

Improved Protein Sequencing Reagents for Proteomics

Proteomics – the Challenge for the New Decade

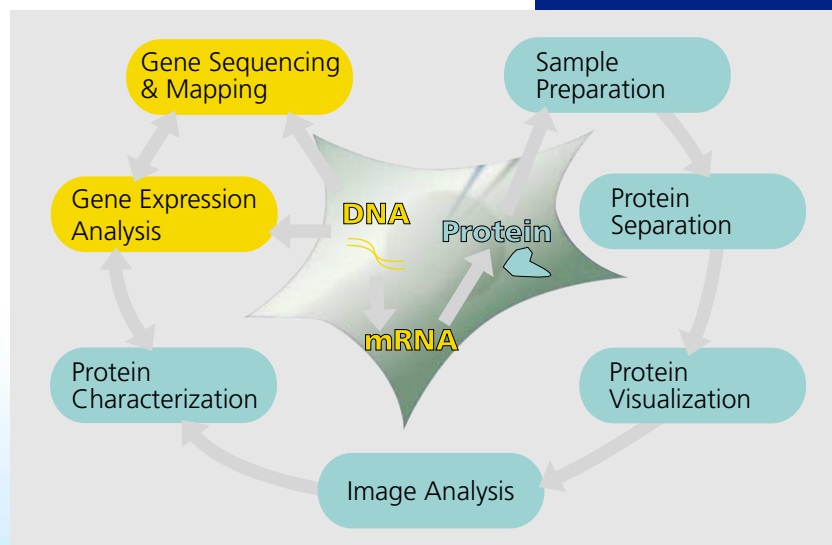
Understanding biological systems initially requires thorough knowledge of the chemical composition of cellular systems. While genomics is moving forward in leaps and bounds and more and more of DNA sequence information becomes available, the proteome is still somewhat of a mystery. The proteome, the entire set of proteins present in a cell, tissue or organism, is equivalent to the total expressed genome. Most of the proteins are alternatively spliced and post-translationally modified. Post-translational modifications include acetylation, phosphorylation, methylation, glycosylation and ubiquitination, just to mention a few. Besides that it is varying over the time course. Due to these facts and to the diversity of chemical properties between different types of proteins, the analysis of the protein is believed to be much more complicated than that of the genome. But only the knowledge of the proteome will really enable the complete understanding of biological systems.



The complexity of cellular protein patterns requires the separation and identification of thousands of proteins. High performance separations, first of all 2D-electrophoresis, but also high performance liquid chromatography and capillary electrophoresis, are used to resolve the separation issues. Identification and

characterization of separated proteins is achieved by mass spectrometry and N-terminal Edman sequencing.

Figure 1:
Proteomics – an overview



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- **Edman Sequencing – a Well-known Technology with ever Increasing Sensitivity**

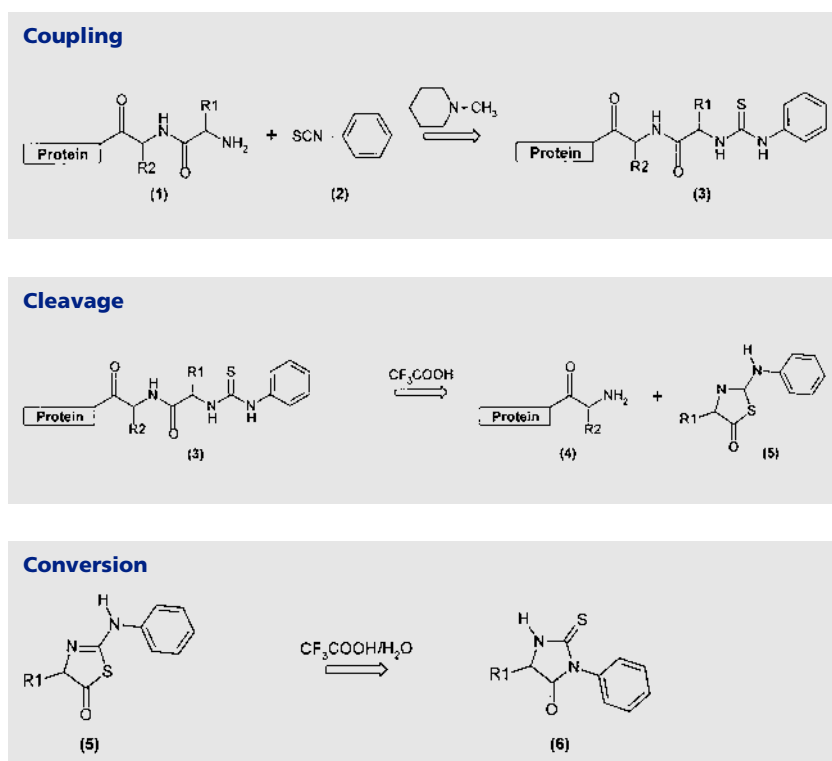
Edman Sequencing – a Well-known Technology with ever Increasing Sensitivity

Due to the progress of instrument manufacturing, the traditional Edman chemistry has gained extremely high sensitivity, allowing sequencing of proteins in the lower pico- or upper femtomol level. An important precondition for successful protein sequencing of such low sample sizes is also the use of ultra pure high quality reagents. It is obvious, that a contamination of solvents or reagents with just some picomols of reactive impurities significantly lowers the quality of Edman sequencing.

Figure 2 shows a simplified scheme of the Edman chemistry. Following coupling of N-terminal amino groups of protein (1) with phenylisothiocyanate (PITC, Edman reagent) (2) forming an open chain thiourea derivative (3) the N-terminal amino acid is split off as anilinothiazolinone (ATZ) derivative (5) with trifluoroacetic acid. This unstable ATZ-derivative is transferred into a more stable phenylthiohydantoin (PTH) derivative (6), which is separated by reversed phase HPLC and identified by comparison with a standard.

The yield of PTH-amino acid is highly dependant on the quality of solvents and reagents used. Especially lysine and tryptophan are extremely sensitive and may be degraded even by the smallest amounts of oxidizing agents. This is demonstrated for lysine in figure 3, which shows two sequencing cycles representing the sequence – M-K- of a standard peptide. Sequencing was per-

Figure 2:
Reaction scheme of
Edman sequencing



formed with a series of improved Fluka reagents specially designed to meet current quality requirements of protein sequencing. These chromatograms are characterized by a very low background and especially a strong signal of lysine, see figure 3 (b). In comparison figure 4 reveals the sequencing of the same peptide using ethyl acetate for protein sequencing offered earlier. Although this ethyl acetate contains DTT as a protecting agent [recognizable by the PITC-DTT adduct peak (*)] the yield of PTH-lysine is significantly reduced.

Figure 5 demonstrates the influence of PITC quality on sequencing results. Besides the high DPU peak, which complicates the detection of tryptophan, the chromatogram shows a disturbing artificial peak in the area of PTH-glutamic acid. Additionally both initial yield (2.9 pmol instead of 5.1 pmol) and repetitive yield (small peak of K) are significantly lowered.

The conversion of unstable ATZ-amino acids to PTH-derivatives takes place in the presence of aqueous acetonitrile and trifluoroacetic acid solution. The quality of these reagents also influences the yield of PTH-amino acids. For high quality reagents the so called blank (without cartridge reagents) does not show any peak. Usage of highest HPLC gradient grade acetonitrile instead of sequencing grade quality results in a characteristic artificial peak after approx. 7.5 min running time (figure 6).

The range of high quality reagents required for automated sequencing reactions is accomplished by ultra pure acetonitrile (with DTT) suitable for the preparation of PTH amino acid standards. Only specifically purified acetonitrile ensures the stability of the standard for a sufficient time. This is demonstrated in figure 7. 7 (a) shows a standard prepared with ultra pure acetonitrile after 1 month storage. Figure 7 (b) shows a standard prepared in acetonitrile, HPLC-quality with addition of DDT after 2 days storage. In the case of HPLC-quality after two days His, Arg, Met and Lys have already decreased obviously and several additional peaks have appeared.

The above mentioned examples demonstrate the requirement for an extraordinary high quality of reagents to meet current needs for protein sequencing. Fluka reworked its reagents for protein sequencing and is actually offering a set of reagents specifically designed for routine automatized sequencing. These reagents are prepared, specified and tested to meet sample sizes in the femtomol range. The packaging enables direct use in current ABI sequencers.

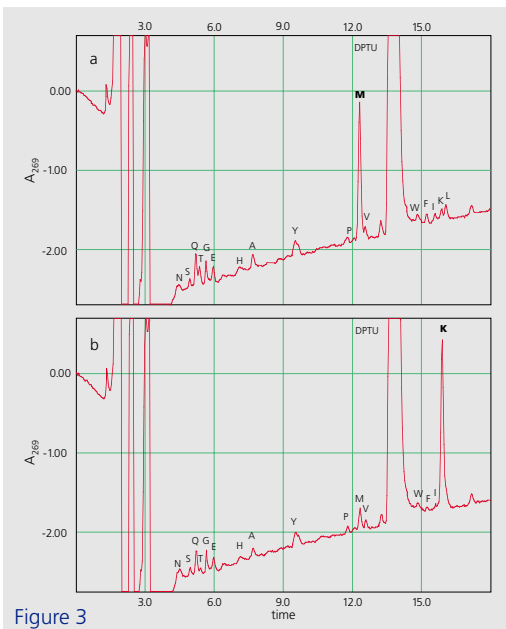


Figure 3

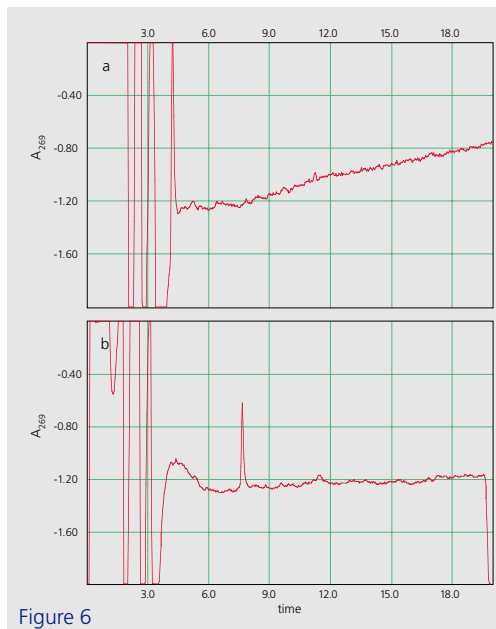


Figure 6

Figure 3:
Edman determination of sequence – M-K- using current Fluka reagents
Yield: M: 5.1 pmol; K: 5.5 pmol

Figure 4:
Edman determination of sequence – M-K- with ethyl acetate as offered earlier
Yield: M: 4.5 pmol; K: 3.0 pmol
(*)= PITC-DTT adduct peak

Figure 5:
Edman determination of sequence – M-K- with insufficient quality of PITC
Yield: M: 2.9 pmol; K: 1.8 pmol

Figure 6:
Blank using specified Fluka acetonitrile (a) and highest HPLC gradient quality (b)

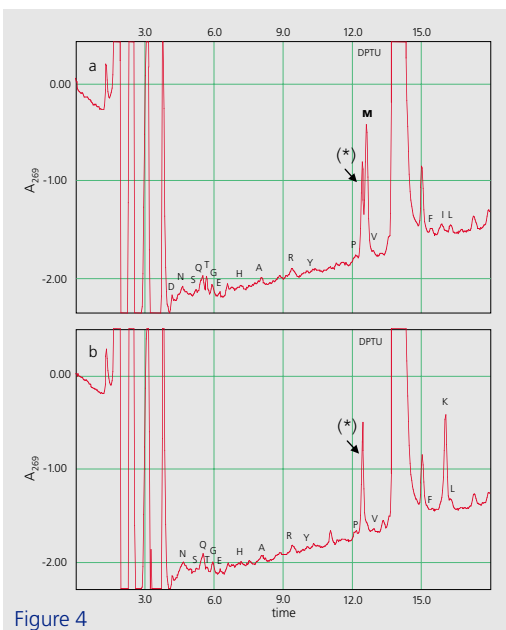


Figure 4

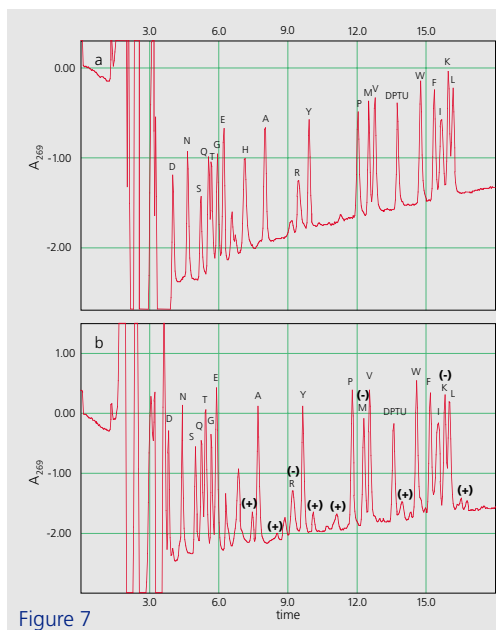


Figure 7

Figure 7:
PTH amino acid standard (a) prepared with specified Fluka acetonitrile, 1 month storage (b) prepared with HPLC acetonitrile and DDT, following 2 days storage
Quantity of PTH amino acid standard: 4 pmol

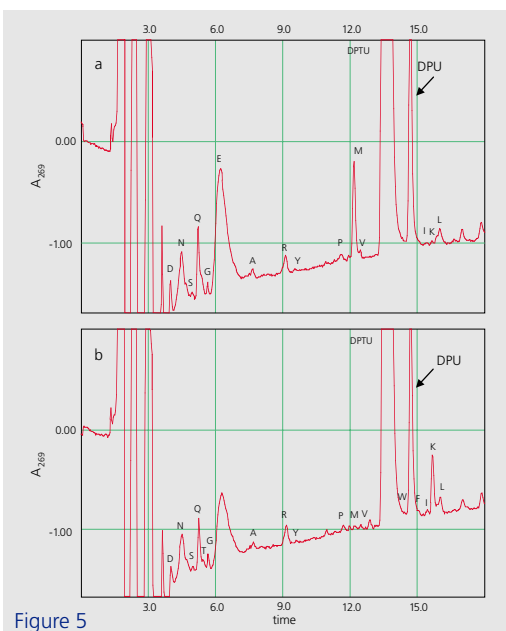


Figure 5

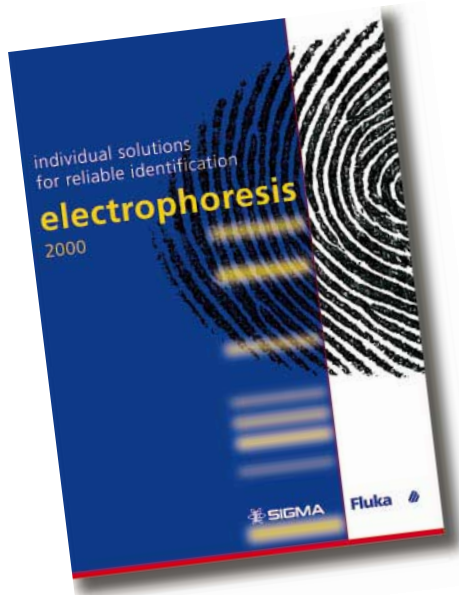
Fluka offers the following core products and packaging sizes:

Reagent	Product no.	Package size
TFA	09653	40 ml
25% TFA solution	91709	40 ml
20% Acetonitrile	00712	200 ml
Acetonitrile (with DTT)	00682	40 ml
Heptan	09642	100 ml
Ethylacetate	09652	450 ml
Butylchloride	09651	200 ml
5% PITC solution	78787	40 ml
Methylpiperidine solution	68835	40 ml

Besides these we also offer many special reagents for less widely used sequencing applications.

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