

ANALYSIS OF PHEROMONAL LIGAND BINDING TO CARRIER PROTEINS USING ION TRAP AND FT-ICR MASS SPECTROMETRY

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The sex pheromone of the Asian elephant, Z-7-dodecen-1-yl acetate, is bound to serum albumin (a 68 kDa alpha helical protein) in the blood of preovulatory females and is excreted in this sequestered form in urine. Male elephants sample the alkaline urine deposits by mixing the urine with acidic trunk mucus thereby releasing volatile pheromone in a pH-mediated fashion that transitions eventually to olfactory receptors in the vomeronasal organ. A portion of this free pheromone is “mopped-up” by copious odorant binding protein (OBP, an 18 kDa beta-barrel lipocalin) a major protein in the secreted mucus. Binding has been demonstrated to both proteins by passive attachment using both a GC-based volatile odorant binding assay and on polyacrylamide gels using a radiolabeled pheromone analogue and autoradiography. We have also used covalent attachment to binding proteins with a photoaffinity analogue, Z7-dodecen-1-yl diazoacetate, using both cold and tritiated forms of this ligand. Archival SDS polyacrylamide gels of several years standing provided the sample source for a proteomics analysis of excised Coomassie-stained protein spots of OBP and albumin samples reacted with the diazoacetate pheromone analogue. Using New Zealand-based Finnigan™ LCQ Deca and LTQ FT™ ion-trap mass spectrometers operating in nanoelectrospray mode, we have now examined peptides from trypsin digestion of protein bands searching for covalently attached adducts. Pheromone analogue fragments were found covalently bound to specific serine, threonine and tyrosine residues on several peptides following SEQUEST® and SALSA analysis of the data. These modified peptides map in close proximity to suspected binding site residues based on 3-D homology models of these proteins. This finding demonstrates the applicability of mass spectrometry to help analyze ligand-protein associations working with archival gel material.

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