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Secondary structure and thermal stability of feruloylated *Rapana thomasiana* hemocynanin

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Hemocyanins (Hcs) are large metalloglycoproteins that are responsible for the oxygen transport in many spices of mollusks and arthropods [1]. Up to date only *Keyhole limpet* hemocyanin is implemented in the practice as an immunotherapeutic in bladder cancer and as a protein carrier in vaccines for breast and prostate cancers, melanoma and non-Hodgkins lymphoma [2]. However, recent studies reveal that Hcs from other sources have also potential as vaccine adjuvants and carriers, anti-tumoral and anti-viral agents. Chemical modification of Hcs may result in changes in their structure and can alter or enhance their biological activities.

This is the **first report** on the conjugation of a hemocyanin from *Rapana thomasiana* (RtH) with ferulic acid, which itself also has immunostimulatory activity. The reaction was conducted in two steps. At first, N-hydroxysuccinimide ester of ferulic acid was obtained, and then the activated ester was reacted with the RtH molecules. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was used to assess the differences in the secondary structures of the native RtH and the feruloylated-RtH. Baseline corrected ATR-FTIR spectra were processed by means of 25-points smoothing filter and a second order polynomial applying the Savitzky–Golay algorithm to obtain second derivatives. Then, the relative contribution of each band component of the Amide I band (1700 cm^{-1}–1600 cm^{-1}) was determined by curve fitting following the procedure of OPUS program. Rearrangement in the RtH, decrease in α-helices and coiled structures in favour of the β-structures, and no aggregation due to the feruloylation in comparison to the native RtH. The effect of modification of RtH with ferulic acid on the thermal unfolding was monitored using a high-sensitivity differential scanning microcalorimeter DASM-4 (Biopribor, Pushchino, Russia), with a sensitivity > 0.017 mJ K^{-1} and a noise level < ±0.05 μW. The DSC curves of the feruloylated-RtHs characterize with an asymmetric shape, which is an indication for the existence of more than one structural unit in the analyzed samples. The protein thermal stability is not affected by the modification, however the profiles of the DSC curves of the feruloylated-RtH and the native RtH differ, which imply the reorganization in the protein molecule and is in correlation with the secondary structure analyses.

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