Abstract

Name of the Candidate: MUMTAZ JABEEN

Title of Ph.D. Thesis:

Identification of a transferrin like protein in cDNA clones of Channa gachua Ham. and significance of its isoforms as genetic markers.

Transferrin (Tf), a monomeric protein belonging to the family of iron-binding proteins, occurs in high concentrations in sera of vertebrates. Structurally, it folds into two, an N- and a C-lobe; each having one binding site for an iron atom. Certain sequences in each one of the two lobes have been highly conserved during evolution, whereas positioning of Cys residues has been important phylogenetically. It ferries free iron from sera and transfers it to hematopoietic and variety of other tissues. Receptors located on the surface of the target cells (e.g., reticulocytes) mediate the process of iron transfer. Transferrin, therefore, has two categories of binding sites. One category binds and carries iron (one atom each on N and C lobes), while the other has to bind the receptor on the site of unloading. Since its function is physiologically important, critical differences in the capacities to transfer iron are most likely to occur between different animal species.
In case of fish species, due to recognition and applicability of Tf variants as biochemical genetic markers, its polymorphism drew most of the early attention. Biochemical characterization, encompassing interspecies variations among molecular weights, pI values and carbohydrate contents were to follow. With the shift in focus to Molecular Genetics, cloning the cDNA of structural genes of some fish Tf's has also been achieved. The last and the least attention has, however, been paid to iron binding and virtually none to releasing capacities, despite its significance outlined in the first paragraph.

The present investigations were envisaged as a contribution to the information on transferrins of Channa gachua and its sister species. Fishes belonging to the genus Channa are commonly known as snakeheads or murrels. They are of considerable economic importance as food fishes and characterized by their bimodal respiration. Falling among the group of teleosts where adaptive radiation has lead to evolution of air breathing, they are of specific interest.

The following aspects of Tf of C. gachua were investigated: (i), Attempting cloning of cDNA of at least one of the Tf isoforms; (ii), phenotyping Tf polymorphs and extent of the polymorphism; (iii),
purification of the most abundant isoform and its biochemical characterization including M, and pI values, presence or absence of sialic acid in carbohydrate moiety, iron binding capacities and the pH dependent release of iron from diferric Tf species.

To expand the scope of the information, one Tf each from other three species of the genus, *Channa punctatus*, *C. striatus* and *C. marulius* have also been included. Human Tf obtained from commercial source (also purified to homogeneity) was used as the standard reference.

An abbreviated version of the obtained results and discussion is being presented below:

**Specificity of cDNA clone of Tf-like protein of *C. gachua***:

(1) Efforts to clone cDNA of a Tf isoform were partially successful. The cloned sequence, however, shows homology of varying percentage to conserved region of N as well as C domains of accessed sequences of four teleosts of different classes and origin (namely: *Oncorhynchus mykiss*, *Oryzias latipes*, *Paralichthys olivaceus* and *Salmo trutta*).

(2) Partial clone, any how, provided some specific information. A comparison of the portion of the accessed sequences of four teleosts,
with the homologies of different degrees in N and C domains, reveals differences in AT:GC contents. cDNA sequence of *C. gachua* Tf-like protein is AT rich. Occurrence of 30 Asn residues in a single stretch and specific positioning of four Cys residues is also species specific. Locations of Cys residues carry specific significance in the evolution of vertebrate transferrin.

**Biochemical Characterization of Tf isoform of *C. gachua* and Its Comparison with Tfs of Other Sister Species:**

(1). Phenotyping of Tf variants by PAGE showed the presence of one banded homozygote BB and two banded pattern represented by both BB and AA. Due to co-dominance inheritance of Tf alleles, their genotype is also the same. Out of the two phenotypes, fast migrating BB, is the most abundant phenotype. Phenotype BB has been reported for the first time. It is obviously in higher frequency than the two banded AA-BB phenotype. No heterozygote has been discovered so far.

Bands initially identified in PAGE as those of Tf, could unambiguously be identified as iron binding proteins by incubating with FENTA (ferric nitrilotriacetic acid). Following this treatment, with the exception of Tfs, all the remaining protein bands in sera or tissues take
up coomassie brilliant blue (CBB) stain. Thus, in superimposed or duplicate gels run under identical conditions, Tf bands can be identified in CBB stained gels by directly comparing with FENTA-incubated CBB stained replicas. The method is non-hazardous and even specific staining with Nitroso-R can be bypassed.

Comigrating bands in liver homogenates and their ammonium sulfate cuts gave positive results with this method.

(2). Biochemically, highly purified TfB isoform of *C. gachua* has all the attributes of a typical transferrin molecule: it is a glycoprotein of $M_r = 72$ kD with sialic acid as the constituent of carbohydrate moiety, typically binds 2 atoms of iron per molecule and diferric protein releases iron as the pH values decline along a semi-biphasic course.

(3). One Tf isoform each, purified from three other species of genus *Channa*: *C. punctatus, C. striatus* and *C. marulius*, show close resemblance in most of the above attributes (*e.g.* $M_r$, presence of sialic acid, pI values and conversion to diferric iron and its pH dependent release). In the absence of any previous published report on iron release from diferric fish Tfs, it may be inferred that semi-biphasic course of release is at least typical of channids Tfs, if not that of all the teleosts.
Some interspecies differences do exist between biochemical properties of Tfs. For instance: (i), Tfs of *C. gachua* and *C. punctatus* retain immunological cross-reactivity even after electrophoresis in SDS-PA gels, whereas Tfs of *C. marulius* and *C. striatus* do not; (ii), above noted distinction between Tfs of channid species into two subgroups is apparent from their IEF values; and (iii), also from their iron binding capacities and pH dependence of iron release.

The differences in pi values reflect the differences between the primary sequences or/and amino acid composition of Tfs of *C. gachua* and other sister species. Therefore, *C. gachua* and *C. punctatus* (which form a pi similarity pair) are likely to exhibit more interspecies affinities in this respect, while the same statement will hold for Tfs of *C. striatus* and *C. marulius*.

(3). The occurrence of a minimum number of Tf loci (two only) in *C. gachua*, in spite of being polyploid, has to have evolutionary implications. The available literature favours the origin of polymorphism of Tf by gene duplication; but the ultimate number of loci in a species may be determined by the selection pressure exerted by the critical physiological role of transferrin alleles. This might result in
deletion of loci instead of retention of all duplications. *C. gachua* may be representing such a case, since among the channids in spite of it being a polyploid, so far the least number of Tf alleles have been detected in its sera.

(4). The results, thus, establish that though Tfs of *C. gachua* as well as three other species of genus *Channa* are similar to other teleost Tfs in several respects, they do possess a few specific characteristic of their own. The specific biochemical and immunological characteristics may be correlated with the differences between the primary functions of iron binding capacities and release.

High iron binding capacities may also have a direct bearing upon the better chances of post-hatching survival that may, in turn, determine the genetic composition that is observed in a surviving population and discerned by Tf polymorphism. As already described, inter- and intraspecies differences in these crucial physiological properties have evolutionary implications also.