Hemoglobin variations in some tilapiine species (Teleostei, Cichlidae) of the genera *Oreochromis* and *Sarotherodon*

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Hemoglobin multiplicity in its broad sense has been well demonstrated in freshwater and marine teleost fishes including tilapias (CHEN and TSUYUKI, 1970; HINES *et al.*, 1971; FYHN *et al.*, 1979; PEREZ and RYLANDER, 1985; VAL *et al.*,1987; OBERST *et al.*, 1989; WEBER, 1990; MACARANAS *et al.*, 1996; FALK *et al.*, 1996, FALK *et al.*, in press). Our present studies on these oxygen binding molecules of 5 tilapiine species of the genera *Oreochromis* and *Sarotherodon* (*Oreochromis andersonii*, *O. aureus*, *O. niloticus*, *Sarotherodon galileus*, *S. melanotheron*) provide evidence indicating the occurrence of hemoglobin and globin chain variations within and among the species under study. In addition, some basic biochemical characteristics of these molecules, like their molecular weights (mws), isoelectric points (pIs), N-terminal globin chain sequence data and subunit compositions are presented.

Characteristically, all species investigated have been found to reveal highly heterogeneous hemoglobin phenotypes. On average about 23 distinguishable hemoglobins were detected per species by thin layer isoelectric focusing indicating the presence of multiple globin chain variants involved in the composition of different tetrameric hemoglobin types. Their estimated pIs ranged between pH 5.94 and pH 8.06. Moreover, variations of hemoglobin types among and within tilapia species have been observed resulting in the identification of species (*Oreochromis andersonii*, *O. aureus*, *O. niloticus*, *Sarotherodon galileus*, *S. melanotheron*), subspecies (*O. niloticus sugutae*) and hybrid (*O. niloticus* x *O. andersonii*) characteristic hemoglobin patterns.

This diversity of tilapia hemoglobins was shown to result from the occurrence of different types of globin chains. By acidic urea PAGE a total of seven major α-chains and five major β-chains could be identified and characteristic globin chain variants were found to occur. The species involved each showed two major α-chains and four (*O. andersonii*, *S. galileus*, *S. melanotheron*) or five (*O. aureus*, *O. niloticus*) β-chain variants. The mws of these monomeric hemoglobin types were estimated to range between 16.3 and 17.6 kDa indicating a molecular mass of about 65 to 70 kDa in their originally tetrameric form. These estimates were in agreement with results obtained by gel filtration chromatography for the tetrameric molecules (67 and 69 kDa).

According to MIED and POWERS (1978) the theorical number of hemoglobin tetramers of a given species may be calculated by combination of possible α/β-dimers, although some of them may be expected to exhibit unstable quaternary structures. Considering the relatively high number of postulated hemoglobin tetramers in the tilapiine species studied here one could expect the occurrence of pairs of identical α/β-dimers (symmetric tetramers) as well as associations of differently composed α/β-units (asymmetric tetramers) comprising a maximum of 4 different globin chains. This assumption was supported by extraction of particular hemoglobins obtained by isoelectric focusing of hemolysates and their subsequent analysis by acidic urea PAGE. Tetrameric hemoglobin variants were found to consist of doublets of identical α- and β-chains ($\alpha_2\beta_2$, symmetric tetramers), or combinations of three

($\alpha_2\beta\beta^*$; $\alpha\alpha^*\beta_2$) or four ($\alpha\alpha^*\beta\beta^*$) distinct chains (asymmetric tetramers). It is interesting to note that the majority of tilapia hemoglobins analysed could be considered to be asymmetrically composed.

Finally, globin chains of *O. niloticus* were isolated and subjected to partial N-terminal amino acid sequencing (pos. 1-40). The N-termini of both major α-chains were found to be blocked, a known charasteristic feature for a-chains of teleost fishes (HILSE and BRAUNITZER, 1968; POWERS and EDMUNDSON, 1972a,b; BOSSA *et al.*, 1976; D'AVINO *et al.*, 1990). Among the three major β-chains investigated (β_{2ONi}, β_{4ONi}, β_{5ONi}) amino acid replacements have been observed in positions 9, 12, 21 and 29 and a microheterogeneity has been found at amino acid position 12 of the β_{4ONi}-chain where Thr and Ala were detected in equal amounts. In comparison to known β-globin chain sequences of different fish species (GRUJIC-INJAC *et al.*, 1980; RODEWALD and BRAUNITZER, 1984; PETRUZELLI *et al.*, 1984) our data obtained for the β-chains of *O. niloticus* confirmed their β-chain identity and thus, supported our α/β chain notation for the globin chain variants of tilapias.

Our findings outlined here also demonstrate some interesting applications for hemoglobin and globin chain studies. First of all, the basic taxonomic issue of morphological species characterization and discrimination (THYS VAN DEN AUDENAERDE, 1970; TREWAVAS, 1983; TEUGELS and THYS VAN DEN AUDENAERDE, 1992) could be supported or refined by standardized hemoglobin and globin chain analysis techniques, in particular in case of morphologically similar species. Secondly, natural (POUYAUD, 1994) or artificial (TREWAVAS, 1983) interspecific hybridization events could be recognized and verified by hemoglobin and globin chain studies, an important application for fisheries management, aquaculture and the protection of small endangered tilapia populations. Finally, a further completion of population genetic studies and researches on aquacultural strains by hemoglobin and globin chain data could be of great future interest, since population characteristic differences have recently been described in tilapias and striking divergent functional properties among individual hemoglobin components have already been demonstrated in fish (HASHIMOTO *et al.*, 1960; BINOTTI *et al.*, 1971; POWERS, 1972;

POWERS and EDMUNDSON, 1972a,b; GILLEN and RIGGS, 1973b; BRUNORI, 1975; WEBER and DE WILDE, 1976; DI PRISCO and TAMBURRINI, 1992). This probably adaptive feature should be considered important especially, in regard to the variety of tilapia culture systems used to date. Moreover, it remains to be elucitated if these multiple hemoglobin types are an expression of optimum physiological adaptation to varying environmental conditions. As pointed out by PEREZ and MACLEAN (1976) it has to be considered that in tropical aereas water temperature and oxygen tensions are very variable and often fishes have to compete with an increasing osmotic pressure.

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