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The use of Ferritin-based Protein Nanocages in Targeted Therapy of Breast Carcinoma

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Extended Abstract

The use of nanocarriers in cancer therapy has growing interest. Nanocarriers are able to encapsulate drugs and deliver the cargo directly to malignant tissue while avoiding toxic effects to healthy cells. This study is focused on ferritin (FRT) representing ubiquitous biocompatible nanocarrier. FRT is a self-assembly icosahedron-shaped protein composed of 24 light and/or heavy subunits, with disassociation/reassociation properties dependent on pH. The quaternary structure of FRT is stable over a broad range of neutral, slightly acidic or slightly basic pH [1]. There are many studies focused on targeted transport of drugs, which are using mainly horse spleen FRT [2, 3]. However, possible immunogenicity of non-human FRT in patient's organism needs to be taken into account. Therefore, we focused on recombinant production of FRTs from alternative organisms and their assumptions to become a suitable drug nanocarrier.

We tested four different FRTs, namely: bacterial FRT (PFU), human FRT composed of only heavy subunits (HsaH), horse FRT composed of only light subunits (EcaL) or composed of 22 light and 2 heavy subunits (EcaLH). To fully understand the structure dependence on pH values native gel electrophoresis was performed. The results showed that EcaL and HsaH disposed the most stable structure. The structure was fully assembled in pH range 4.0 - 12.5 (EcaL) or 4.5 - 12.5 (HsaH). On the other hand, the EcaLH structure was the less stable, since it became fully assembled only between pH 4.0 - 9.0. The structure of PFU remains assembled in pH range 3.5 - 10.0.

The reassociation of FRTs was also studied *via* native gel electrophoresis in order to test the ability to completely assemble all 24 subunits into the icosahedral shape of the FRT protein cage. EcaLH and PFU showed the most effective reassociation process, while in the case of EcaL and HsaH free subunits were visible, pointing at incomplete reassociation.

The disassociation and reassociation mechanism of EcaLH was verified *via* transmission electron microscopy (TEM). Incomplete spherical shape was observed at pH 4.0, while the complete disassociation was observed at pH 2.0, which corresponds with results obtained from native gel electrophoresis.

The internalization kinetics into four breast carcinoma cell lines (MDA-MB-468, MDA-MB-231, MCF-7 and T-47D) and non-malignant HBL-100 cell line was tested in eight different time points and two different serum conditions (*in situ* formed protein corona with 50 % human serum and naked FRT - 0% serum) *via* flow cytometry. The fastest internalization was noticed in naked HsaH, whose uptake was determined already after 5 min of treatment in all tested cell lines except T-47D. Whereas EcaL disposed the slowest internalization kinetics. The results also showed that formed *in situ* protein corona led to highest decrease in the internalization of HsaH, whose uptake was, after 24 h treatment, decreased by 98 % compared to naked HsaH. Taking together, the use of HsaH as nanocarier appeared as the most promising. However surface modification of HsaH ensuring decreased protein corona formation is necessary for further use of HsaH in nanomedicine.

References

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