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Activation of human pepsinogens

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Human pepsinogen A3 and A5 have been purified to chromatographic and electrophoretic homogeneity. At pH 2 pepsinogen A3 activates at a much faster rate than pepsinogen A5. Leu-23 – Lys-24 is the first bond cleaved during activation of pepsinogen A3. This bond is also cleaved in pepsinogen A5, but together with the cleavage of Asp-25 – Phe-26. Amino acid sequencing shows that pepsinogen A3 has Glu at position 43, whereas pepsinogen A5 has Lys.

Pepsinogen; Activation; Amino acid sequencing

1. INTRODUCTION

In the human gastric mucosa several isozymogens for pepsin A are found; these are numbered after their anodal electrophoretic mobilities (reviews [1–3]). The heterogeneity arises both from genetic variations and from posttranslational modifications. The organization of 3 genes corresponding to PgA3, -4, and -5 has been mapped [4,5]. Two of the genes have been investigated by nucleotide sequencing; a few differences were observed, e.g. Glu or Lys at position 43 [6,7]. But the electrophoretic mobilities of the corresponding pepsinogens have not yet been published.

The first step in the activation of the gastric zymogens consists of a conformational change in which the active site is uncovered [8,9]. The reaction proceeds by autocatalytic limited proteolyses which finally remove 42 to 47 amino acid residues from the N-terminal end of the zymogens, but intermediates occur in which only 16 to 26 residues are cut off (reviews [2,3]).

In this communication it is shown that the predominant isozymogens PgA3 and PgA5 have different activation patterns, and the major cleavage site differs from that reported previously [10]. Furthermore, the known N-terminal sequences are allocated to electrophoretically characterized isozymogens.

2. MATERIALS AND METHODS

2.1. *Pepsinogens*

Human gastric mucosa was obtained at autopsy from patients without gastric disorders (Bispebjerg Hospital, Copenhagen). The pepsinogens were extracted and purified by chromatography on DEAE-cellulose as described previously [11]. The individual isozymogens were isolated from pools of the DEAE-chromatography by fast protein liquid chromatography (Mono Q, Pharmacia). Elution with a linear gradient from 0.05 M ammonium acetate, pH 5.6 to 0.5 M of the same buffer. PgA3 and PgA5 were eluted at buffer concentrations of 0.33 M and 0.40 M, respectively. Pools of pure zymogens were dialyzed against 0.01 M sodium phosphate, pH 7, before activation.

2.2. *Activation*

Temperature, 0°C; concentration of pepsinogen, 0.3 mg/ml; addition of 0.2 M HCl to pH 2. During the experiments, aliquots were brought to pH 9 with 1 M ammonia, the samples were concentrated by freeze-drying.

2.3. *Digestion with* Armillaria mellea *proteinase [12]*

Enzyme/substrate ratio 1 : 1000, digestion for 4 h in 0.2 M *N*-ethylmorpholine acetate, pH 8, at 37°C.

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Abbreviations: PgA, pepsinogen A (EC 3.4.23.1); SDS-PAGE, SDS-polyacrylamide gel electrophoresis

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2.4. *Electrophoreses*

Agar gel electrophoresis was carried out in 0.05 M sodium phosphate, pH 6.0, the zymograms were developed with haemoglobin [13]. SDS-PAGE was carried out with the Laemmli buffer system [14]: gel concentration, 15% T and 0.5% C; length of separation gel, 15 cm. Coomassie brilliant blue G-250 was used for staining.

2.5. *Sequencing*

For sequence analyses, the polyacrylamide gel was electroblotted on a membrane of polyvinylidene difluoride (Immobilon Transfer, Millipore) [15]. Sequencing was carried out with a 477A Applied Biosystem analyzer equipped with on-line HPLC.

3. RESULTS AND DISCUSSION

The isopepsinogens were purified to electrophoretic homogeneity as tested by agar gel electrophoresis at pH 6.0. The pattern of isozymogens was the same as that found previously at pH 8 [1], but the lower pH allows characterization of development of enzymic activity during the activation. These results will be reported in a following communication.

Fig.1A shows the cleavage pattern observed by SDS-PAGE during activation of PgA3. Concentration of polyacrylamide and degree of crosslinking were chosen for maximum resolution among pepsinogen, intermediates and pepsin: the actual distance between the bands of pepsinogen and pepsin was 10 mm. The liberated peptides were not observed under these conditions. An intermediate is observed from 0.5 to 6 min, and the conversion into pepsin is completed after 15 min of activation. In a preparative experiment, 100 μg PgA3 (2.5 nmol) was activated for 2 min; after electrophoresis the zones were electroblotted on Immobilon, and sequenced with the results shown in fig.2. The sequence of the intermediate shows that cleavage has occurred between Leu-23 and Lys-24.

In all investigations on the specificity of pepsin A, a preference for apolar side chains at binding site S'1 is reported, e.g. [16]. Cleavage in front of Lys has never been found as a major cleavage site. In the present case other subsite bindings may be important, and the side chain of Lys may fold with the alkaline part (C_β to C_δ) to fit the apolar S'1 binding site, and with the ϵ-amino group outside the substrate binding cleft. Future model building may solve the question.

Sequencing of the intermediate was continued

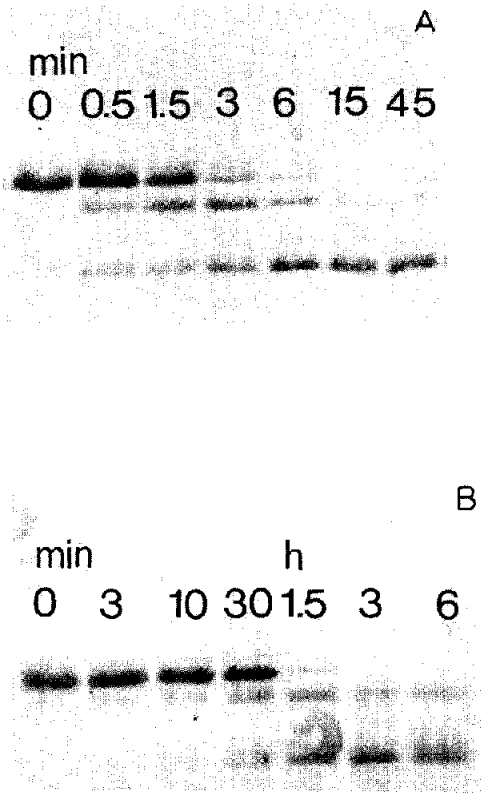

Fig.1. Activation of human isopepsinogens analysed by SDS-PAGE. (A) Pepsinogen A3; (B) pepsinogen A5. Duration of activation indicated above the lanes. Upper bands, pepsinogen; middle, intermediate; lower, pepsin. Samples equivalent to 8 μg of pepsinogen were applied to each slit, pepsin has a lower colour value than that of pepsinogen.

for 30 steps. At step no.20, corresponding to residue no.43 in human pepsinogen, Glu was seen without any trace of Lys. Thus, the propart of PgA3 corresponds to the N-terminal amino acid sequence reported by Sogawa et al. [6].

The conversion of PgA5 to pepsin takes place at a much slower rate than that of PgA3. In the experiment shown in fig.1B, the zone of pepsinogen persists for 1.5 h, and a faint zone of intermediate appears after 30 min. A preparative activation was carried out with 100 μg of PgA5 for 45 min. The zones were electroblotted and sequenced. The results are summarized in fig.2. The intermediate zone produced a double sequence, indicating cleavage in front of Lys-24 and Phe-26. The two sequences occurred in the ratio 2:1, and were iden-

Fig.2. N-terminal amino acid sequences of human isopepsinogens. PgA3 sequence according to Sogawa et al. [6]. PgA5 sequence according to Evers et al. [7]. (AM Dig.) Sequences after digestion with *Armillaria mellea* proteinase. The horizontal arrows indicate sequences determined in the present investigations. Vertical arrows indicate cleavage points during the activation. The amino-terminus of pepsin is Val (underlined).

tified through 5 steps (Lys-28 and His-30, respectively). Preparative activation of PgA5 was repeated with electrophoresis, electroblotting and sequencing after 2 h of activation. In this case the sequences starting with Lys-24 and Phe-26 occurred in the ratio 1:10.

In order to investigate if PgA5 has Lys at position no.43 the zymogen was digested with *Armillaria mellea* proteinase which cleaves in front of Lys with a high degree of specificity [12]. A precipitate which remained after 5 h of digestion was analysed by sequencing. A double sequence appeared, but through 6 steps it was easy to identify the N-terminal sequence of pepsinogen together with a sequence starting with Lys and continuing to the second residue in pepsin (Val). Thus, though the reaction was incomplete the results show that PgA5 has Lys at position 43 as found in [7], and the present investigations support previous preliminary results [3].

By amino acid sequencing of human pepsinogen Kageyama and Takahashi [10] found mostly the same sequence as that deduced from nucleotide sequencing, but Arg-Lys at position 42–43. Their activation experiments were stopped by addition of pepstatin, and cleavage of the bond Asp-25–Phe-26 was observed.

Electrostatic interactions are important for stabilizing the tertiary structure of pepsinogen [9]. Substitutions of basic residues may therefore influence the conformational changes which make the peptide bonds available for cleavage. But in addition to the substitutions in the propart of human PgA mentioned here, changes may also occur in the enzyme moiety of the zymogens. The nucleotide sequencings are carried out with DNA from embryonic [6] and placental [7] libraries. Such genes do not necessarily correspond to those that are dominantly expressed in the gastric mucosa. Amino acid analyses of the chromatographically purified PgA3 and PgA5 show minor differences from the nucleotide derived compositions. The problem is under further investigation with fingerprint analyses.

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NOTE ADDED IN PROOF

The substitution of Glu to Lys in PgA3 and PgA5 was recently reported in another paper: Bank, R.A., Crusius, B.C., Zwiers, T., Meuwissen, S.G.M., Arwert, F. and Pronk, J.C. (1988) FEBS Lett. 238, 105–108.

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