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RECOMBINANT HUMAN LACTOFERRIN FROM MILK OF TRANSGENIC GOATS: ISOLATION AND PHYSICOCHEMICAL PROPERTIES

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Electrophoretically homogeneous preparations of recombinant human lactoferrin (rhLF) from milk of transgenic goats, natural human lactoferrin (nhLf) from woman milk and natural goat lactoferrin (gLf) from milk of non-transgenic goats were obtained using cation-exchange chromatography. Biochemical and physicochemical characteristics of rhLF were similar to nhLf; this included molecular mass, immunochemistry, iron-binding/releasing ability, thermal stability, glycosylation and proteolysis.

Purified transgenic and natural LFs were subjected to chromatography on Mono S column to estimate identity and integrity of N-terminal amino acid fragments. Chromatography showed equal cationic properties for rhLF obtained in the laboratories of Belarus and Russia. Retention time typical for rhLFs differed from the ones of nhLf and gLf. This denotes certain heterogeneity in N-terminal fragments of recombinant and natural lactoferrins.

Preparations of iron-unsaturated (apo-) and iron-saturated (holo-) forms of rhLF were analyzed by spectrophotometry, electron paramagnetic resonance (EPR) and differential scanning calorimetry (DSC).

It was demonstrated that apoforms of rhLF and nhLF lacked maximum absorption at 465 nm typical for their holoforms.

In order to identify the nature of the iron complex, the holoforms of rhLF and nhLF were subjected to EPR analysis. Using a spectrometer operating in the microwave radiation X-range (ν=9.3 GHz) intensive paramagnetic absorption at about 1637 gauss was registered for holoforms of rhLF and nhLF. Such a prominent absorption derivative near g=4.3 is typical of high-spin Fe^{3+} in a centre of rhombic symmetry. Spectrum intensity and breadth practically coincide for both proteins. EPR spectra of lactoferrin apoforms lost resonance lines that are typical of iron-saturated samples.

DSC curves showed higher value of denaturation temperatures and enthalpy changes when rhLF was saturated with iron. This indicates that the binding of iron to lactoferrin is an important factor in the stabilization of its structure. Similar results were obtained for nhLF, indicating a high degree of resemblance between both proteins.