

Chang J-Y, Brauer D & Wittmann-Liebold B. Micro-sequence analysis of peptides and proteins using 4-*NN*-dimethylaminoazobenzene 4'-isothiocyanate/phenylisothiocyanate double coupling method. *FEBS Lett.* 93:205-14, 1978.

[Max-Planck-Institut für Molekulare Genetik, Berlin (Dahlem), Germany]

This paper describes a manual method for protein sequence analysis using a colored Edman reagent. The method allows sequence information to be obtained from low nanomole to picomole quantities of peptides and proteins with only simple facilities. [The *SCI*® indicates that this paper has been cited in more than 830 publications.]

Protein Sequencing at the Picomole Level Using Color Edman Reagent

J.-Y. Chang

Pharmaceuticals Research Laboratories
Ciba-Geigy Ltd.
Basel CH-4002
Switzerland

In 1972, I started my postgraduate studies in the lab of J.-K. Lin, at the National Taiwan University, Taipei. The research project assigned to me was the synthesis of an analog of dansyl chloride (dimethylaminonaphthalene sulfonyl chloride), which was both fluorescent and colored. Dansyl chloride was then a widely used fluorescent reagent for N-terminal analysis of proteins.¹

To start the project, I was given 8 NT\$ (30 cents US) to purchase a half kilogram of toilet deodorant (the naphthalene ball) from a nearby store. The naphthalene was crystallized, nitrated, reduced, methylated, and then coupled to a diazonium salt. The color appeared, but the fluorescence disappeared as a result of diazotization. The experiment was considered a failure, and I was told to switch the project to study the carcinogenic effect of azodye on chicken embryos.

I have always disliked doing experiments on animals. Determined to stay away from the chicken egg, I suggested to Lin that we modify our original goal by synthesizing only the color version of dansyl chloride. We prepared dimethylaminoazobenzene sulfonyl chloride (dabsyl chloride),² a reagent that turned out to be very useful for amino acid analysis.

During the decade of the 1970s, protein chemists began to encounter the pressing need for

sensitive protein sequencing methods. The only practical method available then was Edman (phenylisothiocyanate) degradation.³ As a result of my postgraduate work, I conceived the idea of synthesizing a color Edman reagent to improve the sensitivity of manual sequencing. However, I was only able to put this idea into practice two years later, when I undertook my PhD studies at the Australian National University, Canberra. In the end, the synthesis and application of a color Edman reagent, dimethylaminoazobenzene isothiocyanate (DABITC), was to become the major content of my PhD thesis.

With DABITC, it became possible to monitor visually each step of the manual sequencing. More importantly, as little as 10 picomoles of the released N-terminal amino acids could be identified on a stamp-sized thin-layer plate with the naked eye.

I was genuinely delighted by these results and believed that a method had been found for protein sequencing at the picomole level. I was eager to find an application to prove it. In 1978, I brought the DABITC method to the lab of B. Wittmann-Liebold at the Max Planck Institute in Berlin. There, I refined a protocol by adapting the DABITC/phenylisothiocyanate double coupling. The usefulness of the DABITC technique was convincingly demonstrated as it facilitated the completion of the sequence analysis of ribosomal proteins.

In my opinion, the popularity of the manual DABITC technique stems primarily from the ease and high sensitivity of analyzing the colored amino acid derivatives on thin-layer chromatography. To date, the DABITC manual sequencing method is still used by many labs that cannot afford to install an automatic sequencer. In our lab, the DABITC technique is routinely applied for one step quantitative N-terminal analysis (QNA)⁴ of polypeptides—an extremely useful technique that was widely used in the 1960s and 1970s, but somehow got lost in the 1980s.

1. Gray W R. End-group analysis using dansyl chloride. *Meth. Enzymology* 25:121-37, 1972. (Cited 1,120 times.)
2. Lin J-K & Chang J-Y. Chromophoric labeling of amino acids with 4-dimethylaminoazobenzene-4'-sulfonyl chloride. *Anal. Chem.* 47:1634-8, 1975. (Cited 70 times.)
3. Edman P & Begg G. A protein sequenator. *Eur. J. Biochem.* 1:80-91, 1967. (Cited 2,265 times.) [See also: Begg G. Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 2: the molecules of life.* Philadelphia: ISI Press, 1986. p. 203.]
4. Chang J-Y. A complete quantitative N-terminal analysis method. *Anal. Biochem.* 170:542-56, 1988. (Cited 10 times.)

Received October 22, 1990