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CARBOXYL-TERMINAL SEQUENTIAL DEGRADATION OF PEPTIDES

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Summary. A method is presenting for degrading carboxyl-terminal peptide amides sequentially and in high yield. Combined with the procedure of the previous paper, these results constitute a sequential carboxyl-terminal peptide degradation.

Our previous paper described the efficient removal of the carboxyl-terminal residue of peptides to produce the primary amide of the shortened peptide (1), eq (1).

$$\text{Pep}^{N}\text{-C(=O)-NH-CHR-CO}_2\text{H} \xrightarrow{H_2O} \text{Pep}^{N}\text{-C(=O)-NH}_2 + \text{RCH=O} + \text{CO}_2 \qquad (1)$$

1

Of several ways of extending this result to a sequential carboxyl-terminal peptide degradation, a direct, Hofmann-type degradation of primary amide 1 is a logical candidate for investigation. Previous attempts to deal with peptide-derived primary amides by hydrazinolysis (2) or direct hydrolysis (3) are less than optimally effective. We here disclose our method for a residue-by-residue degradation of peptide-derived primary amides. This and the procedure of the preceding paper (1) provide the basis for the development of a sequential carboxyl-terminal peptide degradation.

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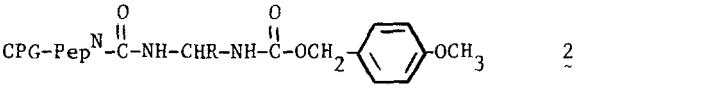
Materials and Methods

Preparation of CPG(O)* Underivatized CPG* (Pierce Chemical Company) was heated under reflux with water (100 mL) for 3 hr with mechanical stirring. The glass was then heated at 60° in concd HNO_3 for 3 hr (4) and washed with 1-2 L of water An aqueous solution (100 mL), 10% in γ-glycidoxy-trimethoxypropylsilane, 1, (Petrarch Systems, Levittown, PA 19059) and 10% in acetic acid was adjusted to pH 1.3-1.5 with concd HCl and allowed to stand 1 hr at room temperature. The pH was then raised to 3.8-4.0 with NaOH and the CPG was then heated in this solution for 1.5 hr at 85-90°, then washed thoroughly and heated in water for 30 min at the same temperature. This silylation procedure was repeated; the resulting derivatized glass was heated in water for 3 hr at reflux, filtered, and dried by aspiration. This glass is a modified form of Glycophase^R-CPG (Corning) except that the silyl linkage has an enhanced stability toward the conditions of our degradation. The derivatized glass was allowed to react with 100 mL of a 0.26M solution of periodic acid overnight at room temperature with mechanical stirring followed by washing with water (1-2 L). This derivative was then allowed to react with 100 ml of a 0.1M solution of $KMnO_4$ for 12 hr with mechanical stirring. The glass was washed with water until the wash solution was colorless, and then suspended in a 10% (v/v) H_2SO_4 solution. Solid $NaHSO_3$ was added until the glass and solution were decolorized, and the glass was washed thoroughly with 2-3L of water. The resulting material was refluxed 3 hr in water, washed thoroughly with water and anhydrous methanol (500 mL), and dried 12 hr in vacuo. The structure of this product is given as 4.

Breakdown of 2. The CPG-peptide carbamate 2 produced in the azide degradation (1) was treated with 3 bed volumes of anhydrous TFA* under N_2. This mixture was allowed to stand at room temperature for 30 min with occasional swirling. The glass was then filtered and washed thoroughly with water and anhydrous methanol, then dried by aspiration on a sintered funnel.



2

Breakdown of 3. The CPG-peptide derivative 3 was placed in a test tube and three volumes of a 0.01M Na_2HPO_4 buffer, pH 7.0, were added. This mixture was heated in a boiling water bath for 80 min. The glass was then washed thoroughly with water and methanol and dried in vacuo.

3

Bis(I,I-trifluoroacetoxy)iodobenzene (BTI). Phenyliodosyl acetate (5.0 g, Aldrich) was dissolved in 25 ml anhydrous TFA with slight heating and allowed to stand at room temperature for 1 hr. The solvent was removed in vacuo and the process was repeated. The product was recrystallized from hexane-TFA to yield 6.7g (80%) BTI, mp 123-5-126° (lit 119-121°) (5), 122-3° (6).

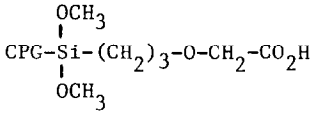
Rearrangement of CPG-Peptide C-Terminal Amide. BTI (0.086 g, 0.2 mmol) was dissolved in 1 ml DMF*, and 1 mL of a 0.01N aqueous solution of TFA was added. This freshly prepared solution was added to the CPG-peptide amide (30-

*Abbreviations: CPG(O) = Controlled Pore Glass derivatized as in 4; CPG = Controlled Pore Glas (Corning); BTI = I,I-bis(trifluoroacetoxy)iodobenzene; TFA = trifluoroacetic acid; DMF = N,N-dimethylformamide; Pep^N = N-terminal peptide; DABA = 2,4-diaminobutyric acid; DAPA = 2,3-diaminopropionic acid.

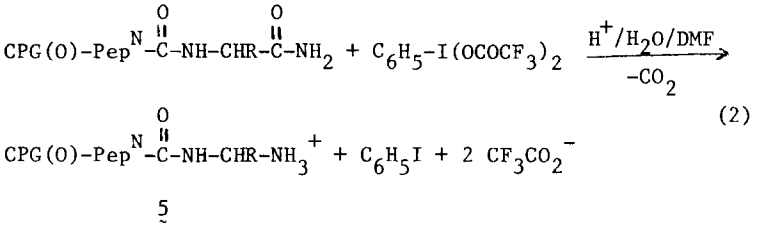
250 mg) in the reaction vessel (1) and allowed to react for 4 hr at 35°. The CPG peptide was washed once with DMF, then with water and methanol, and the glass was dried by aspiration on a sintered funnel.

Results and Discussion

It was found that the aminopropyl-derivatized CPG used in the previous paper gave low degradative yields in the procedure to be described here (1). Therefore, a new glass derivative, CPG(O), 4, was prepared and treated as described in Materials and Methods. This material was equally effective in the azide procedure presented previously (1) and was much superior for the procedure described here. Peptides were immobilized on CPG(O) in the manner described previously (1).



The interesting compound I,I-bis(trifluoroacetoxy)iodobenzene brings about the conversion of peptide amides to isocyanates (5,6). Under the aqueous acidic conditions used, hydrolysis of the isocyanate occurs readily (eq 2).



(2)

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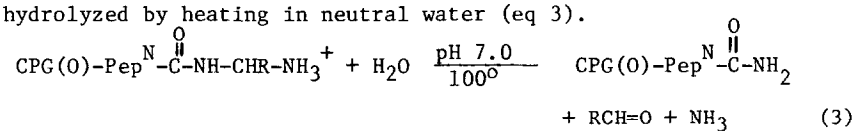
It was found that species 5 was surprisingly resistant to hydrolysis, an observation confirmed in solution studies. However, this derivative could be hydrolyzed by heating in neutral water (eq 3).



(3)

The new amide produced can be degraded by repetition of the degradative procedure. The success of the procedure as applied in a repetitive fashion is evident from results in the degradation of an eledoisin peptide analog (= C-terminal six residues of eledoisin with lysine replacing alanine) shown in Table 1. Note that this peptide terminates in an amide linkage.

Table 1. Sequential Degradation of Eledoisin-Related Peptide Amide from the Carboxyl Terminus

Residue	Amino acid analysis after cycle 0	1	2	3	4	5
Gly	0.99	1.00	0.90	0.37	0.39	0.40
Met	0.66	0.00	0.00	0.00	0.00	0.00
Ile	0.99	1.00	1.05	0.82	0.47	0.43
Leu	1.00	0.88	0.29	0.20	0.19	0.20
Phe	1.00	1.00	1.01	0.99	0.88	0.70
Lys	1.00	1.00	1.00	1.00	1.00	1.00

Residue→ Cycle↓	Met	Leu	Gly	Ile	Phe	Lys
1	-100%	—	—	—	—	—
2	-100%	-71%	—	—	—	—
3	-100%	-80%	-63%	—	—	—
4	-100%	-81%	-61%	-53%	—	—
5	-100%	-80%	-60%	-57%	-30%	—

From the data, the sequence of this peptide is readily read correctly as Lys-Phe-Ile-Gly-Leu-Met-amide.

The sequential degradation appears to work with a repetitive yield of about 80%. If a residue is degraded less than quantitatively, subsequent degradation appears to be relatively free from "overlap error;" that is, little or no loss of that residue occurs in subsequent degradative steps. The reason for this observation may be that intermediates -- possibly the isocyanate -- in the degradation may be reacting with functional groups on the glass support in such a way that further degradation becomes impossible. Consistent with this view is the observation that peptide degradations with aminopropyl-CPG (1) gave repetitive yields of about 30%; residual amino groups present on the latter support are expected to be superior nucleophiles relative to the hydroxyl groups present on CPG(0).

Under the conditions of this procedure, side-chain amides of asparagine and glutamine residues are converted, respectively, to DAPA and DABA (eq 4).

$$\text{CPG(O)-Phe(1.0)Gln(1.02)Asn(1.02)} \rightarrow \text{CPG(O)Phe(1.0)Gln(0.03)Asn(0.17)DABA(0.95) DAPA(0.86)} \quad (4)$$

That the procedure is compatible with the azide degradation of the previous paper (1) in a sequential process is illustrated by the following result (eq 5).

$$\text{CPG(O)-Gly(1.0)Leu(0.99)Ala(0.95)} \xrightarrow[\text{radation}]{\text{azide deg-}} \xrightarrow{CF_3CO_2H} \xrightarrow[100^{\circ}]{0.01N\ NaP_i,\ pH\ 7.0} \text{CPG(O)-Gly(1.0)Leu(0.98)Ala(0.02) [98\%]} \xrightarrow[H^+/H_2O/DMF]{BTI} \text{CPG(O)-Gly(1.0)Leu(0.12)Ala(0.03) [88\%; 90\% of available Leu]} \quad (5)$$

To continue sequentially after the azide process, one need only add to the experimental manipulations brief successive treatments with TFA and boiling water buffered at pH = 7.

The results reported here have encouraged us to enter a phase of our program in which the effects of functionalized amino acid side chains on the degradation are being investigated in detail, and a positive aldehyde identification method is undergoing development. Although protection of some side chains will certainly be required, the requirements for this should be less restrictive than those of peptide synthesis, since our protecting groups need not be removed. Although efforts to improve the degradative yield the remaining 15-20% are currently underway, the yields of the existing procedure are about as good as those of the manual Edman procedure, and the procedure should be useful in situations in which difference amino acid analysis is an acceptable method for assaying results. Finally, the amide procedure reported here can provide a degradative procedure for C-terminal residues blocked by amidation.

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