Structural and functional studies of trypsin digested C-lobe and other major fragments from bovine lactoferrin

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Lactoferrin (Lf) is multifunctional protein; fragments of intact protein are more effective in many biological functions as compared to native protein. It is imperative to study structures and functions of these fragments, to get a clear understanding about this multifunctional and bioactive protein. Lf was hydrolysed with trypsin and three functional fragments (with Mol. Wt. 45kDa, 38kDa, and 21kDa) were purified through Ion Exchange and Gel Filtration Chromatography and characterized using N terminal sequencing. Two of these three fragments, retain iron binding capacity while all the three fragments retain antimicrobial activities suggesting that LF evolved as antimicrobial defence molecule which resist complete hydrolysis and breakdown of its activity.

Iron saturated 38kDa was crystallized at pH 4.0 and pH 6.8. The structure obtained at pH 4.0 shows that the iron atoms are absent and iron binding sites are wide open while at pH6.8 iron was present, His595 appears to be the first residue to dissociate from ferric ion when pH is lowered and it may be the intermediate state between iron saturated and iron free form.

The antimicrobial activities of fragments were examined against *S.pyogenes* M49, *L. monocytogenes* MTCC839, *E.coli* EDL 933 and *Y. enterocolitica* 8081 and *C. albicans* ATCC 90028. The iron binding property was analysed through pH induced release of Iron from LF and its fragments. Silver nanoparticles of fragments were produced through biological method and were characterised by spectroscopy, TEM and FTIR. These nanoparticles were found to be anti-cancerous against various cancer cell lines (such as A549, HepG2 etc.). Efficiency of nanoparticles coated with these fragments, was found to be much higher than free fragments and the concentrations, required, were drastically reduced. The silver nanoparticles of these fragments are able to evade various physiological barriers, assisting in targeted delivery and may emerge as potential anti-cancerous therapeutic agent.