## Analysis of human milk protein complexes containing biologically active proteins and peptides

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Milk is not only a unique and universal source of essential nutrients for newborns, but also contains a number of biologically active components necessary for the protection, growth and development of infants. Some functionally significant milk proteins and peptides can save their biological activity even in the aggressive environment of the gastrointestinal tract due to the formation of high-molecular protein complexes that can protect biologically active components from proteolysis while preserving their biological properties.

We studied the presence and distribution of innate immunity proteins and peptides of human milk serum and high-molecular fractions of serum (more than 50 kDa) obtained by ultrafiltration. The studied preparations were characterized by a number of electrophoretic methods under denaturing and non-denaturing conditions in an alkaline, acidic and neutral environment and also by two-dimensional electrophoresis. Individual biologically active proteins and peptides (human neutrophil defensins HNP 1-3 and HNP 4, lactoferrin (LF), lactoperoxidase (LPO), myeloperoxidase (MPO)) were detected in the analyzed samples and also the main chromatographic fractions obtained by RP HPLC using Western blotting and Dot-ELISA.

It has been shown that high-molecular and low-mobility proteins, such as LF, LPO and MPO, and low-molecular-weight peptides with higher electrophoretic mobility, HNP 1-3 and 4, are detected in the composition of high-molecular complexes in the studied preparations. Two different types of high molecular weight complexes of human milk have been isolated and characterized which are stable under aggressive conditions at pH 3 and high ionic strength (2M sodium chloride). Complex No.1 contains mainly LF and other biologically active proteins and peptides, such as HNP 1-3 and HNP 4, LPO, MPO and lysozyme, whereas protein complex No.2 contains mainly  $\alpha$ -lactalbumin, as well as LF and HNP 1-3, and does not contain HNP 4, LPO, MPO and lysozyme.

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