Short Communications

Naphthyl Isothiocyanate as a Reagent for the Edman Degradation of Peptides

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Naphthyl isothiocyanate reacts with hexosamines smoothly and gives well-defined derivatives (Scott, 1964). As an extension of this study, we found that naphthyl isothiocyanate in place of the phenyl derivative can be employed in the Edman degradation of peptides for sequential amino acid analysis.

NTH* derivatives of amino acids were synthesized in a manner similar to that described by Edman for the PTH derivatives (Edman, 1950). Details peculiar to the syntheses of PTH derivatives from amino acids containing amide, thiol and hydroxyl groups (Edman & Lauber, 1956; Levy & Chung, 1955) also applied to NTH syntheses. We have synthesized NTH derivatives with the following amino acid residues: glycine, alanine, valine, leucine, isoleucine, threonine, dehydrothreonine, serine, dehydroserine, aspartic acid, asparagine, glutamic acid, glutamine, lysine (ϵ -naphthylthiocarbamoyl-), phenylalanine and cysteic acid. These NTH derivatives were all easily crystallizable and the crystals met criteria for purity by elementary analyses and infrared spectra. In the crystal state NTH derivatives were as stable as PTH derivatives. Solutions of NTH derivatives in 90% acetic acid could be stored for a month at -5° , but during storage there was slightly faster decomposition of NTH derivatives than of PTH derivatives.

The solubility characteristics of NTH derivatives are not markedly different from those of PTH derivatives, but NTH derivatives are generally more soluble than PTH derivatives in organic solvents. This results in higher R_F values of NTH derivatives than those of PTH derivatives in partition chromatography. For paper chromatography, solvent D of Edman & Sjöqvist (1956) and solvent H of Blombäck & Yamashina (1958) for PTH derivatives could be used for the NTH derivatives. Likewise, ultraviolet lamp and iodine–azide reagent were useful for the detection of spots on paper. The R_F values of NTH-glycine, for example, were 0.15 in solvent D and 0.65 in solvent H, and of PTH-glycine 0.09 in solvent D and 0.37 in solvent

H. For quantitative analysis, the solvents of Sjöqvist (1960) for chromatography and 95% (v/v) ethanol for elution from paper could be used without difficulty.

The most favourable property of NTH derivatives resides in their ultraviolet-absorption characteristics. Besides an absorption peak at 270–272 m μ due to thiohydantoin, NTH derivatives possess another absorption maximum at 222–222.5 m μ due to naphthyl, and common to all NTH derivatives including those with dehydrothreonine and dehydroserine residues. At this wavelength, the molecular extinction coefficient (ϵ) of NTH derivatives was much higher than that of PTH derivatives at 270 m μ , which is the characteristic absorption maximum for PTH derivatives. Typical spectra with some ϵ values are shown in Fig. 1. The ϵ values of NTH derivatives at 222.5 m μ are roughly five times those of PTH derivatives at their maximum, 270 m μ . The ϵ values of NTH derivatives at 222 m μ are also much less variable from

Fig. 1. Ultraviolet-absorption spectra of thiohydantoin. Curve I, NTH (ϵ -naphthylthiocarbamoyl)-lysine; curve II, NTH-alanine; curve III, NTH-dehydrothreonine; curve IV, PTH-alanine. The ϵ values at 222.5 m μ are 148000 for curve I, 80700 for curve II and 76000 for curve III.

* Abbreviations: NTH, naphthylthiohydantoin; PTH, phenylthiohydantoin.

one amino acid to another as compared with those of PTH derivatives at 270 m μ , being almost entirely a property of the naphthyl group.

The splitting off of naphthylthiazolinones in the stepwise degradation of peptides proceeds more slowly, 45 min. being required for completion of the reaction instead of 15 min. for the corresponding reaction with phenylthiocarbamoyl-peptides. Otherwise, the procedure of Edman's new principle as published by Doolittle (1965) could be followed.

It was found that naphthyl isothiocyanate worked well in the determination of *N*-terminal amino acids of A and B chains of bovine insulin and of yeast cytochrome *c*. The reaction with naphthyl isothiocyanate was even more efficient than that with phenyl isothiocyanate. Thus from yeast cytochrome *c* 0.80 mol. of NTH-threonine/mol. of protein could be recovered in the eluate from the paper of the final chromatogram, whereas 0.70 mol./mol. was found with the original Edman procedure (Yaoi, Titani & Narita, 1964). In this analysis, the greater part of threonine was identified as NTH-dehydrothreonine. Similar results were obtained for threonine and also for serine in other polypeptides.

In the reactions for the sequential amino acid analysis of peptides, we have found no disadvantage in the NTH method compared with the PTH method.

With a dipeptide, glycylthreonine, the NTH method worked perfectly. With the *N*-ethylmaleimide derivative of cytochrome *c* from yeast, the *N*-terminal sequence, Thr-Glu-Phe-Lys-Ala-Gly-Ser, was confirmed. The NTH of each step appeared as a single spot, which made the identification unequivocal. The yield of NTH fell as the number of steps increased and the fifth amino acid, alanine, could be recovered only with a yield of 0.2 mol./mol.

of protein. However, if (Doolittle, 1965) yields are expressed as percentages found in the preceding step, then the average yield, exclusive of the first step, was 72%. This is somewhat lower than that reported by Doolittle (1965) (88%) for lamprey fibrinopeptide. This low yield is probably mainly due to insolubility of the protein, since during the sequential analysis this protein turned out to be insoluble from the second step onwards.

In the analysis of A and B chains of oxidized bovine insulin, insolubility of these polypeptides was not encountered. For the A chain, we followed the sequence up to nine, and the average yield was 85%. For the B chain, in the analysis up to six, the average yield was 81%. These values are comparable with those reported by Doolittle (1965). As with cytochrome *c*, the NTH derivative appeared as a single spot on paper chromatography in each step of the sequential analysis.

In conclusion, the NTH method is as effective as the PTH method in the reactions for sequential analysis of peptides, and is much superior to the latter in sensitivity of spectrophotometric analysis.

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- Blombäck, B. & Yamashina, I. (1958). *Ark. Kemi*, **12**, 299.
 Doolittle, R. F. (1965). *Biochem. J.* **94**, 742.
 Edman, P. (1950). *Acta chem. scand.* **4**, 277.
 Edman, P. & Lauber, K. (1956). *Acta chem. scand.* **10**, 466.
 Edman, P. & Sjöqvist, J. (1956). *Acta chem. scand.* **10**, 1507.
 Levy, A. L. & Chung, D. (1955). *Biochim. biophys. Acta*, **17**, 454.
 Scott, J. E. (1964). *Biochem. J.* **92**, 57F.
 Sjöqvist, J. (1960). *Biochim. biophys. Acta*, **41**, 20.
 Yaoi, Y., Titani, K. & Narita, K. (1964). *J. Biochem., Tokyo*, **56**, 222.

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Evidence that the Oxidation of the Side Chain of Cholesterol by Liver Mitochondria is Stereospecific, and that the Immediate Product of Cleavage is Propionate

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When chemically synthesized [26 (or 27)-¹⁴C]-cholesterol is incubated with rat-liver mitochondria under conditions in which ¹⁴CO₂ is formed, [¹⁴C]-

propionic acid can be extracted from the incubation mixture by steam-distillation (Mitropoulos & Myant, 1965). This provides confirmatory evidence