Interaction of the $GroEL_{14}/GroES_7$ chaperonin complex with amyloidogenic proteins

[a]S. KUDRYAVTCEVA, [b,c]Y. STROYLOVA, [a]T. STANISHNEVA-KONOVALOVA, [d]E. PICHKUR, [a]A. MOISEENKO, [b]V. MURONETZ

[a] *Lomonosov Moscow State University, Moscow, Russia*
[b] *A.N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia,*
[c] *Institute of Molecular Medicine, Sechenov First Moscow State Medical University, Moscow, Russia*
[d] *National Research Center «Kurchatov Institute», Moscow, Russia*

Nowadays there are a number of serious diseases, which are caused by the accumulation of protein aggregates in the nervous tissue. For example, Parkinson's disease or prion diseases. This process activates cellular chaperone system, which starts to interact with the assembled aggregates. Under normal conditions chaperones regulate the folding of proteins and prevent their aggregation. However, the result of the interaction of this cellular system with a normal form of protein and its aggregates may be different and has not yet been fully studied [1].

During this work we investigated the role of chaperones in amyloidogenic transformation of proteins. To achieve our aims, we used two model systems: ATP-dependent bacterial chaperonin complex $GroEL_{14}/GroES_7$, whose 3D structure is now being actively studied by cryo-electron microscopy, and sheep prion protein as a capable of aggregating agent. We assumed the possibility of inhibition of chaperonin complex with amyloid proteins. To test our hypothesis, we obtained various forms of prion protein (PrP): monomeric PrP, small oligomers of PrP, "protofibrils" and fibrils of PrP. The affiliation of molecules to a specific form of prion protein was verified by dynamic light scattering (DLS). The amyloid fibrillation of prion protein was also indicated by the increase in ThioflavinT fluorescence intensity in its presence.

We showed by ELISA that chaperonin complex $GroEL_{14}/GroES_7$ bound different forms of prion protein. Then we studied the outcome of such interaction. We pre-incubated $GroEL_{14}/GroES_7$ complex with different forms of PrP and then measured the fluorescence emission spectra of ThT of this samples. Eventually we found out that chaperonin stimulated not only further amyloid aggregation of already formed oligomers and fibrils but also amyloidogenic transformation of monomeric prion protein. The same results were also obtained by the dynamic light scattering [2]. Our further plans include studying the interactions of $GroEL_{14}/GroES_7$ with PrP using negative stain electron microscopy.

We investigated the possibility of blocking the main function of the chaperonin $GroEL_{14}$– its ability to reactivate different proteins - by different forms of prion protein. To indicate changes in the work of chaperonin we used a marker – a complex glycolytic enzyme glycerol-3-phosphate dehydrogenase. Firstly, we completely denatured GAPD with the addition of 4 M guanidine hydrochloride. Then we incubated denatured GAPD with chaperonin complex and observed the recovery of the enzyme activity. When chaperonin complex $GroEL_{14}/GroES_7$ was co-incubated with one of the prion protein forms and with denatured GAPD, the functional activity of the chaperonin complex was reduced to almost complete loss of its activity.

The similarity of bacterial chaperonin to some mammalian chaperonin complexes (i.e. cytoplasmic chaperonin TRiC), the key role of GAPDH in glycolysis and the presence of different forms of amyloid-like proteins in the neural tissue allows to suggest the involvement of the described mechanism into development of amyloid neurodegenerative diseases.

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References:

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