STABILIZATION MECHANISMS OF AIMP1/p43 PROTEIN IN NANOCOMPOSITE COMPLEXES WITH 2-HYDROXYPROPYL-β-CYCLODEXTRIN

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Introduction

Recombinant proteins are widely used as therapeutic agents at the current stage of medicine. There are many prerequisites for the use of proteins as immunomodulatory and antitumor drugs. Protein drugs as therapeutic agents are more specific to targets and less toxic to healthy cells, so they are promising potential drugs in the fight against severe chronic and oncological diseases. Cytokines can be used in war and post-war times as mediators for the treatment of diseases.

Aminoacyl-tRNA synthetase multifunctional protein 1, also known as AIMP1/p43 and proEMAP-II, is a non-catalytic component of the mammalian multi-tRNA synthetase complex (MSC) and has both tRNA-binding and cytokine activities. AIMP1/p43 is characterized by pro-apoptotic and anti-angiogenic activity, participation in glucose homeostasis, coordination of the development of axonal processes of nerve cells. The effectiveness of the AIMP1/p43 protein preparation as an antitumor agent was established in mouse xenograft models containing human gastric cancer and breast cancer cells [1,2]. The protein is a precursor of the endothelial monocyte-activating polypeptide EMAP-II, the essential role of which has been recorded in pro-inflammatory and antitumor processes. The antitumor effect of the latter was demonstrated on pancreatic cancer and glioma cells.

Aggregation of protein macromolecules remains one of the main problems in the development and commercialization of biotechnological products. The presence of an intrinsically disordered region (IDR) in the central part of the AIMP1/p43 protein (P81-D146) leads to instability and a tendency to aggregation. Protein aggregation in the composition of a therapeutic drug can lead to a decrease or complete absence of its biological activity and increase of the potential immunogenicity or other side effects. Overcoming this problem consists in the use of additional stabilizers of protein molecules, in particular cyclodextrins. These compounds are able to form inclusion complexes with various molecules or their fragments, enclosing them in an internal hydrophobic cavity, thereby changing the properties of the latter. In order to stabilize AIMP1/p43, we used a more soluble analogue of natural β-cyclodextrin – 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). HP-β-CD increases the stability and solubility of drugs and increases their shelf life. HP-β-CD is also characterized by the absence of toxic effects.

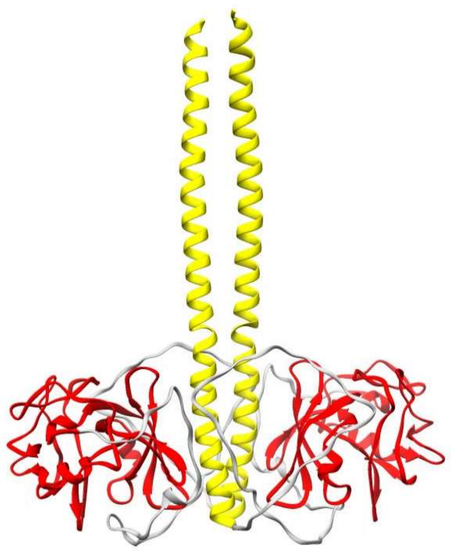

Figure 1. Model of the spatial structure of the AIMP1/p43 dimer after optimization. The sequence of the N-terminal module M1-E70 is highlighted in yellow, the unstructured region I71-D146 is gray, and the sequence of the C-module S147-K312 is red.

Methods

The effect of HP-β-CD on the stability of the recombinant AIMP1/p43 protein expressed in *E. coli BL21(DE3)pLysE* cells transfected with *pET-28b(+)-p43* plasmids by fluorescence spectroscopy at increasing temperature was studied. The dependence of the fluorescence emission maxima of AIMP1/p43 protein and in its nanocomposite complex with HP-β-CD (in a ratio of 1:10) at the fluorescence excitation wavelength of 280 nm was demonstrated.

Results and discussion

The temperature of the local conformational transition of AIMP1/p43 protein to the denatured state was found to be 43±1°C, while for the protein in the nanocomposite complex with HP-β-CD this value ranged from 47±1°C. The wavelength of the maximum fluorescence emission for AIMP1/p43 is 346 nm, for the complex with HP-β-CD – 342 nm, indicating the thermal stabilization of the protein in the complex.

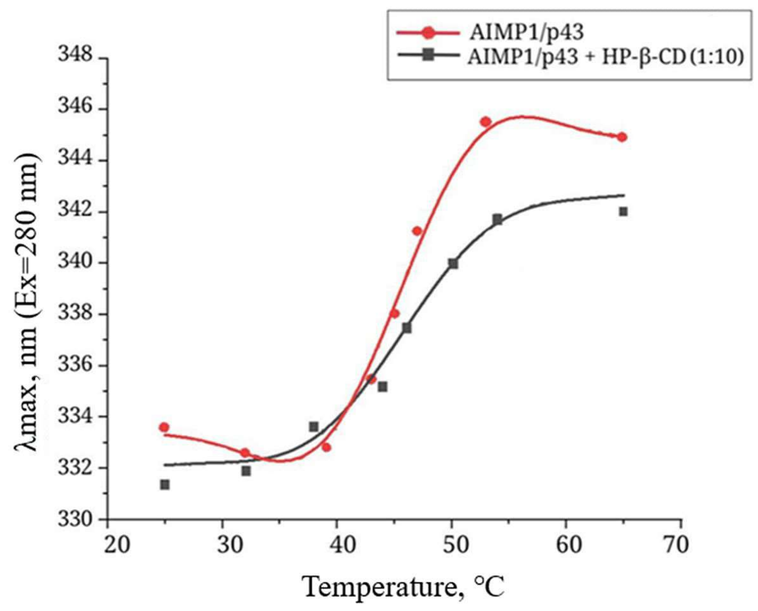

Figure 2. Temperature dependence of the fluorescence emission maxima of AIMP1/p43 protein in the free state and as a part of a nanocomposite complex with HP-β-CD.

According to the computer modeling of the complex [3], the stabilization mechanism is based on the binding of HP-β-CD in the IDR of AIMP1/p43 to Trp271 and its microenvironment.

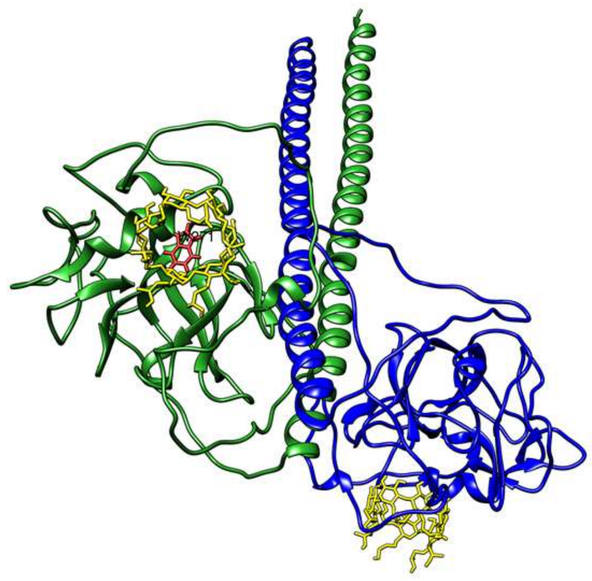

Figure 3. Computational docking of hydroxypropyl-β-cyclodextrin molecules with AIMP1/p43 dimer. Cyclodextrins molecules are stained in white, and Trp271 residue is shown inside of HP-β-CD.

Conclusions

The structure of AIMP1/p43 protein is stabilized in nanocomposite complex with HP-β-CD stabilize and protect it from the denaturation at high temperatures.

This creates the prerequisites for the effective use of AIMP1/p43 protein in nanocomposite complexes with HP-β-CD as putative antitumor agents in anticancer therapy.

References

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