CHAPTER I

LACTOFERRIN: AN UNFINISHED AGENDA

Lactoferrin (Lf), an 80Kd mammalian secretory glycoprotein, along with serotransferrin (STf), ovotransferrin (OTf) and human melanotransferrin (HMTf) constitute the transferrin family of proteins (Karthikeyan et.al., 1999a). Despite having a four decade-old history, the perception of lactoferrin remains elusive spanning all the key aspects. The nineties have seen burgeoning investigative reports concomitant to the accelerated attempts to unleash the functional roles and the structural intrigues of Lf. (Lonnerdal and Iyer 1995; Hutchens and Lonnerdal 1997; Karthikeyan et.al., 1999a; Baker et.al., 2000). However, all these studies failed to yield any convincing lead towards the goal, perplexing the understanding further. Identification of novel functions or proposals on the structural basis of functioning only elevated the ambiguity, leaving the query on the "pivotal function(s)" and "structural mechanism(s)" unanswered.

On the structural front, a large wealth of reports on different members of transferrin family are available, both in native and recombinant forms. Though the metal free apo-structure clearly demonstrated the domain flexibility as a function of metal-coordination (Anderson et.al., 1990), it is still debated whether the different conformations adopted by apo-forms as revealed in crystal structures are species specific features or due to packing preferences for one form over the other from a number of possible conformations due to hinge flexibility (Baker et.al., 2000). Mutational analyses gave promising insights into the role of various residues in dictating the properties of Lf/HMTf (Peterson et.al., 2000); but they also relinquished short of delineating anything concrete on the exact mechanism of iron-binding/release. Prominent structural ambiguities include:

1. the molecular mechanism/pathway of iron binding and release.
2. structural basis of the variation in iron-binding strength between lactoferrins/serotransferrins, and each site within the same molecule, and
3. why do apo-form of lactoferrins adopt different conformations.

Recent demonstrations on the mechanism of non-synergistic anion mediated metal ion release seem to testify the original interlocking hypothesis proposed by Bates and coworkers (Cowart et.al., 1986). Mutational studies have minimized the over emphasis on "dilysine trigger" residues as the sole structural differentiating factor explaining the metal ion release between Lf and STf (Peterson et.al., 2000).

Lf is presumed to serve a plethora of functions, ranging from antimicrobial activity to regulation of the immune system (Lonnerdal and Iyer 1995). Most of these have been proved unambiguously, qualifying Lf for inclusion into the category of "moonlighting proteins" (Jeffery 1999). Two obvious questions still remain in this aspect:

1. what is the major role(s) of Lf ***in vivo***, and
2. what is the mechanism of action, in the identified area(s).

In compliance with the presumption of direct interactions of Lf with target cells, receptors have been identified in a variety of tissues which may trigger a set of Lf-dependent reactions downstream in the cells (Bennatt and McAbee 1997). This latter observation, along with the detection of its specific DNA-binding property (He and Furmanski 1995), strongly hints at the involvement of Lf in more complex physiological circuitries.

Distribution of the transferrin family across the chordates and non-chordates – all phyla of vertebrates to many of the studied invertebrates through the urochordata – points at a common origin, and a concomitant divergence over time (Baldwin 1993). Remarkable conservation of selected residues of Tf-family members across phyla indicates a stringent regulation rendered by nature during evolutionary proliferation.

In toto, all the unresolved queries make lactoferrin an "unfinished agenda", warranting further, significant and extensive studies covering all its realms – functional, structural as well as evolutionary. The post genomic

revelation of "limited genes-more proteins-much more functions" dilutes the canonical views on protein functioning by conceiving more than one function to each (Lander et.al., 2001). While all the new reports on lactoferrin structures brought novel features, none of the mutational studies with recombinant-Lf resolved conclusively any of the underlying mechanisms of iron-binding and release. A precise and comprehensive sequence analysis of all Tf-family members as well as its related proteins is expected to yield insights into the divergence-tree of Tf-class itself.

This dissertation reports:-

1. **Comprehensive Sequence Analysis of the Transferrin Family of Proteins.**
2. **Porcine Lactoferrin: Purification, Preparation of Monoferric Functional Lobes and Reassociated N_{Fe}-C_{Fe} Lobe Complex.**
3. **Iron-Release Behaviour and the Nature of Domain-Domain Interactions of Porcine Lactoferrin.**
4. **Crystallization of Porcine Lactoferrin.**
5. **Thermal Unfolding of Porcine Lactoferrin: Differential Scanning Calorimetric Studies.**