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A fourth trypsinogen (P23) in the rat pancreas induced by CCK

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P23 is a secretory protein synthesized by the unstimulated rat pancreas in relatively small amounts (0.4% of total exocrine product). By non-reducing 2D-IEF/SDS-PAGE it has an estimated Mr of 23 kD, similar to the known acidic ($T_{1,2}$) and basic (T_3) forms of trypsinogen, and a pI of 6.2, between those of the trypsinogens (4.3, 4.4 and 8.0). Caerulein, an analog of CCK/PZ (Cholecystokinin/Pancreozymin), stimulates its absolute synthesis 14-fold (1). By this criterion, P23 is the most strongly regulated protein of the rat exocrine pancreas.

Using a polyclonal rabbit antiserum raised against P23 isolated from 2D gels, four recombinant plaques were identified in a λ zap (Stratagene) cDNA expression library made with poly(A)$^+$-enriched RNA from CCK-stimulated rat pancreas. Recombinant plasmids were excised *in vivo* with helper phage R408 (Stratagene) from the λ clones and sequenced on both strands using Sequenase (USB Corporation). The sequence of the longest clone (P23/2) is shown with its deduced amino acid sequence.

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                                                                                                         1  GCACTAGGAACACC

     1                         10             V          20            V               30                                  40
     MetLysIleSerIlePhePheAlaPheLeuGlyAlaAlaValAlaLeuProValAsnAspAspAspLysIleValGlyGlyTyrThrCysProLysHisLeuValProTyrGlnValSer
  15 ATGAAGATCAGCATCTTCTTTGCTTTTCTCGGAGCTGCTGTTGCTCTCCCTGTTAATGATGATGACAAGATTGTTGGAGGCTACACATGTCCGAAGCATTTGGTTCCTTACCAGGTGTCT

                               50                         60                         70                                  80
     LeuHisAspGlyIleSerHisGlnCysGlyGlySerLeuIleSerAspGlnTrpValLeuSerAlaAlaHISCysTyrLysArgLysLeuGlnValArgLeuGlyGluHisAsnIleHis
 135 TTGCATGATGGCATTAGCCACCAGTGTGGTGGCTCCCTTATCAGTGATCAGTGGGTACTGTCTGCTGCTCATTGCTATAAAAGGAAACTCCAGGTTCGCCTTGGTGAACACAATATTCAT

                               90                        100                        110                                 120
     ValLeuGluGlyGlyGluGlnPheIleAspAlaGluLysIleIleArgHisProGluTyrAsnLysAspThrLeuAspAsnASPIleMetLeuIleLysLeuLysSerProAlaValLeu
 255 GTTCTTGAGGGTGGAGAGCAATTCATTGATGCAGAAAAGATCATTCGACACCCTGAGTATAACAAGGACACTCTGGACAATGACATCATGCTGATTAAATTGAAGTCACCTGCCGTCCTT

                              130                        140                        150                                 160
     AsnSerGlnValSerThrValSerLeuProArgSerCysAlaSerThrAspAlaGlnCysLeuValSerGlyTrpGlyAsnThrValSerIleGlyGlyLysTyrProAlaLeuLeuGln
 375 AACTCCCAAGTATCTACGGTCTCTCTGCCCAGATCCTGTGCATCTACAGATGCTCAGTGCCTTGTGTCTGGCTGGGGAAACACTGTGAGCATTGGTGGTAAATACCCAGCACTCCTTCAA

                              170                        180                        190                                 200
     CysLeuGluAlaProValLeuSerAlaSerSerCysLysLysSerTyrProGlyGlnIleThrSerAsnMetPheCysLeuGlyPheLeuGluGlyGlyLysAspSerCysAspGlyAsp
 495 TGCCTGGAAGCCCCTGTCCTCTCTGCTAGTTCTTGCAAAAAATCCTACCCAGGCCAGATCACCAGCAATATGTTCTGCCTGGGCTTCCTGGAGGGTGGAAAGGACTCCTGTGATGGTGAC

                              210                        220                        230                                 240
     SERGlyGlyProValValCysAsnGlyGluIleGlnGlyIleValSerTrpGlySerValCysAlaMetArgGlyLysProGlyValTyrThrLysValCysAsnTyrLeuSerTrpIle
 615 TCTGGTGGCCCTGTTGTCTGCAATGGAGAGATCCAGGGCATTGTCTCCTGGGGTTCAGTCTGTGCAATGAGAGGGAAGCCTGGTGTTTACACCAAAGTCTGCAACTACCTGAGCTGGATT

     GlnGluThrMetAlaAsnAsn
 735 CAGGAGACTATGGCGAACAACTGAGTTCTCTTACCTTTCGTAAATCACCGGTTCACTATCCATTTCCCTTTCTTCCTATACCTGAAATGAGGTTAAAATAAATAATTTTCTCCTGCTCT-
                                                                                                                    poly(A)
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Nucleotide sequence of a cDNA encoding P23 (EMBL Accession no. X15679), a novel rat pancreatic trypsinogen

All of the shorter clones contained codons of the signal peptide or the activation peptide on their 5' ends and were identical in the remaining sequence except for one lacking a poly(A) tail and containing a single point mutation. The nucleotide homology to $T_{1,2}$ and T_3 is 71% and 78%, respectively, with resulting non-conservative amino acid changes mainly applying to charged residues. The net charge of -3 of the proenzyme aggrees with the observed pI of 6.2. We propose that P23 is a fourth trypsinogen (T_4) because (i) its amino acid sequence contains all the major structural features of other trypsins (2-4): the signal peptide (up to left V) followed by an activation peptide with a typical polyanionic charge cluster preceding the activation cleavage position (right V); an obligatory Asp at position 195; the catalytic triad of HIS_{64}, ASP_{108} and SER_{201}; and finally, six conserved disulphide bonds (Cys-Cys: 30-161, 49-65, 133-234, 140-207, 172-186 and 197-221) which explain the faster mobility of P23 in non-reducing SDS-PAGE (Reduced, the apparent Mr corresponds more closely to the calculated Mr of 26.6 kD). (ii) The trypsin activity of renatured P23 cut out from a 2D gel was found to be comparable to those of $T_{1,2}$ and T_3. (iii) The polyclonal antiserum used for the screening cross-reacted with T_3 and weakly so with $T_{1,2}$ in protein blots.

The physiological role of this fourth trypsinogen is unknown as are the function and the level of its hormonal regulation.

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