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The detection of peptides on paper with phenyl isothiocyanate

In the past few years several methods for the separation of peptides on paper have been developed by using a combination of electrophoresis and chromatography[1-4]. These techniques have been very useful, not only for mapping proteins after proteolytic digestion but also as a first step in structural analysis of proteins. In general, the location of the peptides on paper has been detected by wetting the paper in a weak ninhydrin solution. Following development of the spots, they are cut out and eluted prior to analysis. However, ninhydrin causes a destruction of the peptides despite the very low concentration of reagent used. For this reason, the detection of peptides on paper, followed by elution and analysis has presented some technical difficulties. Some workers prefer to make two parallel runs, one of which can be developed with ninhydrin and used as a map for the location of peptides on the second paper. The difficulty in obtaining exact duplicates, however, makes this technique more or less insensitive. This is particularly apparent when one attempts to separate a complex peptide mixture for subsequent analyses.

Studies in our laboratory on the structure of peptides[5,6] have demonstrated the efficiency of the EDMAN procedure[7] which utilizes phenyl isothiocyanate as a reagent. Phenyl isothiocyanate reacts with the free α- and ε-amino groups of free or peptide-linked amino acids yielding phenylthiocarbamyl derivatives. The reaction is quantitative and does not destroy the peptides. The phenylthiocarbamyl derivatives have a high absorption in the ultraviolet which permits the detection of minute quantities of these derivatives on paper with the use of a fluorescent screen[5,8].

In initial studies with the phenyl isothiocyanate reagent, it was found necessary to wash the paper with a strong salt solution followed by several water and ethanol rinses prior to use. This procedure effectively removes the intense background reaction obtained with some batches of paper when treated with phenyl isothiocyanate. The technique finally adopted was the following: Whatman No. 1 or No. 3 MM paper is soaked for about 12 h in 0.5 M NaCl with two changes of salt solution. The paper is then thoroughly washed with water followed by 50 % ethanol and acetone and dried in air prior to use. After the peptide separation by finger-printing is carried out, the paper is dried at 60° for several hours and then left at room temperature for 30 min. Subsequently the paper is laid flat over a bed of glass spikes contained in a suitable glass dish. Freshly prepared buffer (pyridine–water–triethylamine–phenyl isothiocyanate, 150:50:6:2) is added to the bottom of the dish to saturate the atmosphere. The paper is thoroughly sprayed with the same buffer and the dish is then covered with a glass plate and left at room temperature for 3 h. This interval permits the quantitative conversion of the peptides to their phenylthiocarbamyl derivatives. Excess reagent is rinsed off with three washings in benzene and two washings in ethylene chloride. The paper is dried for about 30 min at room temperature, and the phenylthiocarbamyl peptides are subsequently located with ultraviolet light by the use of a fluorescent screen. A simple method for making such a screen follows: A slurry of phosphor (Phosphor, color television, green, type No. 161; Sylvania Electric Products, Inc., Towanda, Pa., U.S.A.) in isoamyl alcohol or chloroform is sprayed onto a Lucite plate. The phosphor layer is protected by applying a thin cover of a methylmethacrylate from a spray bottle (Plastik 707, National Radiac., Inc., Newark, N.J., U.S.A.).

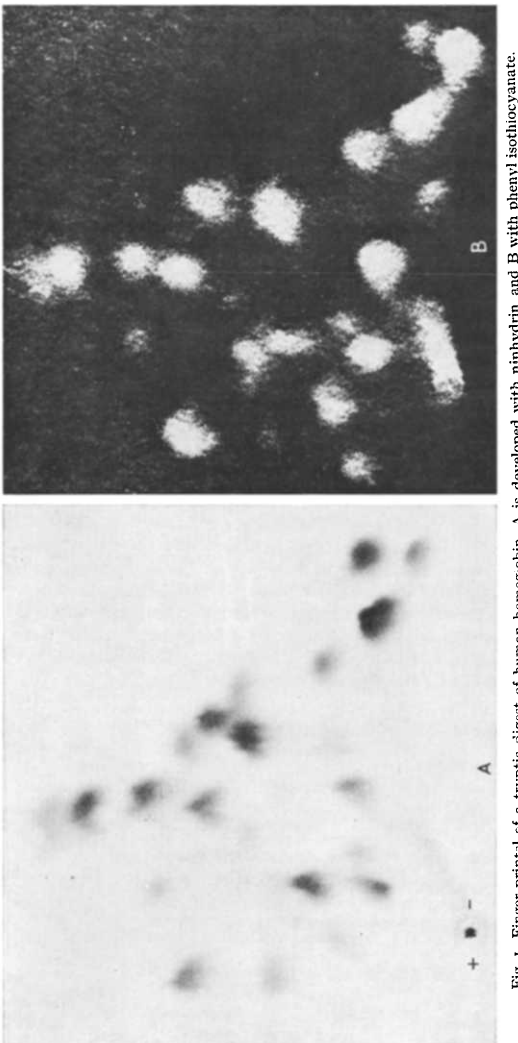

Fig. 1. Finger-prints[1] of a tryptic digest of human hemoglobin. A is developed with ninhydrin and B with phenyl isothiocyanate.

Fig. 1 shows 2 finger-prints on Whatman No. 3 MM of a tryptic digest of normal human hemoglobin. Approx. 2 mg is applied to each paper. One of the finger-prints (A) is developed by ninhydrin and the other one (B) is developed according to the technique described above. A photocopy of the phenylthiccarbamyl-coupled finger-print is made using ultraviolet light.

It was observed that some peptides stained better with one method than the other. Despite small variations, however, it can be seen that comparable patterns are obtained. It is also clear that the phenylthiocarbamyl method seems to be at least as sensitive as the ninhydrin one.

This method has some applications which could be useful in protein chemistry.

Recent experiments indicate that phenylthiocarbamyl peptides obtained from a tryptic digest of hemoglobin can be eluted quantitatively from a finger-print with 50% ethanol and determined spectrophotometrically by measuring their ultraviolet absorption at 243 mμ. At this wavelength the phenylthiocarbamyl component has a molar extinction coefficient of approx. 15000. Hence, a simple method for quantitative determination by optical methods of these derivatives is provided.

As the peptides are converted to phenylthiocarbamyl derivatives the N-terminal amino acids are split off as phenylthiohydantoins following cyclization in an acid medium[7]. The reaction can be performed directly on the paper[8] or on the eluted phenylthiocarbamyl peptides in solution. The N-terminal amino acids released as their phenylthiohydantoin derivatives can be separated by paper chromatography and determined spectrophotometrically following elution from the chromatograms with ethanol[5]. The remainder of the intact peptides can then be further analyzed for amino acid sequences or total amino acid composition.

The N-terminal analysis of the phenylthiocarbamyl peptides determines whether there is more than one peptide in each spot on the finger-print, providing the N-terminal amino acid of each peptide is different. Hence, the application of this method also improves the efficiency of the finger-printing technique[1].

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Department of Physiological Chemistry, University of Lund, Lund (Sweden)

INGA SJÖQUIST
JOHN SJÖQUIST*

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* Present address: Division of Biochemistry, Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass. (U.S.A.).