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Chromatographic and electrophoretic separation techniques for detection of protein post-translational modifications

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Modern bioinformatics has facilitated the production of databases of protein families, protein domains, and functionally active and modified amino acid residues. However within a tissue, only a proportion of protein family members may be expressed and/or be active at a given time. Hence suitable screening methods are required to monitor specific protein enzymatic activity, and this may be provided by native protein or synthetic peptide substrates. The provision of a specific and sensitive substrate enables detection but moreover selective enrichment and purification by column chromatography of the bioactive enzyme. Rational peptide design can provide suitable peptide target substrates to also monitor defined post-translational modifications. Kinases constitute an extensive family of proteins that post-translationally modify target substrates directly via the transfer of phosphate. Detection and purification of kinases has been facilitated by the commercial availability of radiochemicals such as the phosphate donor, [g-32P]-ATP. Herein I describe the use of rationally designed peptide sequences to track and purify kinases activated by inflammatory cytokines. I will also detail electrophoretic methodology that can be exploited to enable removal of extraneous unincorporated radiolabel, to enable visualization of phosphorylation by autoradiography, or phosphorylation quantitation using liquid scintillation counting. Hence suitably designed peptide targets can provide a means to track an enzyme of interest, and quantify the stoichiometry of protein modification at a pre-defined site(s).

Biography

Wayne Grant Carter received his Honours degree in Biochemistry with Nutrition from the University of Southampton. He then completed a PhD at the University of Southampton and post-doctoral studies at the Babraham Institute, Cambridge; Imperial College, London; University of California at Irvine; and University of Oxford, before taking up an industrial post with Mobious Genomics, Exeter. In 2003, Dr Carter joined the staff of the University of Nottingham, where he is currently a Principal Investigator and Lecturer. His research studies pioneered the use of proteomic purification techniques coupled to novel imaging of radiolabels to detect and purify proteins that are modified post-translationally.

He has received external funding for his studies from the Wellcome Trust, UK Sport, the , and Syngenta, UK. He has peer-reviewed manuscripts for numerous academic journals, and is currently an editorial board member of the World Journal of Biological Chemistry, Current Chemical Research, Journal of Analytical Sciences, Methods and Instrumentation, and executive editor for the Journal of Chromatography & Separation Techniques.

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