The dried peptides were stored at –80°C. To obtain a fresh 5 mM iso$A\beta_{42}$ solution, 25 μL 100% anhydrous DMSO (Sigma Aldrich) were added to 0.56 mg of the peptide and incubated for 1 h at room temperature. Cells were treated with peptides dissolved in serum-free growth medium.

The aggregation status of the peptide was analyzed by polyacrylamide gel electrophoresis (PAGE) in 15% gel, in Tris/glycine buffer under native conditions, then the gel was stained with silver using Pierce Silver Stain kit for mass spectrometry (Thermo, United States) according to the manufacturer's protocol (Fig. 1a). The human recombinant HSP70 was obtained according to [31]. The protein was expressed in armyworm cells (*Spodoptera frugiperda*), purified on Ni-NTA-agarose column (Qiagen, Germany) according to the manufacturer's instructions. Protein quality and purity was estimated in 4–20% gradient SDS-PAGE in Tris/glycine buffer. The gel was stained with Roti-Blue quick (Roth, Germany) (Fig. 1b).

The SK-N-SH cells were harvested from culture flasks using trypsin-EDTA solution (Sigma-Aldrich) and washed twice with culture medium. The pellet was resuspended and the cells were counted, diluted in serum-free culture medium, and transferred into glass tubes. Then, the cells were treated with either HSP70 (5 μg/mL) or iso$A\beta_{42}$ (5 μM) for 24 h. Combined treatment of cells with HSP70 and iso$A\beta_{42}$ was performed as follows: cells were incubated for 2 h with 5 μg/mL HSP70, then 5 μM iso$A\beta_{42}$ were added, and cells were incubated for 24 h at 37°C and 5% CO_2. Following incubation, cells were placed in an ice bath for 15 min, centrifuged, and washed tree times in Hanks' Balanced Salt solution (HBSS) (Sigma-Aldrich). The pellet was resuspended in HBSS; cells were counted and centrifuged; and the supernatant was collected, frozen in liquid nitrogen, and stored at –80°C.

To measure proteasomes activity and determine their subunit content cells were thawed and homogenized as follows: 3×10^6 cells in 200 μL homogenization buffer (50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 10 mM $Na_2S_2O_5$ and 2 mM ATP). The homogenate was incubated on ice for 10 min, then centrifuged (30 min, 16000 *g*), and the supernatant was collected. The total protein content was measured by Lowry assay [32]. Chymotrypsin-like and caspase-like activities of proteasomes were measured by hydrolysis of fluorogenic substrates Suc-LLVY-AMC and Z-LLE-AMC (both from Enzo, United States) using VersaFluor Fluorometer (Bio-Rad, United States). Activity was estimated in samples that contained 2, 4, 6, 8, and 10 μL of clarified homogenate in the activity assay buffer [33]. At the same time, activity was estimated in samples pretreated with 10 μM of the proteasome inhibitor MG132 (Sigma-Aldrich). The obtained results were scaled to 1 μL, multiplied by the equalisation factor (calculated regarding to the total protein concentration in samples), then mean value and standard deviation were calculated. The subunit content of proteasomes in cell lysates was analyzed by the immunoblotting. Proteins were separated by the 12% SDS-PAGE in Tris/glycine buffer and transferred onto the nitrocellulose membrane (Bio-Rad). The efficiency of transfer was verified by staining of the membrane with 0.1% Ponceau Rouge (Sigma-Aldrich). To reveal the proteasome subunits, the membranes were incubated with primary antibodies (see table) for 2 h, then washed with 0.1% Tween-20 (Fisher, United States) in PBS, and incubated with the appropriate secondary antibodies conjugated to horseradish peroxidase (see table). Detection was performed with ECL Prime (GE Healthcare, United States). To normalize signals the membranes were washed with the antibody detachment buffer (PBS, 2% SDS, 100 mM β-mercaptoethanol), and with PBS afterwards. The membranes were incubated with anti-β-actin primary antibodies (see table), then washed and incubated with the appropriate secondary antibodies conjugated to HRP (see table).

RESULTS AND DISCUSSION

In the presence of exogenous HSP70 chymotrypsin- and caspase-like activities of the proteasomes in SK-N-SH cells did not differ from the activities in control cells (Fig. 1c). Treatment with iso$A\beta_{42}$ increased both activities by ~18–20% compared to control (Fig. 1c). In SK-N-SH cells iso$A\beta_{42}$ is known to induce oxidative stress [14]. One of the mechanism of cell's adaptation to the oxidative stress is the de novo synthesis of the proteasome subunits and subsequent assembly into catalytically active complexes [21, 34]. To characterize the alterations of proteasome quantity in response to iso$A\beta_{42}$ treatment the immunoblotting with antibodies to a set of proteasome subunits was performed (Fig. 1d). The iso$A\beta_{42}$ peptide was shown to have almost no effect on either the quantitative or subunit content of proteasomes. Previously, we revealed that iso$A\beta_{42}$ could directly regulate the activity of proteasomes [29]. Interactions of the 26S and 20S proteasomes with the 11S regulator iso$A\beta_{42}$ increases their activity and reduces the activity of free 20S proteasomes. The cells have more proteasomes with regulators than free 20S proteasomes [35], which might cause the observed integral effect of increased proteasome activity in the presence of iso$A\beta_{42}$.

In cells pretreated with HSP70, iso$A\beta_{42}$ reduces the chymotrypsin- and caspase-like activity of proteasomes by approximately 20% compared to the control. However, the reduction of the caspase-like activity compared to the control was not statistically significant. Compared to cells treated with only iso$A\beta_{42}$, the reduction of activities was 33 and 32%, respectively (Fig. 1c). The 26S proteasomes were recently shown to

Antibodies

Antibodies	Manufacturer
Mouse monoclonal antibodies against α-subunits of the proteasome α1,2,3,5,6,7	Enzo, United States
Rabbit polyclonal antibodies against β-5 subunit	GeneTex, United States
Rabbit polyclonal antibodies against β-6 subunit	Sigma-Aldrich, Germany
Mouse monoclonal antibodies against β-1i subunit	Enzo, United States
Rabbit monoclonal antibodies against β-5i subunit	Cell signaling, United States
Mouse monoclonal antibodies against 19S Rpt6 subunit	Enzo, United States
Rabbit monoclonal antibodies against β-actin	Sigma-Aldrich, Germany
HRP-conjugated goat anti-mouse IgG antibodies	Enzo, United States
HRP-conjugated goat polyclonal anti-rabbit IgG antibodies	Abcam, United Kingdom

temporarily dissociate into the 20S proteasomes and 19S regulators under oxidative stress, which results in the rapid increase in proteasomes capable of degrading damaged and oxidized proteins [21]. HSP70, which stabilizes free 19S regulators, plays an important role in this process [21]. Furthermore, exogenous HSP70 rapidly penetrates into the cells [36]. We suggest that 26S proteasomes dissociate in cells pretreated with HSP70 in the response to oxidative stress induced by iso$A\beta_{42}$. Therefore, the ratio of 20S proteasomes with regulators and free proteasomes shifts towards free 20S proteasomes. According to our data, iso$A\beta_{42}$ reduces activity of 20S proteasomes [29]. Therefore, the observed reduction of proteasome activity in cells under combined treatment with HSP70 and iso$A\beta_{42}$ might be explained by the increase of 20S proteasome content and reduction of their activity in response to the peptide treatment. The obtained results demonstrate that HSP70 is involved in the regulation of UPS in neuroblastoma cells in response to the amyloid peptide treatment.

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REFERENCES

1. Huang Y., Mucke L. 2012. Alzheimer mechanisms and therapeutic strategies. *Cell.* **148** (6), 1204–1222.
2. Shimizu T., Watanabe A., Ogawara M., Mori H., Shirasawa T. 2000. Isoaspartate formation and neurodegeneration in Alzheimer's disease. *Arch. Biochem. Biophys.* **381**, 225–234.
3. Querfurth H.W., LaFerla F.M. 2010. Alzheimer's disease. *N. Engl. J. Med.* **362**, 329–344.
4. Fossati S., Todd K., Sotolongo K., Ghiso J., Rostagno A. 2013. Differential contribution of isoaspartate post-translational modifications to the fibrillization and toxic properties of amyloid beta and the Asn23 Iowa mutation. *Biochem. J.* **456**, 347–360.
5. Xin F., Radivojac P. 2012. Post-translational modifications induce significant yet not extreme changes to protein structure. *Bioinformatics.* **28**, 2905–2913.
6. Tomiyama T., Asano S., Furiya Y., Shirasawa T., Endo N., Mori H. 1994. Racemization of Asp23 residue affects the aggregation properties of Alzheimer amyloid beta protein analogues. *J. Biol. Chem.* **269**, 10205–10208.
7. Kummer M.P., Hermes M., Delekarte A., Hammerschmidt T., Kumar S., Terwel D., Walter J., Pape H.C., Konig S., Roeber S., Jessen F., Klockgether T., Korte M., Heneka M.T. 2011 Nitration of tyrosine 10 critically enhances amyloid beta aggregation and plaque formation. *Neuron.* **71**, 833–844.
8. Roher A.E., Palmer K.C., Yurewicz E.C., Ball M.J., Greenberg B.D. 1993 Morphological and biochemical analyses of amyloid plaque core proteins purified from Alzheimer disease brain tissue. *J. Neurochem.* **61**, 1916–1926.
9. Mitkevich V.A., Petrushanko I.Y., Yegorov Y.E., Simonenko O.V., Vishnyakova K.S., Kulikova A.A., Tsvetkov P.O., Makarov A.A., Kozin S.A. 2013. Isomerization of Asp7 leads to increased toxic effect of amyloid-β42 on human neuronal cells. *Cell Death Dis.* **4**, e939.
10. Kulikova A.A., Cheglakov I.B., Kukharsky M.S., Ovchinnikov R.K., Kozin S.A., Makarov A.A. 2016. Intracerebral injection of metal-binding domain of abeta comprising the isomerized asp7 increases the amyloid burden in transgenic mice. *Neurotox. Res.* doi 10.1007/s12640-12016-19603-y
11. Kozin S.A., Cheglakov I.B., Ovsepyan A.A., Telegin G.B., Tsvetkov P.O., Lisitsa A.V., Makarov A.A. 2013. Peripherally applied synthetic peptide isoAsp7-Abeta(1-42) triggers cerebral beta-amyloidosis. *Neurotox. Res.* **24**, 370–376.
12. Butterfield D.A. 2002. Amyloid beta-peptide (1-42)-induced oxidative stress and neurotoxicity: implications for neurodegeneration in Alzheimer's disease brain. A review. *Free Radic. Res.* **36** (12), 1307–1313.
13. Yurinskaya M.M., Mit'kevich V.A., Barykin E.P., Garbuz D.G., Evgen'ev M.B., Makarov A.A.,

Vinokurov M.G. 2015. Heat-shock protein HSP70 protects neuroblastoma cells SK-N-SH from the neurotoxic effects hydrogen peroxide and the β-amyloid peptide. *Mol. Biol.* (Moscow). **49** (6), 924–927.

14. Yurinskaya M.M., Mitkevich V.A., Kozin S.A., Evgen'ev M.B., Makarov A.A., Vinokurov M.G. 2015. HSP70 protects human neuroblastoma cells from apoptosis and oxidative stress induced by amyloid peptide isoAsp7-A*β*(1–42). *Cell Death Disease.* **6** (11), e1977.
15. Mayer M.P., Bukau B. 2005. Hsp70 chaperones: Cellular functions and molecular mechanism. *Cell. Mol. Life Sci.* **62** (6), 670–684.
16. Hall L., Martinus R.D. 2013. Hyperglycaemia and oxidative stress upregulate HSP60 & HSP70 expression in HeLa cells. *Springer Plus.* **2**, 431.
17. Evgen'ev M.B., Garbuz D.G., Zatsepina O.G. 2014. *Heat Shock Proteins and Whole Body Adaptation to Extreme Environments.* Springer.
18. Perez N., Sugar J., Charya S., Johnson G., Merril C., Bierer L., Perl D., Haroutunian V., Wallace W. 1991. Increased synthesis and accumulation of heat shock 70 proteins in Alzheimer's disease. *Brain Res. Mol. Brain Res.* **11** (3–4), 249–254.
19. Bobkova N.V., Garbuz D.G., Nesterova I., Medvinskaya N., Samokhin A., Alexandrova I., Yashin V., Karpov V., Kukharsky M.S., Ninkina N.N., Smirnov A.A., Nudler E., Evgen'ev M. 2014. Therapeutic effect of exogenous hsp70 in mouse models of Alzheimer's disease. *J. Alzheimer's Dis.* **38** (2), 425–435.
20. Evans C.G., Wisén S., Gestwicki J.E. 2006. Heat shock proteins 70 and 90 inhibit early stages of amyloid beta-(1–42) aggregation in vitro. *J. Biol. Chem.* **281** (44), 33182–33191.
21. Grune T., Catalgol B., Licht A., Ermak G., Pickering A.M., Ngo J.K., Davies K.J. 2011. HSP70 mediates dissociation and reassociation of the 26S proteasome during adaptation to oxidative stress. *Free Rad. Biol. Med.* **51** (7), 1355–1364.
22. Bobkova N.V., Evgen'ev M., Garbuz D.G., Kulikov A.M., Morozov A., Samokhin A., Velmeshev D., Medvinskaya N., Nesterova I., Pollock A., Nudler E. 2015. Exogenous Hsp70 delays senescence and improves cognitive function in aging mice. *Proc. Natl. Acad. Sci. U. S. A.* **112** (52), 16006–16011.
23. de Vrij F.M., Fischer D.F., van Leeuwen F.W., Hol E.M. 2004. Protein quality control in Alzheimer's disease by the ubiquitin proteasome system. *Prog. Neurobiol.* **74**, 249–270.
24. Keller J.N., Hanni K.B., Markesbery W.R. 2000. Impaired proteasome function in Alzheimer's disease. *J. Neurochem.* **75** (1), 436–439.
25. LaFerla F.M., Green K.N., Oddo S. 2007. Intracellular amyloid-beta in Alzheimer's disease. *Nat. Rev. Neurosci.* **8**, 499–509.
26. Zhao X., Yang J. 2010 Amyloid-beta peptide is a substrate of the human 20S proteasome. *ACS Chem. Neurosci.* **1**, 655–660.
27. Schmidt M., Finley D. 2014. Regulation of proteasome activity in health and disease. *Biochim. Biophys. Acta.* 1843 (1), 13–15. doi 10.1016/j.bbamcr.2013.08.012
28. Pickering A.M., Davies K.J.A. 2012. Degradation of damaged proteins: The main function of the 20S proteasome. *Prog. Mol. Biol. Translat. Sci.* **109**, 227–248.
29. Morozov A.V., Kulikova A.A., Astakhova T.M., Mitkevich V.A., Burnysheva K.M., Adzhubei A.A., Erokhov P.A., Evgen'ev M.B., Sharova N.P., Karpov V.L., Makarov A.A. 2016. Amyloid-β increases activity of proteasomes capped with 19S and 11S regulators. *J. Alzheimer's Dis.* **54** (2), 763–776. doi 10.3233/JAD-160491
30. Klein W.L. 2002. Aβ toxicity in Alzheimer's disease: Globular oligomers (ADDLs) as new vaccine and drug targets. *Neurochem. Internat.* **41**, 345–352.
31. Rozhkova E., Yurinskaya M., Zatsepina O., Garbuz D., Karpov V., Surkov S., Murashev A., Ostrov V., Margulis B., Evgen'ev M., Vinokurov M. 2010. Exogenous mammalian extracellular HSP70 reduces endotoxin manifestations at the cellular and organism levels. *Ann. NY Acad. Sci.* **1197**, 94–107.
32. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.
33. Karpova Ia.D., Lyupina Iu.V., Astakhova T.M., Stepanova A.A., Erokhov P.A., Abramova E.B., Sharova N.P. 2013. Immune proteasomes in the development of rat immune system. *Russ. J. Bioorg. Chem.* **39** (4), 356–365.
34. Pickering A.M., Koop A.L., Teoh C.Y., Ermak G., Grune T., Davies K.J.A. 2010. The immunoproteasome, the 20S proteasome, and the pa28αβ proteasome regulator are oxidative-stress-adaptive proteolytic complexes. *Biochem. J.* **432** (3), 585–594.
35. Tanahashi N., Murakami Y., Minami Y., Shimbara N., Hendil K.B., Tanaka K. 2000. Hybrid proteasomes. Induction by interferon-gamma and contribution to ATP-dependent proteolysis. *J. Biol. Chem.* **275**, 14336–14345.
36. Yurinskaya M., Zatsepina O.G., Vinokurov M.G., Bobkova N.V., Garbuz D.G., Morozov A.V., Kulikova D.A., Mitkevich V.A., Makarov A.A., Funikov S.Y., Evgen'ev M.B. 2015. The fate of exogenous human HSP70 introduced into animal cells by different means. *Curr. Drug Deliv.* **12** (5), 524–532.

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