

Physico-chemical stability of protein-polymer conjugates in solution

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Protein-polymer conjugates are a new class of biohybrids materials with high potentialities for biomedical applications.¹ Most of the works reported in the literature are focused on their synthesis and their characterization, either *in vitro* or *in vivo*. Beyond these aspects, their biophysical evaluation is an important point to deeper understand their microscopic behaviour and to orientate the design of future candidates.

In this frame, we evaluated the physical and thermal stability protein-polymer conjugates in solution. We prepared a novel set of conjugates using the protein myoglobin (intended as a simplified model of haemoglobin), and the polymers polyphosphoesters (PPEs), a novel class of biocompatible and biodegradable polymers, promising substitutes of the gold standard poly(ethylene glycol) in the biomedical field.² We focus our attention on the observation of the protein stability during a heating scan, observing the protein unfolding process using Circular Dichroism, UV-Vis spectroscopy, nano-Differential Scanning Calorimetry and Fluorimetry. Besides, we observed the protein degradation induced by proteolytic enzymes.

Overall, we observed that the increasing in the attached polymer hydrophobicity brought a decrease in the protein stability.³ More in detail, calorimetry showed that in the conjugates the protein unfolding enthalpy and temperature decrease, but the more hydrophilic polymers enhance the protein thermal reversibility, reducing thermally induced aggregation phenomena. This behaviour was confirmed by CD measurements, used also to evaluate protein denaturation at different pHs or urea concentrations. The measurements made by UV-Vis absorbance confirmed the protective action of hydrophilic polymers toward the action of proteolytic enzymes, while the more hydrophobic polymers enhance the protein degradation rate.

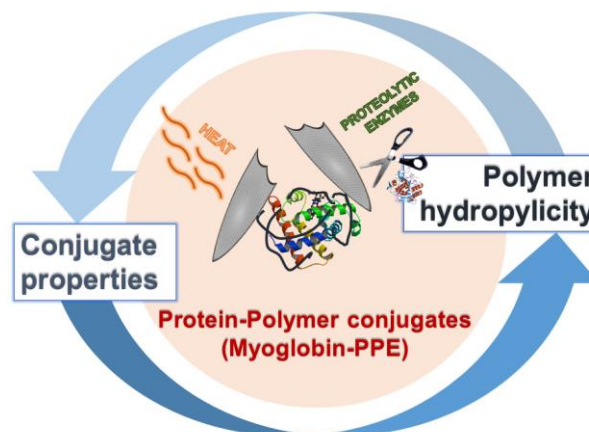


Figure 1. Scheme of protein-polymer conjugates, and the influence of the polymer hydrophilicity of the protein properties.

References

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