Molecular cloning, purification and *in situ* localization of human colon kallikrein

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We have cloned and characterized a full-length cDNA encoding tissue kallikrein from a human colon carcinoma cell line (T84). The nucleic acid sequence of the colon kallikrein cDNA is identical to that of renal/pancreatic or tissue kallikrein cDNA. Reverse-transcription PCR followed by Southern-blot analysis using specific oligonucleotide probes showed expression of tissue kallikrein in human colon, pancreas and kidney. Tissue kallikrein mRNA was localized in glandular epithelial cells (goblet cells) in colon by *in situ* hybridization histochemistry. Human colon kallikrein was purified to apparent homogeneity by DEAE-Sepharose Cl-6B, aprotinin-affinity, and HQ/M perfusion chromatography. The purified colon kallikrein migrated as a broad, 40–45 kDa band in SDS/PAGE and was recognized by antibodies to human tissue kallikrein. The linear displacement curves for the colon kallikrein in an RIA were parallel with the human tissue kallikrein standard curve, indicating their immunological identity. The N-terminal sequence of the purified colon kallikrein matches completely with that of purified urinary or tissue kallikrein. These results indicate that human colon kallikrein is transcribed from the tissue kallikrein gene.

INTRODUCTION

Tissue kallikreins (EC 3.4.21.35) are a group of highly homologous serine proteinases with different substrate specificities. The well-recognized biological function of tissue kallikrein is to release vasoactive kinins from kininogen substrates. Tissue kallikreins represent the products of large families of tandemly arranged genes in the rat and mouse (Mason et al., 1983; Ashley and MacDonald, 1985; Gerald et al., 1986; Wines et al., 1989). The size of the kallikrein gene family varies from species to species: three genes have been identified in the human, 13 in the rat, and 25 in the mouse (MacDonald et al., 1988; Murray et al., 1990; Berg et al., 1992). The three characterized human kallikrein-like genes, *hKLK1*, *hKLK2*, and *hKLK3*, are clustered on the long arm of chromosome 19q13.2-13.4 (Riegman et al., 1992). The *hKLK1* gene encodes a trypsin-like kininogenase (true tissue kallikrein), which is expressed in the submandibular gland, pancreas and kidney (Evans et al., 1988). The *hKLK2* gene (*hGK1*), whose mRNA has been detected in the prostate gland, may encode another trypsin-like proteinase (Schedlich and Bennetts, 1987); however, its encoded protein has not been isolated. The *hKLK3* gene encodes a chymotrypsin-like enzyme (prostate-specific antigen, PSA), which is expressed in the prostate gland (Chapdelaine et al., 1988). Kallikreins are expressed in tissue-specific patterns and are known to be regulated at both transcriptional and post-translational levels (reviewed in MacDonald et al., 1988 and Murray et al., 1990).

Tissue kallikrein-like activities in the gastrointestinal tract have been described in the rat, cat and human (reviewed in Clements, 1989). In these species tissue kallikrein has been localized in the goblet cells of the colon by immunocytochemical techniques (Schachter et al., 1983, 1986). It has been suggested that gastrointestinal kallikrein could be of pancreatic or submaxillary origin (Skagen and Andersen, 1986). In the rat, tissue kallikrein has been shown to be expressed in the colon by Northern-blot analysis (Fuller et al., 1989). In the human, it has been demonstrated by RIA that colon kallikrein cross-reacts with human urinary kallikrein (HUK) (Zimmermann et al., 1979). Although recent studies using an S1 nuclease digestion assay (Baird et al., 1991) showed that a human colon epithelial carcinoma cell line (T84) produces tissue kallikrein, its true identity in normal human colon remains to be established.

The function of tissue kallikrein in colon is not known. It has been suggested that colon kallikrein may serve as a processing enzyme for mucoproteins (Schachter et al., 1983). Previous studies have shown that kinins stimulate ion transport across the epithelium of the gastrointestinal system (Cuthbert and Margolius, 1982; Cuthbert and MacVinish, 1986). Clements (1989) suggested that gastrointestinal kallikrein may be involved in the regulation of local blood flow or chloride ion transport. In order to better understand the role of colon kallikrein, it is important to establish the identity of kallikrein in human colon.

In this study, we have purified and sequenced colon kallikrein, cloned its cDNA and localized its mRNA by *in situ* hybridization. Sequence analysis of the protein and its cDNA indicates that it is encoded by the tissue kallikrein gene (*hKLK1*). Cellular localization showed that colon tissue kallikrein mRNA is localized in glandular epithelial cells (goblet cells).

MATERIALS AND METHODS

Total RNA extraction

Human tissues were minced and homogenized in GIT solution (4 M guanidine isothiocyanate, 0.12 M 2-mercaptoethanol, 0.025 M sodium acetate, pH 6.0) for 1 min with a polytron homogenizer. Human colonic adenocarcinoma cells (T84, Baird et al., 1991) were washed three times with diethyl pyrocarbonate (DEP)-treated PBS (10 mM sodium phosphate, pH 7.0, 0.14 M NaCl) and lysed by adding GIT solution directly to the cell culture flask. The colon homogenate and the cell lysate were ultracentrifuged at 174 000 *g*, at 20 °C for 21 h over a CsCl

Abbreviations used: DEAE, diethylaminoethyl; AFC, 7-amino-4-trifluoromethylcoumarin; DEP, diethylpyrocarbonate; NBT, Nitroblue Tetrazolium salt; X-phosphate, 5-bromo-4-chloro-3-indolyl-phosphate; PSA, prostate-specific antigen; RT-PCR, reverse-transcription PCR; HUK, human urinary kallikrein; BLOTTO, 5% (w/v) non-fat dry milk in 10 mM sodium phosphate (pH 7.4)/0.14 M NaCl/1 μM *p*-phenylmethanesulphonyl fluoride/1 mg/l thimerosal/0.01% (v/v) Antifoam A.

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solution (5.7 M CsCl, 0.025 M sodium acetate, pH 6.0). The resulting RNA pellet was dissolved in DEP-treated water and the RNA concentration was determined spectrophotometrically by absorbance at 260 nm.

Oligonucleotide primers

Oligonucleotides were synthesized using an automated DNA synthesizer. The specific primers were designed according to the human tissue kallikrein gene sequence (Fukushima et al., 1985). The sequences of these primers are identical to the tissue kallikrein cDNA sequence, but are substantially mismatched to *hKLK2* (*hGK1*) and *hKLK3* (PSA) sequences. These primer sequences are specific for tissue kallikrein and do not anneal to either *hKLK2* or *hKLK3* under our test conditions. The upstream and downstream primers for amplification of a full-length tissue kallikrein cDNA were: 5′-AGT TCC TCC ACC TGC TG-3′ and 5′-CAC GGA TCC ACA TTT GAT TTT-3′ respectively. Bases 1, 2, 3 and 8 in the downstream primer, which are different from the published sequence, were introduced to create a *Bam*HI site for cloning (for other studies). The upstream and downstream primers for amplification of the tissue kallikrein cDNA fragment in the tissue distribution analysis were: 5′-CAT TTC AGC ACT TTC CA-3′ (exon 2, bases 166–182) and 5′-GCC ACA AGG GAC GTA GC-3′ (exon 5, bases 728–744) respectively. The oligonucleotide used for probing the Southern blot of the RT-PCR product had the sequence 5′-ACG ACC TTC ACA GAG TC-3′ (exon 3, bases 439–455).

RT-PCR

The first strand of the cDNA was synthesized using the Murine Leukaemia Virus reverse transcriptase (GIBCO/BRL, Gaithersburg, MD, U.S.A.) in a 20-μl reaction mixture containing 1 × reverse transcription buffer (50 mM Tris/HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$), 1 mM of each deoxynucleoside triphosphate (dNTP), 0.4 μM downstream primer, 0.01 M dithiothreitol, and 1 μg of total RNA. The reaction mixture was incubated at 37 °C for 60 min, then heated at 95 °C for 5 min and chilled on ice. The subsequent PCR reaction was performed using the 'hot start' method with Ampliwax according to the manufacturer's instructions (Perkin-Elmer Cetus Corp., Norwalk, CT, U.S.A.). The 50-μl reaction mixture contained the total product of the reverse-transcription, 1 × PCR buffer [10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001 % (w/v) gelatin (Sigma)], 0.4 μM upstream primer and 2.5 units of AmpliTaq DNA polymerase (Perkin-Elmer Cetus Corp.). The amplification was carried out in a GeneMachine II apparatus (USA/Scientific Plastics, Ocala, FL, U.S.A.) with the following cycling program: 30 cycles of 1 min at 94 °C, 2 min at 55 °C, and 3 min at 72 °C followed by a 5 min extension at 72 °C.

Southern-blot hybridization

Blot analysis was performed by the method of Southern (1975). A portion of the RT-PCR products was resolved in a 1.2 % agarose gel in 1 × TAE buffer (0.05 M Tris/HCl, pH 8.0, 0.02 M sodium acetate, 0.002 M Na_2EDTA). The DNA fragments were transferred to Immobilon-N membranes (Millipore Corporation, Bedford, MA, U.S.A.) via capillary action according to the method of Maniatis et al. (1982). After UV cross-linking (Hoefer Scientific Instruments, San Francisco, CA, U.S.A.), the filters were prehybridized in a solution containing 5 × SSPE (20 × SSPE = 3 M NaCl, 0.2 M sodium phosphate, pH 7.4, 20 mM Na_2EDTA), 0.5 % SDS, 5 × Denhardt's solution (50 × Denhardt's solution = 1 % Ficol, 1 % polyvinylpyrrolidone, 1 % BSA), and 0.1 mg/ml herring sperm DNA and then hybridized at 42 °C for 18 h in the same solution with an oligonucleotide probe, which was end-labelled with [γ-^{32}P]ATP (New England Nuclear, Boston, MA, U.S.A.). After hybridization, the filters were washed to a final stringency of 6 × SSC (10 × SSC = 3 M NaCl, 0.3 M sodium citrate, pH 7.0) at 42 °C, and exposed to Kodak AR film at −80 °C.

Cloning of a full-length tissue kallikrein cDNA from T84 cells

A full-length cDNA encoding tissue kallikrein was generated by RT-PCR from T84 cell RNA. The PCR product was analysed by agarose gel electrophoresis and visualized after ethidium bromide staining. The DNA was gel purified, phosphorylated, and cloned into the pSP73 vector through blunt-ended ligation (Maniatis et al., 1982). Positive clones were selected by colony hybridization (Maniatis et al., 1982) using a monkey kallikrein cDNA probe (Murray et al., 1990; Lin et al., 1993) and were confirmed by DNA sequencing.

Synthesis of antisense and sense riboprobes

A human tissue kallikrein cDNA fragment (186 bp, 686–871) was cloned into the pSP73 transcription vector, which contains promoters for SP6 and T7 RNA polymerases adjacent to the cloning site. After the linearization of the template DNA, RNA polymerases were used to create 'run off' transcripts using a RNA Labelling Kit (Boehringer-Mannheim Corporation, Indianapolis, IN, U.S.A.) according to the manufacturer's instruction. Digoxigenin-UTP was incorporated into the transcripts during the *in vitro* transcription.

In situ hybridization histochemistry

Sections (5 μm) of paraffin-embedded human colon tissue were cut and mounted on gelatin-coated slides. For the hybridization, colon sections were deparaffinized with xylene, rehydrated through a series of decreasing ethanol solutions, digested with 5 μg/ml proteinase K and dehydrated through a series of increasing ethanol solutions. Sections were incubated with a labelled antisense tissue kallikrein riboprobe in a hybridization buffer containing 0.3 M NaCl, 20 mM Tris/HCl, pH 8.0, 5 mM EDTA, 50 % formamide, 5 × Denhardt's solution, 10 % dextran sulphate, 100 μg/ml of herring sperm DNA and yeast tRNA. Hybridization was carried out at 42 °C overnight in a humid chamber. After hybridization, the colon sections were washed to a final stringency of 0.2 × SSC at room temperature and then were processed by immunodetection using a Nucleic Acid Detection Kit (Boehringer-Mannheim Corporation). Briefly, the sections were blocked in buffer A (100 mM Tris/HCl, 150 mM NaCl, pH 7.5) containing 2 % (v/v) normal sheep serum and 0.3 % Triton X-100. Diluted anti-digoxigenin antibody conjugate (1:500) was applied to the sections and incubated in a humid chamber for 3–5 h. The colour reaction was carried out by incubating the sections with the cocktail solution containing 4.5 μl of Nitroblue Tetrazolium salt (NBT), 3.5 μl of 5-bromo-4-chloro-3-indolylphosphate (X-phosphate), and 0.24 mg of levamisole in 1 ml of buffer B (100 mM Tris/HCl, 100 mM NaCl, 50 mM $MgCl_2$, pH 9.5). The control sections were either incubated with a labelled sense riboprobe or pretreated with RNase A before incubating with a labelled antisense riboprobe.

Tissue extraction and protein determination

Normal human colon was minced and homogenized in 0.9 % NaCl using a polytron homogenizer. The homogenate was

centrifuged at 600 *g* for 20 min at 4 °C. Sodium deoxycholate (Sigma) was added to the supernatant to a final concentration of 0.5% (w/v). The solution was incubated at room temperature for 30 min followed by centrifugation at 20000 *g* at 4 °C for 30 min. The supernatant was collected and dialysed against 10 mM Tris/HCl, pH 8.0, containing 1 mM EDTA. The protein concentration was determined according to Lowry et al. (1951) using BSA as the standard.

Purification of human colon kallikrein

The dialysed human colon extract was applied to a DEAE-Sepharose Cl-6B column (2.2 cm × 15 cm) equilibrated with 10 mM Tris/HCl, pH 8.0, containing 1 mM EDTA. After washing with the same buffer, elution was performed with a linear salt gradient (0–500 mM NaCl; 1200 ml) in the same buffer at a flow rate of 25 ml/h and 8.0 ml fractions were collected. Kallikrein activity of each fraction was monitored by measuring the cleavage of a synthetic kallikrein substrate, Z-Phe-Arg-AFC (Sigma) (Morita et al., 1977). Fractions with kallikrein activity were pooled, concentrated with polyethylene glycol compound (Sigma), dialysed against 10 mM Tris/HCl, pH 8.0, and applied to an aprotinin-affinity column (Sigma) which was equilibrated with the same buffer. The column was washed with 10 mM Tris/HCl, pH 8.0, containing 0.1 M NaCl and the bound protein was eluted with 0.1 M glycine/HCl, pH 3.0, at a flow rate of 45 ml/h. The sample in each fraction was neutralized with Tris base. The fractions containing kallikrein activity were combined, concentrated and desalted with a Centricon concentrator (Amicon, Beverly, MA, U.S.A.). The concentrated protein was further purified through an HQ/M anion-exchange column (4.6 mm × 100 mm) by perfusion chromatography (PerSeptive Biosystems, Cambridge, MA, U.S.A.). The HQ/M column was equilibrated with 10 column vol. of 20 mM Tris/Bis-Tris propane at pH 8.0 followed by sample loading and washing with 5 column vol. of the same buffer at a flow rate of 10 ml/min. Protein was eluted with the same buffer over a linear NaCl gradient from 0–600 mM. The peak fraction containing kallikrein activity was identified using Z-Phe-Arg-AFC.

Western-blot analysis

Samples were analysed on SDS/PAGE and then electrotransferred to Hybond-ECL nitrocellulose membranes (Amersham, Arlington Heights, IL, U.S.A.). The immunoblotting procedures were carried out using the Enhanced Chemiluminescence (ECL) Western Blotting Kit (Amersham) with the following modifications. The membrane was blocked with BLOTTO for 1 h at room temperature and then incubated with rabbit anti-HUK (Shimamoto et al., 1980) diluted 1:1000 in BLOTTO for 1 h. After washing with three changes of BLOTTO for 30 min, the membrane was incubated with a 1: 1000 dilution (in BLOTTO) of horseradish peroxidase-conjugated secondary antibody for 30 min. The membrane was washed a second time with three changes of BLOTTO for 30 min and then with TBST (20 mM Tris/HCl, pH 7.6, 0.14 M NaCl, 0.02% Tween-20) for 5 min. Detection was performed by overlaying the membrane with a luminol-based detection reagent for 1 min, blotting the excess reagent and exposing the membrane to Kodak X-Omat film at room temperature for 15–30 s.

N-terminal sequence analysis

Purified kallikrein was first subjected to SDS/PAGE under reducing conditions and then electrotransferred to a poly(vinylidene difluoride) membrane. The transferred protein was stained with 0.2% (w/v) Ponceau S in 3% (v/v) trichloroacetic acid for 1 min and destained with distilled water for 2 min. A single band was cut out and subjected to N-terminal sequence analysis using a gas-phase protein sequencer equipped with an on-line narrow-bore phenylthiohydantoin-amino acid analyser (ABI model 470A, Applied Biosystems Inc.).

HUK RIA

Purified HUK was labelled with ^{125}I using the Iodogen (Pierce) method. RIA was carried out according to the protocol of Shimamoto et al. (1980). Briefly, standard purified HUK (8–1000 pg), colon extract (1:100–1:800 dilution), and purified colon kallikrein (1:8000–1:128000 dilution) in a total volume of 100 μl were mixed with 200 μl of rabbit anti-HUK (1:400000 dilution) and 10000 c.p.m. of ^{125}I-HUK in 100 μl of PBS containing 1% BSA. Following incubation, antibody-bound kallikrein was precipitated with polyethylene glycol and separated from free kallikrein by centrifugation.

RESULTS

Cloning, characterization and expression of tissue kallikrein cDNA in human colon

A cDNA fragment corresponding to the full-length tissue kallikrein cDNA was generated from T84 colonic tumour cell RNA by means of RT-PCR. The DNA band was gel-purified and cloned into the pSP73 vector. Sequence analysis of the positive clone showed that it is identical to the renal/pancreatic kallikrein cDNA (Evans et al., 1988). The full-length colon kallikrein cDNA encodes the complete protein of tissue kallikrein which contains the catalytic triad, His, Asp and Ser. Expression of the colon kallikrein transcript was analysed by Southern blotting following RT-PCR as shown in Figure 1 (lanes 3 and 4). PCR products generated by two kallikrein-specific oligo-primers were probed by a third kallikrein-specific oligo-probe. Expression of the human tissue kallikrein gene in the pancreas (lane 1) and kidney (lane 2) is consistent with the previous report (Evans et al., 1988). Human tissue kallikrein expression was not detected in the liver (data not shown).

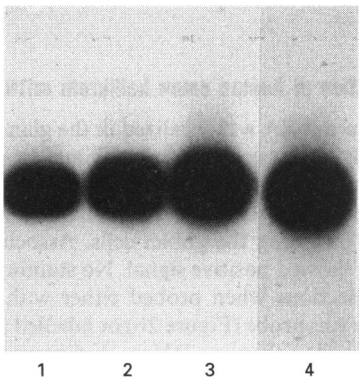

Figure 1 Expression of tissue kallikrein mRNA determined by RT-PCR–Southern blot analysis

Southern blotting was carried out in 5 × SSPE containing 0.5% SDS, 5 × Denhardt's solution, 100 μg/ml herring sperm DNA at 42 °C for 18 h with ^{32}P-end-labelled oligonucleotide probes. The blots were washed to a final stringency of 6 × SSC at 42 °C before autoradiography at −80 °C for 16 h. One μg of total cellular RNA was used in a 50 μl RT-PCR reaction mix and a portion of the PCR products was loaded on the agarose gel: lane 1, pancreas (3 μl); lane 2, kidney (15 μl); lane 3, colon (15 μl); lane 4, T84 (15 μl).

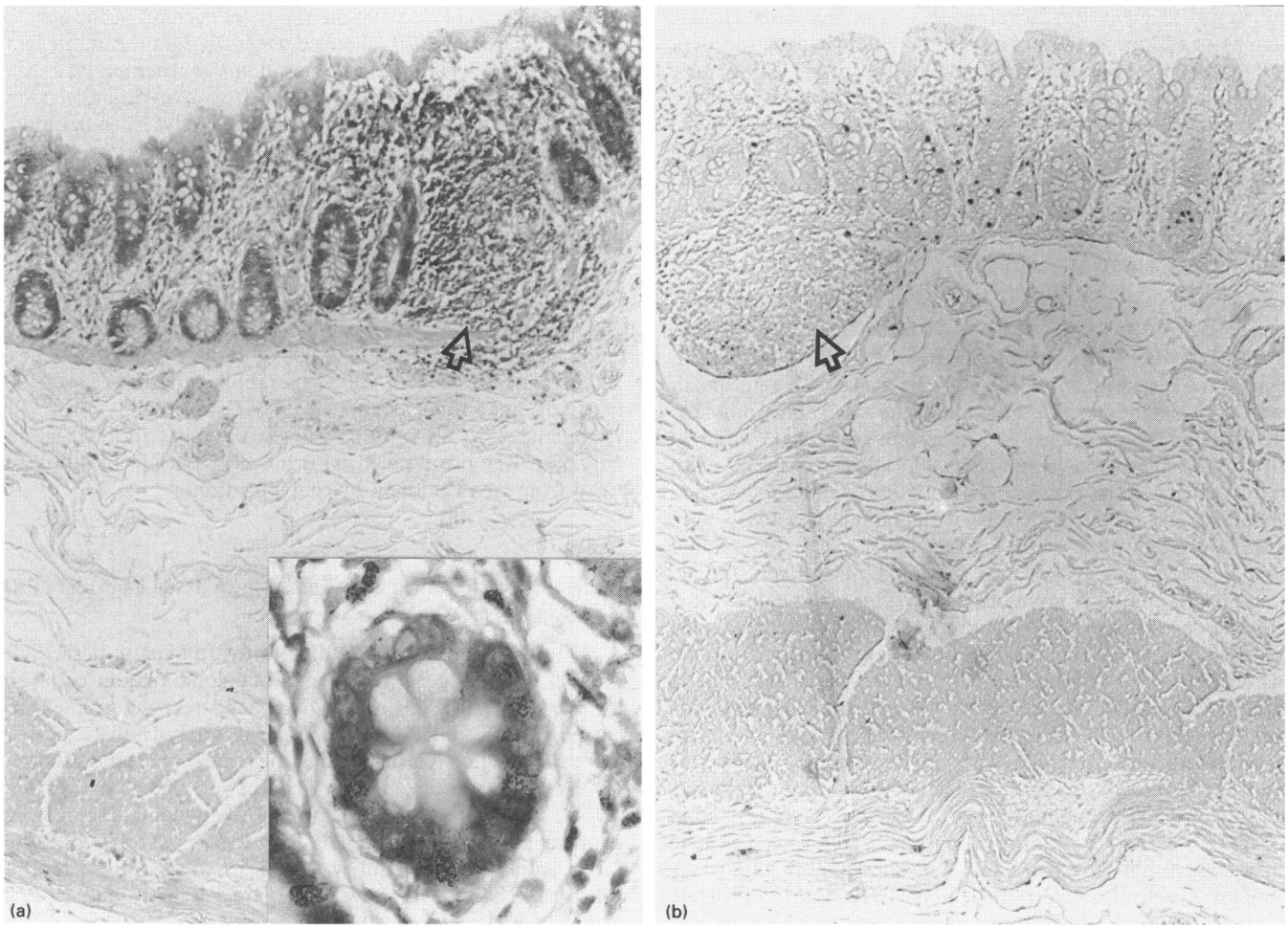

Figure 2 Cellular localization of tissue kallikrein mRNA in human colon by *in situ* hybridization histochemistry

Paraffin-embedded human colon sections (5 μm in thickness) were hybridized with a digoxigenin-labelled tissue kallikrein riboprobe. After hybridization, the labelled probe was detected by incubating with sheep anti-digoxigenin Fab fragments conjugated to alkaline phosphatase followed by colour detection with NBT and X-phosphate. (**a**) Colon section probed with a labelled antisense tissue kallikrein riboprobe (50 × magnification). Tissue kallikrein mRNA is seen in glandular epithelial cells (most likely goblet cells). Positive staining was also observed in lymphatic aggregates (arrow). Insert shows cellular distribution of the message in the cytoplasm of some goblet cells of the glands (150 × magnification). (**b**) Colon section probed with a labelled sense tissue kallikrein riboprobe, showing no localization in goblet cells or lymphatic aggregates (50 × magnification).

Cellular localization of human colon kallikrein mRNA

Colon kallikrein mRNA was localized in the glandular epithelial cells, most likely goblet cells, in the neck and base of the crypts in colon using a digoxigenin-labelled antisense tissue kallikrein riboprobe (Figure 2a). Less staining was seen in the surface epithelial cells, including the goblet cells. Associated lymphatic aggregates also showed positive signal. No staining was observed in the control sections when probed either with labelled sense tissue kallikrein riboprobe (Figure 2b) or labelled antisense tissue kallikrein riboprobe following RNase A digestion.

Purification and immunoreactivity of human colon kallikrein

The final stage in the colon kallikrein purification involved perfusion chromatography through an HQ/M anion-exchange column. Results demonstrated a broad peak eluting at 300–450 mM NaCl which was subsequently identified as colon kallikrein. The purified colon kallikrein was subjected to SDS/PAGE and Western-blot analysis. Figure 3 shows that the

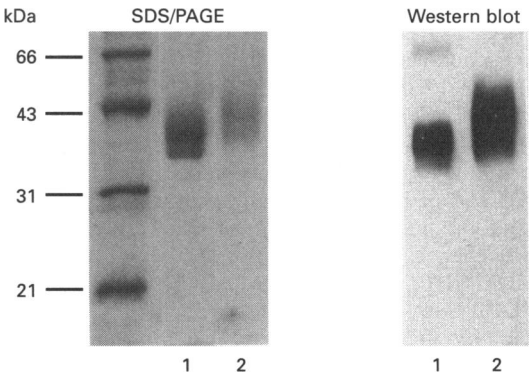

Figure 3 SDS/PAGE and immunoblot

Samples of HUK and purified human colon kallikrein were resolved on an SDS/10%-polyacrylamide gel. Lane 1, HUK; lane 2, colon kallikrein. Left-hand panel: SDS/PAGE followed by protein staining with Coomassie Blue; right-hand panel: SDS/PAGE followed by Western blotting with polyclonal antibodies against HUK.

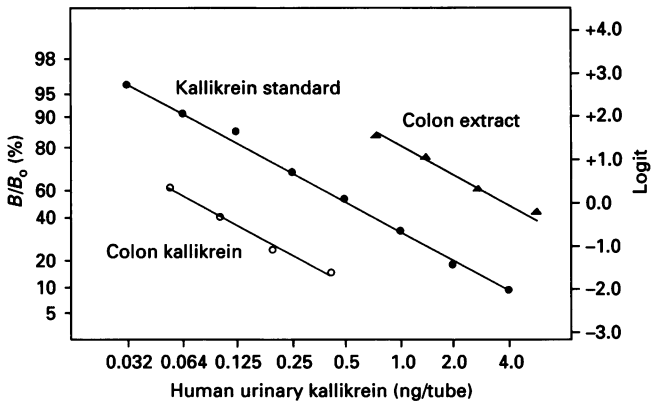

Figure 4 Log-logit transformation of HUK RIA

Serial dilutions of HUK standard (●), human colon extract (▲), and purified human colon kallikrein (○) are parallel to each other, indicating their immunological identity. B/B_0 is the percentage of the bound radioactivity in the presence and absence of unlabelled human tissue kallikrein using rabbit anti-(human tissue kallikrein) serum.

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1  M  W  F  L  V  L  C  L  A  L  S  L  G  G  T  G
2 ATG TGG TTC CTG GTT CTG TGC CTC GCC CTG TCC CTG GGG GGG ACT GGT

1  A  A  P  P  I  Q  S  R↓ I  V  G  G  W  E  C  E
2 GCT GCG CCC CCG ATT CAG TCC CGG ATT GTG GGA GGC TGG GAG TGT GAG
3                          I  V  G  G  W  E  C  E

1  Q  H  S  Q  P  W  Q  A  A  L  Y  H  F  S  T  F
2 CAG CAT TCC CAG CCC TGG CAG GCG GCT CTG TAC CAT TTC AGC ACT TTC
3  Q  H
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Figure 5 Sequence alignment

The alignment shows the colon kallikrein cDNA sequence (line 2) representing exon 1 and part of exon 2; the deduced amino acid sequence from the colon kallikrein cDNA (line 1); and the N-terminal amino acid sequence of the purified colon kallikrein (line 3, bold). The arrow (↓) indicates the cleavage site between signal peptide and mature peptide.

purified kallikrein migrates as a broad band corresponding to approx. 40–45 kDa on an SDS/polyacrylamide gel under reducing conditions (lane 2, left panel) and it was recognized by antibody against HUK in Western-blot analysis (lane 2, right panel). Purified HUK is shown in lane 1. Figure 4 shows a log-logit transformation of an RIA standard curve of human tissue kallikrein and serial dilutions of colon extract and purified colon kallikrein. Linear displacement curves of purified colon kallikrein and colon extract are parallel to the HUK curve, indicating their immunological identity. Furthermore, the assay indicates that the crude colon extract contains 48.6 ng of immunoreactive kallikrein/mg of protein.

N-terminal sequence analysis of human colon kallikrein

Figure 5 shows the sequence alignment of the 5′ region of the human colon kallikrein cDNA (2), its deduced amino acid sequence (1), and the N-terminal amino acid sequence of the purified colon kallikrein (3). N-terminal sequence analysis of 10 amino acids of the colon kallikrein gives IVGGWECEQH, which is identical to that of the purified HUK or tissue kallikrein gene product (Lu et al., 1989; Lin et al., 1993).

DISCUSSION

This is the first study to describe the purification, molecular cloning and mRNA localization of human colon kallikrein. Purified colon kallikrein exhibits properties similar to those of tissue kallikrein with respect to molecular mass, immunological characteristics, and ability to cleave a synthetic peptidyl substrate for kallikrein. A comparison of the translated amino acid sequence of the full-length cDNA encoding human colon kallikrein and the N-terminal sequence of the purified colon kallikrein shows that colon kallikrein is the product of the human renal/pancreatic kallikrein gene (*hKLK1*). Ten amino acid residues from the N-terminus are sufficient to distinguish the kallikrein gene family members and the N-terminal residues of colon kallikrein confirms the identity of colon kallikrein as tissue kallikrein.

Cellular localization of tissue kallikrein mRNA in colon was identified by *in situ* hybridization histochemistry. Tissue kallikrein mRNA was localized in the glandular epithelial cells (most likely goblet cells) in colon (Figure 2a). This result is in agreement with the findings of Schachter et al. (1983), who showed that immunoreactive tissue kallikrein was localized in the goblet cells of the glandular mucosa of human colon. Thus far, only three human tissue kallikrein genes have been identified and two of these genes, *hKLK3* (PSA) and *hKLK2* (*hGK1*), were found only to be expressed in the prostate (Sutherland et al., 1988; Morris, 1989). The signals detected by the tissue kallikrein antisense riboprobe in the colon by *in situ* hybridization is attributed to tissue kallikrein mRNA but not to PSA or *hGK1* mRNA. Epithelial cells of the crypts (absorptive or columnar cells, goblet cells and enteroendocrine cells) originate from undifferentiated stem cells. As columnar cells and goblet cells migrate up the crypt wall and then on to the surface between crypt openings, the older cells are removed from the epithelial surface. The whole process takes approx. 6 days. Less tissue kallikrein mRNA staining in the surface epithelial cells may be due to a reduced expression of the tissue kallikrein gene or a higher rate of degradation of the expressed mRNA in senescent epithelial cells. Several physiological functions have been proposed for kallikrein in colon such as cytoprotection (Schachter et al., 1983) and transportation of electrolytes (Cuthbert and Margolius, 1982) or various molecules across the epithelium (Schachter, 1980). Co-localization of kininogens, the natural substrates of tissue kallikrein, in glandular epithelial cells in colon would provide further insight into the physiological functions of tissue kallikrein in colon.

We have also shown that the tissue kallikrein transcript is translated into an active protein in colon. The purified colon kallikrein migrated as a broad band corresponding to approximately 40–45 kDa on SDS/PAGE and was recognized by anti-HUK antibody in Western blot and in a specific RIA for human tissue kallikrein. The broad protein-banding pattern of human colon kallikrein (Figure 3, lane 2, left panel) is similar to that of HUK (Figure 3, lane 1, left panel) on SDS/PAGE and is probably due to differential glycosylation (Lu et al., 1989). Furthermore, the size of newly purified colon kallikrein is slightly larger than that of human urinary (renal) kallikrein. The difference in size between colon and renal kallikrein is also probably the result of varying degrees of glycosylation in different tissues.

In conclusion, our studies provide evidence that human tissue kallikrein is expressed and synthesized in the colon. The present study will pave the way for further analysing the regulation of colon kallikrein gene expression and its physiological function.

Human colon tissue and paraffin sections were obtained from the Department of Pathology, Medical University of South Carolina. T84 cells were obtained from Dr. Donald H. Miller, Department of Pharmacology, Medical University of South Carolina. We thank Dr. Jo Ann V. Simson and Dr. Carmelann Zintz for their helpful advice and criticisms of the manuscript. This work was supported by National Institutes of Health grants HL 29397 and DE 0973.

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Received 27 May 1994/1 December 1994; accepted 9 December 1994

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