Identification of Transmembrane Tryptic Peptides from the Integral Membrane Protein Rhodopsin Using Mass Spectrometry [MAS]

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Rhodopsin was used as a model integral membrane protein for the development of a mass spectrometric technique designed to identify hydrophobic peptides generated by enzymatic digests. Affinity purified rhodopsin, as well as rhodopsin in retinal rod membranes, were digested with trypsin. Tryptic peptides were separated using a modified reverse phase HPLC technique with the detergent octyl-pglucoside in the mobile phase. The fractionated peptides were analyzed by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry using a-cyano-4- hydroxy cinnamic acid (ACHCA) as the matrix. The putative transmembrane regions of rhodopsin contain six tryptic peptides. Four of the six tryptic peptides, ranging in mass from 3,259Da to 6,528Da, were

identified by their molecular weight and by the amino acid sequence for five of their N-ternimal residues found by Edman micro-sequencing. In addition, heterogeneity in the glycosylation of the N-terminal tryptic peptide of rhodopsin was also identified using this method without modifying the carbohydrate prior to analysis by MALDITOF mass spectro-metry. This study demonstrates a new utility for mass spectrometry in the analysis of integral membrane protein structure.