Isolation of a Proinsulin Connecting Peptide Fragment (C-Peptide) from Bovine and Human Pancreas

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Studies of insulin biosynthesis have led to the discovery of proinsulin, a single-chain precursor (1, 2). Proinsulins have now been isolated and characterized from bovine and porcine crystalline insulin (3, 4) and the amino acid sequence of the peptide segment that connects the amino-terminus of the A chain to the carboxyl-terminus of the B chain has been elucidated in both species (4, 5). Further studies in the rat have detected a large fragment from the connecting segment which we have designated the C-peptide. This peptide consists of all the amino acids present in the proinsulin connecting segment except for the two pairs of basic residues through which it is linked to the insulin chains in proinsulin (i.e., residues 33-58 in the case of bovine proinsulin [5]). Labeled C-peptide was found in the insulin peak after gel filtration on Sephadex G-50 of extracts of rat islets incubated with H^3-leucine or H^3-proline (6). It could be separated from the insulin either by electrophoresis on polyacrylamide gels at pH 8.9 or by paper electrophoresis in 30% formic acid (7). Moreover, labeled C-peptide appeared with newly synthesized insulin when the insulin was secreted into the medium

from incubated islets, and the portion remaining in the islets declined only slightly relative to insulin over a period of 16 hours of incubation (6).

It is thus evident that the C-peptide is retained in the islet cells after the transformation of proinsulin to insulin. The C-peptide probably is liberated along with insulin into the circulation. Preliminary experiments with gel-filtered extracts of bovine plasma confirm this possibility, as displacement of ^{131}I-proinsulin from an insulin-absorbed bovine proinsulin antiserum (8) occurred in the fractions containing proinsulin and also in the fraction containing the peptide (9). On the basis of these observations we have now succeeded in isolating the C-peptide from the pancreas of several mammalian species.

Experimental

Fresh-frozen bovine pancreas was obtained from Illinois Packing Company (Chicago). Human pancreas specimens were obtained from post-mortem examinations of patients without a history of diabetes mellitus or pancreatic disease.

The frozen or iced fresh pancreas (200 g) was cut into small slices and homogenized with 3 ml/g wet weight of acid-ethanol (375 ml 95% ethanol; 7.5 ml conc. HCl) at 3°C in a blender. The homogenate was stirred for 4-16 hours at 2° with a magnetic stirrer, then centrifuged and the cold supernatant fluid adjusted to pH 8.0 with conc. NH_4OH. The precipitate was centrifuged off and discarded. The pH of the supernatant fluid was adjusted to 5.3 with 6 N HCl, using methyl red as the indicator, and 1/40th volume of 2 M ammonium acetate, pH 5.3, was added. Two volumes of ethanol and four volumes of diethyl ether were then added to precipitate the protein. After standing overnight at 2°, the precipitate was collected, dissolved in 1 M acetic acid and fractionated on an 8 X 100 cm column of Sephadex G-50 in 1 M acetic acid (4). The fractions containing insulin were determined both by absorbancy at 275 mμ as well as by immunoassay (10). The combined fractions

were concentrated in vacuo and refractionated on a smaller column of Sephadex G-50 (2.5 X 160 cm).

The insulin containing fractions were concentrated in vacuo and small aliquots (ca. 0.1 to 0.5 mg protein) were spotted on sheets of Whatman 3 MM paper moistened with 30% formic acid. After electrophoresis for 5 hours at 5 volts/cm, the paper was dried and sprayed with ninhydrin, Pauli reagent, or Sakaguchi reagent (11). The results are shown diagrammatically in Figure 1A. The numbered fractions were eluted with 50% acetic acid from the paper (12). The amino acid composition (13) of Fraction 1 which had the same mobility as the bovine C-peptide isolated from tryptic digests of the bovine proinsulin intermediate fraction (5) also had a composition similar to the bovine peptide except for excess glycine and small amounts

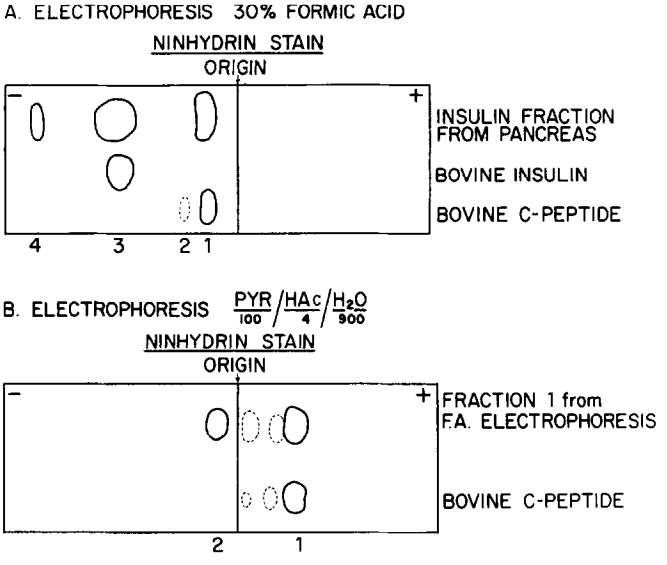

Figure 1A. Diagram of a stained paper electrophoretogram of the insulin-containing fraction separated by gel filtration of bovine pancreas extracts. Fraction 1 did not react with the Pauli or Sakaguchi reagents. Fraction 3 contained both insulin and glucagon, while Fraction 4 was a peptide of unrelated composition. Similar results were obtained with human pancreas extracts.

Figure 1B. Diagram of a stained paper electrophoretogram of the Fraction 1 eluted from the paper after formic acid (F.A.) electrophoresis. Spot 1 corresponds with bovine C-peptide while Spot 2 contains most of the amino sugar and contaminating amino acids found with the C-peptide fraction after the first electrophoresis in formic acid (Fig. 1A).

of aspartic acid, serine, threonine and an amino sugar which was probably glucosamine (14).

Electrophoresis of Fraction 1 on paper in pyridine-acetic acid buffer, pH 6.5, resolved the material into at least four components (Figure 1B). The major component (designated 1) had the same mobility as the bovine C-peptide and an identical amino acid composition (Table 1). A similar peptide isolated in the same fashion from human pancreas differed only slightly in composition from the bovine peptide (Table 1). The N-terminal residue of both peptides was glutamic acid as determined by the dansyl

TABLE 1

Amino Acid Composition of C-Peptides from Bovine and Human Pancreas

	Residues/Molecule		
	BOVINE C-PEPTIDE	BOVINE PROINSULIN CONNECTING SEGMENT†	HUMAN C-PEPTIDE
Asp			1.10 (1)
Ser			1.80 (2)
Glu	5.84 (6)	6	6.85 (7)
Gly	8.08 (8)	8	6.70 (7)
Ala	2.97 (3)	3	2.80 (3)
Val	1.93 (2)	2	1.95 (2)
Leu	3.00 (3)	3	4.75 (5)
Pro	3.48 (4)	4	2.24 (2)
Lys		1	
Arg		3	
Total	26	30	29

†Excess residues over bovine insulin (5). Samples were hydrolyzed 26-30 hours in 5.8 N HCl at 110°.

technique (15). The authentic bovine C-peptide has N-terminal glutamic acid (5).

Discussion

A peptide having many properties identical to the bovine proinsulin C-peptide has been found in suitable extracts of bovine pancreas. A similar peptide fraction has also been separated from human (and monkey) pancreatic extracts. The amount of peptide found appears to correspond roughly on a molar basis with the quantity of insulin present. We interpret these results as showing that the C-peptide, liberated by proteolysis of proinsulin in the islet cells, is retained (presumably within secretory granules) and subsequently liberated into the circulation with insulin when the hormone is secreted.

Complete amino acid sequence analysis of these peptides will be required, of course, to confirm their identity with the proinsulin C-peptides. It is not surprising that the peptides which we have isolated do not contain any of the basic residues, pairs of which are positioned at both ends of the connecting peptide segment of intact proinsulin (3, 5). Since the conversion of proinsulin to insulin may involve proteolytic enzymes having properties similar to trypsin and carboxypeptidase B(4, 5), it would be reasonable to suppose that their action would also divest the peptide of these basic residues.

The availability of the C-peptide fragment which comprises most of the amino acid sequence of the proinsulin connecting peptide segment will permit elucidation of proinsulin structures from a much wider range of species than hitherto has been considered feasible. The fact that this peptide is probably not degraded within the islet cells, but instead is discharged into the circulation, also raises the interesting possibility that it may possess some physiological activity.

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