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APPLICATION NOTE 29

Determining Protein Turnover in Fish with D₇-Leucine

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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-D₇, 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-dependent 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.

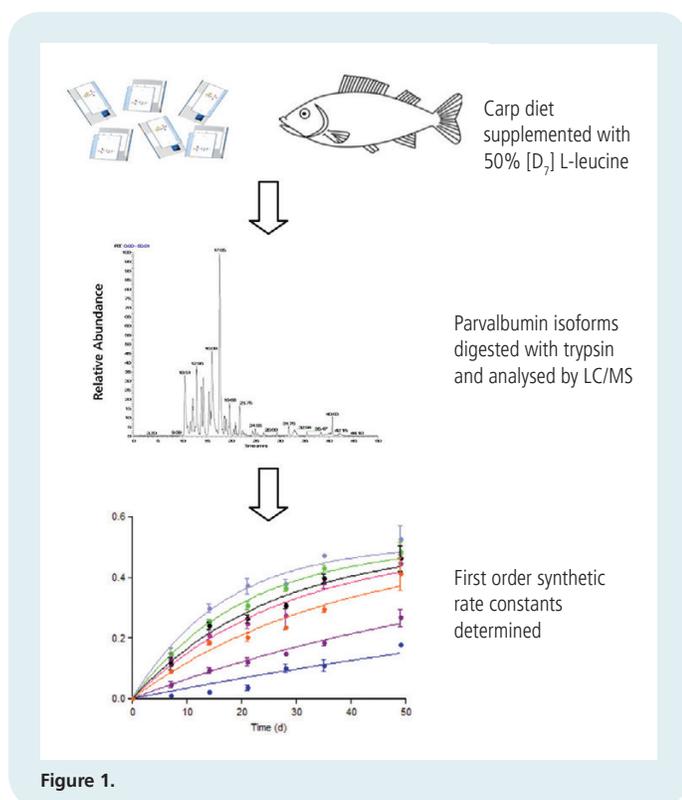


Figure 1.

Related Product

Catalog No.	Description
DLM-4212	L-Leucine (isopropyl-D ₇ , 98%)

References

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