Lactoferrin structural stability in association with enzymatic hydrolysis.

P-02.4-02

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Lactoferrin is an 80-kDa glycoprotein that has an ability to reversibly but strongly binds two iron ions. It is most abundantly presents in mammalian milks but also in other biological secretions and white blood cells. Since its discovery in 1960, it has been widely studied primarily because of its variety of biological functions from antitumor, anti-inflammatory, immuno-modulatory, antioxidant to antimicrobial. Lactoferrin can exist in iron depleted (apo) form and iron loaded (holo) form. Apo form has open conformation and is more prone to denaturation. When incubating lactoferrin in the presents of iron ions, it adopts holo form, which has more closed conformation and exhibit greater resistance to denaturation. In our study, the pH and temperature stability of lactoferrin was measured. Lactoferrin was stable in pH range from 4.0 to 11.0. At pH below pH 4.0 and above pH 11.0 lactoferrin undergoes protein denaturation process. Temperature stability of lactoferrin was measured over a wild pH range. At pH 4.0 lactoferrin exhibited lower temperature stability. The higher temperature stability was observed at neutral pH. However, saturation of lactoferrin with iron thermally stabilize the structure.
As we have observed, lactoferrin unfolds at pH lower than pH 4.0 what makes it more prone to enzymatic proteolysis by pepsin. Using pepsin for lactoferrin hydrolysis, lead to discovery of peptide with higher antimicrobial effect on bacteria. Enzymatic hydrolysis of lactoferrin with pepsin at different pH (1.0-4.0) was studied. SDS PAGE analysis showed peptides smaller than 10 kDa at pH range from pH 1.0 to pH 3.0, while at pH 3.5 and 4.0 peptides larger than 10 kDa were observed. This indicates poor enzymatic activity of pepsin at higher pH. By exposing lactoferrin and proteolytic enzyme to different incubation conditions can affect structure and enzymatic activity of an enzyme or protein witch can reflect in generating new bioactive peptides.