Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 9662–9666, December 1989
Biochemistry

Molecular and functional characterization of amylin, a peptide associated with type 2 diabetes mellitus

(genomic clone/carboxyl-terminal amidation/chromosome 12/glycogen synthesis/insulin resistance)

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Communicated by David C. Phillips, August 21, 1989 (received for review February 13, 1989)

ABSTRACT **The 37-amino acid peptide called amylin is a major component of the islet amyloid deposited in the pancreases of persons with type 2 diabetes mellitus. We report the isolation of a partial cDNA clone and a phage λ genomic clone of the coding region of the amylin gene. The DNA sequence encodes a protein sequence identical to that of amylin isolated from the amyloid found in the diabetic pancreas and shows that amylin is likely to be synthesized as a precursor peptide, now named proamylin. We have demonstrated that the amylin gene is present on chromosome 12 and that it is probably transcribed in the islets of Langerhans. The sequences of the genes for amylin and the calcitonin gene-related peptides (CGRPs) show strong similarity, especially over their 5′ coding regions, where both peptides have a conserved intramolecular disulfide bridge, and also over their 3′ coding regions, where the presence of a glycine codon strongly suggests that the carboxyl-terminal residue of amylin, like that of CGRP, is amidated. To examine the functional relevance of these posttranslational modifications, the biological activity of amylin synthesized with or without the disulfide bridge and/or amidation was measured. It was found that both features are necessary for full biological activity, thereby confirming the functional importance of those regions of the molecule whose sequences are conserved at both protein and genetic levels.**

Type 2 (non-insulin-dependent) diabetes mellitus (NIDDM) is one of the most common chronic diseases. In the United States, some 2.5% of the population has been diagnosed as having diabetes (5.8 million persons based on 1984 estimates), of whom ≈90–95% have NIDDM (1). An equivalent number are undiagnosed (1).

The causes of NIDDM are unknown, but one major component is clearly genetic, as judged by recent studies showing the near 100% concordance for the development of the condition in identical twins (2) and the high incidence of the disease in certain third world groups who appear to be predisposed to diabetes as a result of a change in lifestyle—e.g., change to a Western diet (3). NIDDM currently may be defined as a heterogeneous group of disorders characterized by elevated blood glucose levels, abnormal insulin secretion, and varying degrees of hepatic and peripheral insensitivity to the effects of insulin (insulin resistance). However, despite an enormous amount of research into the problem, the primary lesions responsible for NIDDM remain unknown (4).

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In 1900, discovery of hyaline (amyloid) in the islets of Langerhans of diabetic patients led Opie (5) to suggest that this lesion may be causally related to the type of diabetes now classified as NIDDM. Recent support for this theory has been given by the demonstration that the extent of islet amyloid increases with the severity of NIDDM [as measured by the need for insulin therapy (6)] and the report that >90% of NIDDM patients have amyloid (7).

In addition, studies from our laboratories also implicate islet amyloid as an important pathological feature of NIDDM. The 37-amino acid peptide amylin is a major component of islet amyloid from patients with NIDDM (8). Its amino acid sequence is 46% and 43% identical to those of the calcitonin gene-related neuropeptides CGRP-2 and CGRP-1, respectively, and shows weaker homology with segments of the A chains of the relaxins and insulin (8, 9). Quantitatively, the major site of insulin-stimulated glucose uptake is skeletal muscle where, in humans, it is deposited as glycogen, a polymer of glucose that acts as a fuel source. In type 2 diabetes, insulin-stimulated rates of glycogen synthesis are markedly reduced (10–12), contributing to the occurrence of hyperglycemia.

We have demonstrated that both amylin and CGRP are potent inhibitors of glycogen synthesis in rat skeletal muscle *in vitro* and have proposed that one of the earliest events in NIDDM could be an excessive production of amylin leading to higher circulating levels of the peptide and deposition of islet amyloid (13–15); this would cause impaired glucose clearance from blood into skeletal muscle and hence hyperglycemia. These observations support our earlier hypothesis that amylin is a hormone and that a disorder of amylin and/or CGRP homeostasis underlies the pathogenesis of type 2 diabetes mellitus (13, 14).

The entire sequence of human amylin has so far been determined only from the pancreases of type 2 diabetic patients (8), so the question remained as to whether the amino acid sequence of amylin in a normal person is the same as that previously determined from diseased subjects.

This paper examines the amylin gene and the structure of its product, in particular those features necessary for biological activity. The problem has been assessed by cloning the amylin gene from a nondiabetic source. The derived amino acid sequence of amylin supported the presence of two posttranslational modifications in the peptide as have been

Abbreviations: CGRP, calcitonin gene-related peptide; NIDDM, non-insulin-dependent diabetes mellitus.

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noted (8). We measured the biological activity of synthetic peptides of amylin, with or without these modifications, in an *in vitro* rat skeletal muscle bioassay (13). In addition, the structure of the amylin gene has been investigated by Southern blotting, and the chromosomal location of the gene has been determined. Evidence for transcription of amylin in normal adult human islets of Langerhans is presented.

MATERIALS AND METHODS

Isolation of Human Islets of Langerhans. Human pancreas was obtained (with appropriate permission) from nondiabetic heart-beating cadaver organ donors after *in situ* perfusion via the splenic artery with hypertonic citrate solution at 4°C. Islets were isolated by a modification of an intraductal collagenase injection procedure as described (16).

***In Vitro* DNA Synthesis.** Total and poly(A)$^+$ RNA were prepared as described (17, 18) from human islets isolated as above. cDNA was prepared from this RNA (19) and provided the template for *in vitro* DNA synthesis using *Thermus aquaticus Taq* I polymerase (20) and two oligonucleotides corresponding to the N-terminal and C-terminal portions of the amylin polypeptide as primers (21). After purification through an agarose gel, this DNA band provided the template for a second round of *in vitro* DNA synthesis with the two amylin oligonucleotides and Klenow DNA polymerase. The resultant DNA was purified and subcloned into M13mpRV8.2 (22). Further subcloning used pBluescript KS(+) (Stratagene). This cloned DNA segment is referred to as the 110-base-pair (bp) amylin clone.

Genomic Library. A human λ bacteriophage EMBL3 genomic library, made from DNA isolated from the 48;XXXX cell line GM1416B, was kindly provided by Derek Blake, Oxford University. This was screened at a density of 50,000 plaque-forming units per 150-mm diameter plate with the 110-bp amylin clone. A single positive clone was purified to homogeneity and mapped by standard techniques with restriction enzymes. A 3.5-kilobase (kb) *Bgl* II fragment was subcloned into pUC118 and used for sequencing.

DNA Sequencing. Single-stranded DNA for sequencing was prepared from clones in M13mpRV8.2 or from pUC118 clones after induction with the helper phage M13KO7 (23). DNA was sequenced by the dideoxy chain-termination method (24).

Peptide Synthesis and Analysis. Amylin peptides were synthesized as described (14) according to the known sequence of the peptide (8). The molecule was synthesized with or without an intact disulfide bond (from Cys-2 to Cys-7) and with or without C-terminal amidation to yield four variant amylin molecules: (*i*) linear nonamidated amylin-(1–37); (*ii*) cyclized (i.e., with an intact Cys-2 to Cys-7 disulfide bridge) nonamidated amylin-(1–37); (*iii*) linear amidated amylin-(1–37); and (*iv*) amylin (which we have shown to be cyclized and amidated). The formation of correctly cyclized material by chemical synthesis was difficult partly because the two cysteine residues are the minimum distance apart for the formation of an intramolecular disulfide bridge; therefore, it is unlikely that reoxidation would occur spontaneously in solution. The peptide samples were always purified by HPLC immediately before use; linear and cyclized material displayed distinct retention times, indicating that in the linear molecule the cysteine residues have not reoxidized and cyclized the peptide. The activities of the synthetic peptides were compared to that of amylin extracted from the pancreases of type 2 diabetics according to previously described methods (8, 13). Peptides were analyzed by HPLC (8). Amino acid compositions of all peptides were confirmed by using a Waters Pico·Tag amino acid analysis system (25), and the amino acid sequences of all peptides used in functional assays were checked by protein sequence analysis on an Applied Biosystems 470A protein sequencer with on-line phenylthiohydantoin analysis (26) using the 03PTH cycle in the version 3.0 software (Applied Biosystems). The sequence and quantitative amino acid analysis indicated that all of the synthetic amylin peptides used in the functional assay experiments were substantially pure and had the correct amino acid sequence.

Measurement of Biological Activity of Peptides. Biological activities of synthetic and natural peptides were determined by measurement of insulin-stimulated rates of glycogen synthesis and lactate formation in the isolated incubated stripped soleus muscle of the rat, with materials and methods as previously described (13, 14, 27).

RESULTS

Cloning of the Amylin Gene. Results obtained from the cloning of homologous major histocompatibility complex genes (28) using gene amplification indicated that efficient and specific DNA amplification could be achieved despite mismatches between the target and primer DNA sequences. From the published amino acid sequence of the amylin polypeptide (8), two nondegenerate oligonucleotides were designed, biased for human codon usage (29). Amylin oligonucleotide 1 corresponded to the N-terminal portion of the amylin polypeptide (Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln) and was equivalent to the mRNA strand (5′-AAG TGC AAC ACA GCC ACC TGT GCC ACC CA-3′); amylin oligonucleotide 2 corresponded to the C-terminal end of the amylin polypeptide (Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr) and was complementary to the mRNA sequence (5′-TA GGT GTT GGA GCC CAC ATT GGT-3′). These oligonucleotides were primers for *in vitro* DNA synthesis with cDNA derived from poly(A)$^+$ mRNA prepared from isolated human islets of Langerhans as template. The products of this reaction were run on an agarose gel, and DNA of the expected length (110 bp) was purified, ligated into the *Eco*RV site of M13mpRV8.2 (22), and transformed into *Escherichia coli* JM 101. From the DNA sequence of 12 recombinants picked at random, it was found that 7 of the clones were identical and corresponded to a DNA sequence that encoded the central 19 amino acids of amylin flanked on either side by the sequence of amylin oligonucleotides 1 and 2. The DNA segment of the amylin gene derived from the *in vitro* DNA synthesis reaction was subcloned into pBluescript KS(+) and used to probe a bacteriophage–human genomic library prepared from the 48;XXXX cell line, GM1416B. One positive clone, named λ EMBL-13, was identified and purified after screening approximately 1×10^6 plaques.

Restriction analysis identified a 3.5-kb *Bgl* II fragment within λ EMBL-13 that cross-hybridized with the 110-bp probe. A similar-size band was also identified on genomic Southern blots (see later). Subcloning and sequence analysis with internally priming oligonucleotides that corresponded to the central portion of the 110-bp fragment revealed that the sequence obtained encoded a polypeptide that matched the sequence of amylin. By using a series of synthetic primers, the 474-bp segment of DNA displayed in Fig. 1 was sequenced on both strands.†† Inspection of the DNA sequence revealed an open reading frame encoding a peptide with the same sequence as amylin. This sequence is similar to that reported recently for islet or insulinoma amyloid polypeptide (IAPP) (30) except for a number of differences in the intronic regions. The sequence showed that both amylin oligonucleotides 1 and 2 used in the initial *in vitro* DNA synthesis

††The nucleotide sequence presented here has been submitted to the GenBank data base (accession no. M27503).

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TTTTCATCAATACAAGATATTTGATGTCACATGGCTGGATCCAGCTAAAATTCTAAGGCTCTAACTTTTCACATTTGTTCCATGTTACCAGTCATCAG
                    20                  40                  60                  80

                1                                   10                                  20
 V  E  [K  R] K  C  N  T  A  T  C  A  T  Q  R  L  A  N  F  L  V  H  S  S  N
GTG GAA AAG CGG AAA TGC AAC ACT GCC ACA TGT GCA ACG CAG CGC CTG GCA AAT TTT TTA GTT CAT TCC AGC AAC
100                     120                 140                 160

                        30                  37
 N  F  G  A  I  L  S  S  T  N  V  G  S  N  T  Y [G  K  R] N  A  V  E  V  L
AAC TTT GGT GCC ATT CTC TCA TCT ACC AAC GTG GGA TCC AAT ACA TAT GGC AAG AGG AAT GCA GTA GAG GTT TTA
      180                 200                 220                 240

 K  R  E  P  L  N  Y  L  P  L  *
AAG AGA GAG CCA CTG AAT TAC TTG CCC CTT TAG AGGACAATGTAACTCTATAGTTATTGTTTTATGTTCTAGTGATTTCCTGTATAATT
          260                 280                 300                 320

TAACAGTGCCCTTTTCATCTCCAGTGTGAATATATGGTCTGTGTGTCTGATGTTTGTTGCTAGGACATATACCTTCTCAAAAGATTGTTTTATATGTAGT
340                 360                 380                 400                 420

ACTAACTAAGGTCCCATAATAAAAAGATAGTATCTTT
440                 460
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FIG. 1. The nucleotide sequence of the exon encoding the amylin polypeptide and surrounding DNA contained in λ EMBL-13. The sequence was obtained on both strands and is numbered underneath. The amino acid sequence of proamylin in the one-letter code is shown above, commencing at the nearest potential 3′ splice site to the first residue of amylin. The amino acids of amylin are numbered above. A probable 5′ proteolytic site and the 3′ amidation site are boxed. The asterisk indicates a stop codon. The two potential 3′ splice sites (AG) are underlined.

reaction for the cloning had 17% mismatch with the amylin gene sequence.

The primary amino acid sequence homology between amylin and CGRP has already been noted (8). It is interesting to see that this homology is also found at the DNA level [55% between CGRP-2 (31) and amylin DNA]. This is particularly striking in those regions where the amino acids are most strongly conserved—namely, between residues 2 and 16 and between 30 and 35 (Fig. 2). The result indicates that amylin and the CGRPs may have shared a common ancestral gene and therefore have evolved divergently.

In frame with the amylin peptide sequence are additional amino acids at the N and C termini, indicating a maturation strategy by proteolytic cleavage of the precursor molecule. Until intron and exon boundaries have been mapped, their positions are not known definitively. As found in the CGRP gene (32), the 37 amino acids of the amylin polypeptide are encoded in one exon. At the N terminus, the DNA sequence reveals two 3′ potential splice sites (located at base pairs 89 and 96 in Fig. 1), one of which must define the amylin exon boundary at the 5′ end as there is an in-phase stop codon at nucleotide 54. The splice site nearest to the first amino acid (lysine) of the amylin sequence is delineated in Fig. 1. This segment of DNA encodes an additional four amino acids (Val-Glu-Lys-Arg). The two basic residues (Lys-Arg) immediately in front of the 37 amino acids of amylin are characteristic of a proteolytic cleavage site and are also found in the CGRPs (31). At the C terminus, the DNA sequence encodes an additional 19 amino acids in frame with amylin.

Of direct relevance to this paper are the Gly-Lys-Arg residues next to the C terminus of amylin purified from the diabetic pancreas. These three residues, in particular the glycine, contribute a proteolytic cleavage and amidation signal. Together they identify the putative proteolytic processing site for the formation of amylin from proamylin at the C terminus and strongly suggest that the C-terminal tyrosine is amidated *in vivo*.

Southern Analysis. Human genomic DNA was analyzed by Southern blotting. By using the 110-bp amylin clone as a probe, single cross-reacting bands of ≈25 kb with *Sac* I, 15 kb with *Bcl* I, 14 kb with *Eco*RI, 13 kb with *Sph* I, 10 kb with *Pst* I, 8 kb with *Hin*dIII, 4.1 kb with *Kpn* I, and 3.5 kb with *Bgl* II were identified (not shown). These results suggest that amylin is present as a single-copy gene or as multiple closely positioned genes, within the human genome. There was no evidence of cross-reactivity with the genes for CGRP (results not shown).

Chromosomal Localization. Preparation of DNA and Southern analysis were performed as described (33). Hybridization of the 110-bp amylin probe to Southern blots of *Hin*dIII-digested DNA from a panel of 10 karyotyped human/rodent somatic cell hybrids revealed that the most likely location of the amylin gene is on chromosome 12 (100% concordance; Table 1). Assignment to chromosome 15 (90% concordance) was excluded by absence of a hybridization signal in one hybrid containing this chromosome.

Biological Activity of Amylin Peptides. Previously reported protein characterization experiments (8) suggested that the amylin peptide contains an intramolecular disulfide bond between Cys-2 and Cys-7. C-terminal identification experiments with carboxypeptidase Y provided evidence that the amylin peptide was C-terminally blocked (8). In a recent experiment, we have demonstrated that an amidated phenylalanine residue originating from the C terminus of a synthetic pentapeptide gives the same peak as an unmodified phenylalanine when subjected to standard protein sequenc-

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Position   1                                   10                                  20
Amylin      K   C   N   T   A   T   C   A   T   Q   R   L   A   N   F   L   V   H   S   S
Amylin     AAA TGC AAC ACT GCC ACA TGT GCA ACG CAG CGC CTG GCA AAT TTT TTA GTT CAT TCC AGC
CGRP 2     GCC TGC AAC ACT GCC ACC TGT GTG ACT CAT CGG CTG GCA GGC TTG CTG AGC AGA TCA GGG
CGRP 2      A   C   N   T   A   T   C   V   T   H   R   L   A   G   L   L   S   R   S   G

Position                                30                                  37
Amylin      N   N   F   G   A   I   L   S   S   T   N   V   G   S   N   T   Y
Amylin     AAC AAC TTT GGT GCC ATT CTC TCA TCT ACC AAC GTG GGA TCC AAT ACA TAT
CGRP 2     GGC ATG GTG AAG AGC AAC TTC GTG CCC ACC AAT GTG GGT TCC AAA GCC TTT
CGRP 2      G   M   V   K   S   N   F   V   P   T   N   V   G   S   K   A   F
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FIG. 2. DNA sequence comparison between amylin and human CGRP-2 (31) over the exons encoding the mature peptides. Identical codons are underlined.

Table 1. Correlation of hybridization signal from the 110-bp amylin probe with human chromosomes present in human–rodent somatic cell hybrid

Chromosome	Hybrids, nos.		% concordance
	Concordant	Discordant	
1pter–p34	5	5	50
1p34–qter	6	4	60
2	5	5	50
3	4	6	40
4	7	3	70
5	4	6	40
6pter–q21	5	5	50
6q21–qter	4	6	40
7	6	4	60
8	7	3	70
9	5	5	50
10	4	6	40
11	5	5	50
12	10	0	100
13	6	4	60
14	3	7	30
15	9	1	90
16	6	4	60
17	7	3	70
18	7	3	70
19pter–q13	4	6	40
19q13–qter	5	5	50
20	6	4	60
21	7	3	70
22pter–q13	7	3	70
22q13–qter	7	3	70
Xpter–p21	7	3	70
Xp21–qter	5	5	50
Y	5	5	50

The numbers of somatic cell hybrids which are concordant and discordant with the human amylin 110-bp probe are shown for each chromosome. Some hybrids contain translocated chromosomes, hence concordance figures are shown separately for the chromosomal regions either side of the breakpoint.

ing procedures (A.C.W. & G.J.S.C., unpublished results). Therefore, it is likely that standard protein sequencing would be unable to discriminate between an amidated or nonamidated C-terminal residue (tyrosine) in the amylin molecule (8).

The DNA sequencing experiments reported above and those of others (30) are consistent with C-terminal amidation of amylin. These studies also demonstrate the presence of two regions in the coding sequence for amylin that are nearly identically conserved with corresponding regions in the exons coding for CGRP-1 (32) and CGRP-2 (31). The N-terminal conserved region corresponds to that of the intramolecular disulfide bond in the peptides, and the C-terminal region includes the amidation signal. These findings lend support to our conclusions that both of these regions may be important for the biological activity of the peptides.

The effect of synthetic amylin peptides on the rate of insulin-stimulated glycogen synthesis in the isolated stripped soleus muscle of the rat is shown in Fig. 3. The lowest concentration of the various peptides necessary to significantly inhibit glycogen synthesis was examined. Linear, nonamidated amylin had no biological activity at the supraphysiological concentration of 1 μM (results not shown). When the linear molecule was amidated or cyclized, a significant effect was first seen at 0.1 μM. However, synthetic cyclized, amidated amylin caused an effect at 1 nM, a 100-fold increase in biological activity compared with the peptide with only one modification. The effects of the synthetic amidated cyclized peptide are similar in magnitude to

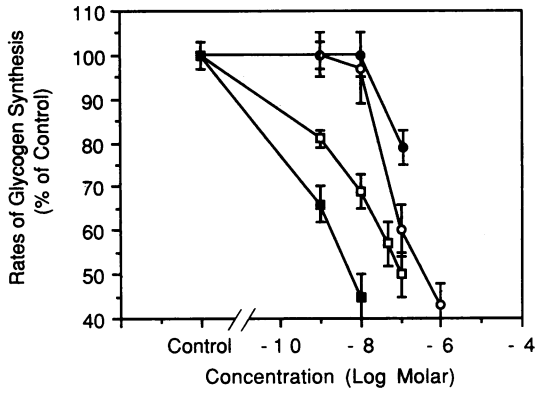

FIG. 3. The effects of human amylin peptides on insulin-stimulated rates of glycogen synthesis, expressed as the percentage of control in isolated incubated soleus muscle of the rat. Concentrations of glucose and insulin in the incubation medium were 5.5 mM and 100 microunits/ml, respectively (1 microunit of insulin per ml ≈ 7.1 pM). ●, Cyclized nonamidated amylin-(1–37); ○, linear amidated amylin-(1–37); □, cyclized amidated amylin-(1–37); and ■, amylin extracted from human type 2 diabetic pancreas. Results of functional assays are expressed as mean ± SEM, and statistical significance was determined by Student's *t*-test.

those of native amylin extracted from the pancreases of type 2 diabetics (13). None of the synthetic peptides were observed to affect insulin-stimulated rates of lactate formation at concentrations of 0.1 μM.

DISCUSSION

The usual method of cloning a gene for which all or partial amino acid sequence is known is to screen cDNA or genomic libraries with mismatched (sometimes degenerate) oligonucleotides. This process is often demanding and laborious but can be eased by using the oligonucleotides as primers in the rapid and sensitive polymerase chain reaction (21). This approach should have a general application for the cloning of genes encoding polypeptides of known amino acid sequence and indeed genes that contain regions of similarity—e.g., related genes and other family members. During the course of the studies reported in this paper, others (34, 35) have cloned genes by a similar strategy using degenerate primers.

The results of the *in vitro* DNA synthesis experiment, which used islet-derived poly(A)$^+$ mRNA as a template, show that the amylin gene is probably transcribed in the human islet of Langerhans and is likely to be expressed in this tissue, as suggested by the results of immunohistochemistry with amylin (36) and insulinoma amyloid polypeptide (7). Thus, there is the strong possibility that amylin is a candidate to be added to the group of peptide hormones—namely, insulin, glucagon, somatostatin, and pancreatic polypeptide—known to be synthesized in the islet of Langerhans (37).

Evidence from genomic and rodent–human hybrid Southern analysis indicates that the amylin gene is present in the human genome as a single-copy gene or as multiple closely positioned genes on chromosome 12. The assignment of amylin to chromosome 12 is of interest because it shows considerable homology to the CGRP family and weaker sequence similarity to the insulin and insulin-like growth factors (IGFs) (8, 9), which (with the exception of IGF-1) have been mapped to chromosome 11 (38). Chromosomes 11 and 12 contain several related pairs of genes, including those for IGF, which are represented in two homologous blocks on the distal short arm of chromosome 11 and the distal long arm of chromosome 12, thus supporting previous observations that chromosomes 11 and 12 share ancestral homology (39,

40). Our data and those of others (30) that map amylin to chromosome 12 may shed light on this relationship.

From the DNA sequence analysis of the amylin gene and deduced amino acid sequence, several important points can be made. First, the amino acid sequence of amylin, which originated either from a 48;XXXX cell line library clone or from islets of a nondiabetic human organ donor, is identical to that isolated and sequenced from five type 2 diabetic pancreases (ref. 8; G.J.S.C. and A.C.W., unpublished data). The fact that the amino acid sequences of amylin from diabetic and nondiabetic sources are the same implies that the lesion responsible for the deposition of high levels of amylin in individuals suffering from NIDDM is not an alteration in the primary sequence of the peptide. Second, the DNA sequence reported here differs in seven places from that of the related peptide IAPP (islet or insulinoma amyloid polypeptide), isolated from insulinoma (30). These differences are all located in the noncoding part of the sequence and correspond to positions 74, 297, 302, 305, 318, 349, and 354 in Fig. 1. Third, the DNA sequence has revealed that amylin is almost certainly synthesized as a precursor molecule, proamylin, that is processed at both the N and C termini by proteolytic cleavage to form the 37-amino acid molecule identified from the diabetic pancreas.

Our studies show that both an intact intramolecular disulfide bond and C-terminal amidation are necessary for full biological activity of the amylin peptide. The introduction of either modification alone restored only moderate activity, whereas introduction of both dramatically increased activity to approach that of the native molecule (13). These results indicate that experiments designed to test the role of amylin in both normal physiology and disease states, such as type 2 diabetes, should be performed with amylin that contains both an intact disulfide bridge and a C-terminal amide group.

Note. We originally assigned the name "diabetes-associated peptide" (DAP) to the molecule now known as amylin (8, 14). We selected the name "amylin" rather than DAP after conclusively demonstrating that the peptide is found in the pancreas of nondiabetics as well (36). The name was selected to indicate the presence of the peptide in amyloid and to reflect its biological activity. Others have written of a peptide having nearly the same primary structure but termed "islet" or "insulinoma amyloid polypeptide" (IAP/IAPP) (41).

Note Added in Proof. Since the submission of this paper, cDNA clones have been isolated and sequenced from human insulinomas (42, 43). The sequence of comparable regions of DNA were identical with the one reported in the present study.

We thank Dr. R. D. Campbell for helpful criticism of the manuscript. G.J.S.C. holds Oxford Nuffield and Girdler's Fellowships.

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