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Detection and partial sequence analysis of gastrin mRNA by using an oligodeoxynucleotide probe

(oligonucleotide synthesis/mRNA/cDNA/nucleotide sequencing/oligonucleotide hybridization)

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ABSTRACT We have used a specific deoxyoligonucleotide probe to detect gastrin mRNA in poly(A)-enriched RNA preparations from hog antrum. The nucleotide sequence of the oligonucleotide, d(C-T-C-C-T-C-C-A-T-C-C-A), was deduced from the unique amino acid sequence Trp-Met-Glu-Glu of gastrin. When used with hog antral RNA, the dodecanucleotide is an effective primer for the synthesis of gastrin-specific cDNA as judged by nucleotide sequence analysis of cDNA isolated by polyacrylamide gel electrophoresis. We have determined an 81-nucleotide sequence corresponding to the region of the gastrin mRNA that codes for the known amino acid sequence of the G34 progastrin intermediate species, and we have demonstrated the presence of two consecutive basic residues preceding the G34 sequence in the prohormone. Hybridization of gastrin cDNA or synthetic dodecanucleotide to hog antral RNA separated by gel electrophoresis on agarose gels in the presence of methylmercuric hydroxide indicates that the mRNA coding for gastrin is about 620 nucleotides long. These results suggest that the gastrin precursor peptide contains 110–140 amino acids. This method should be of general application for detection and characterization of mRNAs corresponding to proteins of known amino acid sequence.

Isolation and characterization of eukaryotic mRNAs and possible mRNA precursors is important in the development of our understanding of eukaryotic gene expression, regulation, and evolution. Many mRNAs studied have been obtained from highly specialized tissues in which a given mRNA occurs as a very abundant, if not single, species. Examples include hemoglobin, ovalbumin, and immunoglobulin light chain mRNAs. Study of mRNA species present in much lower amounts requires more sensitive detection and isolation procedures. Although immunoradiometric assays used in conjunction with cell-free translation systems have proved valuable in studies on several mRNAs, including insulin mRNA (1), this approach is dependent upon the availability of antibody capable of recognizing an initial translation product produced *in vitro*. This is a severe limitation for study of hormones such as gastrin for which available antibodies are directed primarily against the processed hormone. For example, the best available gastrin antibodies are very specific for the amidated carboxyl-terminal pentapeptide of the active hormone or the amino-terminal 13 amino acids of heptadecapeptide gastrin (2, 3). However, gastrin is synthesized as a precursor, progastrin, with an amino-terminal extension of 17 amino acids (4), and even larger precursors have been postulated (5–8). The presence of a carboxyl-terminal amide in gastrin suggests that processing may also occur at the carboxyl terminus of the initial translation product, as Suchanek and Kreil have proposed for melittin (9). Our unpublished results support the supposition that available antibodies will not precipitate a gastrin-specific translation product produced *in vitro*.

In this communication we describe an approach for detection and characterization of the mRNA for gastrin using an oligonucleotide probe whose sequence is deduced from a unique amino acid sequence of the hormone. The procedure allows the detection and partial sequence analysis of a mRNA species present as about 0.6% by weight of the poly(A)-enriched RNA preparation from hog antral mucosa, or about 0.005% of the total RNA extracted.

MATERIALS AND METHODS

Enzymes and Reagents. Reverse transcriptase (RNA-dependent DNA polymerase) from avian myeloblastosis virus was provided by J. W. Beard (Life Sciences, Inc., St. Petersburg, FL), and exonuclease-free T4 phage polynucleotide kinase (32,000 units/mg) was isolated by a modified procedure of Panet *et al.* (10). Oligo(dT)-cellulose was prepared as described by Gilham (11).

Preparation of Poly(A)-RNA. Antral mucosa from freshly slaughtered hogs was dissected from the pyloric gland area, wiped free of mucus, and immediately frozen in liquid nitrogen. The frozen tissue (15 g obtained from one stomach) was added to 80 ml of phenol/chloroform/isoamyl alcohol (50:48:2, vol/vol) and mixed with 80 ml of 0.2 M Tris·HCl (pH 9.0)/0.1 M LiCl/25 mM EDTA/1% sodium dodecyl sulfate. The mixture was homogenized with a Polytron (Brinkmann) operated at high speed for several short bursts totaling 1 min, and the phases were separated by centrifugation for 15 min at 15,000 × *g*. The aqueous phase was removed, and the organic phase and a large interphase were reextracted with an additional 80 ml of buffer. Finally, the aqueous phases were combined and reextracted with 40 ml of phenol/chloroform/isoamyl alcohol. Nucleic acids were precipitated from the aqueous phase at −20°C for 16–24 hr after addition of 0.1 vol of 2 M sodium acetate and 2.5 vol of 95% (vol/vol) ethanol. RNA was further purified by CsCl centrifugation as described by Glisin *et al.* (12) followed by chromatography on oligo(dT)-cellulose (13). Generally, material from 10 hog stomachs (150 g of tissue) was chromatographed on a 7.5-ml (2-g) column of oligo(dT)-cellulose in four batches. The poly(A)-enriched RNA from all four runs was pooled, chromatographed a second time on oligo(dT)-cellulose, concentrated by ethanol precipitation, and stored at −20°C in 2 mM Tris·HCl, pH 7.4, at 1–2 mg/ml.

Deoxydodecanucleotide Primer. The deoxydodecanucleotide d(C-T-C-C-T-C-C-A-T-C-C-A) was chemically synthesized by using the diester approach (14), and its nucleotide sequence was confirmed by two-dimensional homochromatography (15). [γ-^{32}P]ATP (specific activity 1475 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was synthesized as described by Maxam and Gilbert (16) and used for 5′-phosphorylation of the dodecanucleotide in the presence of T4 polynucleotide kinase

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(17). Generally 1 or 2 nmol of primer was labeled and separated from ATP and inorganic phosphate by chromatography on a 10-ml column of Sephadex G-50 superfine in 50 mM triethylammonium bicarbonate, pH 7.8. The dodecanucleotide pool was lyophilized, suspended in water, and relyophilized to remove all traces of triethylammonium bicarbonate, and finally dissolved in 50–100 μl of 15 mM Tris·HCl, pH 7.8.

cDNA Synthesis. cDNA was synthesized in 50 mM Tris·HCl, pH 8.3, containing 10 mM dithiothreitol, 50 mM NaCl, bovine serum albumin at 60 μg/ml, poly(A)-RNA at 130–150 μg/ml, [^{32}P]dodecanucleotide primer at 7–10 μg/ml, and 600 μM each of the four unlabeled deoxynucleoside triphosphates. Reverse transcriptase (lot G1377, 8 units μl, or lot G478, 4 units/μl) was added to give a final concentration of 400 units/ml and the reaction mixture was incubated at 37°C for 40 min. Reactions were stopped with the addition of EDTA to 12 mM followed by phenol/chloroform (50:50, vol/vol) extraction. The aqueous phase was washed with ether and adjusted to 0.3 M sodium acetate, and the [^{32}P]cDNA was precipitated by addition of 2.5 vol of 95% ethanol. The precipitate was washed with 95% ethanol, dried briefly under vacuum at room temperature, dissolved in 0.1 M NaOH/1 mM EDTA (25 μl per 50 μg of poly(A)-RNA in the original reaction mixture) and incubated at 37°C for 30 min. An equal volume of 10 M urea/0.05% xylene cyanol/0.05% bromphenol blue was added, and the mixture was heated to 90°C for 30 sec before being layered on a 12% polyacrylamide/7 M urea slab gel (38 × 24 × 0.3 cm) prepared as described by Maxam and Gilbert (16). Electrophoresis was carried out at 600 V until the xylene cyanol marker had migrated about 33 cm. [^{32}P]cDNA products were visualized by autoradiography (Kodak No-Screen film) and eluted from the gel (16).

DNA Sequence Analysis. 5′-^{32}P-Labeled cDNA prepared and isolated as described above was subjected to five of the base-specific cleavage reactions described by Maxam and Gilbert (16) with the following modifications. Methylations of adenine and guanine were performed in 54 μl, hydrazinolysis of cytosine and cytosine plus thymine were in 29 μl, and only 1 μg of carrier DNA was used in each cleavage reaction. In addition, equal aliquots were withdrawn from reaction mixtures after 5, 10, and 20 min and combined with the appropriate stop solution for further processing. For guanine-only and strong adenine–weak guanine cleavages, 14 μl of five times concentrated dimethyl sulfate stop solution was used (16). Aliquots, 9–10 μl each, from cytosine-only cleavage reactions were added to 40 μl of 0.14 mM EDTA containing base-hydrolyzed RNA carrier at 138 μg/ml. Aliquots from cytosine plus thymine reactions were added to 40 μl of stop solution containing 1.4 M sodium acetate at pH 5, 0.14 mM EDTA, and base-hydrolyzed RNA carrier at 138 μg/ml. Strong adenine–weak cytosine cleavage was carried out as described by Maxam and Gilbert (16) except that 8-μl aliquots taken after 5-, 10-, and 20-min reactions with 1.5 M NaOH were combined for further processing. Cleavage products from the five reactions were dissolved in 95% (vol/vol) formamide/10 mM EDTA/0.0012% each bromphenol blue and xylene cyanol FF, heated for 3 min at 90°C (18), and layered on a 20% acrylamide/7 M urea slab gel (16). Gels were 60 × 24 × 0.05 cm, and electrophoresis was carried out in 50 mM Tris borate (pH 8.3)/1.4 mM EDTA, at 2000 V.

RNA Hybridization. Poly(A)-RNA was electrophoresed on 1.5% agarose gels in the presence of 4 mM CH_3HgOH as described by Bailey and Davidson (19). The RNA was transferred to diazobenzyloxymethyl-cellulose paper prepared from Whatman 540 paper and hybridized with [^{32}P]cDNA probes as described by Alwine *et al.* (20) or with [^{32}P]dodecanucleotide. Hybridization with [^{32}P]dodecanucleotide was performed in 0.6 M sodium chloride/0.06 M sodium citrate containing 0.02% (wt/vol) each of bovine serum albumin, Ficoll, and polyvinylpyrrolidone. Base-hydrolyzed yeast RNA (83 mg/ml) was used in place of denatured sonicated salmon sperm DNA for hybridizations with [^{32}P]dodecanucleotide.

RESULTS

Preparation of RNA. Total RNA was extracted from hog antral mucosa collected from the pyloric gland area, a region of the stomach previously identified by immunochemical staining as a rich source of gastrin (21). After CsCl gradient centrifugation, 1.5 mg of RNA was recovered per gram of original tissue. The RNA had a 260/280-nm absorbance ratio of 2.1, indicating that protein contamination was minimal, and base hydrolysis and DNase digestion demonstrated that the preparation was substantially free of DNA. Poly(A)-enriched RNA obtained after two cycles of oligo(dT)-cellulose chromatography represented 0.8–1% of the total RNA extracted and ranged in size from about 5 to 12 S as judged by sucrose gradient centrifugation and agarose gel electrophoresis (data not shown).

The RNA preparation was effective in directing protein synthesis in both rabbit reticulocyte and wheat germ cell-free translation systems; however, gastrin-specific peptides could not be identified by antibody precipitation or peptide mapping. The yield of poly(A)-RNA from 150 g of hog antral mucosa was 1.5 mg.

Synthesis and Characterization of Gastrin-Specific cDNA. The dodecanucleotide primer d(C-T-C-C-T-C-C-A-T-C-C-A) was chemically synthesized by using the diester approach (14), and its nucleotide sequence was confirmed by two-dimensional homochromatography (15) as shown in Fig. 1. The dodecanucleotide was labeled with [γ-^{32}P]ATP by using polynucleotide kinase and was used as a primer for reverse transcription of hog antral poly(A)-RNA. Several concentrations of reverse transcriptase and deoxynucleoside triphosphates were tested for efficient synthesis, and the best results were obtained with the triphosphate concentration at 400–600 μM and enzyme at

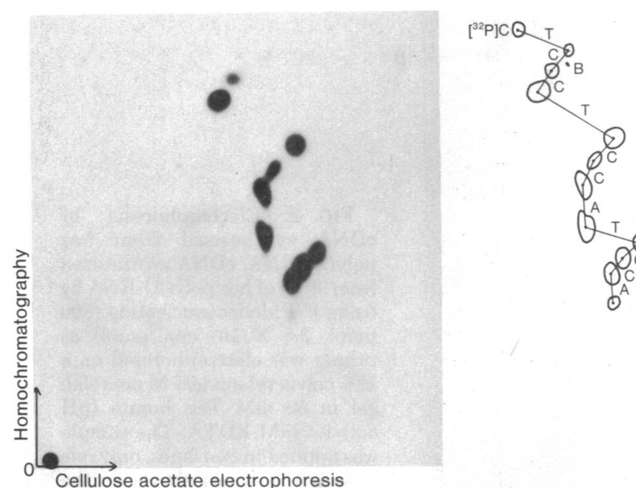

FIG. 1. Autoradiogram of the fingerprint obtained on partial venom phosphodiesterase digestion of the 5′-^{32}P-labeled dodecanucleotide. Oligonucleotide (5 pmol) containing 10 μg of yeast RNA was incubated with 2 μl of snake venom phosphodiesterase at 500 μg/ml in 5 mM Tris·HCl (pH 8.0). After 30 min at 37°C the digest was subjected to two-dimensional fingerprint analysis using homomix-IV of Jay *et al.* (15). The sequence of the dodecanucleotide is derived from the pattern as shown in the reproduction to the right of the fingerprint. B is the blue dye marker.

300–400 units/ml. Terminally labeled cDNA products were separated by gel electrophoresis on a 12% polyacrylamide gel in 7 M urea and visualized by autoradiography as illustrated in Fig. 2. Two prominent bands were reproducibly obtained from three different RNA preparations using two different lots of reverse transcriptase. The sizes of these products are estimated to be (A) 300 and (B) 200 nucleotides as determined from *Hae* III nuclease fragments of single-strand ϕX174 phage DNA used as markers. Minor products also visible in the autoradiogram are probably produced by premature termination of cDNA synthesis or low levels of priming at additional sites.

To determine whether the major cDNA products are synthesized as a result of specific priming of the dodecanucleotide on gastrin mRNA, bands A and B were recovered from the gel and subjected to nucleotide sequence analysis (16). Fig. 3 shows the autoradiogram obtained after electrophoretic separation of the base-specific cleavage products generated from the 300-nucleotide band A. In Fig. 4 the sequence of 81 nucleotides derived from the gel pattern is compared with the amino acid sequence of hog progastrin. Clearly the 300-nucleotide cDNA product (band A) is derived from gastrin mRNA, and the dodecanucleotide is specifically priming the synthesis at the expected position on the mRNA.

Sequence analysis of the 200-nucleotide cDNA product, band B, indicates that it also represents specific priming by the dodecanucleotide (data not shown). However, this product is not derived from a region of the gastrin mRNA for which amino acid sequence data are available. Nor is it obviously derived from a message coding for other proteins whose amino acid sequences are known.

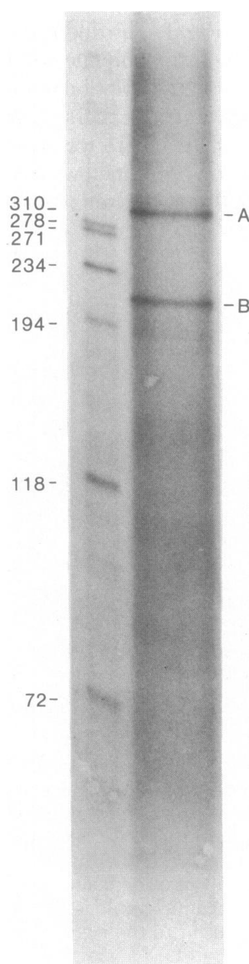

FIG. 2. Electrophoresis of cDNA synthesized from hog poly(A)-RNA. cDNA synthesized from 90 μg of hog poly(A)-RNA by using [^{32}P]dodecanucleotide (800 pmol, 3.5×10^6 cpm/pmol) as primer was electrophoresed on a 12% polyacrylamide/7 M urea slab gel in 50 mM Tris borate (pH 8.3)/1.4 mM EDTA. The sample was applied in two lanes, only one of which is shown above, on the right. The left lane is single-strand ϕX174 DNA digested with restriction endonuclease *Hae* III and end-labeled with [γ-^{32}P]ATP, used for markers of molecular weights (expressed as number of nucleotides). Electrophoresis was at 600 V for 15 hr. Band A cDNA is about 300 nucleotides and band B is about 200 nucleotides long.

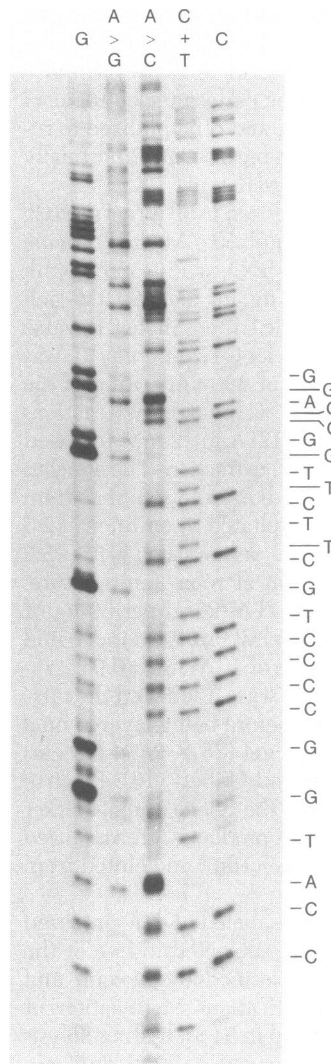

FIG. 3. Nucleotide sequence analysis of gastrin cDNA. 5′-^{32}P-Labeled cDNA (band A) recovered from the gel shown in Fig. 2 was subjected to the five base-specific chemical cleavages described by Maxam and Gilbert (16) and electrophoresed on a $60 \times 24 \times 0.05$ cm slab gel of 20% acrylamide containing 7 M urea. To each gel slot, 8000–10,000 cpm (Cerenkov) was applied in 4–5 μl. Electrophoresis was for 11 hr at 2000 V. Autoradiography was for 4 days at −20°C.

Size Estimation for Gastrin mRNA. To examine in more detail whether both cDNA products are synthesized from the same mRNA and to determine a minimum size for the gastrin mRNA, we carried out hybridization experiments, using the [^{32}P]cDNA products and [^{32}P]dodecanucleotide with hog poly(A)-RNA electrophoresed in the presence of 4 mM CH_3HgOH. As shown in Fig. 5, the 300-nucleotide cDNA (band A), which is synthesized from gastrin mRNA, hybridizes specifically to RNA about 620 nucleotides long. On the other hand, the 200-nucleotide cDNA (band B) hybridizes specifically to an RNA about 930 nucleotides long. [^{32}P]Dodecanucleotide hybridizes only with the 620-nucleotide RNA species. It does not hybridize with the 930-nucleotide species under the conditions used in this experiment. Presumably the dodecanucleotide is partially complementary to the 930-nucleotide RNA and binds to it only when reverse transcriptase is present to stabilize the interaction.

DISCUSSION

The closely related gastrointestinal hormones gastrin and cholecystokinin provide an interesting system for studies of eukaryotic gene expression and evolution. The two hormones possess an identical carboxyl-terminal pentapeptide amide essential for their biological activity, and Larsson and Rehfeld (23) have suggested a common ancestry for the peptides. Gastrin is released from the G cells of the antral mucosa and the

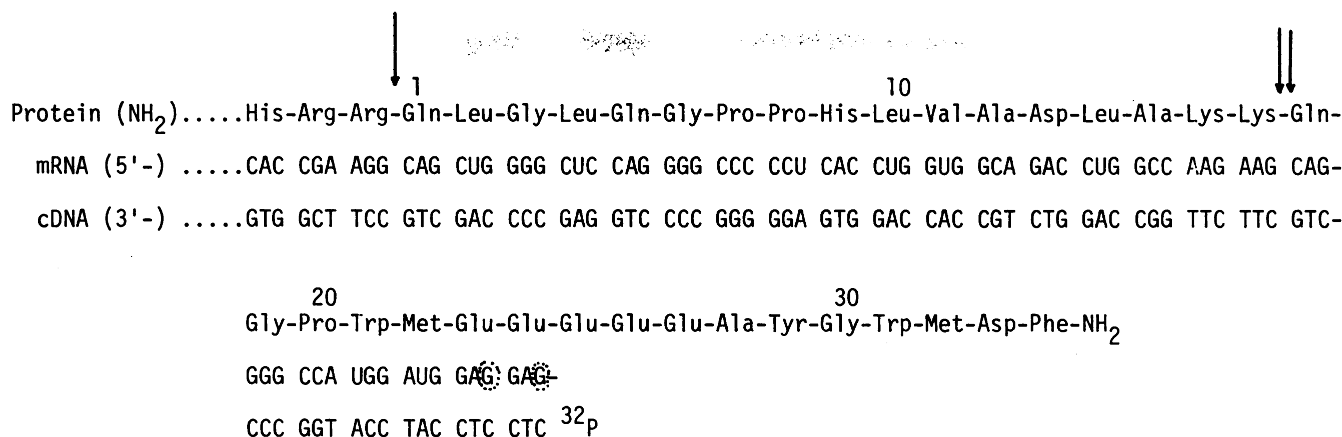

FIG. 4. Comparison of the amino acid sequence of hog progastrin with the nucleotide sequence of its mRNA. The lower line indicates the nucleotide sequence of cDNA (band A) derived from hog antral RNA using [^{32}P]dodecanucleotide as a primer for reverse transcription. The dodecanucleotide sequence is underlined. The mRNA sequence corresponding to this cDNA is given above the actual sequence determined. The circled nucleotides are tentative and represent positions of possible mismatch between the primer and the mRNA. The amino acid sequence derived from the mRNA is depicted in the upper line and corresponds to that previously determined for progastrin (ref. 22; R. A. Gregory, personal communication). On the basis of the nucleotide sequence, three additional amino acids of a preprogastrin are deduced at the amino terminus of progastrin. An arrow indicates the proposed site for trypsin-like cleavage that would generate progastrin from a longer prepropeptide. The cleavage site for conversion of progastrin to gastrin, indicated by double arrows, is similar.

duodenum and stimulates secretion of gastric acid and pepsin [see review by Walsh and Grossman (24)], while cholecystokinin is released from the intestinal mucosa and regulates secretion of pancreatic enzymes and emptying of the gall bladder (25). To investigate the synthesis of these related gastrointestinal hormones and to generate probes for studying the genes responsible for their synthesis, we have begun to develop procedures for the isolation and characterization of their mRNAs. A major consideration in this endeavor is the low level of gastrin- or cholecystokinin-specific mRNA present in cells producing the hormones. Because current methodology lacks the sensitivity required, we have initiated a different approach for the detection of specific mRNAs present at very low levels within a heterogeneous population of RNAs. The data presented above represent initial results with gastrin mRNA, but the approach should apply to other mRNAs for which a nucleotide sequence of sufficient length can be deduced.

To detect gastrin mRNA we synthesized a small oligonucleotide probe whose nucleotide sequence was deduced from the unique amino acid sequence Trp-Met-Glu-Glu of gastrin (see Fig. 4). The sequence of the dodecanucleotide d(C-T-C-C-T-C-C-A-T-C-C-A) could contain a maximum of two mismatched base pairs because there are two possible codons for glutamic acid, GAA and GAG. We selected the codon GAG in designing the gastrin primer because G in the third position is statistically favored for all codons (26), and, in particular cases in which known amino acid sequences have been correlated with determined mRNA sequences, Glu is usually coded for by the triplet GAG (27). If one or both of the glutamic acids in gastrin are coded for by GAA, the synthetic oligonucleotide would carry one or two mismatches located near the 5′ end of the dodecanucleotide. This should not significantly alter the effectiveness of the oligonucleotide as a primer for reverse transcription. As shown in Fig. 5, the chemically synthesized dodecanucleotide does hybridize to a single size class of poly(A)-RNA prepared from hog antral mucosa. In addition, it functions effectively as a primer for specific cDNA synthesis from a mixture of RNAs (see Fig. 2). The results demonstrate clearly that a dodecanucleotide can be successfully used to detect and study a low level of RNA within a heterogeneous population of RNAs. We estimate that gastrin mRNA in preparations from hog antral mucosa represents 0.6% (by weight) or less of the poly(A)-enriched RNA, or less than 0.005% of the total RNA extracted. This estimate is based on the assumptions that: (*i*) although primer is present in large excess over RNA, only 10% of the gastrin mRNA present is used in the synthesis of cDNA, and (*ii*) that gastrin message is about 620 nucleotides in length. If the amount of cDNA synthesized during reverse transcription is actually 50–100% of the mRNA present, the level of gastrin mRNA in hog antral mucosa would be 1/5th to 1/10th of the amount calculated above. Further work is necessary to establish quantitative assays for gastrin mRNA, using both [^{32}P]dodecanucleotide and gastrin cDNA.

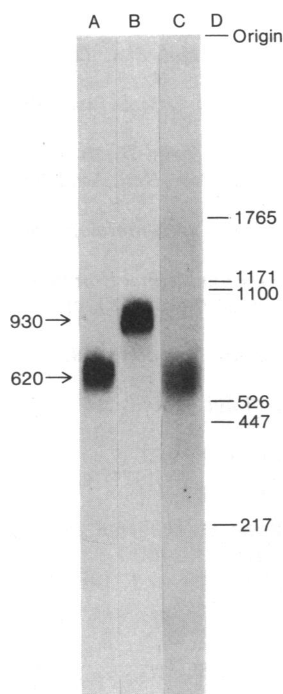

FIG. 5. Hybridization of [^{32}P]cDNA bands A and B and [^{32}P]dodecanucleotide to hog poly(A)-RNA. Poly(A)-RNA from hog antral mucosa (10 μg/lane) was electrophoresed on a 1.5% agarose gel in the presence of 4 mM CH_3HgOH for 15 hr at 40 V (19). The RNA was transferred to diazobenzyloxymethyl-cellulose paper and hybridized to ^{32}P-labeled probes. Lane A shows the results of hybridization with cDNA band A (1×10^5 cpm; 2.5×10^6 cpm/pmol) and lane B, with cDNA band B (1×10^5 cpm; 2.5×10^6 cpm/pmol) isolated from the gel shown in Fig. 2. Lane C shows the result of hybridization with [^{32}P]dodecanucleotide (1×10^5 cpm; 2.5×10^6 cpm/pmol). Simian virus 40 DNA digested with restriction endonuclease *Hin*dIII was run on the same gel and stained with ethidium bromide for use as molecular weight markers. The positions of these fragments are marked in lane D.

It is interesting that in 0.6 M NaCl dodecanucleotide apparently hybridizes to only one RNA, one that is about 620 nucleotides long (Fig. 5). Yet it reproducibly primes the syn-

thesis of cDNA from both 620- and 930-nucleotide RNAs. Sequence analysis indicates that both cDNA products represent specific extensions of the dodecanucleotide. We assume that dodecanucleotide priming on the larger RNA arises from interaction of a non-gastrin-specific RNA and primer that is stabilized in the presence of reverse transcriptase.

The only ambiguities we find in nucleotide sequence analysis of the gastrin cDNA are in the 5′-end region. This may be due to some heterogeneity in the dodecanucleotide primer¶ or to side products generated during the base cleavage reactions. For example, in the experiment shown in Fig. 3 methylation of the guanine-only sample was too extensive so that no large products were visible in the upper portion of the gel (not shown) and minor bands appear at successive positions in the low molecular weight region. Nevertheless, repeated analyses of the 300-nucleotide cDNA have shown unambiguously that this product is complementary to the mRNA coding for gastrin.

The partial nucleotide sequence for gastrin mRNA, which is given in Fig. 4, confirms the amino acid sequence of the porcine G34 intermediate species. Originally amino acid sequence analysis indicated the sequence His-Pro-Pro for residues 7–9 (22). Subsequent work by R. A. Gregory and coworkers (personal communication) established the sequence Pro-Pro-His, which the nucleotide sequence presented here confirms. Larger precursors of gastrin, such as "component I" of Rehfeld (7) and "Big-Big gastrin" of Yalow and Berson (5) have been proposed, but have not been isolated or characterized. The nucleotide sequence presented here predicts that the 34 amino acid progastrin intermediate, which possesses an amino-terminal pyroglutamyl residue, is generated from a still larger peptide through trypsin-like cleavage at the Arg-Arg-Gln sequence with subsequent cyclization of the glutaminyl residue (see Fig. 4). This is similar to the processing proposed for other peptide hormones such as insulin, glucagon, β-lipotropin, and parathyroid hormone (28), and parallels the process proposed for conversion of progastrin to gastrin by cleavage at Lys-Lys-Gln (residues 16–18 of progastrin, Fig. 4) (22).

Although the gastrin-specific cDNA generated by using the synthetic dodecanucleotide hybridizes to an RNA about 620 nucleotides long, the cDNA is only 300 nucleotides long. This cDNA is obtained reproducibly as the major product of reverse transcription and thus may be a near complete copy of the gastrin mRNA from the priming site through the 5′ end of the molecule. Because fewer than 100 nucleotides are required to code for the 34 amino acid progastrin, at least 200 nucleotides remain at the 5′ end of the mRNA. Thus the coding capacity of the gastrin message is sufficient to code for a precursor peptide with about 50 additional amino acids at the amino terminus of progastrin. Moreover, the data indicate that approximately 250–300 nucleotides of the gastrin mRNA are 3′ to the region coding for the carboxyl terminus of the gastrin peptide. It is thus possible that gastrin is first synthesized as a large peptide that is processed at both the amino and carboxyl ends to generate active hormone. Recently Suchanek and Kreil (9) reported that melittin, which terminates in Gln-Gln-NH_2, may be generated from a peptide with carboxyl-terminal Gln-Gln-Gly via a transamidase-like reaction. Gastrin, which possesses a carboxyl-terminal Phe-NH_2, could be produced in a similar fashion. Certainly the size of the gastrin mRNA is consistent with this possibility, but further nucleotide sequence analysis toward the 3′ end of the message is needed. This should be possible through molecular cloning of the hog cDNA, because gastrin-specific cDNA or dodecanucleotide can now be used for screening clones.

¶ High-pressure liquid chromatography on μ-bonded C_{18} silica gel (Waters Assocs., Milford, MA) indicates that the dodecanucleotide is >95% homogeneous.

This work is dedicated to the late Prof. G. W. Kenner, who made major contributions towards our understanding of the chemistry of gastrin.

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