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EDMAN STEPWISE DEGRADATION OF POLYPEPTIDES: A NEW STRATEGY EMPLOYING MILD BASIC CLEAVAGE CONDITIONS

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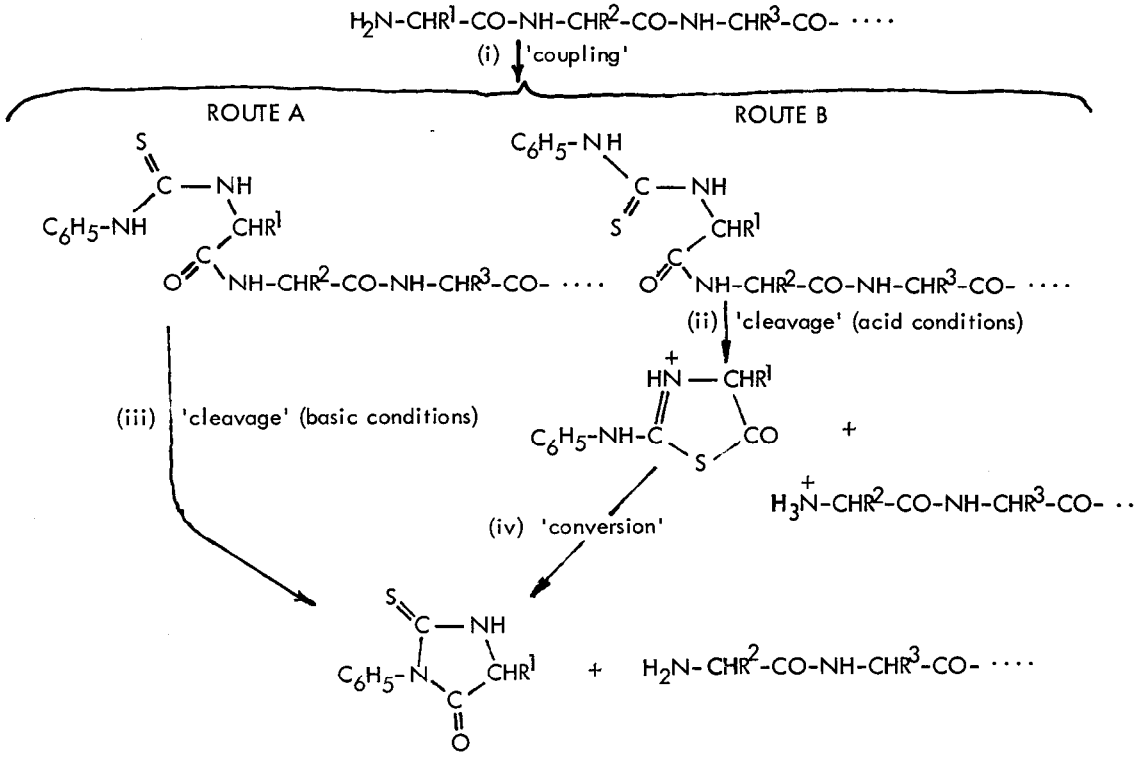
Summary: Terminal N-(phenylthiocarbamoyl)peptides, formed under mild basic conditions in the first stage ("coupling") of the Edman degradation of peptides, are shown to undergo cleavage at the first peptide bond under similar conditions at higher temperatures. The usual products of the Edman degradation are formed (the phenylthiohydantoin of the N-terminal amino acid, and the correspondingly shortened peptide)in one step.

The Edman degradation is widely-used in routine sequence analysis of peptides,[2] and is valuable in protein semi-synthesis.[3] Its limitations are taken into account when it is used for routine sequence analysis, but its repeated application to a peptide can lead eventually to unreliable assignments as artefacts are introduced, arising from 'pre-cleavage' and 'overlaps'.[4] These, and other limitations associated with the use of reagents that are not wholly compatible with the functional groups encountered in side-chains of some protein amino acids,might be overcome by the use of alternative strategies. Whereas the literature continues to reflect continuing intensive development of Edman sequencing methodology,[4] surprisingly little attention has been given to the essential chemistry of the process[5] (carried out still as originally proposed[2]).

We have discovered[6] a simple variant (SCHEME 1; Route A) that involves mild basic conditions throughout, and that achieves the same purpose as the cycle of reactions constituting the usual Edman degradation (SCHEME 1; Route B) using one fewer step. Hitherto, the cleavage step of the Edman degradation has been conducted in an acidic medium, so as to lead to the 2- anilino-thiazolin-5(4H)-one derived from the N-terminal amino acid. This is separated from the reaction mixture containing the shortened peptide, and rearranged in aqueous acid or thermally, into the N-phenylthiohydantoin (the 'PTH'; the conversion step of the Edman degradation). Under the basic reaction conditions that we now propose, the cleavage reaction leads directly to the PTH and the shortened peptide.

The conditions required for cleavage by base are effectively the same as (though considerably more vigorous than) those used to bring about the coupling step that precedes it. An explanation can be found for the losses that occur in the coupling step, as well as a potential source of 'overlaps' that are responsible for diminishing confidence in the results of an extended sequence determination, with the knowledge that cleavage can be brought about by base. Some shortened peptide will form during the coupling step, and then undergo phenylthiocarbamoylation, to be carried forward to cleavage and then introduce contaminants in all succeeding cycles by providing the PTH of the penultimate and N-terminal amino acid residues.[7]

SCHEME 1



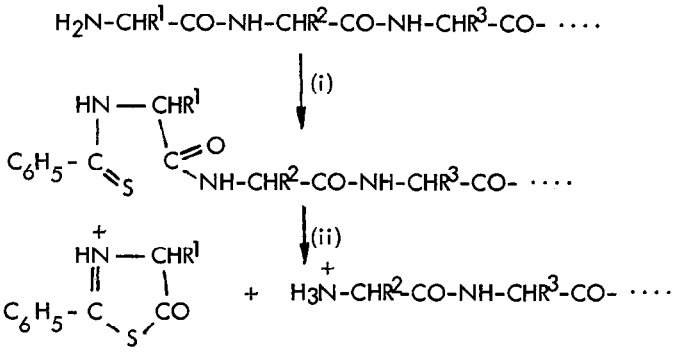
Reagents: (i) $C_6H_5N{=}C{=}S/py/R_3\overset{+}{N}H\ CF_3CO_2^-/H_2O/40\text{-}50^\circ C/20\text{-}60$ min; (ii) anhydrous CF_3CO_2H; (iii) $R_3\overset{+}{N}H\ MeCO_2^-/MeCN/75^\circ C/10\text{-}150$ min; (iv) H_3O^+ or heat.

Base cleavage of terminal N-(phenylthiocarbamoyl)peptides was first studied[6] using a tertiary amine alone, but low cleavage yields were encountered with tripeptide derivatives in contrast with the efficient cleavage achieved with dipeptide analogues. In particular, N-(phenylthiocarbamoyl)prolylglycine was completely cleaved within ten minutes in triethylamine at 60°C (whereas N-terminal proline is cleaved

sluggishly and with reduced yields in the usual Edman procedure[8]). Anchimeric assistance by the C-terminal carboxy group is implicated, since reduced yields were obtained in comparative experiments when this group was esterified. We have now found that with the addition of one equivalent of acetic acid to the cleavage reagent (triethylamine, or N,N-dimethylallylamine in MeCN), efficient cleavage of the N-terminal residue can be accomplished through this modified Edman procedurewith a range of representative peptides that include alanine, leucine, glycine, and phenylalanine residues. Studies have included longer peptides whose N-phenylthiocarbamoyl derivatives, though insoluble in the reagent, underwent cleavage (e.g. insulin).

The mechanism indicated in Route A of SCHEME 1 implicates a de-protonated thiourea nitrogen atom in the initiation of the cleavage step. This is confirmed by the failure of terminal N-(thiobenzoyl)peptides (SCHEME 2) to undergo cleavage under basic conditions, although these[9] and related[10] thioacylated peptides have been used in sequencing procedures employing acidic cleavage.

SCHEME 2



Reagents: (i) $C_6H_5.CS.S.CH_2CO_2^-Na^+/H_2O$; (ii) anhydrous CF_3CO_2H.

In model sequence determinations carried out on a preparative saale, the shortened peptide separated out within 10 minutes at 70-75°C, from a solution of the N-(phenylthiocarbamoyl)peptide in the minimum amount of MeCN containing one equivalent of triethylammonium acetate. Complete disappearance of the starting material occurred within 2½ hours (monitoring by t.l.c. and h.p.l.c.), though in some cases the reaction is considerably faster (vide supra). We have encountered no difficulties in continuing the degradation of the shortened peptide through the same base cleavage procedure (or through the normal Edman procedure or through the use of thiobenzoyl derivatives).

Tangible benefits are anticipated from the use of the base cleavage regime in sequencing peptides

containing acid-sensitive amino acid residues, especially when these appear at the N-terminus in a sequence determination and then undergo reactions which effectively prevent continued sequencing (e.g. glutamine to pyroglutamic acid). The methodology is compatible with existing automated sequence instrumentation and is particularly attractive for use in solid-phase[11] and gas-phase[12] techniques since volatile reagents are used. Further experiments are being carried out to confirm the possible advantages of this new strategy, but we have established a viable alternative to current methodology, and have also demonstrated a source within the current methodology for the introduction of artefacts, to which attention should be given in order to improve further the established Edman procedure.

As noted above, both coupling and cleavage steps employ effectively the same reaction conditions in the modified Edman procedure employing base cleavage (SCHEME 1; Route A). There are two important implications: (i) that a rapid N-terminal amino acid residue analysis can be accomplished simply by warming (10 min) a mixture of peptide, phenyl isothiocyanate, tertiary ammonium acetate, and MeCN, followed by identification of the PTH by h.p.l.c.; (ii) that prolonged treatment of a peptide in this way will bring about progressive degradation (in principle, total degradation from the N-terminus and therefore exploitable for sequence analysis). These implications have both been realised[13] and will be described in later publications.

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7 The observation that degradation of peptides carrying N-terminal histidine leads to release of both the terminal and penultimate amino acid residues in some cases (Ref.2) might be explained by the enhanced cleavage during the coupling step promoted by the basic histidine side-chain.

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