CHAPTER -1
INTRODUCTION

Transferrin (Tf) is a monomeric protein characterized by the function of binding specifically and reversibly most of the acid soluble iron in plasma (Davis et al., 1962). Two other synonyms of it, siderophilin and β1 metal-binding protein are no more in use. Whereas it unloads bound iron in liver, it is unrelated to ferritin, which is an iron storage protein of this organ (Fletcher and Huehns, 1968).

The following vital functions of iron regulation are attributed to Tfs:

i) The iron derived from catabolism of hemoglobin and other proteins is conserved by its quantitative return through Tf to hematopoietic tissues (Fletcher and Huehns, 1968).

ii) The buffering action of Tf on the free iron concentration of plasma prevents the ferric concentration from elevating to toxic levels and it also impedes the possible oxidative or inhibitory effects of ferric ions (Laurell, 1960).

iii) Lastly, by holding the concentration of free iron in plasma it checks the loss of iron by urinary excretion. Experiments further indicate that during cyclic processing of iron by Tf, it binds $\equiv 2$ iron atoms per molecule (Palmour and Sutton, 1971; Welch, 1990).

Apparently, each human Tf monomer has two homologous iron binding sites incorporated in each of the two domains (MacGillivray et al., 1983) specified as N (residues 1-336) and C (residues 337-679). In addition, for the binding of ferric ion at each of the two iron binding sites of Tf, a concomitant binding of a bicarbonate ion is also essential (Aisen et al., 1973). The complex that is formed between purified
Tf and ferrous ion is not chemically or spectrometrically different from that prepared using ferric salts. Loci for Tfs map on positions 21-25 on short arm of chromosome 3 of human karyotypes (Yang et al., 1984).

It has also been suggested that by competing for iron and keeping the plasma concentration low Tf protects against infections. This suggestion is supported by the fact that patients with atransferrinemia (the congenital absence of Tf) suffer from recurrent infections (Heilmeyer, 1966). Furthermore, patients with hypotransferrinemic conditions are more predisposed to infections than those with normal Tf levels (Bernstein, 1987). There are some evidences which suggest a correlation between Tf levels in sera of fish species and certain clinical conditions (Pratschner, 1978; Winter et al., 1980; Hirono and Aoki, 1995).

Though Tf can also combine loosely with the metals of transition and lanthanide series including manganese, copper, cobalt, chromium, scandium, terbium and zinc (Charlwood 1963; Aisen et al., 1969; Gafni and Steinberg, 1974) only the transport of iron is known to have a physiological significance.

Earlier estimates of molecular weights of vertebrate transferrins had settled to a value of ≈90 kD (Koechlin, 1952; Bezkorovainy et al., 1963 Aisen et al., 1966). Charlwood (1963), first disputed this figure and instead determined a value of 68 kD for human, monkey and rat Tfs. Later on, further low values for human Tfs close to 76.5 kD were reported by several workers (Roberts et al., 1966; Mann et al., 1970, Palmour and Sutton, 1971; Welch, 1990). No differences in molecular weights of apo-Tf or those binding either one or two iron atoms has ever been detected.

Values (68-85 kD) similar to those agreed upon for human Tfs have been
recorded for Tf's of other vertebrates (Hara 1984; Welch, 1990). Fish Tf's are also
known to follow this pattern with a value of 77 kD reported for hagfish (Aisen et al.;
1972) and Hershberger (1970) reported a value of 78 kD for brook trout Tf's. In
contrast, Stratil et al. (1985) calculated rather a low value of 68 kD for catfish
against 86.8 kD for pike Tf. This range of molecular weights in vertebrate stand
confirmed by SDS-polyacrylamide gel electrophoresis (Welch 1990).

For the purification of Tf numerous strategies making use of ammonium sulfate,
rivanol (2-ethoxy-6,9-diaminoacridine lactate) or ethanol as precipitants, and
purification by column chromatography or gel filtration were attempted. Out of them,
the use of rivanol (originally proposed by Boettcher et al., 1958) has proved most
effective and rapid. The ratio of rivanol to serum, however, may vary from case to
case. The documented ratios for different vertebrates are: 1:3 for cattle (Patras and
Stone, 1961) 1:3.5 for human (Sutton and Karp, 1965), and 1:2 for brook trout
(Hershberger, 1970) Tf's.

Another important characteristic of Tf's which has drawn considerable attention
is the high degree of polymorphism it displays in most of the vertebrate species
examined so far. Such data is extremely useful in working out the biochemical genetics
of the concerned species. Whereas for human beings due to a correlation with clinical
aspects Tf data on polymorphism carries additional importance, as well as in case
of other vertebrates it may find applications in identification, breeding and maintenance
of animal stocks. The discovery of first Tf polymorph was made by Smithies (1957)
who identified a heritable variant Tf-D in the sera from Negroes and Australian natives.
However, the most common of them is Tf-C which was originally detected as the
third component in the β-globulin region in starch gel electropherogram of human serum (Smithies, 1957). Further studies demonstrated that genetic polymorphism at Tf loci of vertebrates may be a rule rather than an exception. The cited examples include, zebras (Stratil et al., 1992); rat (Nagabuchi et al., 1993); brown hare (Hartle and Ferrand, 1993); hounds (Sherer and Kluge, 1993); donkey (Bell, 1994) and cattle (Blott et al., 1998).

So far as fishes are concerned, a high degree of Tf polymorphism has been reported in several species such as tunas (Barrett and Tsuyuki, 1967; Fujino and Kang, 1968); brook trouts (Hershberger 1970); carps (Valenta et al, 1976). Some species of the family cyprinidae (Valenta and Stratil, 1977); European hake (Mangaly and Jamieson, 1979); sturgeons (Keyvanfar, 1986); Channa sp. (Hasnain et al., 1981; Sahoo and Khuda-Bukhsh, 1989) and coho salmon (Utter et al., 1970; Hirono and Aoki, 1995; Van-Doornik and Milner, 1996).

Phylogenetic relationships of a number of organisms have been worked out by making immunological investigations on several proteins. There exists published evidence which deals with glycerol 3-phosphate dehydrogenase (Fink et al., 1970); lysozymes (Arnheim and Wilson, 1967; Prager and Wilson, 1971); albumins (Prager et al., 1974); histones (Bustin and Stollar, 1973); azurins (Champion et al. 1975). The reports dealing with phylogenetic relationship of Tf's where immunological approach was applied, are scarce. Among them is the report of Prager et al. (1976) on phylogenetic correlation of flightless land birds. As for fish species, only one report was found where phylogenetic interrelationships between Tf's of a number of elasmobranchs were determined by immunological methods (Lawson et al.; 1995).
Thus, there exists a paucity of information on immunological interrelationships of proteins of vertebrates in general and on Tfs in particular.

STATEMENT OF THE PROBLEM

Channa punctatus Bloch is among important food fishes of India as well as most of South Asian countries. It makes a significant contribution to fresh and brackish water capture fisheries of these countries beside being harvested as a component of paddy-cum-fish culture. The information about population structure, in general, is important from the viewpoint of better management of natural resources as well as aquaculture practices. In biochemical genetics, polymorphic protein and isozyme markers have been extensively employed to achieve the above objectives. Unfortunately, very little information is available on these aspects of transferrin (Tf) isoforms of Channa punctatus Bl. and their biochemical characterization remains virtually unattended.

An interesting aspect of the fish species belonging to genus Channa is the adaptation to accessory airbreathing habit for which, specialized organs exist in them. This divergence justifies additional interest in these species because fishes already occupy a central position in the evolution of vertebrates.

Taking the above considerations into account, the present investigations on Tf isoforms of Channa punctatus Bl. were envisaged so as to include the following aspects:

1) To determine the extent of polymorphism at transferrin locus by electrophoretic typing of transferrin isoforms and assess the significance of obtained data in
discerning population genetics of *C. punctatus* Bl.

2) To purify its Tf isoforms to homogeneity and biochemical characterization of them by monitoring functional property of iron binding and estimation of molecular weights.

3) To trace evolutionary relationships among Tfs of four available species of genus *Channa* by microcomplement fixation (MC'F) analysis using purified Tf isoform of *C. punctatus* as reference immunogen.