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Structure and mechanisms of stabilization of cytokines EMAP II and AIMP1/p43 in complexes with ligands for biomedical use

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Aim. The protein AIMP1/p43, which is a component of the high molecular weight complex of aminoacyl-tRNA synthetases in higher eukaryotes, and its C-terminal domain is the cytokine EMAP II (endothelial and monocyte-activating polypeptide II) are known for their ability to inhibit neoangiogenesis and induce apoptosis of cancer cells. A significant role of AIMP1/p43 and EMAP II in pro-inflammatory processes has been identified, which have become a prerequisite for their use as immunomodulatory and anticancer drugs for targeted therapy. The development of pharmaceutical dosage forms involves the use of additional chemical components and excipients, such as 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). **Methods.** The recombinant cytokines AIMP1/p43 and EMAP II were obtained by bacterial expression in the *Escherichia coli BL21(DE3)pLysE* system and purified by affinity metal-chelating chromatography on Ni-NTA-agarose. The formation of nanocomposite complexes of AIMP1/p43 and EMAP II proteins with HP-β-CD was studied and the thermostability of the prepared preparations was analyzed by fluorescence spectroscopy and the spatial structure of proteins and their nanocomposite complexes with ligand was determined by computational modelling and the mechanism of stabilization of AIMP1/p43 and EMAP II was proposed. **Results.** The stoichiometry of inclusion complex formation for both proteins was found to be 1:1. According to *in silico* modelling, the largest contribution to the protein fluorescence of AIMP1/p43 is made by the exposed tryptophan Trp271, and in the composition of EMAP II by Trp128, so the proposed mechanism of protein stabilisation is the interaction of the ligand with the aromatic tryptophan residues and their amino acid environment. As the temperature increases from 25 °C to 65 °C, the conformation of the protein molecule inevitably changes, which affects its fluorescence due to the release of the exposed tryptophan residues to the outside during denaturation of the protein globule. Thus, it has been shown that the formation of a complex with HP-β-CD promotes the thermal stabilization of AIMP1/p43 and EMAP II, since the half-transition temperature between the native and unfolded states of the protein globule shifts from 43±1 °C to 47±1 °C for AIMP1/p43 in the complex with cyclodextrins and from 42±1 °C to 48±1 °C for EMAP II in the nanocomposite complex. **Conclusions.** To sum up, the nanocomposite complexes with biomedical ligands stabilize the molecules of AIMP1/p43 and EMAP II proteins. The created nanocomposite complexes are promising therapeutic agents, which requires further research on the possibility of their implementation in clinical practice for the treatment of cancer.

Keywords: AIMP1/p43, EMAP II, 2-hydroxypropyl-β-cyclodextrin, nanocomposite complex, protein stabilization, computational modelling.