CHANGING OF LACTOFERRIN IRON-BINDING CAPACITY IN INFLAMMATION

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In accordance with the program of World Health Organization new drug development, aimed to prevention of infectious diseases and increasing humans' lifespan and level of living, is underway around the world. Lactoferrin (Lf), non-hemic iron-binding protein of the transferrin family, is the promising protein for this aim because of its many physiological functions, including regulation of iron absorption and immune responses; it also exhibits antioxidant activity and has both anticarcinogenic and anti-inflammatory properties. The antimicrobial activity of Lf, most widely studied function, is driven generally by two mechanisms: 1) direct interaction positively charged amino acids in Lf with anionic molecules on certain microorganism surface causing cell lysis and 2) iron sequestration in site of infection, which deprives microorganisms of this nutrient and causes a bacteriostatic effect [1].

Ceruloplasmin (CP) is a serum ferroxidase that contains greater than 95 % of the copper found in plasma, this protein is an acute phase reactant part of the organism's defense in inflammation. Lf concentration highly increases in inflammation focuses because of neutrophil degranulation. Due to this fact it was found the complexes Lf-CP in blood serum of patients with pleuritis of different etiology [2]. As is known, complexes creation can influence on biological properties of the composing proteins. It already has been revealed [3, 4] that ferroxidase activity of Cp can be stimulated by Lf. But it's still unknown whether iron-binding property of Lf will be changed in case of CP influence by complexes formation in inflammation focuses or not. ROS and RHS formed in inflammatory focuses are high reactivity oxidants modifying biomolecules (lipids, proteins, nucleic acids) and resulting in changing of their properties. Thus in the previous work [5] we showed the reduction of iron-binding capacity of Lf in case of modification by HOBr and HOCl. The present work is aimed to study modification of iron-binding capacity of Lf, which is crucial and necessary for antibacterial property, in case of formation complexes Lf-CP in inflammatory foci.

Lf-CP complex formation was performed by mixing and incubation Lf and CP in equal concentration (i.e. molar ratio 1:1) for 30 min at 37 °C. CP was modified by HOCl in a molar ratio CP:HOCl 1:50, modification lasted for an hour. Iron-binding capacity of Lf was investigated using spectrophotometric analysis. Addition of Fe^{3+} (salt – (NH_4 $Fe(SO_4)_2$)) to Lf solution leads to change in absorption at λ=465 nm due to transition of apo-state of Lf (i.e. iron-unbound form) to holo-state of Lf (i.e. iron-bound form).

It was found that formation of Lf-CP complexes causes reduction of 30 % of iron-binding capacity of Lf. Lf has two iron-binding sites per one molecule, which comprise four protein sidechains (2 Tyr, 1 Asp, and 1 His), side chain of an Arg residue, balanced the negative charge of a CO_3^{2-} anion. Moreover iron binding is cooperative process, at first Fe^{3+} fits into N-lobe of Lf, after that due to conformational reorganization C-lobe becomes open and let Fe^{3+} fits into lobe. It's necessary to mention that when the N-lobe site was knocked out, the C-lobe site behaved as normal (closed form) [6]. Formerly it was showed that cationic N-lobe of Lf is responsible for interaction between Lf and CP [4,7]. Due to complexes formation iron-binding site becomes partially inaccessible because of interruption of conformation alteration, those processes lead to decreasing of number binding ions (10 molecules of Lf bind 14 ions of Fe^{3+}). Nevertheless the modification of CP by HOCl, which leads to structure and activity modification of CP [8], causes formation of slightly remodeled complexes (Lf-CP-Cl). This results in decrease of 39 % of iron-binding capacity of Lf.

Thus it was showed that creation of the complexes Lf with native CP (Lf-CP) and CP under halogenative stress (Lf-CP-Cl) in inflammation focuses leads to decrease Lf iron-binding capacity, which can influence on antibacterial property of Lf.

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