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R. Kellner,[1] T. Houthaeve,[1] T. Kurzchalia,[2] P. Dupree,[2] and K. Simons.[2] Internal Fragmentation of Proteins in Polyacrylamide Matrices for Microsequencing. ([1] Biochemical Instrumentation Programme, [2] Cell Biology Programme, European Molecular Biology Laboratory, Meyerhof-Str. 1, D-6900 Heidelberg, Germany)

Direct N-terminal sequencing is not applied to proteins available in extremely small quantities because of the risk of a blocked N-terminus and therefore loss of the rare sample. Instead, it is preferable to use the entire, minute amount of sample for internal fragmentation and subsequently for microsequencing. One- or two-dimensional gel electrophoresis is the most general method as a final purification step within a protein isolation protocol. For characterization, it is then necessary to get rid of the polyacrylamide matrix, and this is usually done by electrotransferring the protein onto an appropriate membrane, such as PVDF, glassfiber, or nitrocellulose. The yields for blotting differ greatly. Thus, the most straightforward way is to digest the protein directly within the polyacrylamide matrix (Eckerskorn and Lottspeich, 1989), elute the fragments, fractionate them by microbore-HPLC, and sequence them. We have successfully applied this strategy in several investigations on cellular proteins.

In the first application described, we identified the cytosolic protein elongation factor-1a (EF-1A), actin, vimentin, and desmin as putative cytosolic interaction partners of Rab5 (Kurzchalia *et al.*, 1992*a*). Rab5 is a small GTP-binding protein of the *ras*-super family and has been shown to be involved in early endosome fusion. *In vitro* synthesized Rab5 was cross-linked in the presence and absence of ATP to cytosolic proteins. Unexpectedly, ATP depletion of cytosol led to *de novo* formation of filaments with which crosslinked and noncrosslinked Rab5 was strongly associated. Coomassie staining after 1D-PAGE showed that the filaments contained two dominant proteins of 42 and 48 kD. The tryptic digest *in matrix* of these peptides and subsequent microsequencing of resulting fragments identified them as actin and EF-1a, respectively. A similar procedure after 2D-gel analysis of the sample enabled identification of two further proteins as desmin and vimentin. Our data suggest that cytosolic Rab proteins interact with the cytoskeleton.

In a different study, we characterized a new vesicular membrane protein that is proposed to be a component of the molecular machinery of vesicular transport (Kurzchalia *et al.*, 1992*b*). In polarized epithelial cells, apical and basolateral proteins are sorted into separate vesicular carriers before delivery to the appropriate membrane domains. In order to dissect the putative sorting machinery, we have solubilized Golgi-derived transport vesicles with the detergent CHAPS and shown that an apical marker, influenza haemagglutinin (HA), formed a large complex together with several integral membrane proteins. Remarkably, a similar set of CHAPS-insoluble proteins was found after solubilization of a total cellular membrane fraction. A 21 kD protein-band of this complex was cut out after 1D-SDS-PAGE. The Coomassie-stained band was digested with trypsin overnight and the resulting fragments were extracted from the gel matrix. Peptide fragments were separated on a 1.6 mm ID column in the RP-HPLC mode and subjected to microsequencing. The sequence information obtained in this way enabled us to design a degenerate oligonucleotide and finally to isolate the full-length cDNA clone. The cDNA encoded a protein containing the peptide fragments already identified and was in good agreement with the estimated molecular weight from SDS-PAGE. The cDNA was confirmed to encode the investigated protein by *in vitro* transcription and translation of the clone, yielding a labeled product that showed identical mobility in the 2D-gel system. We named the protein VIP21 (Vesicular Integral membrane Protein of 21 kD).

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Tomas Bergman. Application of Capillary Electrophoresis for Protein Sequence Analysis. (Department of Chemistry I, Karolinska Institutet, S-104 01 Stockholm, Sweden)

Capillary zone electrophoresis offers an interesting analytical approach for peptides (Karger *et al.*, 1989) with high sensitivity, short separation time, and low consumption of material. Although the high resolution would make the technique extremely powerful for peptide purifications in structural analyses, the small amounts (fmol) and volumes