Riassunto. Gli acidi tetraidrotrisporici e gli acidi trisporici A, B e C inibiscono la germinatione di semi di varie specie di piante. Il loro effetto è antagonizzato da acido gibberellico. Questo fatto e l'analogia strutturale fra gli acidi trisporici e l'acido abscissico suggeriscono che gli acidi trisporici, oltre ad unaattività ormona le nei funghi, possano anche svolgere una attività regolatrice nelle piante superiori analoga a quella esercitata dall'acido abscissico.

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The Identification of εN-(β-Aspartyl)-L-Lysine in Native and Heated Keratin

The presence of an ω-ε isopeptide link between the γ-carboxyl group of a glutamyl residue and the ε-amino group of a lysyl residue has been reported by LORAND et al.[1] and PISANO et al.[2] in polymerized fibrin. Studies on heated keratin indicated that some 'binding' of the ε-amino groups of lysine was occurring[3]. Subsequent work by the present authors, using an enzymic method resulted in the identification and isolation of this moiety in both native[4] and heated keratin[5]. In the case of the heated protein, a greater concentration of γ-glutamyl lysine were found than in the native keratin.

The enzymic method used to isolate this moiety would also leave intact a similar isopeptide link between the β-carboxyl group of any aspartyl residues and the ε-amino groups of lysine. It was observed on amino acid analysis of the keratin digests, that 2 small peaks between leucine and tyrosine did occur (Figure). The slower moving peak was identified as ε(γ-glutamyl) lysine, whereas the more rapid moving peak was suspected to be ε(β-aspartyl) lysine. Subsequent work has now confirmed the identity of this moiety.

Two samples of Merino wool keratin, one of which had been heated for 100 °C for 48 h, were reduced with tributylphosphine, and the thiol groups blocked with acrylonitrile. The resulting proteins were enzymically digested using pepsin, pronase, aminopeptidase M and prolidase as described by COLE et al.[6]. The resulting digests were fractionated by ion-exchange chromatography using the Technicon amino acid analyzer system with stream splitting to moinitor the fractions.

A wide fraction was collected from the column containing leucine, tyrosine, ε(γ-glutamyl) lysine and the unknown. After desalting and concentration this fraction was separated by paper chromatography using butan-1 ol-acetic acid-water (4:1:5 by vol.) as the eluent. By this means the band containing the isopeptide was located and excized. This band was eluted with water and split into 2 fractions, the first portion being hydrolyzed, the second was treated with 1-dimethyl amino-napthalene-5-sulphonyl chloride[7] and then hydrolyzed. The resulting hydrolysates were examined by high voltage electrophoresis and thin layer chromatography. The first hydrolysate showed the presence of aspartic, glutamic acids and lysine thus indicating that as well as the ε(γ-glutamyl) lysine there was also some peptide material containing an aspartyl residue. On analyzing the second hydrolysate the fact that the aspartic acid and lysine are not involved in a normal peptide link was established as DNS-aspartic and DNS-glutamic acids were located along with α DNS lysine thus proving the existance of εN-(β-aspartyl) lysine in wool keratin.

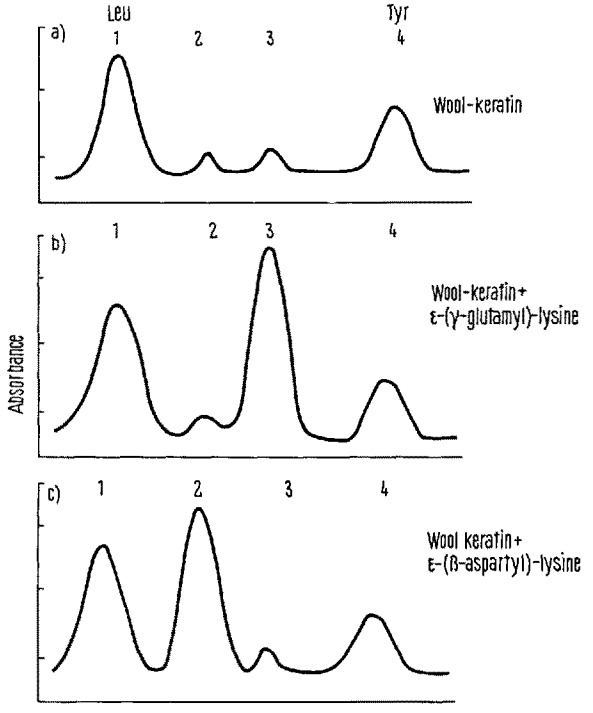
On studying the heated sample of keratin it was found that the amount of εN-(β-aspartyl) lysine had increased (Table).

As a final confirmitory test a sample of synthetic εN (β-aspartyl) lysine was run on the Technicon auto-

Quantities of ε-(β-aspartyl)-lysine in heated keratin

Temperature for 48 h (°C)	ε-(β-aspartyl)-lysine
Control	15
60	17
100	20
120	25

Concentrations in μmoles/g.



Amino acid profiles of wool keratin digests. a) without b) with added ε(γ-glutamyl)-lysine, c) with added ε(β-aspartyl)-lysine. 1. leucine. 2. ε(β-aspartyl)-lysine. 3. ε(γ-glutamyl)-lysine. 4. Tyrosine.

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analyzer, separately and in admixture with the enzymic digests. The position of this peak was co-incident with peak 2 as is shown in the Figure.

Thus from this work it has been shown that the isopeptide εN (β-aspartyl) lysine occurs in native wool keratin and has probably the function of a crosslink cf. εN (γ-glutamyl) lysine. Also it appears from this work that this moiety is formed during the heating of the keratin, as is reflected in the increased amounts found in digests of heated protein.

Zusammenfassung. Chromatographisch (Ionenaustausch, Papierchromatographie, Dünnschichtchromatographie und Hochspannungselektrophorese) wird nach enzymatischer Hydrolyse von nativem und denaturiertem Wolle-Keratin ε(γ-Glutamyl)-lysin und ε(β-Aspartyl)-lysin identifiziert und so die in Proteinen postulierte anomale Peptidverknüpfung nachgewiesen.

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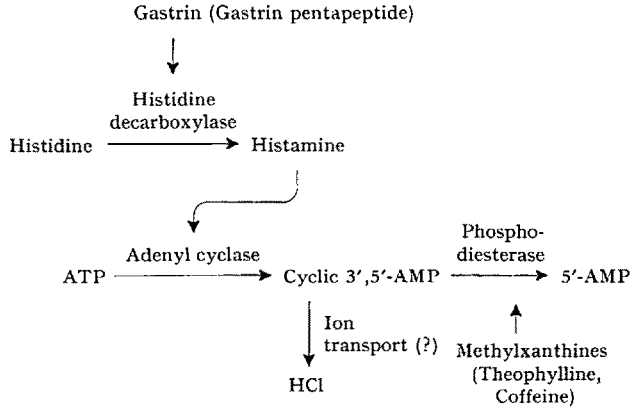
The Stimulating Action of Gastrin Pentapeptide and Histamine on Adenyl Cyclase Activity in Rat Stomach

Experimental data obtained recently[1] suggested that gastrin pentapeptide, and most probably complete gastrin also, cause transcription of DNA regions responsible for the synthesis of histidine decarboxylase. It was assumed that the enzyme induced provides a supply of histamine acting as physiological mediator of gastrin pentapeptide effect on gastric acid secretion. Increasing evidence has shown that histamine enhances adenyl cyclase activity in many tissues[2–4], and it is believed that cyclic adenosine 3′,5′-monophosphate (cyclic AMP) is directly responsible for the physiological effects of histamine. It is well known that cyclic AMP is a 'second messenger' in the action of many hormones and biogenic amines[5,6] and is able to mimic their action on target cells.

We supposed that histamine, formed due to action of gastrin (or its pentapeptide), activates adenyl cyclase in oxyntic cells and cyclic AMP in its turn affects ion transport to result in HCl secretion. The purpose of the present study was to verify this hypothesis.

Fasted male Wistar rats (200–250 g body wt.) received s.c. gastrin pentapeptide (t-butoxycarbonyl-β-Ala-Try-Met-Asp-PheNH$_2$; ICI 50, 123) twice at 20 min intervals in a dose of 0.4 μg or histamine i.m. in a dose of 200 μg per 100 g body wt. Control rats were injected with saline. 40–60 min after the injections the rats were sacrificed and the stomachs removed, rinsed in icecold saline and homogenized in 0.25 *M* sucrose to a final concentration of 50 mg tissue per ml. The stomach tissue homogenates for adenyl cyclase estimation were prepared according to Streeto and Reddy[7].

Adenyl cyclase activity was determined by the method of Weiss and Costa[8] modified by Rosen and Rosen[9]. The incubation mixture (0.2 ml) contained 0.05 *M tris* – HCl buffer pH 7.8, 0.01 *M* theophylline, 0.01 *M* NaF, 3 m*M* $MgSO_4$, 0.02 *M* mercaptoethanol, 0.2 μ*M* ^{14}C–8 ATP (specific activity 0.135 μC/μ*M*) and 0.05 ml of gastric tissue suspension (0.1-0.2 mg of protein) as a source of adenyl cyclase. 1 ml of final supernatant solution obtained after precipitation of ATP, and other metabolites of ATP except the cyclic AMP[10] by $ZnSO_4$–$Ba(OH)_2$[8,9], was added to 10 ml Bray's[11] scintillation fluid and radioactivity measured with Nuclear–Chicago Mark 1 scintillation counter. The activity of adenyl cyclase was expressed as nmoles of cyclic AMP formed per mg of protein per min. Protein was estimated by the method of Lowry et al.[12].



The scheme of the regulation of HCl secretion by gastrin.

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