Determining Protein Turnover in Fish with D_7-Leucine

Mary K. Doherty,[1] Iain S. Young,[2] Simon J. Davies,[3] Phillip D. Whitfield[1]

1 Department of Diabetes and Cardiovascular Science, University of the Highlands and Islands, Inverness, UK
2 Institute of Integrative Biology, University of Liverpool, Liverpool, UK
3 Fish Nutrition and Health Research Group, School of Biological and Biomedical Sciences, University of Plymouth, Plymouth, UK

The proteome of a biological system is a dynamic entity and in constant flux (Doherty and Beynon, 2006). Different proteins turn over at distinctly different rates and even in a position of apparent steady-state, the protein complement is constantly changing. Moving from a static "snapshot" of a proteome to a dynamic view presents a considerable technical challenge, however, the utilization of stable isotope labeling of organisms in conjunction with mass spectrometry has led to considerable advances. These novel proteomic technologies have introduced the possibility of determining the turnover rates of multiple proteins in intact animal species including chicken and mice (Doherty, et al., 2005; Price, et al., 2010; Claydon, et al., 2011). We have extended this experimental strategy to measure the rates of synthesis and degradation of individual proteins in the skeletal muscle of fish (Doherty, et al., 2012) (Figure 1). In particular, we were interested in whether it was possible to distinguish the rates of synthesis of a family of isomeric proteins, β-parvalbumins. In our study, common carp were fed with an experimental diet in which 50% of the L-leucine in the diet was replaced with crystalline L-Leucine (isopropyl-D_7, 98%) (DLM-4212). Leucine was used as this is an essential amino acid and abundant in the carp proteome (Murai and Ogata, 1990). Importantly, the signature tryptic peptides from the individual parvalbumin β-isoforms all contain a leucine residue. The time-dependant incorporation of the isotope into parvalbumin isoforms was monitored by LC/MS analysis of the signature peptides and the data deconvoluted using mass isotopomer distribution analysis (Hellerstein, et al., 1992). Our data showed that the absolute rate of synthesis of parvalbumin β-isoforms in the skeletal muscle of common carp differed by an order of magnitude under steady-state conditions. Whilst the focus of our work was on specific isoforms, this approach can be used to determine the turnover of multiple proteins in carp tissues. The methodology may also be adapted to study proteome dynamics in different species of fish.

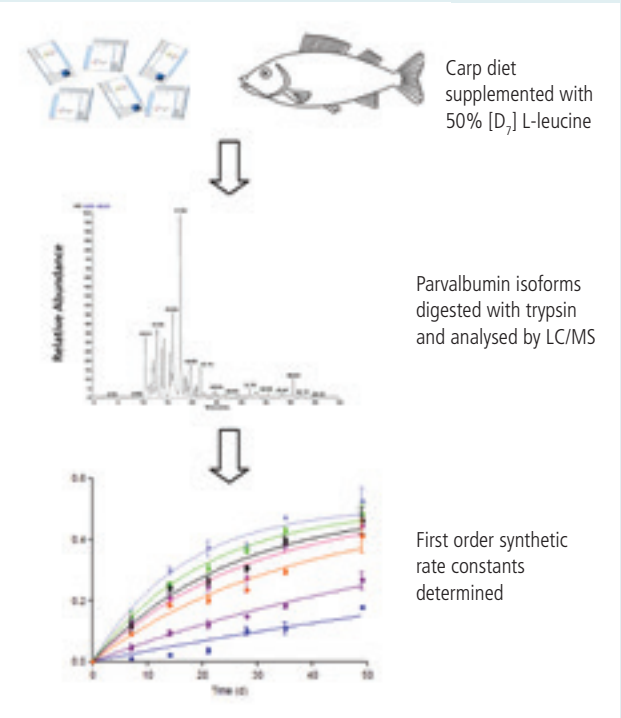

Figure 1.

Related Product

Catalog No.	Description
DLM-4212	L-Leucine (isopropyl-D_7, 98%)

References

Claydon, A.J.; Thom, M.D.; Hurst, J.L.; Beynon, R.J. **2012**. Protein turnover: measurement of proteome dynamics by whole animal metabolic labeling with stable isotope-labeled amino acids. *Proteomics, 12*: 1194-1206.

Doherty, M.K.; Beynon, R.J. **2006**. Protein turnover on the scale of the proteome. *Expert Rev Proteomics, 3*, 97-110.

Doherty, M.K.; Brownridge, P.; Owen, M.A.; Davies, S.J.; Young, I.S.; Whitfield, P.D. **2012**. A proteomics strategy for determining the synthesis and degradation rates of individual proteins in fish. *J Proteomics, 75*, 4471-4477.

Doherty, M.K.; Whitehead, C.; McCormack, H.; Gaskell, S.J.; Beynon, R.J. **2005**. Proteome dynamics in complex organisms: using stable isotopes to monitor individual protein turnover rates. *Proteomics, 5*, 522-533.

Hellerstein, M.K.; Neese, R.A. **1992**. Mass isotopomer distribution analysis: a technique for measuring biosynthesis and turnover of polymers. *Am J Physiol, 263*, E988-1001.

Murai, T; Ogata, H. **1990**. Changes in free amino acid levels in various tissues of common carp in response to insulin injection followed by force-feeding an amino acid diet. *J Nutr, 120*, 711-718.

Price, J.C.; Guan, S.; Burlingame, A.; Prusiner, S.B.; Ghaemmaghami, S. **2010**. Analysis of proteome dynamics in the mouse brain. *Proc Natl Acad Sci USA, 107*, 14508-14513.