

GENE EXPRESSION AND PROTEIN DEGRADATION PATHWAYS

IN RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

The efficiency with which fish utilise their food is related not only to food consumption, but is also influenced by rate of protein turnover. There is evidence that fish that have lower protein turnover for a given amount of protein consumed are able to convert more of the ingested food into growth. Protein synthesis rates appear relatively similar between animals when variation in food consumption is allowed for, but protein degradation seems to hold the key in explaining the efficiency of conversion of food to growth (Houlihan et al., 1995). Protein degradation rates are generally derived from protein growth and synthesis measurements. This indirect approach is necessary partly due to the difficulties in measuring a process that is carried out by a variety of enzyme pathways. In order to make progress in this difficult but key area we are using several molecular approaches.

Both non-lysosomal and lysosomal pathways of protein degradation are involved in the control of the amino acid regulation, both these pathways are highly controlled and regulated (Attaix *et al.*, 1999). The non lysosomal ATP dependant ubiquitin proteasome pathway, is the major route of degradation of muscle protein in mammals that releases amino acids and is dramatically upregulated during acute wasting diseases (Wing *et al.*, 1995). During this route of proteolysis targeted proteins are ubiquitinated prior to proteolytic cleavage by the proteasome. This is a tightly regulated process and mRNA levels for the

ubiquitin proteasome pathway reflect the changes in proteolytic activity. In liver tissue the major route of protein breakdown is via the lysosome where cathepsins are especially important. The pathways that regulate protein turnover have not been studied in detail in fish. We have investigated pathways of protein breakdown in fed and starved rainbow trout using a variety of molecular approaches.

Results and Discussion

Northern blot analysis of RNA extracted from rainbow trout muscle tissue demonstrates an increase in abundance of polyubiquitin mRNA in fish that have had food withdrawn when compared to fed controls (figure 1). There is a corresponding increase in mRNA abundance of the proteasome subunit N3. These results confirm a general increase in gene expression for components of the ATP dependant protein degradation pathway (Wing et al., 1995). No up regulation for these genes was observed in RNA extracted from liver.

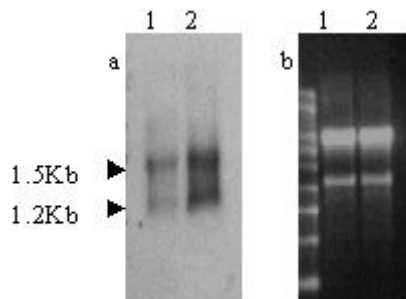


Figure 1. (a) Northern blot analysis of rainbow trout polyubiquitin mRNA in muscle tissue. 10 μ g of total RNA was separated on a 1.2% agarose gel, lane 1, fed fish, lane 2, food withdrawn. The RNA was transferred to nylon membrane and fixed by UV light. The membrane was probed with a trout polyubiquitin cDNA probe labelled with 32 P-dCTP, and washed to high stringency (0.2 x SSC, 65°C). (b) Ethidium bromide stained gel to demonstrate equal loading of RNA.

To identify the proteins / genes differentially expressed in the liver during starvation we are using proteomics to examine changes in protein expression patterns. Protein profiles generated by high resolution 2 D PAGE, coupled with digitised gel image analysis software allows the position and intensity of hundreds to thousands of proteins to be monitored, giving a global picture of these in proteins in a tissue under different metabolic states. In-gel trypsin digests of individual protein spots, combined with mass spectrometry to accurately size the tryptic peptides produces characteristic peptide fingerprints. The peptide masses were used to search the NCBI data base using the MASCOT search program (Perkins, *et al.*, 1999).

One protein spot that was found to consistently more abundant in the starved fish was cut from the gel and the trypsin digest finger print produced allowed the protein to be identified as cathepsin D (Brooks *et al.*, 1997). Figure 2 shows the abundance of the cathepsin D in six individual fish, three fed and three starved. This enzyme, a member of the pepsin family is a lysosomal endopeptidase involved in proteolysis.

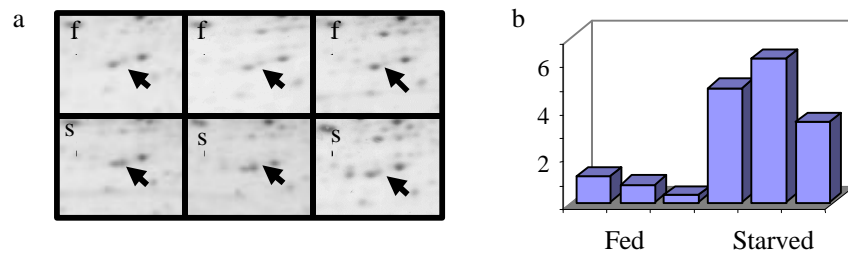


Figure 2.D protein patterns (a) were digitised using Phoretix 2-D software. Relative intensity of the cathepsin D protein in livers of fed and starved trout the protein spots were normalised for each gel (b).

In this study we have demonstrated that components of both the lysosomal and non lysosomal pathways of protein degradation are enhanced after a period of food withdrawal. Questions can now be addressed as to how these pathways are regulated in normal growth and if they can be correlated to feeding efficiency.

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