Rapid Papers

Quantitative Determination of *N*-Terminal Amino Acids of Peptides and Proteins with Cobalt(III) Chelates

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Quantitative *N*-terminal peptide-bond hydrolysis with the *cis*- β -hydroxyaquo(triethylenetetramine)cobalt(III) ion, i.e. β -[Co(trien)(OH)(OH₂)]²⁺, is reported. The method has been demonstrated with 20 small peptides, a hexapeptide, bradykinin, insulin A chain (oxidized), glucagon and insulin. The procedure involves no acidic hydrolysis step and thus no destruction of labile amino acids.

Substitution-labile cobalt(III) ions of the type cis -[Co(N)₄(OH)(OH₂)]²⁺ [where (N)₄ represents the nitrogen donor atoms in either bidentate or tetradentate ligands] have been shown to be capable of promoting the hydrolysis of peptide bonds in aqueous solution, under relatively mild conditions of temperature and pH. This results in hydrolysis of the *N*-terminal amino acid, which appears in the final products chelated in the co-ordination sphere of cobalt(III):

(Collman & Buckingham, 1963; Buckingham *et al.*, 1967; Kimura *et al.*, 1970).

Bentley & Creaser (1973) compared the efficiency of various *cis*-[Co(N)₄(OH)(OH₂)]²⁺ species and concluded that *cis*- β -[Co(trien)(OH)(OH₂)]²⁺ (*k*_{obs.} = $1.42 \times 10^{-2} s^{-1}$) was the species of choice. A method of qualitative *N*-terminal amino acid identification was reported. Quantitative peptide-bond hydrolysis of glycyl dipeptides and dipeptide esters has been used as a means of confirming that both the condensation reaction and subsequent peptide-bond hydrolysis are stoichiometric and specific for the *N*-terminal residue (Bentley & Creaser, 1974)

Materials and Methods

Peptides were of the highest purity obtainable from normal commercial sources. Other chemicals were of A.R. grade. The *cis*- β -diaquo(triethylenetetramine)-cobalt(III) perchlorate was prepared from the carbonato derivative immediately before use as described by Bentley & Creaser (1973). Quantitative identification of the *N*-terminal amino acid after displacement of the cobalt(III) moiety by CN⁻ ion from the base-hydrolysed samples was performed by amino acid analysis according to the method of Moore *et al.* (1958). Amino acid determinations were

performed with a Beckman model 120C amino acid analyser with norleucine as internal reference standard.

Quantitative identification of *N*-terminal amino acids was achieved by the following procedure. To 10 nmol of peptide in 0.2 ml of 0.1 M-sodium Hepes [2 - (*N* - 2 - hydroxyethylpiperazin - *N'* - yl)ethane - sulphonic acid] buffer, pH 8.0, was added 0.2 ml of 0.2 M- β -[Co(trien)(OH)(OH₂)]²⁺, pH 8.0, and the solution was kept at 45°C for 2 h. Then 0.3 ml of 0.5 M-sodium phosphate buffer, pH 10.5, was added and the solution was left for 40 min. Then 0.2 ml of 1.0 M-NaCN and 0.2 ml of conc. HCl were added and the solution was left for 40 min. The reaction products were diluted to 3 ml and the pH was adjusted to about 2, before adsorption on Beckman AA-15 cation-exchange resin (H⁺ form). The resin was washed twice with 10 ml of 0.2 M-sodium citrate buffer, pH 2.0, before removal of the bound amino acid with 0.1 M-NH₃. The ammoniacal solution was freeze-dried and the amino acid sample was prepared for analysis by dissolving it in 1.1 ml of 0.2 M-sodium citrate buffer, pH 2.2.

The results are presented in Table 1.

Discussion

The results presented in Table 1 demonstrate that the chelated cobalt(III) species β -[Co(trien)(OH)(OH₂)]²⁺ is a suitable reagent for detection of the *N*-terminal residues of a variety of peptides and proteins. Its main advantage is that it can be used for acid-labile residues. I have used it to identify *N*-terminal tryptophan, methionine and glutamine. The method cannot quantitatively identify aspartic acid because reaction of the cobalt(III) species with aspartyl residues gives both the five- and six-membered chelated products; subsequent hydrolysis with base releases only that aspartic acid bound into the five-membered chelate. Cysteiny residues require con-

Table 1. *Peptides from which the N-terminal amino acids have been quantitatively removed and identified by cobalt chelation*

	% recovery of N-terminal residue
(a) Di- and tri-peptides	
Glycylglycine	107
Glycyl-L-alanine	98
Glycyl-DL-leucine	98
Glycyl-L-phenylalanine	103
L-Histidylglycine	97
L-Tryptophylglycine	89
L-Asparaginylglycine	76*
L-Glutamylglycine	98
L-Methionyl-L-histidine	88
L-Alanylglycine	102
L-Phenylalanylglycine	100
L-Leucyl-L-tyrosine	96
L-Prolyl-L-hydroxyproline	104
L-Leucylglycyl-L-phenylalanine	101
Glycyl-L-leucyl-L-tyrosine	98
Glycylglycyl-L-arginine	98
(b) Larger peptides	
Leu-Trp-Met-Arg-Phe-Ala	102
Bradykinin	97
Insulin A chain (oxidized)	100
Glucagon	96
Insulin (bovine)	(Phe 103 Gly 98)

* See the text.

version into cysteic acid in order to obtain quantitative recovery. Both aminoethylcysteinyl and carboxymethylcysteinyl derivatives react with the cobalt reagent two form to products and hence are unsuitable for quantitative analysis.

The cobalt reagent was used in the present work under conditions where only the N-terminal amino acid is totally complexed. If the concentration of the cobalt reagent is raised and the time of incubation is prolonged, other amino acids are sequentially released from the peptide chain.

Within the limitations indicated above, total peptide-bond hydrolysis for amino acid analysis of peptides containing acid-labile residues can be accomplished.

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