STRUCTURAL PROPERTIES AND IRON-BINDING CAPACITY OF LACTOFERRIN DURING OXIDATIVE/HALOGENATIVE STRESS

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Lactoferrin (Lf) is an iron-binding glycoprotein, contained in most external secretions: milk, saliva, tears, etc., and in polymorphonuclear leucocytes [1]. Main property of Lf is antimicrobial activity, which depends on protein iron-binding capacity. Apart from that, Lf possesses a number of important properties, among them antiviral, antifungal, immunomodulatory and even anticancer activity [2, 3]. One of the promising application of this protein is development of the drugs, especially using recombinant human lactoferrin (rhLf), produced from the milk of transgenic animals [2]. Lf is released from the secondary granules of neutrophils during degranulation. This process is accompanied by production of reactive oxygen and halogen species (ROS and RHS), which leads to the development of oxidative and halogenative stress [4]. Thus, a large number of ROS and RHS surround Lf and other proteins. We showed previously that this oxidants can affect structure and properties of myeloperoxidase, protein which is closely associated with the production of RHS [5], and that modification of rhLf by HOCl changes it's structure, as well as ability to activate neutrophils.

Little is known about the effect of other ROS and RHS, such as HOBr, Tau-Cl, Tau-Br, H_2O_2 and HOSCN, on the structure and other important biological and biophysical properties of Lf molecule. Thus, this work aims to study the structural properties of Lf and it iron-binding capacity during modification by different ROS and RHS.

It was showed that modification of rhLf by HOCl, HOBr and Tau-Br led to the destruction of tryptophan residues, that was measured by intrinsic fluorescence of tryptophan ($\lambda_{ex.}$=285 nm, $\lambda_{em.}$=340 nm). Modification of rhLf by RHS in molar ratios from 1:10 to 1:50 showed that HOBr and Tau-Br had more potent effect then HOCl, but at the molar ratio of 1:100 HOCl, HOBr, Tau-Br all destroyed tryptophan residues. H_2O_2, HOSCN and Tau-Cl had no significant effect on tryptophan fluorescence.

Modification of primary amines (Lys and Arg) by ROS and RHS was studied with the help of fluorescent probe fluorescamin ($\lambda_{ex.}$=390 nm, $\lambda_{em.}$=490 nm). Only modification by HOCl at the molar ratios 1:50 and 1:100 showed significant effect on the change of the structure of primary amines of rhLf.

Structural properties of the whole protein globe were studied by the fluorescent probe ANS ($\lambda_{ex.}$=350 nm, $\lambda_{em.}$=510 nm). Modification of rhLf by HOCl have showed sights of protein unfolding, whereas modification by Tau-Br likely led to the formation of aggregates of rhLf molecules.

Changes of iron-binding capacity of rhLf, which is the key factor for many biological activities of this protein, were studied by monitoring the absorption at λ=465 nm while adding the Fe^{3+} ($NH_4Fe(SO_4)_2$) salt to the suspension. Modification of rhLf by HOBr, HOCl and Tau-Br led to the loss of the iron-binding capacity of this protein.

In this work we showed that modification of rhLf molecule by different forms of RHS (HOCl, HOBr, Tau-Br) led to the loss of antimicrobial activity of rhLf, but at the same time mechanisms of this effect are vastly different, as shown by analysis of change of protein structure by different fluorescent probes and techniques.

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