Hans-Arthur Bradaczek and Brigitte Wittmann-Liebold. The Development of a C-Terminal Sequencer. (Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, Ihnestraße 73, D-1000 Berlin 33, F.R.G.)

With modules of the Knauer N-terminal sequencer, equipment for the solid-phase C-terminal degradation has been designed (Wittmann-Liebold *et al.*, 1991). Several modifications of the Schlack-Kumpf chemistry have been applied on this machine (Schlack *et al.*, 1926). The activation of the carboxyl group of the peptide as a mixed anhydride has been performed with acetic anhydride and Fmoc-Cl (unpublished results). It was found that acetic anhydride drastically alters proteins and larger peptides. On SDS-page, the spot of treated protein disappears. Therefore, we looked for a new activation reagent. An organic solution of Fmoc-Cl is suitable to activate the carboxyl group under mild conditions (reaction temperature lower than room temperature). For the coupling of thiocyanate to form the thiohydantoin amino acid, several isothiocyanates have been tested: the free acid, HSCN (Inglis *et al.*, 1990), silylated thiocyanates (Bailey *et al.*, 1990), and guanidine thiocyanate (Wittmann-Liebold *et al.*, 1991). Good results were achieved employing Fmoc-Cl for the activation and guanidine thiocyanate for the coupling. An aqueous solution of KOH is used to cleave the thiohydantoin from the peptide (Inglis *et al.*, 1991). After the degradation, the thiohydantoin amino acids are detected online in the machine using a reversed-phase gradient HPLC-system (Inglis *et al.*, 1990). Further, a new method for the covalent attachment of peptides and proteins to succinic glass beads, using PyBOP as activation reagent, as employed in peptide synthesis (Le-Nguyen *et al.*, 1987), has been developed (unpublished results). Under these conditions, a C-terminal degradation of more than three residues has been performed in the sequencer.

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Kiyoshi Nokihara,[1] Jun Kondo,[2] Rintaro Yamamoto,[1] Akira Ueda,[1] and Makoto Hazama.[1] Instrumentation and Applications of a Novel C-Terminal Peptide Fragment Fractionator. ([1] Biotechnology Instrument Department, Shimadzu Corp., Kyoto, Japan; [2] Mitsubishi Kasei Corporation Research Center, Yokohama, Japan)

For the primary structure elucidation, C-terminal sequence information is very important. Over the last few years much effort has been devoted to the C-terminal cleavage followed by identification of the resulting amino acid derivatives (Inglis, 1991). This methodology is, however, still limited. Several amino acids such as His, Gln, and Pro were difficult to identify. Ishii and his coworkers have presented a method for the fractionation of the C-terminal fragment using anhydrotrypsin (Kumazaki *et al.*, 1986). However, this method requires considerable experience; in addition, affinity of the resulting tryptic fragments against this particular ligand is sequence-dependent. Recently, Tsugita and his coworkers presented a simple C-terminal sequencing method using mass spectrometry (Tsugita *et al.*, 1992). This method seems to be very powerful. However, instrumentation is expensive, the ionization depends on the nature of the proteins, and difficulties can be envisaged for large proteins, in particular for nonhomogeneous samples.

Today many protein researchers use Edman-type microsequencers. We have developed an automated instrument based on a facile method to separate the C-terminal fragment after endoprotease Lys-C digestion, followed by covalent immobilization of digests on DITC-polymer beads (Kondo and Ohuchi, 1989). After covalent linkage of α and ε amino groups of digests to the DITC-beads of the column, which is disposable, α amino groups can be cleaved by TFA to liberate C-terminal fragments which have no Lys residue. Utilizing this chemical protocol, we have developed a novel automated C-terminal peptide fragment separator with optimized reaction conditions. The resulting C-terminal fragment can be easily sequenced by conventional gas-phase protein sequencers, such as Shimadzu PSQ-1 or ABI-477A, for samples in the 30–500 pmol range.

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