INTRODUCTION
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An Overview of General Properties and Structure of Transferrin:

Vertebrate transferrin (Tf) is a monomeric glycoprotein of plasma that plays a central role in the transport of iron between the sites of absorption, storage and utilization in vertebrates (Welch, 1990; Cnaani et al., 2001). It is a member of the family of non-heme iron binding proteins distributed within the cells and physiological fluids of all vertebrates (Yang et al., 1984). In mammals, this family also includes at least four more members, namely: ovotransferrin (also known as conalbumin), lactotransferrin, melanoma antigen p97 and ChBlym-1.

Three dimensional structure of vertebrate Tf has been worked out completely. According to which, the single polypeptide chain is folded into two globular domains with each domain having a single iron binding site (Welch and Skinner, 1989). Available evidence has also established that ferric ion couples to Tf only in the presence of an anion (usually bicarbonate) that serves as the bridging ligand between metal and protein (Aisen and Listowsky, 1980; Harris and Aisen, 1989; Shongwe et al., 1992). The two iron binding sites, however, differ in
their affinity for iron and their acid stability. The N-lobe looses iron at pH values between 5.5 and 6.0, whereas the C-lobe looses iron between 4.5 and 5.0 (Antonio and Brock, 2001) and thus shows more acid stability. Their primary sequence, however, indicates that the two domains have a homology wherein 40% of the residues are identical in N-lobe (residues 1-336) and C-lobe (residues 337-678) (Mac Gillivray et al., 1983). In view of the considerable sequence homology between the two globular domains, the family of Tf-like proteins is thought to have evolved from a common ancestral precursor (M, 40,000 daltons) as a result of gene duplication (Williams, 1982).

The major site of transferrin synthesis is the liver, but Tf variants may also be produced in non-hepatic tissues also (Baldwin et al., 1990). Essentially, all circulating plasma iron is bound with Tf and this chelation by Tf serves the vital functions of iron regulation; for instance: (i) the iron derived from catabolism of hemoglobin is conserved by its quantitative return through Tf to hematopoietic tissues (Fletcher and Huens, 1968), (ii) the possible oxidation or inhibitory effects of elevated ferric ions are prevented by buffering action of Tf (Laurell, 1960), (iii) it checks the loss of iron by urinary excretion by
holding the concentration of free iron in plasma; and, (iv) it may also
serve as the physiological source of iron to immature red cells which
preferentially bind iron saturated Tf (Jandl and Katz, 1963)
Serotransferrin is, therefore, meant for the efficient and stringent
physiological regulation of iron metabolism in blood.

In human beings, the role of transferrins in cell proliferation,
activation of immune system and resistance to infection in a number of
clinical conditions, including the congenital absence of Tf or
atransferrinemia (Heilmeyer, 1966) has attracted due attention (Aisen
and Listowsky, 1980; Djeha et al., 1992). Studies on functional aspects
of fish Tf, despite being limited, also reveal that it plays an important
role in innate immune system (Yano, 1996) and some evidence suggests
a correlation between Tf levels in sera of fish species and certain
clinical conditions (Pratschner, 1978; Winter et al., 1980; Hirono and
Aoki, 1995). Some other members of the Tf family are also considered
to play an important role in defense mechanisms in salmonids
(Suzumoto et al., 1977; Winter et al., 1980). As for instance, Sakai et
al. (1993) reported that by activation of phagocytosis bovine lactoferrin
enhances the resistance of rainbow trout against bacterial infections.
Gene Cloning Attempts:

Whereas classical approaches of phenotyping Tf continue to make a significant contribution, during recent years attention has shifted to cloning of Tf genes. Complementary DNA (cDNA) of Tfs of a number of higher chordates have been cloned and characterized. The deduced amino acid sequences have helped in establishing already described three dimensional structure, their phylogenetic correlation and the interaction between transferrin and its receptors.

Among mammals, cDNAs of human (Yang et al., 1984; Park et al., 1985); pig (Baldwin and Weinstock, 1988); rabbit (Banfield et al., 1991) and horse (Carpenter and Broad, 1993) transferrins has been cloned, sequenced and compared. The data on cDNA clones of Tf from some quite distantly related species such as an amphibian, Xenopus (Moskaitis et al., 1990); the invertebrates, cockroach, Blaberus discodalis (Jamroz et al., 1993) and tobacco hornworm, Manduca sexta (Bartfeld and Law, 1990) are also available.

Fish species, whose Tf cDNAs have been cloned are: Atlantic salmon, Salmo salar (Kvingedal et al., 1993); medaka, Oryzias latipes
(Hirono et al., 1995); coho salmon, *Oncorhynchus kisutch* (Lee et al., 1995); Atlantic cod, *Gadus morhua* (Denovan-Wright et al., 1996); Japanese flounder, *Paralichthys olivaceus* (Kim et al., 1997) and rainbow trout, *Oncorhynchus mykiss* (Tange et al., 1997).

Tfs in mammals share 25% amino acid positional identity with one another. This estimate is based on the comparison of eleven complete Tf and Tf-related amino acid sequences. The homology was determined either directly from nucleotide sequence data or indirectly by using the amino acid sequence deduced from the corresponding nucleotide sequence (Baldwin, 1993). The homology between amino acid sequences of Tf of medaka and Atlantic salmon ranges from 30-50% (Hirono and Aoki, 1995). In Salmonids, within each Tf cDNA sequence, there are significant sequence identity regions and the phylogenetic relationships determined between ten species by Tf sequences are quite consistent with those derived from classical taxonomy (Lee et al., 1998). Analysis of these structural genes of Tf has also indicated that it has undergone an intragenic duplication (Park et al., 1985). The position of cysteine residues and iron binding amino acid residues are, however, highly conserved.
Comparative Biochemical Characteristics with Specific Reference to Fish Tfs:

Prior to the switchover to submolecular investigations based on molecular biology related techniques, the analysis of protein itself was the principle source of elucidating the functional and biochemical properties of Tf. The study of Tfs isolated and purified from natural sources has not yet lost its relevance. During the last decade, serotransferrins from a number of vertebrates have been purified and characterized to varying degrees. In this respect, the data on fish transferrin is again far from adequate.

Some of the published evidence deals with the Tfs from rabbit (Strickland and Hudson, 1978; Welch and Skinner, 1989); rat (Schreiber et al., 1979; Welch and Skinner, 1989), frog and turtle (Palmour and Sutton, 1971) and Chicken (Williams, 1968). In all these cases, Tfs have been found to have molecular weight (Mₗ) in the range of 75 kD to 82 kD which has also been confirmed by SDS-polyacrylamide gel electrophoresis (Welch, 1990), though some earlier estimates settled for the value of ≈ 90 kD (Koechlin, 1952; Aisen et al., 1966).
Values between 68-85 kD were recorded for Tfs of other vertebrates including those of fish (Hara, 1984; Welch, 1990). A Mr value of 77 kD has been reported for hagfish (Aisen et al., 1972) and 78 kD for brook trout Hershberger (1970), whereas the values of 68 kD for catfish and pike Tfs have been calculated to be 86.8 kD (Stratil et al., 1985). Fish Tfs are also known to have saccharides other than sialic acid as carbohydrate moieties and may even be totally free of such contents (Stratil et al. 1983, 1985).

**Polymorphism and its Genetic Significance:**

One of the best appreciated characteristics of Tfs from the genetic point of view has been its reported polymorphism in most of the vertebrate species. Though Tf variants represent the polymorphism of a single locus of codominant alleles, the data has been extremely useful in discerning the biochemical genetics of several species. It has applications in breeding and maintenance of stocks, determining gene frequencies, identifying races and genetic structure of natural populations of several fish species (Utter et al., 1970; Jamieson, 1990; Stratil et al., 1992; Nagabuchi et al., 1993; Blott et al., 1998; Lacy, 2000; Nabi et al., 2003).
Essentially, most of these studies had a focus on electrophoretic surveys within and among different species such as some species of the family Cyprinidae (Valenta and Stratil, 1977); European Hake (Mangaly and Jamieson, 1979); sturgeons (Keyvanfar, 1986) and coho salmon (Utter et al., 1970; Hirono and Aoki, 1995). In certain instances, extensive polymorphism and heterogeneity has been discovered at the Tf locus. As for instance, in tuna five Tf phenotypes AB, BB, BC, CC and AC were discovered that could be traced to a 3-allele system (Barrett and Tsuyuki, 1967; Fujino and Kang, 1968). Recently, Van-Doornik et al. in 1996 have detected a 4-allele system in coho salmon. In mirror carp, *Cyprinus carpio* L., as many as 7-alleles had been identified by Valenta et al. (1976). So far, the most extensive polymorphism reported has been displayed by Tfs of Amazonian pescada, *Plagioscion squamosissimus*, where 12 genotypes encoded by six co-dominant alleles were detected (Teixeira et al., 2002).

**Research Status in Context of Indian Fish Species:**

Notwithstanding the vastness of the resources and the diversity of fauna, the information on the polymorphism of Tfs of fish species inhabiting Indian subcontinent is scarce. The first report on
polymorphic transferrins was published on airbreathing snakehead or murrel, *Channa punctatus* (Hasnain *et al.*, 1981) that described the existence of five phenotypes in some North Indian districts. The work of another laboratory from an area about 1300 km away from Aligarh reported a two banded pattern both in *Channa gachua* and *C. striatus* (Sahoo and Khuda-Bukhsh, 1989), while two variants with one band and two band phenotypes were reported for Tf of *C. punctatus*. A more detailed study of Tf polymorphism in *C. punctatus* covering an area of ~7381 sq. kms had been undertaken in our laboratory (Nabi *et al.*, 2003). According to it, Tf polymorphism in *C. punctatus* is controlled by three isoforms A, B and C encoded by three loci, which on the basis of codominant inheritance constitutes six phenotypes: single banded homozygotes AA, BB and CC and the double banded Tf heterozygotes AB, BC and AC.

**Aims and Objectives of the Present Work:**

*Channa gachua* Hamilton is among the important food fishes of India as well as of several Asian countries. It makes a significant contribution to fresh and brackish water capture fisheries. One of the very distinct features of it and other channids is the adaptation to
accessory air breathing for which the specialized accessory organs exist in them.

The available literature on the biochemical genetics of *Channa gachua* Ham. is extremely scarce excepting the work on its parvalbumins done in our laboratory (Arif, 2002), no other information on its proteins or biochemical markers has been published, so far. As a result of investigations on biochemical and immunological characterization of Tf's of the most abundant channid *C. punctatus* (Nabi, 1999), the relevant techniques were already established in our laboratory. In the present effort, biochemical characterization was extended to Tf of *C. gachua*. Moreover, an attempt was also made to clone cDNA of Tf of this species.

This thesis, therefore, presents and discusses the information collected on the above two main aspects under the heads and subheads as follows:

(1) Identification of Tf clones in cDNA library of mRNA extracted from the liver of *C. gachua*:

a) By colony screening protocol.

b) By DNA sequencing.
(2) Determining the extent of polymorphism within *C. gachua* itself.

(3) Biochemical characterization of Tf purified from natural sources:
   
   a) Identification of Tf bands in sera and liver by various staining techniques;

   b) Purification by ammonium sulfate fractionation and preparative polyacrylamide gel electrophoresis (PAGE);

   c) Estimation of molecular weights by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

   d) Isoelectric focusing (IEF)

   e) Iron binding capacities and pH dependent release

   f) Interspecies comparison based on the above data and its genetic significance.