TITLE: Molecular cloning and expression patterns of two tumor necrosis factor alpha genes in crucian carp (*Carassius carassius*

Abstract

Tumor necrosis factor (TNF[31]) is a potent inflammatory cytokine produced during inflammation. In this study, two crucian carp *TNF*-α genes, *TNF*α-1 and *TNF*α-2, were cloned and sequenced. The *TNF*α-1 is 720 bp long and consists of a 699 bp opening reading frame (ORF) encoding 232 amino acids and *TNF*α-2 is 793 bp long and contains an ORF of 687 bp, which encodes 228 amino acids. The genomic structure of both genes consists of 4 exons and 3 introns similar to the other known *TNF*-α genes. The amino acid sequences of the crucian carp TNFα-1 and TNFα-2 share the highest identity with common carp, goldfish and the lowest with flounder and humans. The phylogenetic analysis grouped crucian carp TNFα-1 and TNFα-2 with other cypriniformes, which are more closely related to goldfish and common carp TNFα-1 and TNFα-2. Real time PCR analysis showed a constitutive expression of crucian carp *TNF*α-1 and *TNF*α-2 in all seven tissues examined. The *TNF*α-1 mRNA was expressed significantly higher in the liver and kidney than those of *TNF*α-2, while *TNF*α-2 in turn was expressed significantly higher in the muscle. The *Aeromonas hydrophila* BSK-10 strain upregulated the expression level of both *TNF*α-1 and *TNF*α-2 in all of the tested tissues. At 6 h, the expression levels of *TNF*α-1 were increased significantly higher in the muscles, skin, and liver, while the expression levels of *TNF*α-2 were increased significantly higher in the muscles and gills. *TNF*α-1 was expressed much stronger than *TNF*α-2. At 12 h the expression levels started to decline and were even lower at 24 h. These results imply that both *TNF*α-1 and *TNF*α-2 mRNA are distributed differently in tissues and are implicated in the immune response to bacterial infection.