## **Gold-Mediated Modification of Apoferritin Surface with Targeting Antibodies**

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Abstract: Protein apoferritin seems to be a very promising structure for use as a nanocarrier. It is prepared from intracellular ferritin protein naturally found in most organisms. The role of ferritin proteins is to store and transport ferrous ions. Apoferritin is a hollow protein cage without ferrous ions that can be prepared from ferritin by reduction with thioglycolic acid or dithionite. The structure of apoferritin is composed of 24 protein subunits, creating a sphere with 12 nm in diameter. The inner cavity has a diameter of 8 nm. The drug encapsulation process is based on the response of apoferritin structure to the pH changes of surrounding solution. In low pH, apoferritin is disassembled into individual subunits and its structure is "opened". It can then be mixed with any desired cytotoxic drug and after adjustment of pH back to neutral the subunits are reconnected again and the drug is encapsulated within the apoferritin particles. Excess drug molecules can be removed by dialysis. The receptors for apoferritin, SCARA5 and TfR1 can be found in the membrane of both healthy and cancer cells. To enhance the specific targeting of apoferritin nanocarrier, it is possible to modify its surface with targeting moieties, such as antibodies. To ensure sterically correct complex, we used a a peptide linker based on a protein G with N-terminus affinity towards Fc region of antibodies. To connect the peptide to the surface of apoferritin, the C-terminus of peptide was made of cysteine with affinity to gold. The surface of apoferritin with encapsulated doxorubicin (ApoDox) was coated either with gold nanoparticles (ApoDox-Nano) or gold (III) chloride hydrate reduced with sodium borohydride (ApoDox-HAu). The applied amount of gold in form of gold (III) chloride hydrate was 10 times higher than in the case of gold nanoparticles. However, after removal of the excess unbound ions by electrophoretic separation, the concentration of gold on the surface of apoferritin was only 6 times higher for ApoDox-HAu in comparison with ApoDox-Nano. Moreover, the reduction with sodium borohydride caused a loss of doxorubicin fluorescent properties (excitation maximum at 480 nm with emission maximum at 600 nm) and thus its biological activity. Fluorescent properties of ApoDox-Nano were similar to the unmodified ApoDox, therefore it was more suited for the intended use. To evaluate the specificity of apoferritin modified with antibodies, we used ELISA-like method with the surface of microtitration plate wells coated by the antigen (goat anti-human IgG antibodies). To these wells, we applied ApoDox without targeting antibodies and ApoDox-Nano modified with targeting antibodies (human IgG antibodies). The amount of unmodified ApoDox on antigen after incubation and subsequent rinsing with water was 5 times lower than in the case of ApoDox-Nano modified with targeting antibodies. The modification of non-gold ApoDox with antibodies caused no change in its targeting properties. It can therefore be concluded that the demonstrated procedure allows us to create nanocarrier with enhanced targeting properties, suitable for nanomedicine.

Keywords : apoferritin, doxorubicin, nanocarrier, targeting antibodies

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